

# Role of *H. pylori* eradication therapy success on five year dynamics of dysplasia

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## **European Helicobacter Study Group XXIVth International Workshop on Helicobacter and related bacteria in chronic digestive inflammation and gastric cancer**

Dublin, September 11–13, 2011

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**Contents****Workshops**

WS1 Gastric Cancer	79
WS2 Inflammation	81
WS3 Immunity and Extragastric diseases	83
WS4 Paediatrics	85
WS5 Clinical Aspect, Drug Resistance	87
WS6 Microbiology, Molecular Pathology, Virulence Factors	89

**Posters**

P01 Gastric Cancer, Pathology and pathophysiology, Preneoplastic and neoplastic diseases	91
P02 Microbiology, Molecular genetics and genomics	96
P03 Virulence factors and pathogenesis	100
P04 Epidemiology and Transmission	104
P05 Paediatric Issues	109
P06 Diagnosis	112
P07 Drug resistance	117
P08 Inflammation and Host Response	123
P09 Extragastric and Hepatobiliary Diseases	127
P10 Clinical trials & NSAIDs	131
P11 Immunity, Animal Models, Vaccines, Probiotics, and Other Helicobacters	139

# Workshop Presentations

## WS1 Gastric Cancer

Abstract no.: WS1.1

### HELICOBACTER PYLORI INFECTION AND MARKERS OF GASTRIC CANCER RISK IN ALASKA NATIVE PEOPLE

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**Background:** Alaska Native gastric cancer incidence and mortality rates are 3 to 4-times higher than general US population rates. We evaluated pepsinogen I, pepsinogen I/II ratio, anti-*H. pylori* and CagA antibodies, and blood group to determine their association with gastric cancer development in Alaska Native people.

**Methods:** We conducted a retrospective case-control study that matched gastric cancers reported to the Alaska Native Tumor Registry from 1969–2008 to three controls on known demographic risk factors for *H. pylori* infection, using previously collected sera from the Alaska Area Specimen Bank. Conditional logistic regression evaluated the associations between serum markers and gastric cancer.

**Results:** We included 122 gastric cancer cases with sera predating cancer diagnosis (mean = 13 years) and 346 matched controls. One hundred and twelve cases (91.8%) and 285 controls (82.4%) had evidence of previous or ongoing *H. pylori* infection as measured by anti-*H. pylori* antibodies. Gastric cancer cases had 2.63-fold increased odds of positive anti-*H. pylori* antibodies compared with their matched controls ( $p = .01$ ). In a multivariate model, non-cardia gastric cancer ( $n = 94$ ) was associated with anti-*H. pylori* antibodies (adjusted OR 3.92,  $p = .004$ ) and low pepsinogen I (aOR 6.04,  $p = .04$ ). We found no association between gastric cancer and blood group, anti-CagA antibodies, or pepsinogen I/II ratio.

**Conclusions:** Alaska Native people with gastric cancer had increased odds of previous *H. pylori* infection. Low pepsinogen I might function as a pre-cancer marker for non-cardia cancer.

**Impact:** Future research to identify Alaska Native individuals with increased gastric cancer risk includes *H. pylori* genotype and host characteristic studies.

Abstract no.: WS1.2

### CLUSTERING OF HELICOBACTER PYLORI STRAINS FROM GASTRIC CANCER

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Genetic differences between strains play an important role in the determination of clinical outcomes of *Helicobacter pylori* infection. This study aimed to determine the sequencing types of *H. pylori* strains from gastric cancer.

**Materials and Methods:** Twenty-two strains of *H. pylori* were enrolled, including 12 strains from patients with gastric cancer. MLST was used to determine the sequencing type.

**Results:** The seven genetic loci of *H. pylori* were PCR amplified and sequenced. Those sequences of the seven genes were concatenated, and aligned with the sequences of strains from Europe (5), Africa (5), Asia (5) and other parts of China (16) extracted from the MLST database. A neighbour-joining tree with a kimura 2-parameter model was subsequently constructed. The results showed that all 22 strains, as well as Asia strains from database fell into the HpEastAsia haplogroup which could be divided into two groups, groups I and II. Group I consisted of seven cancer strains but only one non-cancer strain of *H. pylori*, in addition to five strains from database. Fisher's exact test revealed a statistically significant difference ( $p = .027$ ).

**Discussion and Conclusion:** The clustering of cancer strains of *H. pylori* is consistent with a recent report showing that the phylogeographic origin of *H. pylori* is a determinant of gastric cancer risk. This may reflect the consequence of long-term interaction of the bacterium with individual hosts of different genetic ground. The results suggested that the sequencing types could possibly be used to predict the clinical outcomes of *H. pylori* infection.

Abstract no.: WS1.3

### LACK OF ASSOCIATION BETWEEN GENE POLYMORPHISMS OF ANGIOTENSIN CONVERTING ENZYME, NOD-LIKE RECEPTOR 1, TOLL-LIKE RECEPTOR 4 AND FAS/FASL WITH THE PRESENCE OF HELICOBACTER PYLORI-INDUCED PREMALIGNANT GASTRIC LESIONS AND GASTRIC CANCER IN CAUCASIANS

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**Background:** Several polymorphisms of genes involved in the immunological recognition of *Helicobacter pylori* and regulating apoptosis and proliferation have been linked to gastric carcinogenesis, however reported data are partially conflicting. The aim of our study was to evaluate potential associations between the presence of gastric cancer (GC) and high risk atrophic gastritis (HRAG) and polymorphisms of genes encoding *Angiotensin converting enzyme (ACE)*, *Nod-like receptor 1 (NOD1)*, *Toll-like receptor 4 (TLR4)* and *FAS/FASL*.

**Methods:** Gene polymorphisms were analyzed in 574 subjects (GC:  $n = 114$ ; HRAG:  $n = 222$ , controls:  $n = 238$ ) of Caucasian origin. *ACE I/II* (rs4646994), *NOD1 796G>A* (rs5743336), *TLR4 3725G>C* (rs11536889), *FAS 1377G>A* (rs2234767), *FAS 670A>G* (rs1800682) and *FASL 844T>C* (rs763110) were genotyped by different PCR approaches and RFLP analysis.

**Results:** Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. There was a tendency for *NOD1 796G/G* genotype to be associated with increased risk of HRAG (62.4% vs 54.5% in controls,  $p = .082$ ). *FAS 670G/G* genotype was more frequent in HRAG when compared to controls, 23.9% and 17.2% respectively, however it failed to reach significance level ( $p = .077$ ). We did not find any significant associations for all examined polymorphisms in relation to GC or HRAG. *NOD1 796G>A* and *TLR4 3725G>C* gene polymorphisms were also not linked with *Helicobacter pylori* seropositivity status.

**Conclusions:** *ACE*, *NOD1*, *TLR4* and *FAS/FASL* gene polymorphisms are not linked with gastric carcinogenesis in Caucasians, and therefore they should not be considered as potential biomarkers for identifying individuals with higher risk for GC.

Abstract no.: WS1.4

### ATROPHIC GASTRITIS BY THE OLGA STAGES AND HELICOBACTER CAGA SEROPOSITIVITY IN GASTRIC CANCER

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**Introduction:** Operative Link on Gastritis Assessment (OLGA) express extent of gastric atrophy in terms of gastritis staging, which severity should be related to gastric cancer.

**Aim:** To study how the OLGA stages of atrophic gastritis are associated with the morphological type, and *Helicobacter pylori* CagA positivity in gastric cancer.

**Patients:** Twenty two gastric carcinoma patients (8 male, 14 female; mean age  $64 \pm 12$ ) were operated on. The intestinal type of carcinoma was diagnosed in 12, diffuse in 8, mixed and indeterminate type in two cases (according to Lauren).

**Methods:** Gastric mucosa samples (altogether up to 15) from the each operation specimen were stained with haematoxylin and eosin. Tissue material was received from the primary tumour and the tumour surrounding antral and corpus mucosa. The stage of atrophy by OLGA was established by combining the extent of histologically scored atrophy with the topography of atrophy. IgG antibodies to *H. pylori* cell surface proteins and CagA were evaluated using ELISA.

**Results:** Of the 12 patients with intestinal type of gastric cancer eight had OLGA stage III or IV, four had OLGA stage II and nobody had OLGA stage I ( $p < .05$ ). Five patients with diffuse cancer had OLGA stage I and II, two had III stage and one had IV stage. There was no association of OLGA stage or cancer type with CagA positivity.

**Conclusion:** Gastric cancer patients represented all stages of gastric atrophy from OLGA stage I to OLGA stage IV which was not associated with cancer type and CagA seropositivity.

Abstract no.: WS1.5

## MICROBIAL DIVERSITY OF GASTROINTESTINAL FLORA INFLUENCES DYNAMICS OF GASTRIC CANCER PROGRESSION IN INS/GAS MICE

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A high percentage (~80%) of *Helicobacter pylori* (*Hp*) infected male INS-GAS mice develop gastric adenocarcinoma at 7 months postinfection (Pi). Germfree (GF) INS-GAS mice did not develop significant gastric lesions until 9 months old and did not develop GIN through 13 months. *Hp* monoassociation caused progressive gastritis, hyperplasia, dysplasia, and tissue proinflammatory immune responses between 5 and 11 months Pi. Eight of 18 male *Hp* monoinfected INS-GAS mice developed low to high-grade GIN by 11 months Pi. (Lofgren et al, 2011). We hypothesized that changes in gastric microbiota composition might promote GIN in achlorhydric stomachs of SPF mice. Three groups of INS/GAS mice were infected with *Hp* SS1 and control mice were sham dosed and followed for 7 months Pi: 1, GF mice (5M, 6F) 2, GF mice associated with three altered Schaedler flora-*Clostridia* spp., *Bacteroides* sp., and *Lactobacillus* sp. (9M, 7F) and 3, SPF mice (15M, 9F). All *Hp* infected groups had significantly ( $p < .05$ ) higher median gastric histology activity index (GHAI) scores than respective controls. SPF infected mice had significantly higher GHAI scores than ASF and GF groups of *Hp* infected mice. Importantly, none of the GF monoassociated *Hp* mice developed any high grade carcinoma when compared to 22% of ASF/*Hp* infected mice and 33% *Hp* infected SPF mice. ASF/*Hp* infected mice had their gastric contents colonized with the three species of ASF when measured by qPCR. Our data demonstrates that specific enteric flora colonizing the achlorhydric stomach influences progression of gastric cancer development.

Abstract no.: WS1.6

ROLE OF *H. PYLORI* ERADICATION THERAPY SUCESS ON 5 YEAR DYNAMICS OF DYSPLASIAT. Filipec Kanizaj, M. Katicic, B. Skurla, M. Prskalo, V. Colic Cvrnje, S. Naumovski Mihalic, A. Mrzljak, T. Bradic and N. Sobocan  
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**Aim:** To investigate the role of eradication therapy success on five year dynamics of dysplasia.

**Materials and Methods:** Study was performed on 41 *H. pylori* positive patients with dysplasia in basic bioptic specimens analyzed according to updated Sydney protocol. All patients received triple eradication therapy. Therapy success and dynamics of dysplasia were evaluated according to 5th year histological finding.

**Results:** Basically 41 patients had dysplasia in corpus and/or antrum. Grade I 7.31%, grade II 39.02% and grade III 53.65% of patients. *H. pylori* was successfully eradicated in 37 patients (90.24%). In successfully eradicated, complete regression of dysplastic changes appeared in 33/37 (90.89%) of patients. In all of rest three eradicated patients regression of grade of dysplasia was observed. In 2 of 4 non-eradicated patients complete regression of dysplasia and for rest 2 only regression of grade of dysplasia appeared. Statistically significant dynamics of grade of dysplasia was observed for successfully eradicated patients (Wilcoxon rank sum test,  $p < .001$ ). The difference in proportion of patients with complete regression of dysplasia between eradicated and non-eradicated is statistically significant.

**Conclusions:** In 5 year interval, proportion of patents with complete regression of dysplasia is statistically significantly higher in successfully eradicated patients than non-eradicated. In both groups regression of grade of dysplasia (and no progression) was observed with application of eradication therapy.

# WS2 Inflammation

Abstract no.: WS2.1

## HELICOBACTER PYLORI-DERIVED HP(2-20) ACCELERATES THE HEALING OF CHRONIC GASTRIC ULCERS

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*Helicobacter pylori* (*H. pylori*)-derived peptide RpL1 aa 2-20 (Hp2-20) interacts with formyl peptide receptors (FPRs) to trigger immunomodulation but Hp2-20 influence on the gastric secretory functions and healing of preexisting gastric ulcers has not been investigated. We determined the effect of Hp2-20 on the gastric acid secretion in rats equipped with gastric fistula (GF) and the healing of acetic-acid-induced gastric ulcers (ulcer area = 28 mm<sup>2</sup>) were treated daily for 9 and 15 days with: 1) vehicle (saline); 2) Hp2-20 (0.5–25 mg/kg/day i.g.) or 3) control peptide Hp1 (10 mg/kg/day i.g.) with or without L-NNA, the non-selective inhibitor of NO-synthase and L-Nil, the selective inhibitor of iNOS combined with L-arginine (200 mg/kg/day). The ulcer area were measured by planimetry, the gastric blood flow (GBF) was determined by H<sub>2</sub>-gas clearance technique and luminal NO<sub>x</sub> concentration, plasma VEGF levels, the expression VEGF and FPR mRNA and protein were assessed. Hp2-20 dose-dependently inhibited the gastric acid secretion and reduced the area of acetic acid gastric ulcers (ID50 = 10 mg/kg) accompanied by the rise in GBF, plasma NO<sub>x</sub> levels and VEGF concentration. Treatment with L-NNA and L-Nil significantly reduced the Hp2-20-induced healing, the increase in the GBF and plasma NO<sub>x</sub> content. The VEGF and FPR mRNA and protein were upregulated at ulcer margin of Hp2-20 rats being significantly attenuated by L-NNA or L-Nil. We conclude that Hp2-20 accelerates ulcer healing via inhibition of gastric acid, the activation of FRP receptors and NOS/NO system and by an overexpression of VEGF responsible for angiogenesis.

Abstract no.: WS2.2

## H. PYLORI INFECTION INDUCES A VITAMIN D IMMUNE RESPONSE

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A vitamin D antimicrobial activity against *Mycobacterium tuberculosis* was confirmed in human monocytes in 1986 and 1987. There are few, if any, reports indicating a vitamin D<sub>3</sub> immune response to *Helicobacter pylori* (*H. pylori*) infection. We used microarray analysis to monitor host responses to *H. pylori* infection and found that the vitamin D receptor gene (VDR) was up-regulated (fold changes >5, *p* < .05), which suggested that VDR may play an important role in immune response to *H. pylori* infection. We tested this observation in the RAW 264.7 cell line using qPCR, and confirmed that VDR, CYP27B1 and Cathelicidin expressions were increased during *H. pylori* infection. We also observed increased CYP27b1 expression, 1, 25-dihydroxyvitamin D<sub>3</sub> (1,25D<sub>3</sub>) levels and Cathelicidin expression in resident macrophages isolated from the peritoneal cavity of C57BL/6 wild type mice. In contrast, CYP27b1 is down-regulated in resident macrophages isolated from VDR-deficient C57BL/6 VDR KO mice. We extended our studies to C57BL/6 wild type and C57BL/6 VDR KO mice to evaluate the role of VDR on the modulation of mucosal immune response to *H. pylori* infection. *H. pylori* colonization in the gastric mucosa of C57BL/6 VDR KO mice was significantly lower compared with wild-type littermates. These observations indicate that vitamin D<sub>3</sub> exerts considerable influence on the host innate immune response against *H. pylori* infection acting via the CYP27b1 response and subsequent roles of VDR and 1,25D<sub>3</sub> on Cathelicidin production. *H. pylori* infection elicits a Vitamin D<sub>3</sub> innate immune response that reduces adaptive immune responses, thus, persistence *H. pylori* colonization.

Abstract no.: WS2.3

## EXPRESSION OF GALECTIN-3 (GAL-3) IN HOST RESPONSE TO HELICOBACTER PYLORI INFECTION

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**Background:** *H. pylori* Cytotoxin associated gene A (CagA) protein was reported to upregulate the expression of intracellular Gal-3, a 31 kDa β-galactoside lectin.

However, the functional characteristics and significance of Gal-3 expression in *H. pylori*-infected host cells have not been well established. The present study aimed to determine the CagA-mediated expression of Gal-3 in response to *H. pylori* infection. The role of Gal-3 in *H. pylori* induced inflammation and apoptosis were also investigated.

**Methods:** AGS cells were infected with *H. pylori* 26695 WT and ΔcagA strains. The subcellular expression and localisation were examined using immunofluorescence microscopy and immunoblot analysis, respectively. Gal-3 was transiently knocked down in AGS cells using targeted siRNA and the resultant effects in inflammation and apoptosis were analysed using, IL-8 and flowcytometric assays, respectively.

**Results and Conclusion:** In untreated AGS cells, Gal-3 is predominantly found to be nuclear confined. Interestingly, in *H. pylori*-infected cells, there was an upregulation of Gal-3 in the nucleus which was exported into the cell cytoplasm and then onto the cell membrane. However, this process was delayed and reduced in the absence of CagA, suggesting its role in the induction of Gal-3 expression. Furthermore, knock down of Gal-3 expression contributed to an increase in apoptosis and reduced IL8 response in *H. pylori*-infected AGS cells, thus strengthening the significance of Gal-3 in host response to infection. Taken together, our data suggest that Gal-3 is an important host factor that may interfere with *H. pylori*-associated pathological events.

Abstract no.: WS2.4

## HOST ADAPTIVE RESPONSE DETERMINED CLINICAL OUTCOME OF HELICOBACTER PYLORI INFECTION

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We hypothesized that adaptive host response after *H. pylori* infection can explain why a few develop clinical diseases while most remain asymptomatic. The changes of damaging factors and defense factors besides of their responsible signal transduction pathways were checked. Significantly coinciding inductions of damaging genes with activation of p-ERK1/2, c-jun, NF-κB, and AP-1 and defensive genes with activation of Nrf2 were noted after *H. pylori* infection. In order to prove that ARE activation might occur after *H. pylori*, we infected *H. pylori* to ARE-hPAP<sup>+/+</sup> wild type and ARE-hPAP<sup>-/-</sup> transgenic mice and found *H. pylori*-induced inflammation also was associated with ARE activation in mouse stomach. To further validate the adaptive engagement of Nrf2 activation after *H. pylori* infection as host defense, we infected *H. pylori* to Nrf2<sup>+/+</sup> and Nrf2<sup>-/-</sup> mice for 20 weeks and found *H. pylori*-induced inflammation was aggravated in Nrf2<sup>-/-</sup> mice compared with wild type littermates. Finally, we checked the expressions of COX-2, HO-1, and Nrf2 in biopsied mucosal samples from patients with chronic gastritis according to *H. pylori* status. Significantly higher expressions of COX-2, HO-1, and Nrf2 were noted in patients with *H. pylori* (+) chronic gastritis than *H. pylori* (-) chronic gastritis. In conclusion, adaptive host response could be the ultimate determinants predicting the progress of *H. pylori*-associated chronic gastritis.

Abstract no.: WS2.5

## CYTOCHROME C RELEASE FROM MITOCHONDRIA IN EPITHELIAL GASTRIC CELLS COINFECTED WITH H. PYLORI

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**Introduction:** *H. pylori* induces apoptosis in gastric epithelial cells through oxidative stress via mitochondrial way. Mitochondrial permeability transition pores (MPTP), Bcl-2 protein family and interactions between them are involved in cytc release during apoptosis, being unknown the role in *H. pylori* infection.

**Aims:** Analysis of MPTP opening and mitochondrial bax translocation to explain cytc release and apoptotic phenomena in gastric epithelial cells infected with *H. pylori*.

**Methods:** AGS cells were coinfectd for 24 hour with *H. pylori* (ATCC 51932) at increasing densities (10<sup>4</sup>–10<sup>8</sup> CFU/mL). We evaluated: Intracellular (ROS) and mitochondrial (O<sub>2</sub><sup>-</sup>) free radicals; Cytc in cytosolic and mitochondrial fractions; Apoptosis. At 10<sup>8</sup> CFU/mL, cocultures were pre-treated with/without Vit.E and we analysed: MPTP; Mitochondrial membrane potential (MMP); Apoptosis; Bax protein. At the same density, a pre-treatment with/without V5 (Bax translocation inhibitor) was performed, and we carried out crosslinking studies (with DSP) to examine Bax dimer-oligomerization (both in mitochondrial and in cytosolic extracts).

**Results:** *H. pylori* increased ROS and O<sub>2</sub><sup>-</sup> proportionally to density. Apoptotic cells were augmented 60%; cytosolic/mitochondrial cytc ratio was higher



( $3.07 \pm 1.30$  vs  $2.03 \pm 0.95$ ). *H. pylori* significantly decreased MMP, caused MPTP opening in 70% of cells and enhanced 3.5-fold Bax amount. Vit.E recovered all these values ( $p < .05$ ). Additionally, bacteria decreased the cytosolic/mitochondrial bax ratio and it was only observed dimer and oligomer bands in the mitochondrial fraction. V5 attenuated all these alterations. ( $p < .05$ ).

**Conclusions:** *H. pylori* induces cytc release through the two pathways analyzed. Antioxidants and bax translocation inhibitors treatment could inhibit the apoptosis and help to reduce the toxic effects of the bacteria on gastric mucosa.

Abstract no.: WS2.6

#### THE INFLUENCE OF BLOCKING TLR4 SIGNAL PATHWAY ON IMMUNE PROTECTION OF *H. PYLORI* VACCINE AND TH IMMUNE RESPOND

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**Objective:** To observe the influence of blocking TLR4 signal on immune protection of Hp vaccine.

**Methods:** BALB/c mice were divided into three groups: 1. Control; 2, Hp vaccine; 3, Anti-TLR4 antibody pretreatment + Hp vaccine. At 4 weeks after immunization, mice from 2 and 3 groups were challenged by Hp. At 4 weeks after

challenge, Sample were collected. Hp, cytokine, Foxp3+Treg in gastric mucosa were determined.

**Results:** 1, Hp colonized in mice of Hp vaccine was lower than that in control ( $p < .001$ ), and was significantly higher in group with anti-TLR4 antibody pretreatment than in group without pretreatment ( $p < .05$ ). 2, Inflammatory degree in mice of Hp vaccine was higher than in control ( $p < .05$ ), and in group with anti-TLR4 antibody pretreatment were lower than group without pretreatment ( $p < .05$ ). 3, Level of Th1and Th17 cytokine in mice of Hp vaccine were significantly higher than that in control ( $p < .05$ ), and in group with anti-TLR4 antibody pretreatment were significantly lower than those in groups without pretreatment ( $p < .05$ ); Level of Th2 cytokine, there were no significant difference between in control and vaccine ( $p > .05$ ), and between in group with anti-Tim-3 antibody pretreatment and without pretreatment ( $p > .05$ ). 4, Foxp3+Treg in mice of Hp vaccine were significantly higher than that in control ( $p < .01$ ), and in group with anti-TLR4 antibody pretreatment were significantly lower than those in groups without pretreatment ( $p < .05$ ).

**Conclusion:** Blocking TLR4 signal can depress Hp vaccine protection and depress Th1 and Th17 respond, and increase the numbers of CD4+CD25+Foxp3+Treg, this could be the mechanism that it destroy Hp vaccine immune protection.

# WS3 Immunity and Extragastric Diseases

Abstract no.: WS3.1

## THERAPEUTIC EFFICACY OF A *HELICOBACTER PYLORI* VACCINE DEPENDENT ON ANTIBODIES AND T-CELLS

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As one of the most prevalent bacterial infections worldwide, *Helicobacter pylori* (Hp) is affecting half of the world's population, causing peptic ulcers and gastric cancer. Until now no human vaccination study was successful, although big efforts have been initiated to develop a vaccine against this pathogen. Thus, an approved vaccine for humans is still not in sight. As this is due to the vaccine formulation – antigen and adjuvant composition, as well as the type of immunity induced – systemic or mucosal has to be figured out. Our group described a virulence factor of *H. pylori*, the Hp gamma-glutamyltranspeptidase (HPgGT) that inhibits the proliferation of T-cells and thus prevents the generation of an effective immune response. We used HPgGT in an experimental mouse infection model for a novel vaccination approach. As HPgGT is a secreted protein, HPgGT specific T-cells can hardly target the pathogen. Therefore HPgGT was combined with outer membrane proteins to induce protective T-cell responses. With different vaccine designs we tested their capability to induce protection, revealing a need for mucosal immunization. Notably, immunization with HPgGT induced a strong antibody response, which blocked its enzymatic activity, thereby counteracting the immunosuppressive activity of HPgGT. In infection experiments this vaccination led to a substantial decrease of bacterial colonization in the stomach (>80% of the mice cleared the infection), making this novel “liberation vaccine” a promising candidate for a new immunization strategy.

Abstract no.: WS3.2

## EFFICIENCY IMPROVEMENT OF A MULTIVALENT DNA VACCINE AGAINST *HELICOBACTER PYLORI*

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*Helicobacter pylori* is the major etiological factor for the development of severe gastroduodenal diseases, namely peptic ulcer and gastric cancer. Although successful in eradicating bacteria, antibiotic therapies present several disadvantages and vaccination is still considered a very attractive approach in infection management.

In this work, eight *H. pylori* proteins were chosen for including in a multiepitope DNA vaccine, namely FlaA, UreA, CagA, VacA, HpaA, KatA, NapA and TsaA. Antigenic sequences of these proteins were obtained using Jameson-Wolf, Rothbard-Taylor and AMPHI methods (DNASTAR Lasergene, Inc), which predict B and T epitopes. We design a DNA vaccine construct containing a ~50 amino acids sequence of each of those proteins. This was codon-optimized, for optimal expression in mammalian cells, and synthesized by GENEART, Inc., and was then cloned in pVAX. The construct also contained a FLAG tag sequence for simplify the detection of the synthetic protein that resulted from its expression. Three additional vaccine constructs containing additional sequences that target protein to MHCII pathway were made, namely using apoptosis, secretion or lysosomal target signals.

In vitro transfection efficacy of each one of these constructions was evaluated using AGS cell line and two different chitosan-based nanoparticles with adjuvant properties, as delivery system. Immunocytochemistry and immunoblotting using anti-FLAG antibody reveal that synthetic protein was expressed for every constructs. We are now evaluating constructs ability for target synthetic protein to the MHCII pathway.

Work supported by PTDC/BIO/69242/2006(FCT) research grant. IV and TC are recipient of SFRH/BD/38634/2007(FCT) and SFRH/BD/23902/2005(FCT) doctoral fellowships, respectively.

Abstract no.: WS3.3

## A NOVEL LINE BLOT SYSTEM DO DETECT INFECTION WITH PATHOGENIC *H. PYLORI*

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*H. pylori* infects half of the world's population, but only a minority of infected individuals develop diseases. To date, it is not possible to identify patients at increased risk for disease. *H. pylori* virulence factors have been associated with disease development, but direct assessment of virulence factors requires invasive methods to obtain gastric biopsies. Our study aimed at the development of a non-invasive serologic test to detect immune responses against important *H. pylori* virulence factors. This immuno-line blot is based on recombinant proteins produced in *E. coli*, which are applied to a solid phase. Seventeen highly immunogenic proteins were selected, some of which are associated with chronic atrophic gastritis, ulcers, or gastric cancer.

The coding sequences were amplified from *H. pylori* strains G27 and 26995 and cloned into the expression plasmid pDestHisMBP. After recombinant expression as soluble His-MBP-fusion proteins, they were purified using affinity chromatography and gel filtration. All proteins (CagA, VacA, GroEL, gGT, BabA, HcpC, UreA, HtrA, NapA, ICD, Omp1, Omp15, Omp18, HpaA, HP231, HP947, HP940) could be expressed and purified. Proteins were bound to nitrocellulose membranes and serologic immune responses were detected by secondary antibodies. For the validation of the prototype a cohort of 1400 patients was established. The assay showed a sensitivity and specificity of >95% compared to histology and ELISA. In direct comparison to older lysate blots, the line blot assay had increased discriminatory power. Prospective studies will be performed to analyse the positive and negative predictive value of the novel line blot assay.

Abstract no.: WS3.4

## SUBJECTS WITH CAGA POSITIVE (CAGA+) *H. PYLORI* (HP) INFECTION HAVE REDUCED CIRCULATING ESTROGENS AND INCREASED POST-PRANDIAL SEROTONIN LEVELS: EFFECTS ON CIRCADIAN RHYTHM OF BONE TURNOVER AND SKELETAL HEALTH

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**Introduction and Aims:** We previously demonstrated that osteoporotic patients show an increased prevalence of CagA+ HP infection. CagA+ patients and subjects have augmented bone resorption and fracture risk. Now, we explored the relationship between HP infection and bone turnover markers and hormones in a large cohort of elderly male and female subjects.

**Subjects and Methods:** Approximately half of the 1109 subjects studied underwent all measurements at 8.00 am in a fasting state, while the remaining half underwent blood sampling post-feeding, at 15.00 pm ca., in order to uncover potential effects on circadian rhythms. We examined the circulating concentrations of bone alkaline phosphatase, serum carboxy-terminal collagen crosslinks (CTX), 25OH vitamin D, PTH, sex hormone and sex hormone binding globulin, adiponectin, ghrelin and serotonin. HP infectious and CagA status were determined serologically.

**Results and Discussion:** We observed reduced total and free estradiol levels in both males and females infected by CagA+ HP, with an increased significance in the fasting state, respect to uninfected and CagA-infected subjects. In CagA+ subjects, the ghrelin systemic levels were significantly lower than in CagA-infected and uninfected subjects. Serum CTX concentrations significantly decreased after feeding in all subjects; however, a significant reduction of bone alkaline phosphatase postprandial levels was observed only in CagA+ subjects, which was associated with augmented serum and plasma serotonin levels. In conclusion, the pathogenetic mechanisms of the association between CagA+ HP infection and osteoporosis may include a reduction of estrogen and ghrelin and a rise of serotonin in the blood stream.



Abstract no.: WS3.5

**HELICOBACTER PYLORI INFECTION AND FUNDIC GASTRIC ATROPHY ARE NOT ASSOCIATED WITH OESOPHAGEAL SQUAMOUS CELL CARCINOMA: A CASE-CONTROLLED STUDY**

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**Objective:** Recent studies from Asia and Northern Europe suggest that apart from alcohol intake and smoking, also fundic gastric atrophy (FGA) may increase the risk of oesophageal squamous cell carcinoma (OSCC). However, due to the wide geographic variation of this cancer and the changing prevalence of the *Helicobacter pylori* infection, these findings need to be confirmed in other ethnic groups. The aim of the present case-controlled study was to investigate whether *H. pylori* infection and FGA carry an increased risk for OSCC.

**Methods:** FGA was evaluated by histology and serology in 75 patients with OSCC, and 75 sex- and age-matched controls. Pepsinogen (PG)-I levels  $\leq 70$   $\mu\text{g}/\text{mL}$  and PGI/II ratio  $\leq 3$  were indicative for FGA. *H. pylori* infection was defined as positivity to at least one test among histology, rapid urease test, and serology for both general anti-IgG and anti-CagA.

**Results:** Overall, the prevalence of *H. pylori* infection was identical high (70.7%) in both patients with OSCC and controls. FGA diagnosed by serology and histology was not associated with an increased risk for OSCC (OR 1.17; CI 95% 0.54–2.56 and OR 1.91; CI 95% 0.6–5.99, respectively). Odds ratios (CI 95%) for hazardous alcohol consumption, smoking, and the presence of both risk factors were 5.75 (2.20–15.05), 22.18 (9.41–52.28), and 31.69 (8.39–119.67) respectively.

**Conclusions:** Hazardous alcohol consumption and smoking increase synergistically the risk for developing OSCC. In our population neither *H. pylori* infection, nor FGA was associated with an increased risk for OSCC.

Abstract no.: WS3.6

**IS THERE AN ASSOCIATION BETWEEN HELICOBACTER PYLORI INFECTION AND INFLAMMATORY BOWEL DISEASE: A META-ANALYSIS**

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**Background:** There are epidemiologic data suggesting a protective effect of *Helicobacter pylori* (*H. pylori*) infection against the development of autoimmune disease. In addition laboratory data illustrate *H. pylori*'s ability to induce immune tolerance and limit inflammatory responses. Numerous studies have examined the association between *H. pylori* infection and inflammatory bowel disease (IBD).

**Aim:** The aim of this study was to perform a meta-analysis on the association between *H. pylori* infection with Crohn's disease (CD) and ulcerative colitis (UC).

**Methods:** Extensive Medline and EMBASE English language medical literature searches for human studies were performed through April 2010, using suitable keywords. Pooled estimates were obtained using fixed or random-effects models as appropriate. Heterogeneity between studies was evaluated with the Cochran Q test whereas the likelihood of publication bias was assessed by the Begg and Mazumdar adjusted rank correlation test and by the Egger's regression test.

**Results:** For CD the pooled odds ratio (OR) with 95% confidence intervals (CI) were 0.405 (0.316–0.520), test for overall effect  $Z = -7.124$ ,  $p < .0001$ . The heterogeneity Q value was 48.118,  $I^2 = 60.514$ ,  $p < .0001$ . For UC the pooled ORs were 0.516 (0.403–0.660),  $Z = -5.62$ ,  $p < .0001$ . The heterogeneity Q value was 28.059,  $I^2 = 50.105$ ,  $p < .0001$ . There was no publication bias.

**Conclusions:** These results suggest a protective role of *H. pylori* infection against the development of IBD. Therefore, further studies investigating the effect of eradication of *H. pylori* on the development of IBD are warranted and also studies in *H. pylori* experimental models are necessary to further define the mechanism of this negative association.

# WS4 Paediatrics

Abstract no.: WS4.1

## PROSPECTIVE EUROPEAN MULTI-CENTRE EPIDEMIOLOGIC CASE-CONTROL STUDY ON RISK FACTORS OF GASTRIC AND DUODENAL ULCERS OR EROSIONS IN CHILDREN

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**Aims:** To analyse the risk factors associated with gastric and duodenal ulcers or erosions among paediatric patients. A pilot study suggested that *H. pylori* infection (27%) and gastrototoxic medications (23%) were less frequently implicated than expected.

**Methods:** Open, Prospective, multi-centre, case-control study. Consecutive patients presenting gastric or duodenal ulceration/erosions and 2 age-matched controls were included between January 2008 and December 2009.

**Results:** 244 patients (153 with erosions alone and 91 with ulcer (s)) and 488 controls were included. Ulcer and/or erosions were more frequent in children older than 10 year (95/244 vs 149/244 –  $p < .0001$ ). Peptic lesions were significantly related to male gender (57.7% vs 49.6%,  $p = .04$ ), use of non-steroidal anti-inflammatory drugs (NSAIDs –  $p = .05$ ), alcohol consumption ( $p = .05$ ) and tobacco use ( $p < .0001$ ). *H. pylori* infection was present in 63/244 (25.8%) patients and 81/488 (16.6%) controls ( $p < .001$ ). However, *H. pylori* status was considered as not valid in 26 patients and 34 controls because of recent use of antibiotics. *H. pylori* infection was strongly related to duodenal ulcer (20/40 –  $p < .0001$ ) and duodenal erosion (14/45 –  $p = .02$ ) but not to gastric lesions. No known risk factors for PUD were observed in 141/244 (57.8%) cases.

**Conclusion:** This study confirms that *H. pylori* infection is a risk factor for duodenal, but not for gastric lesions in children. Male gender, age (older than 10 year), NSAID use, alcohol and tobacco use are independent risk factors of gastric and duodenal ulcer/erosions in children. A high proportion of children have primary ulcer/erosions with no identifiable risk factors.

Abstract no.: WS4.2

## SEQUENTIAL THERAPY AS FIRST LINE TREATMENT IN CHILDREN WITH NEW DIAGNOSED SYMPTOMATIC *H. PYLORI* INFECTION

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**Objective:** Sequential Therapy (ST), a two-step 10 day-therapy giving proton-pump-inhibitor (PPI) with amoxicillin for 5 days, followed by triple therapy (PPI, clarithromycin, metronidazole) has been suggested as first-line treatment in *Helicobacter pylori* (*Hp*) infected children making susceptibility testing superfluous.

**Aim:** To evaluate the eradication rate of *Hp* infection with ST in treatment naïve children with antibiotic susceptibility results

**Methods:** A prospective audit on anonymous patient data was performed from nine European centers, where ST was used as first line treatment giving esomeprazole (~1 mg/kg), amoxicillin (~50 mg/kg), clarithromycin (~25 mg/kg)

and metronidazole (~25 mg/kg) per bodyweight in two divided doses. Eradication was assessed 6–8 weeks after treatment.

**Results:** Data of 160 patients with f/u results were analyzed (91 female, mean age 12.3 years). Primary resistance was reported for clarithromycin only in 14 (15%), metronidazole only in 24 (15%), for both in 7 (4%), culture failed in 4. Overall eradication-success reached 82% (131/160), but was significantly better in double susceptible strains (91%) compared to metronidazole resistance only (67%,  $p < .001$ ) or clarithromycin resistance only (70%,  $p < .02$ ). Only 2/7 (29%) infections with a double resistant strain were cleared.

**Conclusion:** In the real-life-situation ST gave low eradication rates in children infected with single or double resistant strains. Our results support ESPGHAN/NASPGHAN recommendations (Koletzko et al JPGN epub 06/05/2011) that treatment tailored to susceptibility testing is the first choice, particularly in populations with high antibiotic resistance rates. ST is a first line option if susceptibility testing has failed or is not available.

Abstract no.: WS4.3

## THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN SYMPTOMATIC CHILDREN – 10 YEARS OBSERVATIONAL STUDY IN LOWER SILESIA REGION

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*H. pylori* infection in Polish children is still a major therapeutic problem. In the nineties the incidence of infection was approximately 30% in our region.

**Aim:** To estimate the occurrence of *H. pylori* infection in children with gastro-duodenal pathologies.

**Material and Methods:** Retrospective analysis was based on the results of 8012 cultures for *H. pylori* in children aged 1.5–18, diagnosed because of recurrent abdominal pain suggesting gastro-duodenal pathology in years 2001–2010. Gastric biopsy specimens were taken from children and were sent for microbiological examination. *H. pylori* infection in children was based on clinical, endoscopic and microbiological diagnosis. *H. heilmannii* infection was identified on the basis of direct microscope examination.

**Results:** Overall, among 8012 cultures analyzed in 10 years time, 1311 (16.32%) were positive for *H. pylori*. The prevalence of *H. pylori* infection in certain years was as follows: in 2001 – 23%, 2003 – 19.3%, 2005 – 17%, 2008 – 9.8% and in 2010 – 8.9%. *H. heilmannii* infection was documented in 15 subjects in examined period. The highest incidence of *H. pylori* infection was noted in children from 15 to 18 years of age (31%), whereas the lowest (2%) in children aged 1.5–4 years old.

**Conclusions:** There has been a decline in incidence of *H. pylori* infection in symptomatic children but it is still high. *H. heilmannii* infection is rare cause of gastric pathology. The highest incidence of *H. pylori* infection is present in children aged 15–18.

Abstract no.: WS4.4

## *HELICOBACTER PYLORI* INFECTION, IL-1B AND IRON DEFICIENCY ANAEMIA IN CHILDREN

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Interleukin (IL)-1 $\beta$ , a potent inhibitor of gastric secretion, is speculated to be responsible for down modulation of gastric acid secretion upon *H. pylori* infection. In turn, infection has been associated with iron deficiency although the mechanisms involved are not yet completely known. Thus, because gastric acidity is essential for the duodenal iron absorption, we investigated whether gastric IL-1 $\beta$  levels were associated with iron deficiency anaemia parameters and *H. pylori* infection in 125 children (59.2% girls, mean age 12.2  $\pm$  2.9 years, 4–16 years). Exclusion criteria included coeliac disease, peptic ulcer, antimicrobial and PPI use 30 days before endoscopy and intestinal parasites. *H. pylori* status was evaluated by culture, urease test, histology, ureA-PCR and <sup>13</sup>C-UBT. Forty-three (37.6%) children were *H. pylori*-positive. Antral gastric levels of IL-1 $\beta$  (ELISA, Biosource) were significantly higher ( $p < .001$ ) in *H. pylori*-positive (333.6 pg/mg of tissue) than in -negative (19.67 pg/mg) children. In the *H. pylori*-positive children, IL- $\beta$  levels negatively correlated with haemoglobin ( $r = -.35$ ,  $p = .004$ ), haematocrit ( $r = -.40$ ,  $p = .008$ ) and serum ferritin ( $r = -.41$ ,  $p = .007$ ). IL-1 $\beta$  levels positively correlated with the serum pepsinogen II ( $p = .002$ ) level which is increased with gastric corpus inflammation. Other cytokines were also signifi-

cantly increased in the gastric mucosa of *H. pylori*-positive children and associated with antral (IL-8 and IFN- $\gamma$ ) and corpus gastritis (IL-1 $\alpha$ , IL-6). However, none of them correlated with the haematological parameters pointing to a putative role of IL-1 $\beta$  in decreasing iron absorption by inhibition of gastric acid secretion. Funded under the Sixth Framework Programme of the European Union, Project CONTENT (INCO-DEV-3-032136).

Abstract no.: WS4.5

#### THE PREVALENCE OF *H. PYLORI* INFECTION REMAINS HIGH IN CHILDREN FROM DEVELOPING COUNTRIES

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Although the prevalence of *H. pylori* is declining globally, it is still one of the most frequent bacterial infections in developing countries where gastric carcinoma remains as an important cause of death. We aimed to evaluate the frequency of *H. pylori* infection in children undergoing endoscopy due to gastric complaints from two developing and one developed countries. Two hundred and ninety seven children (mean age 10.7  $\pm$  3.1 years, 3–16 years, 171 girls) were included: 101 from Santiago/Chile, 125 from Belo Horizonte/Brazil and 71 from London/UK. Among them, 83 (28.0%) were *H. pylori*-positive (positive culture or biopsy urease test or histology) and 214-negative. *H. pylori* positivity rate was significantly higher in Santiago (31/101–30.7%) and Belo Horizonte (47/125–37.6%) than in London (5/71–7.1%) ( $p < 10^{-3}$  for both). There was no difference in mean age ( $p = .11$ ) or in gender ( $p = .89$ ) among the three groups. *H. pylori*-positive status was associated with increasing age ( $p = .006$ ), antral ( $p = .003$ ) and corpus ( $p = .002$ ) enantema and antral nodularity ( $p < 10^{-3}$ ). The infection was not associated with abdominal pain, diarrhea, vomiting, weight loss and fever or with anthropometric data (weight/age, height/age and BMI/age). *cagA*, detected by PCR, was positive in 54.3% and 60.9% of strains from Belo Horizonte and Chile, respectively ( $p = .79$ ). In conclusion, our results confirm the low *H. pylori* prevalence in a developed country. Although quality of life has increased in Brazil, the frequency of *H. pylori* in endoscoped children based on our previous

studies remains unchanged. Funded under the Sixth Framework Programme of the European Union, Project CONTENT (INCO-DEV-3-032136).

Abstract no.: WS4.6

#### INTRAFAMILIAL TRANSMISSION OF *HELICOBACTER PYLORI* INFECTION IN A RURAL AREA OF JAPAN

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**Objectives:** The purpose of this study is to examine the mode of transmission of *H. pylori* infection in a rural area of Japan.

**Subjects and Methods:** An epidemiologic study in Sasayama City has found that 15 of 738 children (probands) were *H. pylori* infection positive by stool antigen test. The subjects are probands and their family members in that study. We collected stool samples from 35 family members (11 families) of *H. pylori* positive probands, as well as 36 family members (12 families) of *H. pylori* negative probands.

**Results:** The prevalence of *H. pylori* infection was 40.0% in 35 family members of positive probands, whereas it was 8.3% in 36 family members of negative probands ( $p = .002$ ). *H. pylori* test results were available for both parents in nine families of positive and in nine families of negative probands. Of the parents of positive probands, only fathers of three probands, only mothers of three probands, and both parents of two probands were *H. pylori* positive, and both parents of one proband were negative. Of the parents of negative probands, only father of one proband, and only mother of one proband were positive. We observed a similar prevalence of *H. pylori* infection between fathers and mothers of positive probands. All siblings of positive and negative probands were *H. pylori* negative.

**Conclusion:** In Japanese children, parent-to-child transmission may be an important route of *H. pylori* infection, and infection between siblings was not observed.

# WS5 Clinical Aspect, Drug Resistance

Abstract no.: WS5.1

**SEQUENTIAL AND STANDARD LEVOFLOXACIN-BASED *H. PYLORI* ERADICATING REGIMENS COMPARED TO QUADRUPLE THERAPY: EFFECT OF LEVOFLOXACIN DOSAGE AND WAY OF ADMINISTRATION**  
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**Background:** Levofloxacin is another option to quadruple therapy as a second line treatment. Whether sequential schemes are superior to linear regimens or different doses affect eradication is unknown.

**Methods:** Five hundred *H. pylori*-positive patients in whom standard first line therapy failed underwent 10 different schemes (50 patients each): lansoprazole 15 mg bid, amoxicillin 1000 mg bid and levofloxacin 500 mg (LAL500x7) or 750 mg (LAL750x7) or 1000 mg (LAL1000x7) for 7 days; lansoprazole 15 mg bid, amoxicillin 1000 mg bid and levofloxacin 500 mg (LAL500x10) or 750 mg (LAL750x10) or 1000 mg (LAL1000x10) for 10 days; lansoprazole 15 mg bid, amoxicillin 1000 mg bid for 5 days followed by lansoprazole 15 mg bid, tinidazole 500 mg bid and levofloxacin 500 mg (LALT500) or 750 mg (LALT750) or 1000 mg (LALT1000) for 5 days; lansoprazole 15 mg bid, tetracycline 500 mg qid, metronidazole 500 mg tid, bismuth salt 120 mg qid for 7 days (TMBL). Patients underwent UBT; adverse effects were also assessed.

**Results:** Eradication rates were 66% in LAL500x7, 90% in LAL500x10 ( $p < .003$ ), 76% in LAL750x7, 90% in LAL750x10 ( $p = ns$ ), 70% in LAL1000x7, 85% in LAL1000x10 ( $p = ns$ ); 82% in LALT500, 80% in LALT750, 72% in LALT1000 ( $p = ns$ ); 70% in TMBL. Efficacy of linear and sequential schemes was similar; LAL500x10 and LAL750x10 were superior to TMBL ( $p < .01$ ). Side effects were increased in TMBL ( $p < .01$ ).

**Conclusions:** Duration, but not dosage of levofloxacin affects eradication rate. Sequential regimens do not add any advantage. LAL500x10 and LAL750x10 are more effective than TMBL.

Abstract no.: WS5.2

**SEQUENTIAL THERAPY FOR *HELICOBACTER PYLORI* ERADICATION IN ADULTS COMPARED WITH TRIPLE THERAPY IN CHINA: A MULTICENTER, PROSPECTIVE, RANDOMIZED, CONTROLLED TRIAL**  
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**Objective:** Sequential therapy is currently suggested as first-line therapy in curing *Helicobacter pylori* infection, but results coming from its use in clinical practice are scarce. We designed a multiple-center (Beijing, Shanghai, Wuhan and Guangzhou), prospective, randomized, controlled trial to determine the effectiveness of sequential therapy for *Helicobacter pylori* eradication in adults compared with 10-day triple therapy in China.

**Methods:** In 624 patients with *H. pylori* infection confirmed with two tests (histopathology WS stain and rapid urease test), we conducted a clinical trial to compare the effectiveness of sequential treatment (amoxicillin 1.0 g bid and esomeprazole 20 mg bid for 5 days followed by clarithromycin 0.5 g bid, tinidazole 0.5 g bid, and esomeprazole 20 mg bid for 5 days) and a 10-day triple eradication regimen (amoxicillin 1.0 g bid, and clarithromycin 0.5 g bid, plus esomeprazole 20 mg bid). At 4–12 weeks after the completion of treatment, 13C-UBT or WS stain was performed to confirm the eradication results.

**Results:** The response rate of EAC and sequential group were 85.9% (293/341) and 87.5% (246/281), respectively. There was no significant differences in age and BMI between two groups. There was no statistical difference ( $p = .528$ ) in the *H. pylori* eradication rate between EAC group (75.1% 220/293) and sequential group (75.2% 185/246). The eradication rate of the two groups had no relationships with gender, smoking or alcohol intake.

**Conclusions:** In adults with *H. pylori* infection, the sequential eradication therapy resulted in the same rate compared with the 10-day triple therapy.

Abstract no.: WS5.3

**ERADICATION THERAPY FOR *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH DUODENAL ULCERS BASED ON FURAZOLIDONE TRIPLE AND QUADRUPLE THERAPY: A MULTICENTER RANDOMIZED CONTROLLED TRIAL**

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**Objective:** To evaluate the efficacy of furazolidone-based triple and quadruple therapy in eradicating Hp.

**Methods:** We enrolled 580 patients referred to ten hospitals in Jiangxi province with duodenal ulcer and Hp infection. Patients were randomly assigned to four treatment groups (A–D): Group A and B, rabeprazole 10 mg, amoxicillin 1000 mg, furazolidone 100 mg, given twice daily for 7 and 10 days respectively; Group C and D, rabeprazole 10 mg, bismuth 220 mg, amoxicillin 1000 mg, furazolidone 100 mg, given twice daily for 7 and 10 days respectively. Hp status was re-assessed with the 14C-urea breath test 4 weeks after the end of therapy.

**Result:** Five hundred and forty-two patients completed the therapy and were re-assessed for *H. pylori* status with the 14C-urea breath test. Thirty-eight patients discontinued. According to the analysis of ITT, the Hp-eradication rate in group A to D were 73.8% (107/145), 79.3% (115/145), 82.8% (120/145) and 86.9% (126/145) respectively, there was significant deviation among all groups ( $p = .035$ ). Hp-eradication rate in group B was significantly higher than that in group A ( $p = .005$ ). According to the analysis of PP, the A to D were 79.9% (107/134), 85.2% (115/135), 88.9% (120/135) and 91.3% (126/138) respectively, there was significant deviation among all groups ( $p = .036$ ). Hp-eradication rate in group C and D were significantly higher than that in group A ( $p = .041, .007$ ).

**Conclusion:** Furazolidone-based quadruple therapy provide higher *H. pylori* eradication rates than triple therapy; But there are no significant deviation between therapy for 7 days and for 10 days.

Abstract no.: WS5.4

**NICKEL FREE-DIET ENHANCES *HELICOBACTER PYLORI* ERADICATION RATE**

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**Background:** *Helicobacter pylori* eradication rate with standard triple therapy is very low. *H. pylori* required the nickel-containing metalloenzymes urease and NiFe-hydrogenase to survive at low pH in stomach. In vitro studies showed that media added with nickel increased *H. pylori* urease activity.

**Aim:** To compare *H. pylori* eradication rate of a nickel free-diet associated to standard triple therapy and standard triple therapy alone in first-line regimen.

**Methods:** Forty sex and age matched patients at first diagnosis of *H. pylori* infection were randomized 1 : 1 into two different schemes: 1, standard LCA: lansoprazole 15 mg bid, clarithromycin 500 mg bid and amoxicillin 1000 mg bid for 7 days in common diet; 2, standard LCA plus a nickel free-diet (NFD-LCA). Patients undergo 30 days of nickel free diet and LCA is performed at the 15th day. Eradication was confirmed by 13C-urea breath test 4 weeks after the end of therapy. Compliance and adverse effects were assessed by validated questionnaire.

**Results:** All pts completed the study. Significant higher eradication rate was observed in LCA: 60% (12/20) versus NFD-LCA: 90% (18/20)  $p < .01$ . A good compliance of the nickel free diet was observed. Mild side effects (diarrhoea and nausea) occur in 3/20 with LCA and 4/20 in NFD-LCA.

**Conclusions:** The add of a nickel free-diet to standard triple therapy significantly increase *H. pylori* eradication rate. The reduction of *H. pylori* urease activity due to the nickel free diet, could expose bacterium to the gastric acid with a decrease of survival. However further studies are necessary to confirm this preliminary result.

Abstract no.: W55.5

**DISTRIBUTION OF GYRA MUTATIONS IN 97 FLUOROQUINOLONE-RESISTANT *HELICOBACTER PYLORI* ISOLATES IN FRANCE**

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*Helicobacter pylori* infection is associated with severe inflammatory gastroduodenal disease including peptic ulcer disease and gastric cancer. Since 2000, antimicrobial resistance of *H. pylori* increased dramatically and evolved to multiresistance particularly to clarithromycin, metronidazole and more recently to fluoroquinolones. We studied 97 isolates of *H. pylori* collected from gastric biopsies of patients from Paris and Poitiers. These strains were all resistant to ciprofloxacin. The MIC was determined using the agar dilution reference method (breakpoint 1 mg/L). The QRDR of *gyrA* gene was sequenced for all the strains. Among the 97 studied isolates, 94 harbored at least one mutation already described in the QRDR region of *gyrA* (T87I n = 23, N87K n = 32, D91N n = 30, D91G n = 7, D91Y n = 6), two harbored a mutation never previously described (D91H and A88P). The role of these two new mutations was assessed by a transformation of the *gyrA* wild type strain J99 with each one of the *gyrA* amplified DNA of the resistant strains. One strain was resistant (ciprofloxacin MIC 8 mg/L) without any mutation in the *gyrA* and *gyrB* genes. The prevalence of *gyrA* mutations conferring fluoroquinolone resistance among 97 French clinical isolates was identified and two new mutations in the QRDR of *gyrA* were reported.

Abstract no.: W55.6

**IS *HELICOBACTER PYLORI* ANTIBIOTIC RESISTANCE SURVEILLANCE NEEDED AND HOW CAN IT BE DELIVERED? A FEASIBILITY STUDY IN THREE UK CENTRES**

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In England in 2008 *Helicobacter pylori* culture and antibiotic sensitivity was only routinely performed in Gloucester and the Helicobacter Reference Unit (HRU). The Maastricht III Consensus recommends surveillance of primary *H. pylori* antibiotic resistance to inform empirical treatment – notably Clarithromycin should not be prescribed if local resistance rates are above 15–20%. Resistance rates in the UK are currently unmonitored.

We aimed to determine the feasibility of *H. pylori* antimicrobial resistance surveillance, using gastric antral biopsy specimens from routine endoscopies cultured in Gloucester and Bangor and referred to HRU. European standard methods were used for culture, and susceptibility by E-tests.

Prevalence was low in Bangor 6.6% and Gloucester 5.5%. Higher prevalence (32.2%) and resistance rates in the HRU reflected a greater proportion of referrals 'post treatment'. Resistance rates were: Metronidazole, HRU 87%, Glos 22%, Bangor 38%; Clarithromycin, HRU 68%, Glos 4%, Bangor 15%; Levofloxacin, HRU 17%, Glos 1%, Bangor 13%; Rifabutin, HRU 0%, Glos 2.8%, Bangor 3.3%; Amoxicillin, HRU 2.8%, Glos 0%, Bangor 1.7%; Tetracycline HRU <1%, Glos 1.4%, Bangor 0%. Patient history data collection retrospectively by endoscopy staff was poor, thus a dedicated staff member in Gloucester PCU completed all data collection.

Surveillance is essential in the UK as Clarithromycin resistance varies significantly between centres and is reaching the Maastricht threshold between centres. There was higher resistance post treatment. Future studies would require dedicated clerical staff to collect all data. Since prevalence was low, large numbers of biopsy specimens would be needed to monitor antibiotic susceptibility in the UK.



# WS6 Microbiology, Molecular Pathology, Virulence Factors

Abstract no.: WS6.1

## PRESENCE OF MANNOSE IN BIOFILMS OF *HELICOBACTER PYLORI*

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*H. pylori* forms biofilms. However, the components of the biofilm have yet to be elucidated. In this study, the carbohydrate and protein compositions of *H. pylori* NCTC11637 biofilms of different ages were investigated. Crystal violet staining showed that biofilm formation increases over time. Scanning electron microscopy shows that biofilms of Day 4, 7 and 14 consisted mostly of spirals, spirals + coccioids and coccioids, respectively. Size exclusion chromatography and nuclear magnetic resonance indicates the presence of proteomannans. Monosaccharide analysis of extracted extracellular polysaccharides of Day 14 biofilms indicated that mannose is the major sugar (80%) followed by glucose (13%) and galactose (7%). GC-MS and Hakomori methylation analyses of sugar linkages of the biofilm revealed that the spiral biofilm contained 1,3-mannosyl,1,4-mannosyl and terminal mannosyl linkages. Interestingly, both the spirals + coccioids biofilm and coccioid-biofilm showed 1,4-mannosyl as the common linkage. Ten differentially expressed proteins were detected in 1D-protein analysis of Day 4 and Day 7 biofilms. Of these, 60 kDa chaperonin (GroEL) and neutrophil-activating protein A (NapA) were found to be upregulated in Day 7 biofilm. Interestingly, culture of napA mutant was observed to form microcolonies that were looser and less compact compared to that of wild-type. Our studies have shown that mannose is a major sugar component of NCTC 11637 biofilms. Additionally, our protein study suggests that NapA plays an important role in adhesion to the substratum follows by formation of biofilm. Studies are on-going to better understand the role of proteomannans play in biofilm formation.

Abstract no.: WS6.2

## COMPARATIVE PROTEOMIC ANALYSES OF *HELICOBACTER PYLORI* STRAINS FROM CHILDREN WITH AND WITHOUT IRON DEFICIENCY

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**Introduction:** *H. pylori* infection is associated with iron deficiency (ID) likely due in part to sequestration of host iron by the pathogen and transient gastric hypochlorohydrin associated with acute infection, which can modulate iron absorption by the host. To colonise successfully *H. pylori* must compete with the host for bio-available iron and iron availability modulates protein expression by the pathogen. The aim of this study was to identify changes to the proteome of ID and non-ID-associated isolates of *H. pylori* when cultured under the iron-restricted conditions encountered in vivo.

**Methods:** Clinical isolates of *H. pylori* were obtained from children referred for upper GI endoscopy. Haematological analyses was undertaken to determine iron status. *H. pylori* was grown in broth culture under iron-replete and iron-restricted conditions. Isolates from subjects with, and without ID, were subjected to 2D SDS-PAGE analysis. Differentially expressed proteins were identified by mass spectrometry.

**Results:** Multiple *H. pylori* proteins were differentially expressed when the pathogen was cultured under iron-restriction compared with iron-replete conditions, including adhesins, OMPs, virulence and colonisation factors. Difference gel electrophoresis (DIGE), in combination with image analysis software, is being used to quantify and determine the significance of the protein expression changes between ID and non-ID-associated strains.

**Conclusions:** These data demonstrate differential protein expression profiles between ID and non-ID associated *H. pylori* isolates when cultured under iron-restricted conditions. Whether or not these differences are linked to clinical outcome remains to be determined. Funded under VITH Framework Programme of the European Union, Project CONTENT (INCO-DEV-3-032136).

Abstract no.: WS6.3

## *HELICOBACTER PYLORI* BCS 100 GENOME VARIABILITY AND POTENTIAL VIRULENCE DETERMINANTS DURING AN EXPERIMENTAL HUMAN INFECTION

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*Helicobacter pylori* shows extremely high allelic diversity. We recently demonstrated extensive genomic changes in sequential isolates obtained from chronically infected Colombians (Kennemann, PNAS 2011). Much less is known about genomic adaptations in the first months of infection of a new human host. Here, we present the complete genome sequence of *H. pylori* strain BCS 100 (H1), a cagPAI deficient isolate that has been used for several human challenge studies in the US and Germany, and draft genome sequences of reisolates obtained from four volunteers after 3 months of infection.

The genome sequence of *H. pylori* H1 was obtained by 454 sequencing and subsequent gap closure. Draft genome sequences were obtained by 454 sequencing of one additional input isolate (H3) and four isolates from four volunteers belonging either to the control group or to the Ty21a (UreA/B) vaccinated group. Additional reisolates from 29 volunteers were analyzed at selected loci by Sanger sequencing.

In contrast to the sequential isolates from Colombia, no evidence of recombination was detected in the reisolates from infected volunteers. 1–2 novel SNPs were identified in each of the reisolated genomes, permitting the calculation of a robust estimate for the *H. pylori* in vivo mutation rate. Unexpectedly, a previously unknown variant of H1 was detected in several volunteers which differed from the 454 sequenced input clone by 86 SNPs and three clusters of nucleotide polymorphisms (CNPs). This variant was commonly detected among the additional reisolates, suggesting strong in vivo selection favoring this subpopulation in some human hosts.

Abstract no.: WS6.4

## WHOLE GENOME SEQUENCE ANALYSIS OF *HELICOBACTER HEILMANNII*

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Although “*Candidatus Helicobacter heilmannii*” has been proposed as a provisional species name years ago, little was known about this species because it was unculturable. Recently, *H. heilmannii* has been cultured in vitro from the gastric mucosa of cats, resulting in the valid description of this microorganism as a novel *Helicobacter* species. This bacterium, naturally colonizing the stomach of cats and dogs, has been associated with gastritis, gastric and duodenal ulcers and low grade mucosa associated lymphoid tissue (MALT) lymphoma in humans. In order to obtain better insights in the genes involved in pathogenicity and the adaptation to the gastric environment, a whole genome sequence analysis of this zoonotic *Helicobacter* species was performed. Several genes encoding homologues of known *H. pylori* virulence factors were annotated. These include the gamma-glutamyl transpeptidase GGT, the immunomodulator NapA, the flavodoxin FldA, the plasminogen binding proteins PgbA and PgbB and the secreted serine protease HtrA. *H. heilmannii* encodes several outer membrane proteins (OMPs), such as HpaA, HorB and 2 haemagglutinin-like OMPs, but lacks the important Bab and Sab adhesins. The genome possesses a complete comB system conferring natural competence but lacks a Cag pathogenicity island as well as homologue genes encoding a vacuolating cytotoxin VacA. However, *H. heilmannii* harbours a paralogue of the *H. pylori* VacA. Although some genes encoding *H. pylori* virulence factors are not detected, homologues of other genes involved in colonization, induction of lesions and inflammation are present in the *H. heilmannii* genome and may contribute to this pathogen’s virulence and carcinogenic properties.

Abstract no.: WS6.5

## *HELICOBACTER PYLORI* CAGL INDUCES GASTRIN EXPRESSION

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Gastrin is mainly required for the regulation of the gastric pH, but is also involved in growth and differentiation of gastric epithelial cells. In *Helicobacter pylori*-



infected patients and Mongolian gerbil model gastrin secretion can be up-regulated by the pathogen, resulting in hypergastrinemia. *H. pylori*-induced hypergastrinemia is described as being a major risk factor for development of gastric adenocarcinoma.

Upstream signaling and bacterial factors involved in *H. pylori*-induced gastrin gene expression were investigated. Gastric epithelial cells which were stably transfected with a human gastrin promoter luciferase reporter construct were stimulated with *H. pylori* wild type (WT) and isogenic *cag* and OMP mutant strains. To identify the binding host receptor siRNA, blocking antibodies, binding experiments, and immunoprecipitation experiments were applied.

Interestingly, adherence of *H. pylori* to epithelial cells is essential for gastrin promoter stimulation but occurs Alp, Sab, and Bab adhesions independent. Transfecting these cells with CagA expressing vector or stimulating with activated VacA revealed no gastrin promoter activation. Out of several *H. pylori* *cag*PAI mutants tested, for the first time, we could show that CagL, which binds at the surface of the T4SS pilus, stimulates the gastrin promoter in a RGD-independent manner. Integrin 1, as a possible interacting partner for CagL gastrin promoter activation, could not be verified neither by siRNA nor blocking experiments. Furthermore, upon interaction of *H. pylori* with gastric epithelial cells, we identified the EGFR/Raf/MEK/ERK downstream signaling cascade, which plays a central role in *H. pylori* gastrin induction.

Abstract no.: WS6.6

#### **HELICOBACTER PYLORI CAGA INHIBITS ENDOCYTOSIS OF CYTOTOXIN VACA AND ITS RECEPTOR RPTP ALPHA IN HOST CELLS**

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*Helicobacter pylori* has evolved to establish persistent infections in the human stomach. Epidemiological evidence suggests that *H. pylori* with both highly active

vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA), the major virulence factors, has an advantage in adapting to the host environment. However, the mechanistic relationship between VacA and CagA remains obscure. We report that CagA interferes with eukaryotic endocytosis, as revealed by genome-wide screening in yeast. Moreover, CagA suppresses pinocytic endocytosis and the cytotoxicity of VacA in gastric epithelial cells without affecting clathrin-dependent endocytosis. Our data suggest that *H. pylori* secretes VacA to attack distant host cells while injecting CagA into the gastric epithelial cells to which the bacteria are directly attached, thereby protecting these attached host cells from the cytotoxicity of VacA and creating a local ecological niche. This mechanism may allow *H. pylori* to balance damage to one population of host cells with the preservation of another, allowing for persistent infection. We also demonstrated that the uptake of VacA receptor RPTP alpha from the host cell membrane were inhibited in CagA expressing cells. CagA might contribute to changes in signalling pathways through modifying endocytosis in gastric epithelial cells infected with *H. pylori*.

# Posters

## P01 Gastric Cancer, Pathology and Pathophysiology, Preneoplastic and Neoplastic Diseases

Abstract no.: P01.01

### DOWN-REGULATION OF ACTIVATION-INDUCED CYTIDINE DEAMINASE BY CURCUMIN

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Aberrant expression of activation-induced cytidine deaminase (AID) in *H. pylori*-infected gastric epithelial cells has been postulated as the key mechanisms in the development of gastric cancer, via the induction of p53 mutation in epithelial cells. The suppression of AID might serve a novel strategy to prevent *H. pylori*-induced gastric cancer. Curcumin, a spice derived polyphenol, has anti-inflammatory activity. In this study, we investigated whether curcumin modify AID expression in *H. pylori*-infected gastric epithelial cells. Gastric cancer cell lines, MKN-28 or MKN-45 cells and *H. pylori* strains were co-cultured. Cells were pretreated with or without non-bactericidal concentrations of curcumin. Apoptosis was determined by DNA fragmentation assay. Real-time PCR were used to evaluate AID, IL-6, IL-8, and TNF- $\alpha$  mRNA. Immunoblot was performed for the analysis of AID, NF- $\kappa$ B, I $\kappa$ B, and IKK. At the concentration of 10  $\mu$ mol/L, curcumin did not show any bactericidal activity to *H. pylori*. Pretreatment of curcumin at  $\leq$ 10  $\mu$ mol/L down-regulated the mRNA and protein expression of AID provoked by *H. pylori*. Similarly, expression of inflammatory cytokines such as TNF- $\alpha$ , IL-6 and IL-8 were also suppressed by curcumin. Moreover, curcumin ( $\leq$ 10  $\mu$ mol/L) suppressed *H. pylori*-induced NF- $\kappa$ B activation via inhibition of IKK activation and I $\kappa$ B degradation. Non-bactericidal concentration of curcumin down-regulated *H. pylori*-induced AID expression in gastric epithelial cells, via inhibiting NF- $\kappa$ B pathway. Curcumin might be a potential chemopreventive candidate against *H. pylori* related gastric carcinogenesis.

Abstract no.: P01.02

### CAN SERUM GASTRIN, PEPSINOGEN I/II LEVEL AND INTRAGASTRIC PH SHOW EXISTENCE OF THE ATROPHY IN GASTRIC NEOPLASTIC LESION BEFORE ENDOSCOPIC SUBMUCOSAL DISSECTION?

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**Introduction:** In general, serum pepsinogen level is a good surrogate marker of atrophic gastritis. The aim of this study is to investigate whether serum gastrin, pepsinogen I/II level and intragastric pH can show existence of the atrophy in gastric neoplastic lesion before endoscopic submucosal dissection (ESD).

**Methods:** From April 2010 to February 2011, we routinely checked serum gastrin, PG I, PG II, PG I/II ratio in 81 patients with gastric neoplastic lesion before ESD. And intragastric pH in gastric juice was measured. The endoscopic still photographs and histopathological finding were reviewed for investigating gastric atrophy. *H. pylori* infection was determined by rapid ureas testing.

**Results:** Thirty-one of low grade dysplasia (LGD), 16 of high grade dysplasia (HGD), 34 patients of early gastric cancer (EGC) were enrolled. Serum concentration of PG I and PG I/II ratio were significantly low in patients with corpus atrophic gastritis compared in patients with non-corpus atrophic gastritis ( $p = .004$ ,  $p = .007$ ). The PG II level was higher in *H. pylori* positive patients than in *H. pylori* negative patients ( $p < .001$ ). PG I of patients with LGC, PG I/II ratio of patients with HGD or EGC was significantly lower in patients with corpus atrophic gastritis than in patients with non-corpus atrophic gastritis. Patients with negative *H. pylori* and corpus atrophic gastritis had significantly lower PG I level than other patients ( $p < .001$ ).

**Conclusion:** We showed that serum pepsinogen I and I/II ratio are a good surrogate marker of atrophic gastritis and can be applied on adenoma and EGC. However, gastrin and intragastric pH did not show existence of the atrophy.

Abstract no.: P01.03

### UPREGULATION OF LEUKOTRIENE RECEPTORS IN GASTRIC CANCER

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**Background:** Leukotrienes (LT) mediate allergic and inflammatory processes. Previously, we identified significant changes in the expression pattern of LT receptors in the gastric mucosa after eradication of *Helicobacter pylori* infection. The aim of the present study was to evaluate the expression of 5-LOX and LT receptors in gastric cancer.

**Methods:** The expression of 5-Lipoxygenase (5-LOX) and receptors for LTB<sub>4</sub> (BLT-1, BLT-2) and cysteinyl-LT (CysLT-1, CysLT-2) were analyzed by immunohistochemistry (IHC) in gastric cancer samples of 35 consecutive patients who underwent gastrectomy and in 29 tumor-free tissue specimens from gastric mucosa.

**Results:** Male-to-female ratio was 24 : 11. The median age was 70 years (range 34–91). Twenty-two patients had gastric cancer of intestinal, six of diffuse, six of mixed and one of undifferentiated type. The IHC analysis showed a nearly ubiquitous expression of studied proteins in gastric cancer (88–97%) and in tumor-free specimens as well (89–100%). An increase in the immunoreactive score of both BLT receptors and CysLT-1 was observed in gastric cancer compared to tumor-free gastric mucosa ( $p < .001$  for BLT-1;  $p < .01$  for BLT-2 and CysLT-1, Mann-Whitney *U*-test). No differences in the IHC expression of 5-LOX and CysLT-2 were observed between gastric cancer and tumor-free mucosa. The expression of BLT-2, CysLT-1 and CysLT-2 was increased in gastric cancer of intestinal type when compared to the diffuse type ( $p < .05$ ; Mann-Whitney *U*-test).

**Conclusions:** LTB<sub>4</sub> receptors and CysLT-1 are up-regulated in gastric cancer tissue. The expression of BLT-2 and both CysLT-receptors is increased in gastric cancer of intestinal type compared to the diffuse type.

Abstract no.: P01.04

### HIGHER FREQUENCY OF CAGA-C PHOSPHORYLATION SITES IN H. PYLORI (HP)STRAINS FROM RELATIVES OF GASTRIC CANCER (GC) PATIENTS

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HP infection is an important risk factor for distal GC being CagA and VacA the major bacterium virulence factors. It is also has been shown that infection with CagA strains with higher number of EPIYA-C segments is a risk for GC. Since the infection is predominantly acquired in childhood and most HP strains are shared between familiar members, we evaluated the frequency of vacA genotypes and CagA EPIYA-C motifs in cagA-positive HP strains isolated from relatives of GC patients (n = 51) and from age and gender matched patients with no family history of GC (n = 49, control group), selected among those undergoing upper endoscopy for investigation of dyspeptic symptoms in Ceará, Northeastern Brazil. The number of EPIYA-C segments was determined by PCR and the results confirmed by sequencing. Data were analyzed by using SPSS, 17.0. Infection with vacA s1m1 genotype was more frequently observed in the relatives of GC patients than in controls. CagA-positive strains possessing >1 EPIYA-C motifs were more frequently observed (OR = 4.23, 95% CI = 1.53–11.69,  $p = .006$ ) in the group of GC relatives (22/51, 43.1%) than in the controls (8/43, 18.6%). Higher number of EPIYA-C segments was also associated with corpus inflammation ( $p = .04$ ), corpus foveolar hyperplasia ( $p = .05$ ) and corpus atrophy ( $p = .05$ ). In conclusion, we found that infection by HP CagA-positive strains harboring multiple EPIYA-C repeats is more frequently observed in relatives of patients with GC. These results, suggests that additionally to familial predisposing factors, GC relatives are infected with more virulent HP strains. Grants: INCT/Brazil

Abstract no.: P01.05

### COMPARATIVE GENOMIC PROFILING IDENTIFIES HDAC6 AND TRAF1 UNDERLYING THE OVERPROLIFERATION OF GASTRIC EPITHELIAL CELL LINE GES-1 IN VITRO INDUCED BY CLINICAL ISOLATES OF HELICOBACTER PYLORI FROM GASTRIC CARCINOMA SPECIMENS

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**Objective:** Persistent *Helicobacter pylori* (Hp) infection is subject to gastric carcinogenesis although the prognosis of Hp infection is highly variable among indi-

viduals. In this study, we examined the effects of *Hp* clinical isolates from gastric carcinoma (GC) on gastric epithelial cell line GES-1 in vitro, in the sense of cell behavior and genomic profiling.

**Methods:** *Hp* isolates were harvested from gastric carcinoma (GC, n = 10) or chronic gastritis specimens (CG, n = 10) were co-cultured with GES-1 cells individually. MTT assay was used to determine the proliferation of GES-1 cells. GES-1 cells that exhibited the most and least significant proliferative effect were harvested for microarray analysis, which was further verified by real-time PCR. Expressions of genes of interest were also examined in GC versus precancerous specimens in the presence of *Hp* infection by using immunohistochemistry.

**Results:** GES-1 cells exhibited a more potent proliferative response to the co-cultivation with *Hp* isolates from GC specimens versus from CG or homo-culture. Microarray analysis identified 2834 and 314 significant differential gene expression profiles in GES-1 cells co-cultured with GC- or CG-derived *Hp* isolates, respectively. Quantitative PCR analyses verified significant up-regulations of HDAC6 and TRAF1 mRNA expressions among GES-1 cells co-cultured with GC-derived *Hp* isolate, cells with CG-derived *Hp* isolate, and homo-cultured control cells. Immunohistochemistry showed that the expressions of TRAF1 and HDAC6 were sequentially up-regulated in chronic superficial gastritis, intestinal epithelial dysplasia, atypical proliferation and gastric adenocarcinoma specimens.

**Conclusions:** HDAC6 and TRAF1 are candidate genes contributing to pathogenesis of *Hp* associated gastric carcinoma.

Abstract no.: P01.06

#### MODULATION OF GATA 5 BY *HELICOBACTER PYLORI* INFECTION, IN VITRO AND IN VIVO

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The aims of this study were 1, to evaluate the effect of *Helicobacter pylori* infection on the expression pattern of GATA-5 in vitro and in vivo and 2, to investigate the methylation profile of GATA-5 in patients with chronic gastritis and gastric cancer. Our preliminary results in human gastric epithelial cells AGS infected with *H. pylori* showed an upregulation of GATA-5 after 6, 24 and 48 hour of infection. We found a decrease on GATA-5 expression in infected cells at 48 hour comparing to 6 and 24 hour. GATA-5 expression levels of gastric mucosa from *H. pylori*-infected mice showed an up regulation after 6 month of infection. The infection has no effect on mRNA levels after 12 month. These results were validated in 103 biopsies samples from individuals with chronic gastritis infected or not by *H. pylori* and patients with gastric cancer. Hypermethylation of the promoter region of GATA-5 was more prevalent among patients with gastric cancer (75%) when compared with *H. pylori* positive (32%) and negative (0%) chronic gastritis patients. Since the infection by *H. pylori* increase the generation of genotoxic compounds as well as the inflammatory response, the up regulation of GATA-5 observed in vitro and in vivo could be correlated with an early effect of *H. pylori* infection. Regarding the results from human biopsies we observed an epigenetic inactivation of this gene which significantly correlates with *H. pylori* infection.

Abstract no.: P01.07

#### EFFECT OF ERADICATION OF *HELICOBACTER PYLORI* ON RECURRENCE AFTER ENDOSCOPIC MUCOSAL RESECTION OF GASTRIC ADENOMA AND EARLY GASTRIC CANCER

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The effect of *H. pylori* treatment on recurrence after endoscopic mucosal resection (EMR) of gastric cancer remain uncertain because of contradictory opinions. A total of 2089 adult patients aged between 28 and 88 years had undergone EMR of gastric adenoma and early gastric cancer from November 1, 2004 to December 31 2008 were investigated retrospectively. Among them, the 521 patients had been excluded from the study because of short follow up duration (<1 year) and short recurrence interval (<3 months) and no diagnostic test of *H. pylori*. We investigated group without *H. pylori* infection (35.4%) and group with *H. pylori* infection (64.6%) for recurrence rate, recurrence interval. Among group with *H. pylori* infection, group without *H. pylori* treatment were 25.8% and group with *H. pylori* treatment were 74.2%. Among total enrolled patients, mean age was 61.6 ± 9 years old, mean follow up durations were 51 months and mean

recurrence interval was 22 months. The baseline parameters of age, sex, depth of invasion, alcohol, smoking, proportion of early gastric cancer, mean follow up duration were homogeneously distributed. Recurrence rate of group without *H. pylori* infection and group with *H. pylori* infection was 6.1% and 14.8%. ( $p < .01$ ) In a subgroup of patients with *H. pylori* infection, recurrence rate of group without *H. pylori* treatment and group with *H. pylori* treatment was 25.9% and 11.2% ( $p < .01$ ). A retrospective study showed that *H. pylori* eradication may reduce the recurrence in the patients received EMR of adenoma and early gastric cancer.

Abstract no.: P01.08

#### SURVEILLANCE OF GASTRIC INTESTINAL METAPLASIA LEADS TO EARLIER STAGE DIAGNOSIS OF GASTRIC ADENOCARCINOMA

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**Introduction:** Gastric cancer is the end result of a series of mutations begun in early life. During the precancerous phase, histological changes takes place from chronic gastritis to intestinal metaplasia (IM), dysplasia and cancer. It is unknown whether endoscopic surveillance of intestinal metaplasia is worthwhile in low prevalence populations. Adelaide and Meath Hospital serves a population of 350,000 which performs approximately 4000 Oesophago-gastro-duodenoscopies (OGD) per year. All patients with IM are offered follow-up.

**Aims and Methods:** We examined all cases of gastric cancer diagnosed between 2005 and 2009 and identified how many were diagnosed having undergone surveillance for IM.

**Results:** There were 46 diagnoses of cancer during the timeframe. 8.7% (n = 4, 95% CI 3.43% > 20.32%) of these occurred in patients with previous diagnosis of IM, three of whom were having endoscopic surveillance. 69.56% (n = 32, 95% CI 55.19% > 80.92%) of all cancers were gastric adenocarcinoma. In two cases of adenocarcinoma patients were asymptomatic and had their tumours found by scheduled surveillance endoscopy. Both of these patients had T1N0M0 lesions. This is compared to 14.28% of the gastric adenocarcinoma that were not in patients with identified previous premalignant lesions were T1N0M0 ( $p$ -value = .0302). The patient with a history of IM not under surveillance (focal IM) was symptomatic and had been re-referred after defaulting to follow-up. This patient had a T2N1M0 adenocarcinoma. A fourth patient with IM under surveillance was found to have a non-MALT gastric lymphoma (focal IM).

**Conclusion:** This study shows an encouraging trend towards earlier diagnosis of malignancy in patients with gastric intestinal metaplasia who undergo surveillance OGD.

Abstract no.: P01.09

#### DEPARTMENTAL ATTITUDES FOR THE AWARENESS AND PREVENTION OF GASTRIC CANCER PREVENT MISSED DIAGNOSES

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**Introduction:** Missed and new gastric cancers occurring after oesophago-gastro-duodenoscopies (OGD) are reportedly frequent. Our endoscopy department employs a "triple-lock" strategy for the prevention of gastric cancer with three principles.

- 1 Biopsies are taken at every OGD for rapid urease testing and at least two for histology.
- 2 *H. pylori* is treated when found and eradication confirmed.
- 3 Premalignant lesions such as intestinal metaplasia are followed up with repeat OGD.

**Aims and Methods:** We aimed to identify if instituting policies aimed at the detection and prevention of gastric cancers was efficacious. We examined all cases of gastric cancer identified in the region in a 5 year period and cross-checked to see how many of these patients had passed through the endoscopy unit in the 7 years before their cancer diagnosis and examined their diagnoses and management from first point of contact.

**Results:** Thirty-three thousand five hundred and fifty-nine OGDs were done between 1998 and 2009. Between 2005 and 2009 19,324 OGD were performed and 46 cases of gastric cancer were detected. Of these, five (10.87%) had had a previous OGD. In four cases the diagnoses were found in asymptomatic patients undergoing surveillance for premalignant lesions. In the other case the patient had been diagnosed with *H. pylori* infection and intestinal metaplasia but defaulted follow-up and was re-referred with symptoms. All five patients remain alive with a mean time since diagnosis of 3.16 years.

**Conclusion:** Endoscopy units offer excellent opportunities for gastric cancer prevention. Institutional policies for gastric cancer prevention reduced missed cancers and can lead to early diagnosis and favourable outcomes in those developing neoplasia.

Abstract no.: P01.10

#### EFFECTS OF *HELICOBACTER PYLORI* INFECTION ON TUMORIGENESIS OF YOUNG AND ELDERLY PATIENTS WITH GASTRIC CANCER

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**Background and Aims:** The incidence of gastric cancer shows younger trend in recent years. *Helicobacter pylori* (*H. pylori*) has been classified as a group I carcinogen of gastric cancer by WHO. *H. pylori* carcinogenic process is long term, is it the major cause of young patients with gastric cancer? In this study, we investigated the efficacy of *H. pylori* on tumorigenesis of young and elderly patients with gastric cancer.

**Patients and Methods:** Hundred and ten tissue sections of gastric carcinomas including 55 young and 55 elderly patients were examined by modified Giemsa stain.

**Results:** 1, The young patients group (Y group) consisted of 26 male, 29 female, the mean age was  $23.87 \pm 3.31$  years (range 18–30). The elderly patients group (E group) consisted of 22 male, 33 female, the mean age was  $78.27 \pm 3.74$  years (range 75–90). 2, No significant difference of *H. pylori* infection was observed between Y group and E group (60.0% vs 65.4%,  $p > .05$ ). 3, In Y or E group: The rates of *H. pylori* infection in Female were not higher than in male (58.6% vs 61.5% and 72.7% vs 60.6%), the difference between the two groups were not significant ( $p > .05$ ).

**Conclusions:** *H. pylori* infection might be a not exclusive carcinogenic cause of young patients with gastric cancer.

Abstract no.: P01.11

#### *HELICOBACTER PYLORI* INVASION AND CYTOGENETIC CHANGES IN EPITHELIAL CELLS OF STOMACH MUCOSA IN CHRONIC GASTRODUODENITIS PATIENTS

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**Objectives:** It is known that *Helicobacter pylori* is a risk factor of stomach cancer, but cytogenetic criteria of its genotoxic action are not well investigated yet. The aim of our study is definition of cytogenetic markers which show cancerogenic properties of *H. pylori*.

**Methods:** We made histologic and citologic analysis of stomach antrum and body biopsies of 183 patients (124 patients with *H. pylori*-positive gastroduodenitis and 59 patients with *H. pylori*-negative gastroduodenitis). We investigated cytogenetic infringements in epithelial cells of stomach mucosa in chronic gastroduodenitis patients with different level of *H. pylori* invasion. Cells with true micronucleuses and cells with morphologic anomalies (chromatin relations “nucleus-nucleus”, nucleus-micronucleus” and “nucleus-micronucleus with tail”) of interface nucleuses were estimated.

**Results:** Frequency of cells with genetic infringements was significantly high in stomach antrum in comparison with stomach body ( $p < .05$ ,  $t = 2.5$ ). In *H. pylori*-positive group frequency of cells with cytogenetic changes in stomach antrum (8.1%) was significant high than in stomach body (4.4%). In *H. pylori*-positive group frequency of cells with genetic infringements in stomach antrum was significantly high in comparison with *H. pylori*-negative group. Level of true micronucleus and morphologic changes of epithelial cells increased significantly that might be associated with direct action of *H. pylori* on epithelial cells.

**Conclusion:** Cells genetic anomalies are associated with chromosome aberration and with activation of oncogenes. We recommend the analysis of cytogenetic changes and nuclear anomalies (“nucleus-nucleus”, nucleus-micronucleus” and “nucleus-micronucleus with tail”) for detection of risk group of stomach cancer among patients with *H. pylori*-associated chronic gastroduodenitis.

Abstract no.: P01.12

#### NUTRITIONAL INTERVENTION TO TACKLE *HELICOBACTER*-ASSOCIATED GASTRIC CANCER

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Key molecular players that link *Helicobacter pylori*-induced inflammation to gastric carcinogenesis are prostaglandins, cytokines, NF- $\kappa$ B, chemokines, angiogenic growth factors, and free radicals, etc, all of which can lead to increased mutations and altered functions of important enzymes and proteins, for instance, the activation of oncogenic products and/or the inhibition of tumor suppressor proteins, in inflamed gastric tissues, thus contributing to multi-stage carcinogenesis process, chronic atrophic gastritis to dysplasia or adenocarcinoma. Furthermore, the elucidation of the exact molecular mechanisms by which chronic inflammation increases cancer risk can make the intervention of targeted drugs or agents during the inflammation-associated carcinogenic process for cancer prevention. In this Far East Symposium of EHS, a wise strategy to prevent inflammation based cancer through efficient control of *H. pylori*-induced inflammation process with the intervention of nutraceuticals will be introduced. For instance, we have very promising results as follows; *Helicobacter pylori* (*H. pylori*)-associated gastric carcinogenesis tackled with the administration of phytochemicals including Korean red ginseng, special extracts of licorice, and *L. plantarum*, Korea Cuisine, Gimch-derived probiotics. Especially, Korean red ginseng is a good example of a natural herb that has ubiquitous properties that are conducive to stopping inflammatory carcinogenesis that is associated with *H. pylori* infection, rendering rejuvenation of chronic atrophic gastritis and our novel extract of licochalcone A was very efficient in blocking the progression of *H. pylori*-associated precancerous lesion.

Abstract no.: P01.13

#### STUDY OF *HELICOBACTER PYLORI* INFECTION AND TISSUE EXPRESSION OF P53, CLEAVED CASPASE-3, BCL-2 AND BAX IN GASTRIC BIOPSIES

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**Background:** *Helicobacter pylori* is the main cause of chronic gastritis, modulating genes controlling apoptosis. *H. pylori* infection has been reported to upregulate caspase-3, Bax and mutated p53, among other proapoptotic factors, and to downregulate Bcl-2 antiapoptotic protein.

**Aims:** To study the expression of p53, cleaved caspase-3 (cCasp3), Bax and Bcl-2, together with *H. pylori* infection, in gastric antrum and body biopsies of dyspeptic patients.

**Methods:** Biopsies from patients subjected to gastroscopy according to clinical criteria were studied by immunohistochemistry (IHC). Exclusion criteria were: previous eradication therapy, ulcer disease, regular non-steroidal anti-inflammatory use, or antibiotic treatment 30 days prior to recruitment. Tissue expression of p53, cCasp3, Bax and Bcl-2 was determined with specific antibodies (R&D Systems; DAKO) and classified as positive versus negative (cCasp3 and p53) or low versus high expression (Bax and Bcl-2). *H. pylori* infection was assessed by <sup>13</sup>C-urea breath test and/or histopathological diagnosis.

**Results:** Twenty-four biopsies (13 antral) from 15 patients were studied. The median age was 62(37–64) years, 40% males, 83% gastritis and/or atrophy and 29% were *H. pylori* positive. *H. pylori* infection showed no effect on studied markers. All infected patients had gastric lesions. All cCasp3 (30%) and/or p53 (35%) positive samples showed gastritis and/or atrophy; Bax expression was high in all samples without alterations and low in 77% of gastritis, the only negative biopsy showed mucosal atrophy; Bcl-2 was not associated with histological findings.

**Conclusions:**

1 *H. pylori* had no effect on the studied factors.

2 p53 or cleaved caspase-3 positive samples had gastric mucosal lesions.

3 Bax expression inversely correlated with the grade of mucosal lesions.



Abstract no.: P01.14

THE BMP PATHWAY MEDIATES THE *HELICOBACTER PYLORI*-DEPENDENT UP-REGULATION OF CDX2 IN AGS GASTRIC CARCINOMA CELL LINE

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*Helicobacter pylori* (*Hp*) colonization of the gastric mucosa increases the risk to develop gastric intestinal metaplasia (IM) and cancer. CDX2 is the key molecular mediator of gastric IM. We have shown that *Hp* upregulates the expression of CDX2 in gastric cells in an in vitro co-culture model. However the mechanisms underlying this regulatory effect are not understood. A candidate pathway is the Bone Morphogenetic Protein (BMP)/SMAD4 pathway since it is strongly activated in IM and upregulates the expression of CDX2 in gastric cell lines. Concordantly, it was shown that *Hp* infection leads to an influx of BMP-expressing inflammatory cells to the stomach. These studies show that *Hp* and the BMP/SMAD signaling pathway upregulate the expression of CDX2 in the gastric context.

Our aim was to clarify if the BMP pathway is the mediator of the *Hp* effect on CDX2 expression.

Upregulation of the BMP/SMAD4 pathway was observed in AGS cells following an 8 hour co-culture with either a CagPAI+ (26695) or a CagPAI- (Tx30) *Hp* strain (MOI 1 : 100). This was demonstrated by increased endogenous expression of BMP2, SMAD4 and pSMAD1/5/8, the hallmark of an active pathway. Concomitant CDX2 upregulation was observed. Furthermore, in AGS cells in which the BMP/SMAD4 pathway was compromised by SMAD4 downregulation using shRNAs, CDX2 upregulation by *Hp* was significantly impaired.

In conclusion, this study shows a pivotal role of the BMP/SMAD4 pathway as a mediator of *Hp* infection and CDX2 expression in vitro which further supports the relevance of this pathway for the development of gastric IM.

Abstract no.: P01.15

*H. PYLORI* ERADICATION REDUCES THE RECURRENCE OF GASTRIC ADENOMA AFTER ENDOSCOPIC MUCOSAL RESECTION (EMR) OR ENDOSCOPIC SUBMUCOSAL DISSECTION (ESD)

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**Background:** *Helicobacter pylori* is the main cause for gastric cancer. Infection with *H. pylori* triggers the carcinogenesis cascade from gastritis into atrophic gastritis, intestinal metaplasia, dysplasia, and eventually, into gastric cancer. Eradication of *H. pylori* is proven to reduce the incidence of gastric cancer, and in some studies, it has been shown to inhibit gastric adenoma progression into gastric cancer.

**Aim:** To investigate whether *H. pylori* eradication prevent the recurrence of gastric adenoma after endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD).

**Methods:** We retrospectively reviewed medical records of 150 patients who underwent EMR or ESD for gastric adenoma. *H. pylori* status was assessed either by biopsy obtained with endoscopy or CLO test. The recurrence rate of gastric adenoma between the eradication group and non-eradication group was compared using the Fisher's exact test.

**Results:** Sixty-six patients positive for *H. pylori* infection were included for analysis. Of these, 42 patients received eradication therapy and 24 patients did not. Sex, mean age and pathologic grade of adenoma did not differ between the two groups. Gastric adenoma recurred in 3 of the 42 patients who received the eradication therapy and in 7 of the 24 patients who did not and this difference was statistically significant ( $p = .029$ ).

**Conclusion:** Although preliminary, the results of this study suggest that *Helicobacter pylori* eradication is associated with the reduced recurrence of gastric adenoma, a premalignant lesion of gastric cancer, after EMR or ESD.

Abstract no.: P01.16

IMPLICATION OF COX-2 ON PREDICTING REGRESSION OF *HELICOBACTER PYLORI* ASSOCIATED GASTRIC HYPERPLASTIC POLYP AFTER ERADICATION

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**Background and Aims:** Recent studies suggest that eradication of *Helicobacter pylori* (*Hp*) may lead to the regression of gastric hyperplastic polyps (GHP). We evaluated clinical parameters and immunohistochemical staining of GHP before and after *Hp* eradication between regression and non-regression group to predict regression of polyp.

**Methods:** We enrolled 187 patients with GHP. The polyps were measured by using biopsy forceps, and endoscopic changes of polyps were assessed by two endoscopists.

**Results:** Total regression was observed in 68 patients of eradicated group and six patients in non-eradicated group (42.5% vs 22.2%;  $p < .05$ ). Non regression rate was significantly higher in non-eradicated group than that of eradicated group (33% vs 10%;  $p < .05$ ). Comparing between regression and non-regression group, incidence of polyps that were smaller than 10 mm in size and sessile were significantly higher in the regression group. And EGFR, P53 and Ki-67 expression was not stained in both groups before and after *Hp* eradication. Cox-2 expression of cytoplasm in stroma cell was disappeared after *Hp* eradication in regression group, but it was still stained in non-regression group.

**Conclusions:** *Hp* eradication could be a therapeutic option for *Hp* positive-hyperplastic gastric polyps, especially <10 mm in size and sessile. And the patterns of Cox-2 expression may be an important predictive value of regression after *Hp* eradication.

Abstract no.: P01.17

CLINICOPATHOLOGIC FEATURES OF ACQUIRED HYPERPLASTIC GASTRIC POLYPS AT THE RESECTION SITE AFTER ESD OR EMR

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**Backgrounds and Objectives:** Endoscopic submucosal dissection (ESD) or endoscopic mucosal resection (EMR) has been widely performed for early gastric cancer (EGC) or gastric adenoma that would result in artificial ulcers. We have found patients with acquired hyperplastic polyps at the healed artificial ulcer site. The aim of this study was to identify clinicopathologic features that might be associated with the development of hyperplastic polyps at the resection site after ESD or EMR.

**Methods:** This was a retrospective study of 1960 patients with hyperplastic gastric polyps at the healed artificial ulcer site from January 2002 to April 2011. Demographic data, polyp characteristics, *Helicobacter pylori* (*H. pylori*) infection status, and change of polyp after eradication of *H. pylori* were analyzed.

**Results:** Hyperplastic gastric polyps were found in 22 of 1960 patients (1.1%). The mean discovering time of acquired hyperplastic polyps were 8.4 months (3–43 months). Sixteen patients had polyps located in the antrum and six had in the corpus (including angle), nine had as a single and 14 had as multiple. Eight patients had Y-I polyps, eight had Y-II, five had Y-III and 1 had Y-IV. Thirteen patients had pathologically confirmed hyperplastic polyps and eight had polyps such as chronic atrophic gastritis (6), inflammatory polyp (1), granulation tissue (1). The overall prevalence of *H. pylori* infection of the patients with hyperplastic polyps was 63.6% (14/22) and almost were (12/14) successfully eradicated. In 12 patients that successfully eradicated, 75% (9/12) of polyps were totally or partially regressed, and 25% (3/12) were not changed or recurrent. In four patients that had no *H. pylori*, on the other hand, all were not changed or recurrent.

**Conclusions:** Acquired hyperplastic gastric polyps that develop after ESD or EMR are likely to be associated with *H. pylori* infection as well as naturally developed hyperplastic polyps. Also, underlying extensive mucosal injury during ESD or EMR and healing process of artificial ulcers by epithelial migration and proliferation might play important role in developing hyperplastic polyps. Further studies are needed to clarify the mechanism of developing acquired hyperplastic gastric polyps.

Abstract no.: P01.18

**HIGH INCIDENCE OF MICROSCOPIC GASTRIC INTESTINAL METAPLASIA IN PATIENTS WITH ENDOSCOPICALLY NORMAL STOMACH IN A LOW PREVALENCE GASTRIC CANCER REGION**

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Gastric intestinal metaplasia (GIM) is a premalignant lesion that leads to 10-fold increased risk of gastric cancer. It is comparable with other premalignant conditions such as Barrett's Oesophagus, but is more difficult to recognise using white light endoscopy. There is no consensus on the routine biopsy of gastric mucosa during endoscopy to diagnose GIM in cohorts with a lower prevalence of gastric cancer. The aim of this report is to examine the presence of GIM in a low prevalence group with macroscopically normal gastric mucosa at white light endoscopy.

**Methods:** Reports of oesophagogastroduodenoscopy (OGD) procedures performed by a single experienced endoscopist over a 6 month period were reviewed to identify patients with macroscopically normal gastric mucosa using a white

light endoscope. Histology of gastric biopsies was reviewed to determine the cases of GIM, characterised by the presence of goblet cells and columnar mucous cells on hematoxylin-eosin staining.

**Results:** Seventy-eight of 94 patients who underwent OGD had macroscopically normal gastric mucosa. The indications were; dyspepsia, non specific abdominal pain, small bowel biopsy and reflux symptoms. All 78 patients had random gastric biopsies taken from the Cardia, Corpus and Antrum. Histology revealed six (7.7%) patients had evidence of GIM, with no dysplasia or malignancy; three in the Antrum, two in the Corpus, and one in the Cardia.

**Conclusion:** About 1 in 12 patients with normal-looking gastric mucosa in low incidence regions may have premalignant gastric lesions. This supports taking routine gastric biopsies from different sites at OGD regardless of the gross findings.



## P02 Microbiology, Molecular Genetics and Genomics

Abstract no.: P02.01

### DIVERSITY AND PHYLOGENY OF THE *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEIN ENCODING GENE HOMC

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The genetic diversity and evolution of the homC gene was evaluated in a panel of approximately 200 clinical and reference strains, isolated from patients from different geographical origins and presenting different gastric diseases. PCR, sequencing and bioinformatics analyses were used.

All the strains tested harboured a complete homC gene at a conserved locus. Phylogenetic reconstruction of homC showed a geographical segregation, with three predominant groups: Western, East Asian/Amerindian and African. A similarity plot analysis suggested a conserved profile of gene segmentation, where three segments were defined. In the first segment (5' end extremity), sequences were separated according to the geographical origin of the strain. A higher level of diversity (<50%) was observed in the middle segment, while the third segment (3' end extremity) was the most conserved (~90%). In the middle segment, eight allelic variants were identified, with geographic specificity regarding the most prevalent ones. The AI allele was predominant and exclusive of Western strains. The AII allele was predominant in African strains and was the only allele present in the three geographical groups. The AIV allele was predominant in East Asian/Amerindian strains and was not observed in Western strains. The Western group showed greater molecular distance while the sequences from the East Asian/Amerindian group were the closest.

Overall, the regular presence of homC and its allelic variability suggest that this gene is a good candidate to be part of the pool of *H. pylori* outer membrane proteins involved in bacterial persistence.

Abstract no.: P02.02

### HIGH WORLDWIDE CONSERVATION OF A *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEIN GENE, HOMD

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The genetic diversity and evolution of homD, coding for *Helicobacter pylori* outer membrane protein (OMP) was investigated in a panel of approximately 200 clinical and reference strains, isolated from patients from different geographical origins and presenting different gastric diseases. PCR, sequencing and bioinformatics analyses were used.

The homD gene was present in all strains, at a conserved locus, and showed a low genomic diversity, displaying high similarity at both nucleotide and amino acid level. A similarity plot analysis also showed a high level of sequence conservation, although a small region (~30 nucleotides) differed between Western strains and the other strains (East Asian/Amerindian and African). This region was also found in some allelic variants of another hom family member, the homC gene, suggesting the existence of recombination events between these two OMP encoding genes.

Sequence analysis of the HomD predicted protein showed a N terminus region with a variable number of KP motif repeats (2-9 KP), with a correlation between the lowest number of KP motif repeats (≤4 KP) and peptic ulcer disease and the highest number of repeats (≥7 KP) and gastritis. In silico analysis of the HomD protein showed that the region of KP motif repeats exhibits a strong hydrophilicity and antigenicity and a high probability of being exposed to the bacterial surface, suggesting that HomD is immunogenic.

These results suggest that homD gene is an important *H. pylori* antigen and, because of its high global conservation, it is likely to constitute a new vaccine target.

Abstract no.: P02.03

### DIVERGENT MECHANISMS OF INTERACTION OF *HELICOBACTER PYLORI* AND *CAMPYLOBACTER JEJUNI* WITH MUCUS AND MUCINS

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*Helicobacter pylori* and *Campylobacter jejuni* are related organisms specifically adapted to colonise the mucus layers of the gastric mucosa and intestine, respectively. This study aimed to examine the interaction of the organisms with mucins from various animal species and how they colonise the adherent mucus layer of mucus secreting cells. Mucus secreting HT29-MTX-E12 (E12) cells, mucin secreting HT29-MTX cells and HT29 cells (non mucin/mucus secretors) were each infected with *H. pylori* and *C. jejuni* organisms. Binding of *H. pylori* and *C. jejuni* to mucins purified from E12 cells and various animal species was assessed. Both *C. jejuni* and *H. pylori* displayed a tropism for chicken or porcine mucin respectively compared to mucins from other natural sources. *H. pylori* colonised E12 and to a much lesser extent HT29-MTX cells but not HT29 cells indicating that the presence of an adherent mucus layer was essential for efficient infection. In contrast, *C. jejuni* infected all three cell lines. However, the presence of an adherent mucus layer in E12 cells enhanced colonisation by *C. jejuni*. *C. jejuni* bound to E12 mucin. However, *H. pylori* bound not to mucin but to Lewis<sup>b</sup> containing non mucin fractions of E12 mucus. Although the presence of mucus was important for effective infection by both *H. pylori* and *C. jejuni* the mechanisms underpinning mucus colonisation by these two organisms differed. This study highlights the role of mucus in promoting bacterial infection and the importance of host glycans in mediating the interaction of bacteria with host tissue.

Abstract no.: P02.04

### USE OF MICROFLUIDIC DEVICES TO INVESTIGATE CHEMOTAXIS OF *HELICOBACTER PYLORI* IN RESPONSE TO GASTRIC CANCER CELLS

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**Background:** Host-pathogen interaction is of increasing interest in the study of infections. In the present study, we use custom-made microfluidic devices to examine chemotaxis of *Helicobacter pylori* to gastric epithelial cells.

**Methods:** Microfluidic devices were prepared using polydimethylsiloxane (PDMS) bound to a glass cover slip, forming three channels. The channels were then coated with Poly-D-Lysine. AGS cells and bacteria were seeded in two separate channels, while the third was filled with culture medium (nutrient). The setup therefore provides the pathogen the choice of interacting with AGS cells or nutrient. The devices were sacrificed by paraformaldehyde fixation at time intervals. The bacteria were first treated with an Anti-*H. pylori* antibody for four hours followed by a Cy3-tagged secondary antibody for another four hours. Cell nuclei were stained using DAPI. The devices were then analysed using Confocal Laser Scanning Microscopy (CLSM).

**Results and Conclusions:** The bacteria were stained red and appeared as bright red dots of high intensity at the interface between the collagen and the channel with the AGS cells. However, the intensity at the interface between the collagen and the culture medium channel was qualitatively low. It shows that *H. pylori* has a predilection for AGS cells than the nutrient. This preliminary study demonstrates that microfluidic device is a potential useful tool for evaluation of chemotaxis of pathogens. Experiments on cell-bacteria interaction and bacteria-bacteria communication are in progress.

Abstract no.: P02.05

### PRODUCTION OF MONOCLONAL ANTIBODY AGAINST ALKYL HYDROPEROXIDE REDUCTASE (AHP) OF *HELICOBACTER PYLORI*

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**Introduction:** Stool-antigen detection kits for diagnosis of *H. pylori* infection have been widely used because of their full non-invasive nature. Because *H. pylori*

strains show a distinctive genetic diversity, it is important to find a protein that is a common antigen of various strains and shows a strong immunogenicity for the development of a stool-antigen detection kit. Alkyl hydroperoxide reductase (AhpC) of *H. pylori* strongly reacts with the sera of patients with gastritis and peptic ulcer. Therefore, AhpC seems to be an excellent candidate as a target protein for this study.

**Method:** Isolation and purification of AhpC were performed by preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroelution. The purified enzyme was used as immunogen. Three BAL/C mice (female, 6-weeks old) were immunized by peritoneal injection of the immunogen mixed with same volume of Freund's complete adjuvant on day 0. Biweekly mice boosted with the immunogen mixed with Freund's incomplete adjuvant. After 6 week, a final injection of the immunogen without adjuvant was administered. Spleen cells and SP2/0 myeloma cells were fused by polyethylene glycol. Hybridoma cells were selected in a hypoxanthine aminopterin thymidine (HAT) medium and were subcloned twice by limiting dilution.

**Result:** Two stable clones produced antibodies (24H2, 27C7) that reacted with the same purified protein antigen and also with whole cell protein extract of *H. pylori* in an indirect enzyme immunoassay system.

**Conclusion:** These monoclonal antibodies can be used for development of immunoassay in order to detection of *H. pylori* antigen in stool of infected patients.

Abstract no.: P02.06

#### A COMPARATIVE STUDY BETWEEN GASTRITIS AND GASTRIC ADENOCARCINOMA SPECIMENS USING PYROSEQUENCING ANALYSIS FOR 16S rRNA-BASED IDENTIFICATION OF *H. PYLORI*

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Bacterial genotyping is important for diagnosis and selection of the most suitable therapy, and for understanding the pathogenesis of the disease. Using the pyrosequencing analysis for 16S rRNA-based identification of *H. pylori* it is possible to analyze bacterial genetic targets in DNA extracted directly from human gastric tissue, without time-consuming culturing of bacteria. We compared the results of gastritis and gastric adenocarcinoma specimens using pyrosequencing analysis for 16S rRNA-based identification of *H. pylori*. DNA was extracted directly from paraffin embedded gastric tissue. PCR primers were designed to amplify the 133-bp PCR fragment in highly conserved regions of the 16S rRNA gene. The sequence of the PCR products was analyzed using a PSQ 96 system with SQA software. *H. pylori* was present in 75 (50%) of the 150 gastritis specimens and 47 (92.2%) of the 51 gastric adenocarcinoma specimens. In the gastritis specimens, *C. hyointestinalis* (12 cases), *C. upsaliensis* (1 case) and *H. cinaedi* (2 cases) were detected in 15  $\alpha$ -*H. pylori* Ab staining (-)/Gimsa staining (+) cases. 60 cases (40%) of 150 gastritis specimens were  $\alpha$ -*H. pylori* Ab staining (-)/Gimsa staining (-). In the gastric adenocarcinoma specimens, *C. hyointestinalis* (1 case), *H. cinaedi* (2 cases) and *H. mustelae* (1 case) were detected in the four *H. pylori*-negative cases. Pyrosequencing analysis was useful in the identification and differentiation of *H. pylori* from other species by analyzing the gene encoding 16S rRNA. All gastric adenocarcinoma specimens contain bacteria and the majority are *H. pylori*. *C. hyointestinalis* was detected in the majority of non-*H. pylori*-related Giemsa stained specimens.

Abstract no.: P02.07

#### SUCCESSFUL LONG DISTANCE SHIPPING OF *H. PYLORI* CULTURES AT ROOM TEMPERATURE

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**Background:** Transport of *H. pylori* in frozen transport media using dry ice is successful but may fail if there is exhaustion of the dry ice and regulations prohibit use of dry ice in many regions.

**Aim:** To investigate whether soft agar stab could be used for long distance transport of *H. pylori* at room temperature.

**Methods:** Screw tip 4 mL transport medium vials containing 2.5 mL of *Helicobacter pylori* Special Peptone Agar (HPSPA) with 0.75% agar, 7% horse serum and 0.5%  $\beta$ -cyclodextrin were inoculated with a 10  $\mu$ L loop-full of fresh *H. pylori* growth by stabbing into the vials near the bottom portion of the agar medium. Vials were than incubated at 37°C in a 12% CO<sub>2</sub> incubator with the tops kept loose. Prior to shipping vials are inoculated and placed in a CO<sub>2</sub> incubator for 2–48 hour and then shipped at room temperature. Upon receipt the vials are placed in a 12% CO<sub>2</sub> incubator for 2 days with caps loosened and cultured after at least 2 days of incubation.

**Results:** *H. pylori* strains remain viable in this media for at least 7 weeks of incubation. Using this media strains have been successfully shipped at room temperature to Japan, Colombia, and Sweden with 100% recovery.

**Conclusion:** The use of a semi-solid agar medium may contributed to the survival of the *H. pylori* as the semi-solid media allows for establishment of an oxygen gradient with the agar matrix to produce an appropriate optimal microaerobic environment for *H. pylori* growth.

Abstract no.: P02.08

#### ELECTROELUTION OF THE STAINED ALKYL HYDROPEROXIDE REDUCTASE OF *HELICOBACTER PYLORI* FROM PREPARATIVE SODIUM DODECYL SULFATE POLYACRYLAMIDE GELS

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**Introduction:** Electroelution is a widely used methodology for protein purification. In this study, a practical and low cost system for alkyl hydro peroxide reductase (AhpC) purification from stained polyacrylamide gels was developed. AhpC of *Helicobacter pylori* has been described as a specific and unique enzyme for *H. pylori* and therefore, both *H. pylori* AhpC and anti-AhpC could be useful in the development of serologic and stool antigen tests, for detecting and monitoring *H. pylori* infection.

**Method:** In order to whole cell protein extraction, the bacterial cells were ruptured by octyl- $\beta$ -D glucopyranoside. AhpC from *H. pylori* isolated and purified by techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroelution.

**Result:** Purification was monitored on the basis AhpC-increased oxidation of NADPH, including the final electrophoretic purification. AhpC was purified 87-fold with an overall recovery of 90% from clinical isolates of *H. pylori*.

**Conclusion:** The present approach is simple, rapid, and low cost and makes it possible to preparation AhpC from *Helicobacter pylori*.

Abstract no.: P02.09

#### PROTEOME ANALYSIS OF CLUSTERED *HELICOBACTER PYLORI* STRAINS ACCORDING TO THEIR GENOMIC-METHYLATION STATUS

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Genomic-methylation typing method, based on strains' Restriction/Modification systems, confirmed the genetic variability of *Helicobacter pylori*. According to this, strains isolated from patients of the same family, or from the same geographic region, cluster together. The analysis of proteome's variability of these clusters has been a missing topic. We applied the Minimum-Common-Restriction-Modification (MCRM) algorithm to genomic-methylation data of 30 *H. pylori* strains, isolated from Portuguese patients, presenting different gastric diseases. 100% of generated dendrograms presented three incipient clusters (C1, C2 and C3), which is characteristic of strains sharing the geographic origin. The same pattern was observed when the MCRM algorithm was applied to a subset of strains (2 of C1, 2 of C2, 4 of C3 and two outsiders). These were heterogeneous regarding their *cagA* and *vacA* genotypes and in terms of patient's age, gender and gastric disease. Comparative analysis of two-dimensional-gel-electrophoresis (2-DE) maps, obtained for total-protein extracts of each strain, revealed that among 70 matched protein spots (in a universe of 300), 16 were differently abundant ( $p < .05$ ) among clusters. These proteins' abundance was then compared having the 2DE-maps re-grouped according to the strain's *cagA*-genotype or its association with gastric disease. We concluded that abundance variations of at least 12 proteins were dictated by differences in virulence, rather than cluster proximity. Therefore, although the genome-methylation typing method discriminates differences in restriction/modification enzymes, strains of each generated cluster do not share a marked particular proteome, arguing that strains with common geographic origin vary greatly in virulence. IV is recipient of SFRH/BD/38634/2007-fellowship.

Abstract no.: P02.10

#### A FUNCTIONAL OUTER MEMBRANE PHOSPHOLIPASE A (OMPLA) IS REQUIRED FOR SURVIVAL OF *HELICOBACTER PYLORI* AT PH 3.5

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*Helicobacter pylori* OMPLA degrades bacterial phospholipids to lysophospholipids. The phospholipase A gene (*pldA*) displays phase variation resulting in variants

with high (*pldAON*) or low (*pldAOFF*) OMPLA activity. We have previously reported that *pldAOFF* phase variants harbouring a truncated OMPLA protein are deprived the ability to survive acid shocks. In connection with an ongoing study of OMPLA protein structure we wanted to examine if the deprived ability to survive was a consequence of protein truncation or if any disturbance of OMPLA activity would give the same effect.

Spontaneous, isogenic pairs of OMPLA variants were selected. The *pldA* gene was sequenced to detect the genetic background for the OMPLA phenotype. Two pairs had missense mutations affecting the proposed active site, three pairs were classical phase variants.

Variants were spread on blood plates (pH 3.5) and incubated under microaerobic conditions for 18 hours and transferred to blood plates (pH 7.4). After 5 days incubation colonies were counted. Variants were also cultured in liquid media at pH 5 and pH 7 for 1 hour before total RNA extraction and cDNA synthesis. Expression of *cagA* was measured by RT-PCR.

Whether the OMPLA turn-off is caused by phase variation resulting in a truncated outer membrane protein or if the phenotype is caused by a point mutation resulting in a full length, but modified and non-functional protein, the result is the same. The bacteria has lost its ability to survive acid shock. Both OMPLA active and inactive variants expressed comparable levels of *cagA* at low pH.

Abstract no.: P02.11

#### GASTRIC MUCOSAL EXPRESSION OF MICRORNA-204 AND -650 IN *HELICOBACTER PYLORI* INFECTED PATIENTS FROM A WESTERN POPULATION

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**Background:** Thousands of genes that produce regulatory noncoding-RNA transcripts have been discovered. MicroRNAs (miRNAs) have garnered considerable attention because of their roles in biological processes. We previously reported a microarray experiment that analysed the profile of miRNAs in non-metaplastic, non-atrophic *H. pylori*-infected patients. A miRNA signature of 20 miRNAs was associated with infection (*Helicobacter*-2010;15(4):352).

**Objective:** To confirm the expression levels of the upregulated mir-650 and the downregulated mir-204 in an independent validation group of dyspeptic patients.

**Methods:** Thirty-five subjects were included. *H. pylori* status was defined as positive if at least two tests of the 13C-UBT, RUT tests or histology were positive. Patients were divided in three groups: without gastritis (n = 8), *H. pylori*-negative with gastritis (n = 11), and *H. pylori*-positive with gastritis (n = 16). Interleukin-8, RN7, mir-204, and mir-650 expression was assessed by real-time PCR.

**Results:** mir-204 was downregulated in *H. pylori*-positive patients. The upregulation of mir-650 did not reach statistical significance. Chronic gastritis in *H. pylori*-infected and uninfected patients was also investigated. IL-8 and mir-650

were upregulated and mir-204 downregulated in *H. pylori*-positive patients with gastritis. This effect was not observed in *H. pylori*-negative gastritis. However, the ratio mir-204/mir-650 significantly discriminated normal mucosa from *H. pylori*-negative and positive gastritis and was correlated to gastritis grade (rs = -.705; p = .000).

**Conclusions:** These preliminary results point to mir-204 as an indicator for the presence of *H. pylori* and the ratio mir-204/mir-650 as a marker for gastritis.

Abstract no.: P02.12

#### *HELICOBACTER PYLORI* TYPE IIG RESTRICTION AND MODIFICATION LOCI

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*Helicobacter pylori* complete sequenced genomes have a large number of putative restriction and modification systems (RMS) between 26 and 34. The major consequence of RMS is keeping the bacterial genomes free from alien DNA, acting as a speciation barrier. RMS are classified into four major types and several subtypes. The *H. pylori* genomes annotated in REBASE were analyzed and it was found that RMS are not randomly distributed over the genome. Using *H. pylori* 26695 as model, four type IIG RMS loci were found: 1st – *glpC-horF*; 2nd – *nadC-HINFIM*; 3rd – *tmk-pola*; 4th – *res-nusA*. Primers were designed for the four loci and tested in 17 different *H. pylori* strains, isolated from asymptomatic patients. PCR products were obtained in all loci but differences were observed among them. All the PCR products were digested with HindIII to evaluate the variability of the amplified genes. Locus 1 and 4 are empty in a large percentage of strains, 64.7 and 52.9 respectively. Locus 2 has a high percentage of strains with unspecific PCR products, 58.8%, but all the others showed PCR products with the expected size. Locus 3 has the highest percentage of PCR products with the expected size, 82.3%. Different HindIII profiles were observed: 2 in locus 1, 5 in locus 2 and 4, and 6 in locus 3. This might correspond to 18 different type IIG RMS in 17 strains, thus being one more example of *H. pylori* genetic diversity although their species genomic organization might be conservative.

Abstract no.: P02.13

#### AKT IS ACTIVATED IN THE EARLY STAGE OF *HELICOBACTER PYLORI* INFECTION

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**Objectives:** To explore the role of Akt activation in the pathopoiesis of *Helicobacter pylori* (*H. pylori*).

**Methods:** 1, 160 pathological specimens of gastric mucosa (*H. pylori*-related or not) with chronic no-atrophic gastritis (CNAG), metaplastic atrophy (MA), dysplasia (Dys) or gastric cancer (GC) were enrolled. Expressions of protein were measured by immunohistochemical technique. 2, Prepare the culture filtrates of type culture strain (NCTC11637 *H. pylori*), concentration is set to be 11 mg/mL, and then dilute it to different concentrations, co-cultured with gastric epithelial cells (GES-1). In control group only *H. pylori* culture medium is added, cells are collected and detected Akt, pAkt protein level with western blotting at different points.

**Table 1** Gene expression of IL-8, the housekeeping gene RN7 and the microRNAs mir-204 and mir-650 in *H. pylori* infected patients

(n)	(n)	IL-8	mir-204	mir-650	mir-204/650	RN7
<b>H. pylori status</b>						
Negative	4.86E + 02	2.54E + 04	5.21E + 02	27.2	6.54E + 05	6.54E + 05
Positive	16	9.97E + 03*	3.24E + 03*	2.69E + 03ns	1.54*	9.37E + 05ns
<b>Gastritis</b>						
Normal stomach	8	3.26E + 02	2.56E + 04	2.13E + 02	137	4.00E + 05
<i>Hp</i> -negative	11	5.15E + 02ns	2.54E + 04ns	3.75E + 03ns	16.2†	7.10E + 05ns
<i>Hp</i> -positive	16	9.97E + 03‡§	3.24E + 03‡	2.69E + 03‡	1.54‡ §	9.37E + 05ns

Units are Medians of Arbitrary Florescence Units calculated and corrected by PCR efficiency by the use of LinReg software. Medians across groups were compared by nonparametric tests.

NS, not significant.

\*p < .001 *Hp* positive versus *Hp* negative.

†p < .01 Normal Stomach versus *Hp*-negative gastritis.

‡p < .01 Normal Stomach versus *Hp*-positive gastritis.

**Results:** 1. In gastric mucosal lesions with CNAG or MA, Dys or GC, pAkt expression in Group CNAG was much higher in *H. pylori*-positive specimens compared to *H. pylori*-negative specimens ( $p < .05$ ); Akt expression showed no significant difference among all the groups ( $p > .05$ ); In *H. pylori* related gastric mucosal lesions with CNAG or MA, DYS or GC, Akt and pAkt expressions showed no significant difference among all the groups ( $p > .05$ ). 2. After the introduction of 1 : 4 dilution of culture filtrate of *H. pylori* to GES-1 cells, Akt changed little ( $p > .05$ ). pAkt increased 1 hour after the introduction, reached the peak at 3rd hour and kept increasing during 48 hours after the introduction.

**Conclusions:** Akt activation is induced by *H. pylori* infection in the early stage, which may play a role in the occurrence of *H. pylori*-related gastric cancer.

Abstract no.: P02.14

**PREVALENCE AND DISTRIBUTION OF DUPA GEN, A *HELICOBACTER PYLORI* VIRULENCE FACTOR, AMONG SPANISH CLINICAL ISOLATES**

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**Objective:** Determine prevalence and distribution of gen dupA (Duodenal Ulcer Promoting gen). Positive association of this gen with duodenal ulceration had been suggested. However, results from different studies not always supported this hypothesis.

**Methods:** Strains were obtained from biopsies of symptomatic patients between 2008 and 2009. Biopsies were cultured in Blood and Pylori Agar plates incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere. DNA extraction of each strain was performed by using the automatic system EasyMag (BioMérieux). After conventional PCR with previously described primers, fragments of bp were detected by electrophoresis with 1.2% agarose gel.

**Results:** Eighty strains from symptomatic Spanish patients were studied. Patients included 43 children (53.75%) and 37 adults (46.25%). The prevalence of dupA gen in the whole population was 35% (28 out of 80). Among pediatric patients results showed a 25.58% of positivity for dupA (11 out of 43), while the prevalence for adult patients was 45.95% (17 out of 37). Distribution of CagA and Vacs1 genes was: 21 CagA (26.25%) and 21 vacs1 (26.25%) positive strains respectively. Relationship between dupA gen with these two virulence factors was:(table).

**Table 1** Relationship between dupA gen and the classical *H. pylori* virulence factors

dupA	
cagA positive	42.86%
cagA negative	32.20%
vacA s1	47.61%
vac s2	39.53%

**Conclusions:** DupA was present in more than 35% of the population studied whereas cagA and vacA s1 prevalence was almost 10% lower. Age seemed to be an important factor for the prevalence of dupA gen with almost double of preva-

lence in adults patients. However, relationship among dupA and cagA and vacA s1 was not statistically significant.

Abstract no.: P02.15

***HELICOBACTER PYLORI*-DERIVED EXTRACELLULAR VESICLES IN THE PATHOGENESIS OF GASTRIC DISEASES**

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**Background and Aim:** Extracellular vesicles are shed by Gram-negative bacteria during normal growth, and have been reported to enter and transport virulence factors into host cells.

The purpose of this study is to elucidate the role of *H. pylori*-derived extracellular vesicle in the pathogenesis of gastritis, gastric ulcer, and gastric cancer.

**Materials and Methods:** Clinically isolated *H. pylori* from those patients was incubated and then centrifugation and extraction of extracellular vesicle from the upper band of fluid were undertaken. Extracted extracellular vesicles were inoculated to U-937 cells and AGS cells, respectively. IL-8 mRNA expression and IL-8 secretion were assessed using RT-PCR, real time RT-PCR and ELISA.

**Results:** IL-8 mRNA expression and IL-8 secretion were increased by *H. pylori*-derived extracellular vesicles from patients with gastritis, gastric ulcer and gastric cancer. Especially, IL-8 mRNA expression and IL-8 secretion by *H. pylori*-derived extracellular vesicle of patients with gastric cancer were increased compared with those by *H. pylori*-derived extracellular vesicle of patients with gastric ulcer.

**Conclusion:** This study suggests that the role of *H. pylori*-derived extracellular vesicle is different according to gastric diseases and that it might act as an important factor in the pathogenesis of gastric diseases.



## P03 Virulence factors and pathogenesis

Abstract no.: P03.01

### IMPLICATION OF CAGA EPIYA-C PHOSPHORYLATION IN IL-8 INDUCTION BY GASTRIC EPITHELIAL CELLS

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New findings suggest that presence of CagA in *H. pylori* strains is required for full IL-8 induction. Our aim was to investigate the impact of CagA tyrosine phosphorylation of repeating EPIYA-C domains, on IL-8 activation and secretion by gastric epithelial cells. Based on *H. pylori* strain P12, we constructed genetically modified isogenic mutants expressing CagA protein with variable EPIYA-C phosphorylation motifs (AB, ABCC, ABCCC) and their respective EPIYA-C phosphorylation deficient counterparts (ABFFF). These strains were used to infect AGS cells for 0, 2, 4 and 24 hours. IL-8 gene activation was quantified by Quantitative Real Time PCR and concentration of secreted IL-8 was determined in supernatants with ELISA. The presence of EPIYA-C phosphorylation, independent of the motifs number (2 or 3) significantly increased the activation of IL-8 gene by approximately 120 times in 2 hours post infection (p.i.). However, strains expressing CagA without EPIYA-C motifs (AB) or carrying phosphorylation deficient motifs (ABFFF), failed to fully activate IL-8 gene transcription. Moreover, the ABFFF strain failed to induce IL-8 protein. At 4 hours p.i. IL-8 gene activation reached background levels in all cases, except for ABCCC type which retained about 50% activation of IL-8 gene. No IL-8 gene activity was detected at 24 hours p.i. but IL-8 concentration in supernatants appeared dependent on the number of EPIYA-C motifs. Time-dependent NF- $\kappa$ B activation analysis was concordant with these findings. In conclusion, phosphorylation of CagA on EPIYA-C motifs, plays an important contributing role in the full transcriptional activation of IL-8 gene and subsequent secretion of IL-8.

Abstract no.: P03.02

### HELICOBACTER PYLORI INFECTION OF GASTROINTESTINAL EPITHELIAL CELLS IN VITRO RECRUITS MESENCHYMAL STEM CELL THROUGH AN NF-KB-DEPENDENT PATHWAY

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The role of bone marrow-derived mesenchymal stem cells (MSC) in the physiology of the gastrointestinal tract epithelium is currently not well established. These cells can be recruited in response to inflammation due to epithelial damage, home, and they participate in tissue repair. In addition, in the case of tissue repair failure, these cells can transform and be at the origin of carcinomas. However, the chemoattractant molecules responsible for MSC recruitment and migration in response to epithelial damage, and particularly to *Helicobacter pylori* infection, remain unknown although the role of some chemokines has been suggested. This work aimed to get insight into the mechanisms of MSC recruitment during in vitro infection of gastrointestinal epithelial cells by *H. pylori*. Using a cell culture insert system, we showed that infection of gastrointestinal epithelial cells by different *H. pylori* strains is able to stimulate the migration of MSC. This mechanism involves the secretion by infected epithelial cells of several chemokines, including TNF $\alpha$ , via a pathway independent of PI3K, Src and MAPK but dependent on Nuclear Factor-kappa B (NF- $\kappa$ B). This study provides the first evidence of the role of *H. pylori* infection in MSC recruitment and paves the way to a better understanding of the role of bone marrow-derived stem cells in gastric physiopathology and carcinogenesis.

Abstract no.: P03.03

### PROTEIN KINASE CK2 MEDIATES EPITHELIAL MESENCHYMAL TRANSITION IN HELICOBACTER PYLORI-INFECTED GASTRIC EPITHELIAL CELLS

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**Backgrounds:** Previous studies have shown that *H. pylori* activates Wnt/beta catenin signaling pathway and epithelial mesenchymal transition (EMT). In

addition, protein kinase CK2 is potentially a highly plausible target for cancer therapy.

**Aim:** To evaluate the role of CK2 in Wnt/beta catenin signaling pathway and EMT in *H. pylori* infected stimulated gastric epithelial cells.

**Methods:** VacA+, CagA+ wildtype *H. pylori* strain 60190 (ATCC 49503, Rockville, MD, USA), CagA-strain 8822, or KO CagA are grown on blood agar plate supplemented with 5% sheep blood for 48 hours at 37 °C in microaerobic system jar (Difco, Sparks, MD, USA). Human gastric epithelial cells (AGS and MKN28) are cultured in RPMI 1640 (Gibco, Grand Island, CA, USA), containing 10% FBS (Gibco). Gastric epithelial cells are pretreated with CK2 inhibitor (TBB) and infected with *H. pylori* for different periods of time. The effects of CK2 inhibitor (TBB) on *H. pylori* stimulated gastric cancer cells are evaluated by using immunoblot, immunoprecipitation, promoter assay and migration assay.

**Results:** Immuno blotting revealed that phosphorylated form of  $\alpha$  catenin (at S641) and dissociation of  $\alpha/\beta$  complex were increased in *H. pylori* stimulated gastric epithelial cells and these increased expression of phospho alpha catenin molecules and dissociation decreased when *H. pylori* stimulated gastric epithelial cells pre-treated with TBB. *H. pylori* 60190 induced  $\beta$ -catenin transactivation while TBB/siRNA inhibited this activation.

**Conclusion:** These data suggest that *H. pylori* activates EMT through the protein kinase CK2 by alpha catenin phosphorylation in part.

Abstract no.: P03.04

### NCK, PLC- $\gamma$ AND C-SRC ARE INVOLVED IN HELICOBACTER PYLORI-MEDIATED HOST CELL INVASION IN A C-MET-DEPENDENT MANNER

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*Helicobacter pylori* infection induces cytoskeletal rearrangements, cell elongation, and scattering via c-Met receptor activation. We previously reported that *H. pylori* strains containing a functional bacterial *cag* T4SS induce cell invasion through c-Met and increased activity of matrix metalloproteases. Our aim was to disclose the signaling molecules downstream c-Met that are implicated in the cell invasive phenotype induced by *H. pylori*.

We transiently transfected AGS cells with siRNA abrogating the expression of the c-Met adaptors c-Cbl, Nck, Gab1, and Shc, and also of other known downstream targets of the c-Met pathway, c-Src, FAK, PLC- $\gamma$ , and Shp-2. The invasive phenotype of non-infected and *H. pylori*-infected cells was evaluated by the matrigel invasion assay.

We found that the silencing of Nck, c-Src, FAK, and PLC- $\gamma$  resulted in a significant decrease in the number of invasive cells in the presence of *H. pylori*, in comparison with non-silenced AGS cells, suggesting that these molecules are involved in *H. pylori*-mediated cell invasion. Further, we evaluated whether *H. pylori* activates Nck, PLC- $\gamma$ , and c-Src and if this occurs via the c-Met receptor. We observed that *H. pylori* induced phosphorylation of all these proteins in non-silenced cells, whereas no phosphorylation was observed in c-Met-silenced AGS cells, suggesting that activation of Nck, PLC- $\gamma$  and c-Src by *H. pylori* are mediated by the c-Met receptor.

The dissection of this pathway will contribute to the understanding of *H. pylori*-mediated cell invasion and disclose molecular targets for therapeutic intervention.

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Abstract no.: P03.05

### THE ROLE OF TFF1 IN MEDIATING HELICOBACTER PYLORI COLONISATION OF THE ADHERENT MUCUS LAYER OF E12 CELLS

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*Helicobacter pylori* colonization of the gastric mucosa of humans is often considered a paradigm for chronic infection of mucosal surfaces. We have previously shown that *H. pylori* interacts with the trefoil peptide TFF1 and binds preferentially to TFF1 dimer. We hypothesised that the interaction of *H. pylori* with TFF1 dimer, which is present in gastric mucus, is mediated by *H. pylori* lipopolysaccharide and promotes mucus colonization.

Polarised HT29-MTX-E12 cells produce an adherent mucus layer that contains TFF1 and the gastric mucin MUC5AC. *H. pylori* co-localized with TFF1 in the

HT29-MTX-E12 cell adherent mucus layer following infection and in gastric biopsies from infected humans.

Culture of HT29-MTX-E12 cells in the presence of copper, which increases TFF1 dimer formation, resulted in a significant increase in colonisation by *H. pylori*.

Isogenic mutants of *H. pylori* with truncated LPS core structures were produced and their binding to TFF1 and ability to colonise adherent mucus determined. One of these isogenic mutants of *H. pylori* was unable to interact with TFF1, and colonization of HT29-MTX-E12 cells was reduced 100-fold as compared to the wild-type strain ( $p < .05$ ).

Using the HT29-MTX-E12 cell model system results indicate that the interaction of *H. pylori* with TFF1 promotes colonization of gastric mucus and that the core oligosaccharide of *H. pylori* LPS is the critical adhesin in this interaction.

Abstract no.: P03.07

#### CHARACTERIZATION OF NEW HUMAN GASTRIC EPITHELIAL CELL LINES DERIVED FROM NCI-N87 CELLS AFTER OVER-EXPRESSION OF HUMAN TELOMERASE CATALYTIC SUBUNIT

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The lack of a cellular model which correctly mimics the natural niche of the pathogen *Helicobacter pylori* is still limitative for the study of this infection. Aiming to overcome this limitation, we have previously isolated clones of a subpopulation of the widely used heterogenic NCI-N87 (ATCC CRL-5822) gastric cell line<sup>1</sup>, those presenting typical epithelial markers and a progenitor-like phenotype (simultaneous synthesis of mucus and zymogens). For that, we stably-transduced the NCI-N87 cells with human telomerase reverse-transcriptase (hTERT) catalytic subunit (pGRN145 plasmid, ATCC MBA-141), using the FuGENE-HD reagent (Roche). The two most promising NCI-N87-derived clones (C5 and C6) were shown to be composed of cells with homogenous phenotype with ability to grow in adherent monolayers, to produce gastric zymogens (hematoxylin staining) and to produce and secrete neutral mucins (Periodic-Acid-Schiff staining). Preliminary results have also shown that they are able to generate transepithelial electrical resistance and the ability of C5 to produce and secrete acidic mucins (Alcian-Blue staining). We are now clarifying the identity of the mucin species C5 and C6 produce by immunohistochemical analysis and zymogens (Pepsinogen) by western-blot. Moreover, the subcellular localization (immunocytochemistry) of adherens and tight-junctions' proteins (*E-cadherin* and *ZO-1*) and the polarization status of both clones is now under evaluation. Due to their improved properties, compared to the heterogeneous parental line, these NCI-N87-derived clones are promising models of the human gastric epithelium.

1. Chailler, P. and D. Ménard. *J.Cell.Physiol.*, 202;263–274, 2005.

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Abstract no.: P03.08

#### HELICOBACTER PYLORI HTRA VIRULENCE FACTOR IS CONSERVED AMONG CLINICAL ISOLATES

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*Helicobacter pylori* virulence factor HtrA, encoded by the *htrA* gene, is a secreted serine protease. It has recently been shown that HtrA cleaves the cell-cell adhesion protein E-cadherin, possibly allowing *H. pylori* to access the intercellular space of epithelial cells.

Our aim was to elucidate whether HtrA is conserved among clinical isolates. For that, we fully sequenced *htrA* in 36 clinical isolates and two reference strains (*H. pylori* 26695 and G27), using primers designed to cover the whole gene.

Using this strategy, we were able to sequence *htrA* in all clinical isolates. All sequences gave rise to open reading frames of 1431 bp (476 amino-acids). Neither insertions nor deletions were observed along the gene. The phylogenetic relationship between *htrA* sequences was analysed using the MEGA 4 software, applying the Neighbour-Joining method. The mean similarity between *htrA* sequences was 96.5% ± 0.3 (mean ± SE), and the mean molecular distance was 0.034 ± 0.003. The nucleotide substitutions were 0.158 ± 0.013 and 0.003 ± 0.001, for the synonymous (Ks) and the non-synonymous (Ka) rates, respectively. The Ka/Ks ratio was 0.019, implying that these sequences are under stabilizing selection. After translation of the nucleotide sequences and using strain 26695 as reference, 17 amino-acid substitutions were found, mainly concentrated at the N- and C-terminus. No mutations were found at the active site (Ser205), suggesting that all strains have an active HtrA.

Our results show that HtrA is highly conserved among clinical isolates, reinforcing its essentiality for *H. pylori* survival.

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Abstract no.: P03.09

#### REGULATION OF MDM2 ONCOGENE BY HELICOBACTER PYLORI LIPOPOLYSACCHARIDE IN GASTRIC EPITHELIAL CELLS

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**Purpose:** Mdm2 is critical regulators of the p53 protein which plays a crucial role in maintaining genomic integrity and tumor prevention. *Helicobacter pylori* is reportedly involved in the development of gastric cancer. We investigated the mechanisms between *H. pylori* and MDM2, focusing on *H. pylori*-derived lipopolysaccharide (LPS).

**Experimental Design:** *H. pylori*-LPS and two gastric cancer cell lines (AGS and MKN28) were used. We examined whether the expression of MDM2 in a dose and time-dependent manner of Gastric epithelial cells, when they are exposed to *H. pylori*-LPS. We also examined if PI3k/Akt/mTOR signaling pathway mediated this expression. Western blotting was employed to evaluate the expressions of MDM2, pAkt-5473 and Akt, and the functionality of the MDM2 promoter is examined by luciferase assay.

**Results:** Gastric epithelial cells express more MDM2 in a dose- and time-dependent manner when they are stimulated with *H. pylori*-LPS. Treatment of Gastric epithelial cells application of LY294002 and Rapamycin caused a dramatic reduction of *H. pylori*-LPS induced MDM2. In addition, *H. pylori*-LPS stimulation increased the MDM2 promoter activity.

**Conclusion:** *H. pylori*-LPS induced MDM2 over expression is mediated by PI3K/Akt/mTOR.

Abstract no.: P03.10

#### DISRUPTION OF TIGHT JUNCTIONS OF GASTRIC EPITHELIAL CELLS INDUCED BY HELICOBACTER PYLORI AS ANALYSED USING REAL-TIME PHASE CONTRAST MICROSCOPY

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*Helicobacter pylori* cytotoxin-associated gene A (CagA) has been regarded as a major player in the disruption of tight junctions. However, the exact mechanism of tight junction disruption induced by *H. pylori* is still not well-established. This study uses a high resolution imaging system that is able to maintain perfect focus and optimal growth conditions for cells to follow live cell observations. Using MKN28 cells, which form functional tight junctions, these cells were infected with *H. pylori* 26695 wildtype or  $\Delta cagA$  separately. The real-time event of tight junction disruption of the gastric cells induced by *H. pylori* was recorded over a period of 44 hours and the images were then analyzed quantitatively using ImageJ software. The images show that the tight junctions of uninfected MKN28 cells remained intact for the entire recording period. Interestingly, tight junction disruption as observed in wildtype-infected and  $\Delta cagA$ -infected host cells began at 4 hours post-infection. The process of tight junction disruption as shown by the real-time microscopy observations is further supported by results obtained from barrier function test. Taken together, our findings show that real-time phase contrast microscopy can provide a highly supportive role on the mechanistic events occurring during host-pathogen interactions.

Abstract no.: P03.11

#### ULCEROGENIC PROFILE OF HELICOBACTER PYLORI PEDIATRIC STRAINS: A CONTRIBUTION TO GET INSIGHT INTO THE VIRULENCE OF THE BACTERIA

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*Helicobacter pylori* infection is the major cause for the development of peptic ulcer disease (PUD). In addition to patient genetic susceptibility, PUD occurrence in



children, with no other etiology for the disease, presumes the involvement of more virulent strains.

Indeed, our in vitro infection assays showed the marked ability of a pool of five *H. pylori* strains isolated from PUD pediatric patients to induce a decrease in host-cells' viability, severe damages in cytoskeleton and impairment in the production/secretion of mucins in NCI-N87 cells, when compared with a pool of five other isolated from non-ulcer dyspepsia (NUD) pediatric patients. Subsequently proteomic comparison of these two groups of *H. pylori* strains revealed 27 differentially expressed proteins between them. In addition to the presence of genes encoding well established virulence factors (*cagA*, *vacAs1*, *oipA* "on" status, *homb* and *jhp562*), these ulcerogenic strains shared a proteome profile characterized by changes in the abundance of: motility-associated proteins, accounting for higher motility; antioxidant proteins, which may confer increased resistance to inflammation; key enzymes in the metabolism of glucose, amino acids and urea, which may be advantageous to face nutrient fluctuations. Therefore, during infection the pediatric ulcerogenic *H. pylori* strains may take advantage of a synergy between their natural ability to better adapt to their hostile niche and the expression of those virulence factors. We are now characterizing the interaction of these strains with human gastric epithelial cells and mucins. Work supported by PPCDT/SAL-IMI/57297/2004 research-grant. IV and KDSP are recipient of SFRH/BD/38634/2007 and SFRH/BD/72849/2011 fellowships, respectively.

Abstract no.: P03.12

#### PREVALENCE OF CAGA AND VACA GENES IN HELICOBACTER PYLORI FROM THE GAMBIA IN RELATION TO DISEASE PHENOTYPE

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*Helicobacter pylori* is common in Africa, whereas *H. pylori*-associated disease is less common than in developed countries. In this study we investigated the prevalence of virulence-related *H. pylori* genotypes and disease phenotype in The Gambia.

Hundred and twenty-one of 169 patients with abdominal pain or dyspepsia, tested for *H. pylori* by PCR of DNA from gastric biopsies, were found to be *H. pylori* positive. The *cagA* gene, *s1*, *m1* alleles of the *vacA* gene were found in 61.2%, 76.9% and 45.5% respectively. The less toxigenic *s2* *vacA* gene allele was found in 19.0% of the patients. *cagA* positive strains were found more frequently in patients with overt gastric diseases compared to patients with non-ulcerative disease (NUD); 85.7% in those with duodenal ulcers, 71.4%, in patients with gastric erosions, 72.7% in those with gastric ulcers and 56.4% in patients with NUD. There was no link between *vacA* allele and disease phenotype. However, we found that the co-existence of mixed *cagA* positive and *cagA* negative strains was more common in patients with non-ulcerative diseases compared to patients with gastric disease (24.5% versus 0%;  $p = .002$ ).

This study indicates that the prevalence of *H. pylori* is high in dyspeptic patients in The Gambia and showed that *cagA+*, *s1* and *m1* are common genotypes. Carriage of *cagA* positive strain was associated with an increased risk of overt gastric disease. In addition, patients who were infected with mixed *cagA* positive and *cagA* negative strains were less likely to have gastro-duodenal diseases than those infected with pure strains.

Abstract no.: P03.13

#### NEUTRALIZING MONOCLONAL ANTIBODIES ARE EFFECTIVE AGAINST HELICOBACTER PYLORI $\Gamma$ -GLUTAMYL TRANSEPTIDASE ACTIVITY

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*Helicobacter pylori*  $\gamma$ -glutamyl transpeptidase (GGT) has been reported to be an important colonizing and apoptosis-inducing factor. Recently, we have also shown that GGT induces reactive oxygen species production, interleukin-8 upregulation and DNA damage in gastric cells. The aim of this study was to investigate if monoclonal antibodies raised against *H. pylori* GGT were able to inhibit its activity. Using recombinant GGT protein purified by affinity chromatography as an immunogen, monoclonal antibodies (MAbs) were generated in mice. Specificity of MAbs was analyzed by immunofluorescence staining of *H. pylori* and Western blot analysis. The MAbs were tested for their ability to neutralize GGT activity of various *H. pylori* strains using GGT assay. One of the

MAbs generated was found to inhibit and neutralize GGT activity by 46–95%. Further characterization of this MAB is in progress to understand the underlying mode of inhibition.

Abstract no.: P03.14

#### NO ASSOCIATION OF THE H. PYLORI VACA, DUPA AND OIPA GENES WITH ATROPHIC GASTRITIS IN DYSPLEPTIC PATIENTS FROM A POPULATION AT HIGH RISK OF GASTRIC CANCER IN COSTA RICA

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**Background and Aim:** Costa Rica is one of the countries with the highest incidence and mortality rates from gastric cancer. Gastric cancer prevalence varies among different regions. However, the prevalence of *Helicobacter pylori* infection is high in the whole country. We have previously shown that *H. pylori* CagA+, as defined by a combination of PCR analysis and serology, is significantly associated with atrophic gastritis of the antrum in a dyspeptic population. The aim of this study was to determine if other *H. pylori* virulence factors are associated with atrophic gastritis.

**Methods:** Seven biopsies and a blood sample were obtained from 501 patients referred to endoscopy for dyspeptic symptoms. In each case, a histopathological examination was performed. The presence of the *vacA*, *dupA* and *oipA* genes was analyzed by PCR in 88 cultured strains. Odds ratio and 95% confidence intervals for atrophic gastritis patients versus non-atrophic gastritis were calculated.

**Results:**

**Table 1** Association of *H. pylori* *dupA*, *oipA*, *vacA s1m1* genes with atrophic gastritis

Gene (n)	OR (AG vs NAG)	95% CI	p
<i>dupA</i> <sup>+</sup> (59)	0.74	(0.27–2.06)	.425
<i>oipA</i> <sup>+</sup> (77)	1.40	(0.15–13.31)	.584
<i>vacA s1m1</i> (57)	2.04	(0.66–6.24)	.296

OR, odds ratio; CI, confidence intervals; AG, atrophic gastritis; NAG, non atrophic gastritis.

**Conclusion:** Infection with *Helicobacter pylori* strains carrying the *dupA*, *oipA*, *vacA s1m1* genes is not significantly associated with atrophic gastritis risk in this dyspeptic population.

Abstract no.: P03.15

#### HELICOBACTER PYLORI INFECTION AND PATHOGENESIS OF PEPTIC ULCER IN EXTREME COLD CLIMATE

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**Objective:** To investigate the relationship between *Helicobacter pylori* (*H. pylori*, Hp) infection and Pathogenesis of peptic ulcer (PU) in extreme cold climate.

**Methods:** Collected gastric mucosa and juice of peptic ulcer patients who were taking endoscopy examination in our hospital and didn't take any stomach-related drugs for a month in extreme cold weather (temperature <10 °C). PH values of gastric juice were obtained on site by precise PH dipstick. Hp infection were determined by modified Giemsa staining. Tregs, macrophages infiltrating and Occludin, HSP70, NOS, EGF and EGFR expression in gastric mucosa were detected by immunohistochemical stain.

**Results:** 1, 82(80.4%) PU were Hp+, PH value of gastric juice in Hp+ PU was significantly lower than that in Hp- (1.00 ± 0.699 vs 1.88 ± 1.193,  $p < .01$ ). 2, There were more Tregs and macrophages infiltrated in Hp+ gastric mucosa than those in Hp- (26.6 ± 10.0 vs 39.3 ± 24.0,  $t = -3.567$ ,  $p = .001$ ; 12.7 ± 11.1 vs 23.4 ± 14.4,  $t = -2.932$ ,  $p = .004$ ). 3, EGFR expression in gastric antrum mucosa of Hp+ was significantly lower than that of Hp- ( $H$  value: 61.44 vs 48.10,  $U = -2.101$ ,  $p < .05$ ). 4, Occludin, HSP70, NOS and EGF expression in gastric mucosa were not significantly associated with Hp infection ( $p > .05$ ).

**Conclusion:** Promoting gastric acid secretion and increasing Tregs and macrophages infiltration might be one of the pathogenesis of Hp associated peptic ulcer in extreme cold conditions.

Abstract no.: P03.16

RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* INFECTION AND GASTRIC ACID OF PEPTIC ULCER IN EXTREME COLD CLIMATE

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**Objective:** To research the relationship *Helicobacter pylori* (Hp) infection and gastric acid of peptic ulcer (PU) in extreme cold climate.

**Methods:** Collected gastric mucosa and juice, blood of peptic ulcer patients who were taking endoscopy examination in our hospital and didn't take any stomach-related drugs for a month in extreme cold weather (temperature <10 °C). PH values of gastric juice were obtained on site by precise PH dipstick. Hp infection were determined by modified Giemsa staining. Gastrin and somatostatin were examined using radioimmunoassay.

**Results:** PH value of gastric juice in Hp+ PU was significantly lower than that in Hp- ( $1.00 \pm 0.699$  vs  $1.88 \pm 1.193$ ,  $p < .01$ ). The results of radioimmunoassay showed that Serum Gastrin and somatostatin concentration of Hp+ group were not significantly different from Hp- group ( $76.0 \pm 64.1$  vs  $82.0 \pm 66.1$  pg/mL,  $t = .34$ ,  $p > .05$ ;  $564.0 \pm 1437.0$  vs  $776.2 \pm 1469.6$  pg/mL,  $t = .547$ ,  $p > .05$ ). There was no association between gastric acid and Gastrin or somatostatin concentration of peptic ulcer.

**Conclusion:** The increasing of gastric acid secretion in Hp associated peptic ulcer might not because of the imbalance of Gastrin and somatostatin.

Abstract no.: P03.17

GENETIC FEATURES (CAG-STATUS) OF *HELICOBACTER PYLORI* PATHOGENICITY ISLAND IN DIFFERENT VARIANTS OF HELICOBACTERIOSIS

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**Objectives:** *cag*-group genes are genes coding synthesis of factors pathogenicity of *Helicobacter pylori*. Presence of these genes in genome of *H. pylori* is a sign of high virulence.

**Aim:** To investigate frequency of *cag*-group genes of *Helicobacter pylori* in different variants of helicobacteriosis: duodenal ulcer, chronic gastroenteritis and healthy microbial carriers.

**Methods:** It has been surveyed 91 person: 38 patients with duodenal ulcer, 39 – with chronic gastroenteritis and 14 – healthy microbial carriers. Gastroscopy

with antrum biopsy was made for each patient. Polymerase chain reaction was made with all bioplates to define *cagA*, *cagC*, *cagE* and *cagH* gene of *H. pylori*.

**Results:** Gene *cagA* was found in 91.3% of ulcer disease patients, in 61.2% of chronic gastroenteritis patients and in 57.1% of healthy microbial carriers ( $p < .05$ ). Gene *cagC* was found in 52.2% of ulcer disease patients, in 35.9% of chronic gastroenteritis patients and in 7.1% of healthy microbial carriers ( $p < .05$ ). Gene *cagE* was found in 78.2% of ulcer disease patients, in 26.2% of chronic gastroenteritis patients and in 42.9% of healthy microbial carriers ( $p < .05$ ). Gene *cagH* was found in 65.2% of ulcer disease patients, in 55.3% of chronic gastroenteritis patients and in 28.6% of healthy microbial carriers ( $p < .05$ ).

**Conclusion:** Duodenal ulcer patients are infected more virulence strains of *H. pylori* than chronic gastroenteritis patients and healthy microbial carriers. Chronic gastroenteritis patients are infected more virulence strains of *H. pylori* than healthy microbial carriers. So virulence of *H. pylori* strains is associated with certain diseases.

# P04 Epidemiology and Transmission

Abstract no.: P04.01

## SYMPTOMATIC MANIFESTATIONS OF *H. PYLORI*-ASSOCIATED DISEASE IN A NORTHERN CANADIAN COMMUNITY

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This analysis describes symptomatic manifestations of *H. pylori*-associated disease in an Aboriginal community in the Northwest Territories. In 2008, we invited participants in the Aklavik *H. pylori* Project to undergo endoscopy with gastric biopsy, without restricting symptom or *H. pylori* status, in temporary endoscopy units at the Aklavik Health Center. Gastroenterologists followed a standard protocol to note endoscopy findings and collect biopsies (2 antrum, 1 incisura, 2 corpus). One pathologist examined all biopsies, using hematoxylin-eosin & Giemsa stains, and scored *H. pylori* density, acute and chronic inflammation, gastric atrophy, and intestinal metaplasia on the updated Sydney System four-point scale (0–3). Each individual was assigned the highest score of examined biopsies for each variable. We interviewed participants to ascertain digestive symptoms using a validated questionnaire. Specific symptoms were grouped into none/any and categorized by the highest severity mentioned. Hundred and eighty-nine participants (10–80 years, 57% female) had complete data. Across all diagnostic categories, 33–50% of participants were asymptomatic. In 22 participants with severe symptoms, 55% were *H. pylori* negative, and 41% had normal histopathology. Participants with endoscopically diagnosed lesions were more likely to have sought medical care for stomach problems, but those with more severe histopathology were, in general, less likely to have done so. Our report reveals a substantial prevalence of severe *H. pylori*-associated disease that would not normally come to the attention of health care providers.

**Table 1** Digestive symptom severity and history of medical attention by diagnostic category

Diagnostic category	n	Symptom severity			Ever sought care for stomach problems %
		None %	Mild-mod %	Severe %	
<b>Endoscopy (few duodenal lesions: 7 duodenitis, 1 duodenal erosions, 0 duodenal ulcers)</b>					
No gastritis	164	37	52	10	37
Gastritis	25	44	36	20	44
Gastric erosions	12	33	50	17	58
Gastric ulcer	6	50	33	17	67
<b>Histopathology (normal = chronic inflammation = 0)</b>					
Chronic inflammation = 0	61	44	39	16	57
<i>H. pylori</i> negative/density = 0	64	42	39	19	56
Density = 1	30	30	60	10	30
2	48	40	50	10	29
3	47	36	60	4	26
Acute inflammation = 1	74	35	54	11	34
2–3	45	38	60	2	16
Chronic inflammation = 1	13	23	54	23	38
2	59	37	51	12	34
3	56	36	61	4	20
Atrophy = 1–3	27	37	52	11	26
Metaplasia = 1–3	16	38	38	25	44

Abstract no.: P04.02

## *H. PYLORI* COLONIZATION DENSITY AND GASTRIC HISTOPATHOLOGY IN A NORTHERN CANADIAN COMMUNITY

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The aim of this study was to estimate the association between *H. pylori* colonization density and gastric histopathological outcomes in a Northern Canadian Aboriginal community. Participants in the Aklavik *H. pylori* Project in the Northwest Territories were invited to undergo upper gastrointestinal endoscopy with gastric biopsy in 2008. Five biopsy specimens (2 antrum, 1 incisura, 2 corpus) were collected from each participant, processed with hematoxylin-eosin and Giemsa staining, and examined microscopically by one pathologist (SG), who scored *H. pylori* density, acute inflammation (neutrophilic activity), chronic inflammation, glandular atrophy, and intestinal metaplasia on a four-point scale (0–3) according to the updated Sydney System. Each individual was assigned the highest score of examined biopsies for each variable. Trend analysis was performed by inspecting the prevalence of histopathologic outcomes across increasing *H. pylori* density grades and conducting  $\chi^2$  tests for trend. *H. pylori* density scores were available for 192 participants (age range = 10–80, 57% female, 91% Aboriginal), 127 of whom had *H. pylori* -positive histology. All participants with density >0 had chronic inflammation and nearly all (except 19% with density = 1) had acute inflammation (Table 1). A strong positive effect gradient was observed for atrophy but not metaplasia. These findings provide evidence of a dose-response effect of *H. pylori* density on gastric atrophy.

**Table 1** Prevalence of histopathologic diagnoses by *H. pylori* density\*

<i>H. pylori</i> density score	Acute inflammation	Chronic inflammation	Glandular atrophy	Intestinal metaplasia
0 (none) n = 65	0%	5%	0%	3%
1 (mild) n = 32	81%	100%	6%	16%
2 (moderate) n = 48	100%	100%	19%	10%
3 (marked) n = 47	100%	100%	35%	9%
$\chi^2$ for trend <i>p</i> -value	<0.001	<0.001	<0.001	0.18

\*Table percentages represent the prevalence of each histopathological diagnosis within an *H. pylori* density score.

Abstract no.: P04.03

## THE OCCUPATIONAL RISK OF *HELICOBACTER PYLORI* IN HEALTHCARE WORKERS

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**Background and Objectives:** *Helicobacter pylori* was discovered in 1984, but up to now its transmission is not clear. Direct person-to-person transmission is thought to be most likely and this could be relevant to occupational transmission particularly in healthcare workers (HCWs).

**Methods:** We used serology to study the occupational risk for *H. pylori* in HCWs in 2 cross-sectional studies and one cohort study.

**Results:** In a cross-sectional study, 587 healthcare workers (HCWs) working in institutions for children with mental disabilities with a documented high prevalence of *H. pylori* infection were compared to non-exposed controls. Using multiple logistic regression to adjust for confounding variables, an OR of 2 (95% CI 1.4–2.7) was found in workers having contact with faeces of inhabitants. In another cross-sectional study in 198 nursing home workers, an OR of 0.9 (95% CI 0.5–1.9) was found in multiple logistic regression compared to non-exposed controls after adjusting for other risk factors.

In the cohort of HCWs and non-exposed controls, workers, seronegative at baseline were followed up for at least 10 years, resulting in 2254 person years (py) in the HCWs group and 1284 py in non-exposed controls. An incidence rate for *H. pylori* infection of 0.53/100 py (95% CI 0.28–0.93) was found in HCWs, compared to 0.39/100 py (95% CI 0.13–0.91) in non-exposed controls, resulting in a rate ratio of 1.36 (95% CI 0.43–4.21).

**Conclusions:** Results of our studies show the difficulty in interpreting cross-sectional studies. Results of the cohort study show a slightly increased incidence in HCWs compared to non-exposed controls.

Abstract no.: P04.04

**DEMOGRAPHIC CHARACTERIZATION OF HIGH RISK POPULATIONS MAY HELP IDENTIFY GASTRIC CANCER HIGH RISK SUBJECTS FOR FURTHER SCREENING PLANS AND CLINICAL FOLLOW-UPS**

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We carried out a hospital based case-control study, including 382 cases with confirmed gastric cancer and 645 gastric cancer free controls. A self-designed questionnaire was filled by trained staff to collect personal, dietary and lifestyle habits. Logistic regression was employed to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) using SPSS 18.0.

We observed that age, gender, smoking and education are the most important demographic factors in our population which affect the risk of gastric cancer. Furthermore, our study demonstrated that Kurdish people (a subgroup of Iranian ethnicity) are at a significantly increased risk of GC development (OR: 7.013, 95% CI (3.965–12.406),  $p = .0001$ ). In this study, we also assessed the joint effect of age, gender and smoking status on the risk of GC development. According to the calculated adjusted OR for ethnicity and education, we found that male smokers over the age of 50 years are at more than six fold increased risk of GC development (OR: 6.281, 95% CI (2.108–18.714),  $p = .001$ ) which is further enhanced in non-cardia subsite category (OR: 7.219, 95% CI (1.766–29.505),  $p = .006$ ). Moreover, stratification based on GC histologic subtypes demonstrated an increased risk for these subjects in development of both intestinal (OR: 5.646, 95% CI (1.175–27.138),  $p = .031$ ) and diffuse (OR: 5.504, 95% CI (1.021–29.666),  $p = .047$ ) type GC. On the other hand, any kind of classical education (particularly above 8 years) reduced the risk of GC development by 70% (OR: 0.307, 95% CI: 0.149–0.633,  $p = .001$ ) in general and noncardia subsite and both subtypes.

Abstract no.: P04.05

**HOUSEHOLD FACTORS ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION IN AKLAVIK, NORTHWEST TERRITORIES, CANADA**

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Concerns raised by residents of Aklavik, Northwest Territories (population = 590, ~90% Aboriginal) about health risks from *H. pylori* infection resulted in the community-driven Aklavik *H. pylori* Project, aimed at reducing health risks from *H. pylori* infection in Arctic Canada. This analysis describes associations of household characteristics with *H. pylori* prevalence among project participants recruited by open invitation disseminated throughout the community.

During 2008–2010, participants were tested for *H. pylori* by urea breath test or histology. To ascertain household characteristics, we interviewed representatives of participating households using a structured questionnaire. We used logistic regression with random effects for household clustering to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for associations of household characteristics with individual *H. pylori* status, adjusting for age, sex and ethnicity. *H. pylori* prevalence among all project participants was 62% (221/355). We collected household data for 296 individuals (*H. pylori* prevalence = 60%) in 145 households.

The most notable effects of household factors were for income, education and household crowding indicators.

Our preliminary analysis of household-level risk factors for *H. pylori* infection in this Arctic Aboriginal hamlet shows low socioeconomic status and household crowding to be associated with increased odds of *H. pylori* infection.

**Table 1** Odds ratios for the association of household factors with individual *H. pylori* status,  $n = 296$

Variable	Unadjusted	Adjusted <sup>a</sup>
	OR (95% CI)	OR (95% CI)
<b>Annual household income (in CAD)</b>		
<\$25,000	1.0	1.0
\$25,000–\$49,999	0.67 (0.26–1.7)	0.74 (0.30–1.8)
\$50,000–\$74,999	0.41 (0.16–1.0)	0.50 (0.21–1.2)
≥\$75,000	0.26 (0.12–0.56)	0.33 (0.16–0.69)
<b>Highest educational attainment by a household member</b>		
<Grade 12	1.0	1.0
Grade 12	0.82 (0.40–1.7)	0.86 (0.42–1.7)
>Grade 12	0.42 (0.19–0.91)	0.60 (0.26–1.4)
<b>Number of children in the house</b>		
0	1.0	1.0
1	0.82 (0.41–1.6)	0.76 (0.36–1.6)
2	0.98 (0.42–2.3)	0.98 (0.38–2.5)
3–6	4.6 (1.4–15)	4.2 (1.2–15)
<b>Number of people per bedroom</b>		
≤1	1.0	1.0
1.01–2	1.5 (0.84–2.8)	1.4 (0.72–2.8)
2.01–3	4.0 (0.85–19)	3.1 (0.63–15)

<sup>a</sup>Adjusted for age, sex, ethnicity, and random household effect.

Abstract no.: P04.06

**THE DECLINE IN PREVALENCE OF *H. PYLORI* INFECTION IN CROATIA AFFECT SIGNIFICANTLY THE INCIDENCE OF ESOPHAGO-GASTRO-DUODENAL (EGD)ENDOSCOPIC FINDINGS**

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**Aim:** We compare incidence of *H. pylori* infection and various endoscopic findings in two time periods of five years (Period-1 1994–1998; Period-2 2006–2010) in patients undergoing first endoscopy for dyspepsia, naive to any *H. pylori* treatment.

**Methods:** From 18147 of all patients in Period-1, 1647 were untreated and examined for the first time. The same number in Period-2 was 1224 from 18529 patients. For the evaluation of *H. pylori* infection, 1–2 biopsy specimens were obtained from the antrum and corpus.

**Results:** The proportion of naive patients in Period-1 was lower for 25.7% ( $p < .0001$ ), as well as number of both ulcers/scars: ventricular (VU) for 40.8% and duodenal (DU) for 50.8%, ( $p < .0001$ ). Difference was not significant for stomach cancers and MALT lymphoma. Number of patients with normal gastroduodenal findings (NUD) and usually GERD symptoms showed clear increase of 65.1%, ( $p < .0001$ ). Incidence of *H. pylori* infection declined significantly; altogether from 76.9% to 38.7% and in all groups; in VU from 80.1% to 37.1%, in DU from 85.0% to 43.2%, in NUD from 61.2% to 39.0%, even in C from 45.7% to 37.1% and in MALT from 100% to 80%.

**Conclusions:** The incidence of *H. pylori* infection among patients undergoing EGD for dyspepsia, naive to anti-*H. pylori* treatment, has decreased markedly in the 15-year follow-up in Croatia. This, and earlier proton pump inhibitor use, may contribute to significant decline in incidence of peptic ulcers and maybe the decline in the prevalence of gastric cancer in the future. As we expected from West World experience, the incidence of NUD/GERD showed clear increase.



Abstract no.: P04.07

IMPACT OF SOCIO-ECONOMICAL CONDITIONS ON *HELICOBACTER PYLORI* STATUS. A CROSS-SECTIONAL STUDY DURING THE YEARS 1996–2005

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**Background:** In developing countries the significant risk factors for acquisition of the *H. pylori* (Hp) infection include low socio-economic status, crowded living conditions, poor sanitation and hygiene.

**Aim:** To find an association of Hp prevalence with socio-economic status in fast developing Poland over the decade of years 1996–2005.

**Methods:** A retrospective population-based study was performed on 11,104 adult inhabitants of Krakow municipal area (aged 18–78, mean 47.2 year: 6491 females, 5290 males), all with upper digestive tract symptoms. Each patient responded to a detailed questionnaire. The Hp status was assessed non-invasively using our urea breath test (UBT) with capsulated low-dose 13C-urea. 6541 patients underwent endoscopy with biopsy for histological and CLO examination. Data for gross domestic product per capita (GDP) for Poland were based on OECD Factbook 2006.

**Results:** The overall mean Hp prevalence over the studied decade was 60.95%; in males 61.9% and 57.9% in females. The highest prevalence was found in the age group of 46–55 year. From 1996 to 2005 year. Hp prevalence decreased from 72.9% to 39.4% whereas GDP increased from 8023 \$ in 1996 year to 14,138 \$ per capita in 2005 year. The observed relationship correlated highly significant ( $p < .0001$ ,  $R^2 = 0.926$ ). Endoscopy revealed an increase of “idiopathic” peptic ulcers (PU) which correlated also significantly ( $p < .0001$ ) with GDP per capita ( $R^2 = 0.859$ ) over the studied decade.

**Conclusion:** One decade of improvement of socio-economic status significantly decreases Hp prevalence of urban population but causes the rise of “idiopathic” peptic ulceration.

Abstract no.: P04.08

STUDY OF THE PRESENCE OF *H. PYLORI* IN UV TREATED WASTEWATER BY FISH AND PCR TECHNIQUES

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Because *H. pylori* is able to survive physical and some disinfection wastewater treatments as chlorination, effluents of waters contaminated with this pathogen could be a potential route of transmission. Treatments, such as UV, could be an alternative to disinfection. We have used a LNA-HPY specific probe for FISH detection and *VacA* DNA PCR, for investigating the presence of *H. pylori* in water samples from a secondary wastewater treatment plant with a UV final effluent disinfection step.

Wastewater samples were obtained from the influent (raw), after secondary treatment, after sand filtration and finally after UV disinfection from a wastewater treatment plant located in Valencia, Spain. Samples were analysed directly and after enrichment. A portion of each sample was fixed with paraformaldehyde and subsequently hybridized with a specific oligonucleotide probe (HPY) designed as LNA/DNA probe. An aliquot of each sample was also processed for specific *H. pylori* PCR with *VacA* primers.

*H. pylori* cells were detected in 12 among 24 samples with specific DNA/LNA probe without enrichment. Four of these eleven samples were taken after UV disinfection treatment. All samples failed to show *H. pylori* cells by FISH after enrichment. Only four samples were PCR positive directly in water. These results demonstrate the presence of *H. pylori* in wastewater samples even after disinfection treatment showing that this pathogen could survive the UV treatment. FISH technique by using LNA/DNA probes has the potential to be used as a specific and effective method for detection of *H. pylori* in environmental samples.

Abstract no.: P04.09

SEROLOGICAL PREVALENCE OF *HELICOBACTER PYLORI*-INFECTION IN SAXONY-ANHALT, GERMANYT. Wex, J. Kreutzer, M. Venerito, T. Götze, A. Kandulski and P. Malfertheiner  
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**Background and Aims:** Epidemiological studies from different countries have shown a steady decline of the prevalence of *H. pylori* infection. In order to investigate the current seroprevalence of *H. pylori* infection in the area of Magdeburg, a city of the former East Germany, *H. pylori* antibodies among patients presenting in our emergency wards with a wide spectrum of different disorders were analyzed.

**Methods:** Two thousand three hundred and eighteen patients (1181 males, 1137 females) who were seen in our emergency wards between September 2009 and August 2010 were tested for immunoglobulin G (IgG) against *H. pylori* and anti-CagA antibodies by specific EIA. Patients with either anti-*H. pylori* IgG or anti-cagA antibodies were classified as “*H. pylori*-positive”, whereas the lack of both antibodies led to the assignment of a “*H. pylori*-negative” status.

**Results:** The overall seroprevalence of *H. pylori* infection was 45.6% (n = 1057 out of 2318). The prevalence of anti-*H. pylori* IgG in males and females was similar (46.3% and 44.9%, respectively). The seroprevalence showed a birth-cohort effect (0–20 year: 18.8%; 21–30 year: 23.2%; 31–40 year: 41.6%; 41–50 year: 47.8%; 51–60 year 51.1%) up to the age of 60, while it remained between 46.1% and 52.3% for the following decades.

Patients younger than 30 year were significantly less “*H. pylori*-positive” (22.5%) than those older than 30 year (48.9%;  $p < .01$ ), whereas the proportion of CagA-positivity was almost identical (49.2 and 49.4%) in these both groups.

**Conclusions:** *H. pylori* infection is still frequent in Saxony-Anhalt, in particular for individuals (>30 year) from those the half is affected by this condition.

Abstract no.: P04.10

*HELICOBACTER PYLORI* INFECTION IN CELIAC DISEASE PATIENTS FROM SAN LUIS, ARGENTINA

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*Helicobacter pylori* is the main etiologic agent of chronic gastritis, peptic ulcer, gastric cancer and MALT lymphoma. Celiac disease (CD) is a constant gluten intolerance that may affect the morphology and function of the entire gastrointestinal tract. The gluten is a second factor stimulating the rise of MALT neoplasm in celiac patients. The aim of this study was to assess the gastric histological pattern in patients with *H. pylori* and celiac disease. Histological studies, culture and urease test were carried out in biopsy samples of one hundred six patients. Analysis of antigliadin (AGA) and antitransglutaminase antibody (ATA) were performed in blood samples. *H. pylori* infection was detected in 52.8% of the patients. The CD was confirmed by positive antibodies and histological studies in 23 patients (22%). The changes in villous area (V) and crypt length (C) (V/C ratio) allowed the CD classification by Marsh’s System. Ten (six children and four adults) celiac patients had *H. pylori* infection associated with chronic gastritis. Regardless of their *H. pylori* status, all pediatric celiac patients had severe atrophy (grade 4). *H. pylori*-positive adult celiacs showed more severe atrophy (grade 4) than *H. pylori*-negative celiac patients (grade 2). Celiac adults infected with *H. pylori* in San Luis Argentina presented more severe histopathological profiles from those *H. pylori*-negative celiac patients. The delay in diagnosis of people infected with *H. pylori* and CD can increase risk of lymphoproliferative lesions.

Abstract no.: P04.11

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION AMONG EMPLOYEES AND STUDENTS IN ST-PETERSBURG, RUSSIA

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**Aim:** To define prevalence of *Helicobacter pylori* infection among employees and students in St-Petersburg and to reveal influence of age, sex and habitual intoxications on the microorganism invasion.

**Methods:** Two hundred persons (119 employees and 81 students) without any gastroenterological complaints have been examined. All examined persons have been divided into six age groups: 15–19, 20–29, 30–39, 40–49, 50–59 years are more senior 60 years. Revealing *H. pylori* has been carried out by means of the respiratory test (“Helik-test”, Association of Medicine and Analytics, St.-Petersburg).

**Results:** *H. pylori* has been revealed at 148 examinees (74%). The microorganism was present in 69.7% of employees and in 81.3% of students. *H. pylori* has been revealed in men more often, than in women (77% and 72% accordingly) and prevailed in age groups of 15–19 years (80%), 30–39 years (86%) and 40–49 years (82%). The prevalence of the infection in other groups of research was significantly lower. It has been revealed that to infection *H. pylori* smokers (77%), than non-smoking (73%) people are more subject. Estimating prevalence *H. pylori* among the persons who are using and not taking alcohol, a significant difference was not revealed – 73% and 74% accordingly.

**Conclusions:** Smoking teenagers and people (especially men) of 30–49 years are in the basic group of risk on *H. pylori* invasion. It dictates necessity of screening of these groups of people for timely administrate of eradication therapy for *H. pylori*-positive persons.

Abstract no.: P04.12

#### FOOD-BORNE SACCHAROMYCES CEREVISIAE HARBORS *H. PYLORI*-SPECIFIC GENE

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**Introduction:** The routes of transmission of *Helicobacter pylori* are unknown. *Saccharomyces cerevisiae* is the most useful yeast in baking and brewing since the ancient time. Investigating the intracellular life of *H. pylori* inside yeast might propose *S. cerevisiae* as an important reservoir and vector of *H. pylori*.

**Material and Methods:** Five *S. cerevisiae* yeasts isolates from yogurt, ice cream, date, bakery leaven and standard *S. cerevisiae*. They were identified by malachite green/safranin staining for observing ascospores when grown on Acetate agar. Light microscopy was performed to see the BLBs (bacterial-like bodies) inside the yeasts vacuoles. Total DNA was extracted from yeasts and PCR was performed for detection of *H. pylori* 16S rRNA gene in yeasts.

**Results:** All the five yeasts were identified as *S. cerevisiae*. Moving BLBs were observed inside the vacuoles of all yeasts. *H. pylori* 16S rRNA gene was amplified from 4/5 yeasts. The size of PCR products was 519 bp and homologous to the one amplified from control *H. pylori*. No band was detected from standard yeast.

**Conclusion:** Occurrence of *H. pylori* 16S rRNA gene and BLBs indicate the likelihood of intracellular existence of *H. pylori* inside yeasts. Endosymbiotic interaction between bacteria and fungi has been described, indicating the important role of fungi in protection and spread of bacteria in the environment. *S. cerevisiae* which has been recruited as a potential tool in production of fermented food could harbor *H. pylori* as an endosymbiont. Accordingly, control of yeast content of food should be regarded as a hygienic measurement to prevent transmission of fastidious bacteria such as *H. pylori*.

Abstract no.: P04.13

#### AMPLIFICATION OF THE *HELICOBACTER PYLORI* SPECIES – SPECIFIC GENE FROM FECAL *CANDIDA ALBICANS*

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**Introduction:** The mechanisms of survival and persistence of *H. pylori* after release from stomach are still unknown. Only few studies demonstrated isolation of *H. pylori* from the intestinal content. In this study we studied fecal *Candida* yeast as the possible reservoir of *H. pylori* in the intestine.

**Material and Methods:** Twelve fecal *C. albicans* were recruited in the study. Yeasts were sub-cultured >10 times on Yeast extract Glucose Chloramphenicol Agar for elimination of any possible bacterial contamination and identified according to green colonies on CHROM Agar. Total DNAs were extracted and PCR was performed to amplify *H. pylori*-16S rDNA gene by designed primers. *H. pylori* and *Escherichia coli* were used as controls.

**Results:** *H. pylori*-specific 16S rRNA gene was amplified from 4/12 fecal yeasts. The size of the amplified products (519bp) of all four *C. albicans* yeasts was similar to those of control *H. pylori*. No band was detected in *E. coli*.

**Discussion:** Antagonistic microorganisms and harsh conditions of gastrointestinal tract provide stressful conditions for fastidious microorganisms such as *H. pylori*. Detection of the *H. pylori*-specific gene in fecal *C. albicans* proposes the possibility of important role of *Candida* yeast in protecting *H. pylori* in the gastrointestinal tract and when released to the environment.

Abstract no.: P04.14

#### DETECTION OF *HELICOBACTER PYLORI* SPECIES-SPECIFIC GENES IN *CANDIDA* SPP OF UPPER GASTROINTESTINAL TRACT

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**Introduction:** Unicellular eukaryotes of normal flora of human mouth and esophagus such as yeasts have been proposed as the reservoirs of *H. pylori* that could transmit the bacteria to the stomach. In this study gastric and esophageal *Candida* isolates from five dyspeptic patients were compared with their oral *Candida* isolates for having the *H. pylori* species-specific genes.

**Material and Methods:** Seven oral, three gastric and three esophageal *Candida* yeasts were isolated from five dyspeptic patients. Yeasts were subcultured >10 times on Yeast extract Glucose Chloramphenicol Agar to eliminate any possible bacterial contamination and were identified as *Candida* spp on CHROM Agar. Total DNAs were extracted and PCR was performed to amplify *H. pylori*-16S rRNA and *vacA* (*m*) genes. *H. pylori* and *Escherichia coli* were used as controls. Oral and gastric yeasts of one patient which were positive for *H. pylori*-specific 16S rRNA were stained by Live/Dead BacLight Bacterial Viability Kit.

**Results:** *H. pylori*-specific 16S rRNA (519bp) was amplified in all three gastric yeasts. Also, amplified products of *H. pylori*-specific 16S rRNA & *vacA* (*m1*:290bp, *m2*:352bp) genes from oral and gastric yeasts of one patient were similar to those of controls. Observations with the fluorescent microscope demonstrated fast moving green fluorescent Bacterium-Like Bodies inside the yeasts vacuoles.

**Discussion:** Symbiosis is one of the old strategies exploited by microorganism for escaping from environmental stresses. Oral normal flora such as *Candida* could provide a safe niche to accommodate bacteria such as *H. pylori*. This could be crucial for bacterial survival and spread to new hosts.

Abstract no.: P04.15

#### A POPULATION-BASED ENDOSCOPIC SURVEY OF *HELICOBACTER PYLORI* INFECTION AND ITS CLINICAL CONSEQUENCES IN BHUTAN

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**Aim:** Bhutan has the population of about 690,000 with area of 38,816 Sq.m. and the land consists mostly of steep and high mountains with elevation from 660–23,000 ft. The aim of this study was to determine the causes of dyspepsia and prevalence of *H. pylori* in this population.

**Method:** We conducted a cross-sectional population-based endoscopic survey of volunteers with and without dyspeptic symptoms during December 2010. The survey took place in Thimphu, Punakha and Wangdue. Complete questionnaire was obtained from each patient. Gastroscopy and biopsies were performed for CLO-test, culture, histopathology. *H. pylori* infection was determined if at least one of the test was positive.

**Results:** Total of 378 subjects were included (159 men (42%) and 219 women (58%), mean age 39.5 years). Endoscopic findings revealed normal 24%, gastritis 60.3%, gastric ulcer 17.2%, duodenal ulcer 6.9% and gastric cancer 2.5%. Overall *H. pylori* infection rate was 71.9%, highest in Punakha (82.1%), Wangdue (72.6%) and lowest in the capital, Thimphu (65.5%). *H. pylori* infection in DU and GU were significantly higher than subjects with normal finding or gastritis (85.7% vs 53%: *p*-value <.05, 84% vs 53%: *p*-value <.05 respectively).

**Conclusion:** Prevalence of *H. pylori* infection is high in Bhutan which could explain the high occurrence of PUD and gastric cancer. It is important to know the disease burden of gastric cancer in relation to *H. pylori* infection in this country in order to implement screening and preventive measures to reduce the risks of *H. pylori* infection and thus gastric cancer in Bhutan.

Abstract no.: P04.16

**COMMUNITY-BASED, PARTICIPATORY RESEARCH ON *H. PYLORI*: MAKING MICROBIOLOGY RESULTS MEANINGFUL TO PARTICIPANTS**A. Colquhoun,\* L. Aplin,\* K. J. Goodman,\* J. Geary,\* R. Munday<sup>†</sup> and M. Keelan\*\*University of Alberta, Edmonton, AB, Canada; <sup>†</sup>Government of the Northwest Territories, Aklavik, NT, Canada

The Canadian North *Helicobacter pylori* (CANHelp) Working Group conducts community-based, participatory research in Arctic Aboriginal communities to address community concerns about health risks from *H. pylori*. While *H. pylori* transmission has decreased in developed countries, evidence suggests that Arctic Aboriginal populations have a disproportionately high prevalence of the bacteria. Our collaborative initiative aims to describe the burden of disease, and seeks to identify effective public health strategies to reduce associated health risks. This research links Northwest Territories and Yukon community representatives, health care practitioners and health care decision makers, with faculty from various disciplines at the University of Alberta.

A component of our research involves culturing *H. pylori* from gastric biopsies obtained from participating community members. From these cultures, house-keeping genes have been sequenced to identify strain types and determine relatedness within households and communities. An important element of this work is the dissemination of research results in a manner that is meaningful to a variety of audiences. Because of differences in knowledge structures and world views between Aboriginal communities, health officials and researchers, the development of effective strategies for the dissemination of meaningful microbiology results is essential to successfully address our community-driven research goals. This process requires collaboration with community representatives to understand which results are of interest to community members and how they

would be best presented. The process through which these decisions were made and the methods of dissemination chosen by community representatives will be described in a case study of the Aklavik *H. pylori* Project.

Abstract no.: P04.17

**HELICOBACTER PYLORI IN CYPRUS**

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We are presenting a pilot study concerning the epidemiology of *H. pylori* in Cyprus. This is the first time that such a study is proposed in our country, a small Mediterranean country of 700,000 population. We started a collection of gastric biopsies from patients with gastroenterological clinical symptoms. The aim is to evaluate the presence of *Helicobacter pylori* in the biopsy and in parallel, in stool samples of the same patient. Every patient has also a CLO test and independently of the answer, the biopsy is analyzed for the presence/absence of *H. pylori* DNA. Every positive sample is characterized further for the presence/absence of *cagA*, and molecular characterization of *vacA* alleles (signal and mid region). Also, for every positive biopsy, we try to standardize a methodology to identify the presence/absence of *Helicobacter pylori* in stool samples. From the above study, preliminary results indicate an approximately 50% of positive biopsies using a PCR test easy to handle in a molecular diagnostic laboratory and that it is also possible even on a diagnostic level (not only research) to detect *H. pylori* in stool samples.

# P05 Paediatric Issues

Abstract no.: P05.01

## PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN IN A RURAL AREA OF JAPAN

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**Objectives:** Acquisition of *H. pylori* infection occurs mainly in childhood. The aim of this study is to estimate *H. pylori* infection prevalence in Japanese children using stool antigen test.

**Subjects and Methods:** One thousand two hundred seventy-seven children from 0 year old to 3rd grade of elementary school in 16 schools (seven elementary schools, six kindergartens, and three nursery schools) in Sasayama-city were invited to this study. In their stool samples *H. pylori* antigen was detected using two methods: original TestMate *Helicobacter pylori* Antigen EIA (Wakamoto Pharmaceutical Co., Ltd., Japan) and its improved type, extraction buffer of which has been improved. According to the manufacturer's instruction, the cutoff value of the original kit was 0.1, which is the same as that of the improved one. It was defined positive when both the original and the improved type kits gave positive results. It was defined negative in the other cases.

**Results:** Participation rate was 54% (689/1277). Stool antigen positive% was 1.9% (13/689) in Total: 0% (0/146) in those aged 0–3, 0.8% (1/120) in 4, 3.7% (5/134) in 5 years, 2.2% (2/89) in 1st grade, 1.8% (2/110) in 2nd grade and 3.3% (3/90) in 3rd grade of elementary schools.

**Conclusion:** Positive rate of *H. pylori* stool antigen was 1.9% in Japanese children. The prevalence of *H. pylori* infection seems to be still declining.

Abstract no.: P05.02

## *HELICOBACTER PYLORI* PREVALENCE OF INFECTION IN CHILDREN: WORLDWIDE ANALYSIS FOR THE PERIOD 2005–2009

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*Helicobacter pylori* infection occurs mainly during childhood, which makes children the principal age group population at risk. Risk factors for infection include absence of sanitary drinking water and of a sewage disposal facility during childhood, among others. According to WHO about 1.1 billion people drink globally unsafe water. The objective of these work was to study the current prevalence of infection by *H. pylori* in children (<18 years) and its correlation with safe drinking water and sanitation level.

Prevalence data on *H. pylori* infection in children for several countries, available in Pubmed, for the five years period (2005–2009) was gathered. During this five-year period it was possible to select information from 42 countries. This study showed that the worldwide prevalence of *H. pylori* in children is 36.7% in the world with a standard deviation (SD) of 22.3%, being higher in Africa (66.0%, SD of 15.5%) and America (41.6%, SD of 21.2%) and lower in Asia (35.7%, SD of 20.6%) and Europe (22.3%, SD of 14.3%).

This data was then correlated with sanitation level and safety of drinking water sources specific for each country (information available from WHO). As expected, there was a clear tendency towards a prevalence decrease with increased access to safe drinking water (correlation  $r = .17$ ) and sanitation (correlation  $r = .21$ ). Introduction of safe drinking water and sanitation will decrease *H. pylori* prevalence, but not immediately observed as in the case of pathogens associated with acute (diarrheal) diseases, since this infection is for life if left untreated.

Abstract no.: P05.03

## EPIDEMIOLOGICAL FACTORS AND FOOD: WHICH IS THE ROLE IN *HELICOBACTER PYLORI* RE-INFECTION IN PEDIATRIC AGE?

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**Background:** *Helicobacter pylori* (Hp) infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis and gastric cancer. Its acquisition is related with poor socioeconomic conditions while the relationship of nutrition and Hp is still a question.

**Aim:** To analyzed if socioeconomic factors and dietary contribute to Hp re-infection in pediatric age.

**Patients and Methods** Hundred and fifty patients (92 males; age range 5–16 years) with Hp infection treated and eradicated in the past. Fifty-five patients with Hp re-infection and 95 patients not re-infected. We interviewed the children with questionnaire about socioeconomics factors, hygiene, living conditions and their dietary habits.

**Results:** A lower frequency of fermented dairy food, fruits and vegetable consumption was registered among children with Hp re-infection as compared to not been re-infected. Among persons with Hp re-infection were noted low socioeconomic markers such as crowded living conditions, a large number of siblings and unclean water.

**Conclusions:** Might decrease the risk of Hp re-infection the use of probiotic, vitamin C, antioxidants contained in fruit and vegetables. Risk factors for Hp re-infection are low socioeconomics factors, hygiene and living conditions.

Abstract no.: P05.04

## *H. PYLORI* STRAINS GENOTYPING AND OUTCOME OF THE TRIPLE ERADICATION THERAPY IN CHILDREN LIVING IN RUSSIA

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**Aim of the Study:** To determine whether outcome of treatment is related to virulence genes presence in *H. pylori* strains isolated from Russian children.

**Patients and Methods:** Seventy three pediatric outpatients (female 41, mean age 13.8, age range 12–17 years) underwent endoscopy for dyspeptic complaints. *H. pylori* was cultured in 41 patients (56.1%) and genomic DNA was extracted. The extracted DNA was subjected to PCR for detection of the following *H. pylori* genes: CagA gene, VacA gene with differentiation of four PCR products (s1 and s2 from s-region and m1 and m2 from m-region), Ice A gene (Ice A1 and Ice A2 alleles) and Bab A2 gene presence. Patients were given a bismuth subcitrate (8 mg/kg/day), nifuratel (30 mg/kg/day) and amoxicillin (50 mg/kg/day) 10-day treatment. Eradication rate was evaluated by standard ammonia breath test (Helic-test, AMA, Russia).

**Results:** Positive CagA status was detected in 19(46.3%) patients, positive VacAs1/VacAs2 – in 18 (43.9%)/23 (56.09%) patients. VacA m1/VacA m2 PCR products were determined in 20 (48.7%)/21(51.2%) children. IceA1/IceA2 genotypes were identified in 27(65.8%)/14(34.1%) children. Positive BabA2 status was revealed in 18(43.9%) outpatients.

*H. pylori* eradication was confirmed in 33(80.4%) of the patients.

There was a strong association between CagA+ genotype ( $\chi^2$ -test,  $p = .0039$ ) and BabA2 + genotype ( $p = .0062$ ) and eradication of *H. pylori*. No associations with eradication were found for IceA alleles ( $p = .423$ ) and VacAs1/s2( $p = .055$ ) and VacAm1/m2 alleles ( $p = .319$ ).

**Conclusion:** Higher cure rates confirmed importance of CagA and BabA2 as predictors of successful therapeutic outcome in pediatric patients from Russia.

Abstract no.: P05.05

## IRON DEFICIENCY ANAEMIA (IDA) AND *H. PYLORI* INFECTION IN CHILDREN: A MULTICENTERED STUDY

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Epidemiological studies have suggested an association between IDA and *H. pylori* infection, which is reinforced by the demonstration of reversal of refractory anaemia after eradication of *H. pylori* in some patients. Mechanisms that can explain this association include decreased iron absorption, blood loss due to gastritis and increased uptake of iron by the bacterium. We aimed to evaluate the association between IDA and *H. pylori* infection in children undergoing upper gastrointestinal endoscopy due to gastric complaints. Two hundred and ninety seven children (mean age  $10.7 \pm 3.1$  years, 3–16 years, 171 female) were included: 101 from Santiago/Chile, 125 from Belo Horizonte/Brazil and 71 from London/England. Among them, 83 (28.0%) were *H. pylori* positive (positive culture or biopsy urease test or histology) and 214-negative. Children who had taken antimicrobials or PPI in the last month, those who had coeliac disease, peptic ulcer or intestinal parasites and female adolescents with menorrhagia were not included. IDA was defined by a hemoglobin value <11.0 g/dL in children 3–5 years, <11.5 g/dL in children 6–11 years and <12.0 g/dL in those 12–16 years and a ferritin value <12 µg/dL in children 3–5 years and <15 µg/dL in those 6–16 years. Although the prevalence of IDA was low (1%), it was associated with *H.*



**Table 1** Patients with intestinal metaplasia: demographic data, diagnostic *H. pylori* tests, and pathology grading. Degree according to Sydney classification

Sex	Age	UBT	Endoscopic	Activity	Inflammation	IM	Atrophy	Dysplasia	<i>H. pylori</i>	LF finding
M	9 year	+	AN	3	3	2	0	0	++	+
F	12 year	+	AN, Ulcer	2	3	1	0	0	+++	+

UBT, urea breath test; AN, antral nodularity; IM, intestinal metaplasia; LF, lymph follicle.

*pylori* infection even after adjustment for age and gender ( $p = .02$ ). In the regression logistic model, iron deficiency, detected in 16 (4.4%) children, was associated with female sex ( $p = .04$ ), increasing age ( $p = .03$ ) and antral nodularity ( $p = .02$ ). Funded under the Sixth Framework Programme of the European Union, Project CONTENT (INCO-DEV-3-032136).

Abstract no.: P05.06

#### INTESTINAL METAPLASIA AND GASTRIC ATROPHY IN CHILDREN

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**Aim:** Atrophic gastritis and intestinal metaplasia are frequent among adult patients and thought to be premalignant. The prevalence of these lesions among children is barely known. Aim of the study is to assess the prevalence of gastric atrophy and intestinal metaplasia in children.

**Material and Methods:** Children with gastrointestinal symptoms were evaluated. Urea breath test, uppergastrointestinal endoscopy macroscopical findings and histopathological evaluation with Sydney classification were made.

**Results:** Three hundred and fifty-seven children underwent uppergastrointestinal system endoscopy. Macroscopically, nodular gastritis was found in 59.57% and peptic ulcers were found in 13.16%. Histopathological evaluation revealed no gastric atrophy 0%, but intestinal metaplasia in two children 0.56%. Both children had positive UBT and *H. pylori* histology. One had ulcer in antrum of stomach. Both children underwent reendoscopy after *H. pylori* eradication therapy. Intestinal metaplasia was not seen on the biopsy materials.

**Conclusion:** Atrophy and/or intestinal metaplasia, considered as preneoplastic lesions, are frequently seen in adults with *H. pylori* gastritis. The frequency and relation with gastric cancer in the pediatric population is controversial. Our results show that the incidences are very low, although this study is made where *H. pylori* gastritis is very frequent among children. Prevalence of atrophic gastritis and intestinal metaplasia among children is lower than in adults. The reason of this is multifactorial.

Abstract no.: P05.07

#### HELICOBACTER PYLORI GENOTYPES IN ROMANIAN CHILDREN WITH CHRONIC GASTRITIS: A SINGLE CENTER STUDY

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**Background:** Although *Helicobacter pylori* has a worldwide distribution, there is substantial genetic variation of the main molecular virulence markers among different geographic regions.

**Aim:** To evaluate the prevalence of selected virulence genes *cag A*, *vac A* (alleles *s1a*, *s1b*, *s2*, *m1* and *m2*), gene *ure A* and its relationship to a specific gastric lesion intensity in the children with chronic gastritis.

**Methods:** Antral biopsy specimens were taken from 111 children (68 girls, age range to 1–18 years) undergoing esophagogastroduodenoscopy in our unit, from January to December 2010. Gastric antral biopsies were obtained for rapid urease test, histopathology, culture and PCR for *H. pylori* virulence markers *cag A*, *vac A* and its allele types. DNA was extracted from cultured strains. PCR for *cag A* and *vac A* alleles were performed using primers previously described (a 16s-23Sr DNA and *ure* markers for species identification).

**Results:** Of 111 children, 55 (49.54%) were rapid urease positive, and 45 of them (81.81%) were culture positive. The presence of the gene *cag A* was detected in 84% *H. pylori* infected children. The predominant combination found for gene *vac A* was *m1s1*, without *s2/m1*. The *H. pylori* strains, *vac A s1b1/m1/cag A*-positive, were associated with an increased antral nodularity and higher degrees of activity of gastritis ( $p < .001$ ).

**Conclusions:** We found a significant relationship between clinical disease manifestations and the putative virulence markers in Romanian *H. pylori* infected children of our endoscopy unit, with a high prevalence of *cag A vac A s1m1* genotype.

Abstract no.: P05.08

#### A 10 YEARS SURVEY OF HELICOBACTER PYLORI INFECTION IN SYMPTOMATIC CHILDREN

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**Introduction:** *Helicobacter pylori* infection is acquired mostly in childhood and leads to prolonged exposure to this potentially carcinogenic agent.

**Aim:** To assess the evolution of *H. pylori* prevalence, the clinical and endoscopic features and the changes in the eradication rates after the first-line therapies, among gastroscopied symptomatic children during the last decade.

**Methods:** This was a retrospective single center study of all esophagogastroduodenoscopies (EGD) performed in symptomatic children (710 girls, age range to 6 months – 18 years) between 2001 and 2010. *H. pylori* infection was assessed before and 4–6 weeks after treatment by urease test, histopathology and sometimes by stool antigen. Infected children received one of the standard three first-line triple therapies for 7–14 days or a 10 day sequential regimen.

**Results:** *H. pylori* infection was documented in 606 of the 1142 studied children (53.06%) respectively in 467 of 802 children at the first gastroscopy (58.22%) and in 139 of 340 control gastroscopies (40.88%). Overall, its yearly prevalence varied from 46.93% in 2001, to 49.54% in 2010 with an unexpected increase between 2006 and 2009 (69.87%–59.64%). The main symptoms were: abdominal pain (89%), vomiting (45%), regurgitations (38%), halitosis (26%). Antral nodularity was identified in 79% of cases. The eradication rate after the first treatment was 70.73% with a decrease from 83.61% in 2001 to 71.18% in 2010.

**Conclusions:** This endoscopic series revealed that the prevalence of *H. pylori* infection in symptomatic children has remained high in our country, despite the recent decline observed in developed countries.

Abstract no.: P05.09

#### GASTRIC ULTRASONOGRAPHIC FINDINGS IN CHILDREN WITH H. PYLORI GASTRITIS

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**Aim:** *H. pylori* is very common in developing countries. Although accurate diagnosis is based on gastric biopsy and histology there exists some noninvasive tests such as urea breath test, stool antigens. We planned to evaluate the gastric wall thickness of children and the clinic applicability. Here we report the preliminary results of the study.

**Material and Methods:** Children with biopsy proven *H. pylori* gastritis underwent ultrasonographic evaluation after 6–8 hours' fasting by a blinded radiologist. After distending the stomach with water anterior and posterior wall thicknesses from corpus and antrum were evaluated transabdominally on left oblique, right oblique and supine positions. Histological Sydney classification and radiological findings were correlated using SPSS.

**Results:** There were 19 children with *H. pylori* gastritis. Corpus posterior measurement was significantly correlated with *H. pylori* density and neutrophil activity; corpus anterior and antrum anterior were significantly correlated with chronic inflammation. Antral and overall nodularity was significantly correlated with chronic inflammation. There was significant correlation between age and *H. pylori* density.

**Conclusion:** Ultrasonographic evaluation of stomach may give hint about *H. pylori* gastritis in children.

Abstract no.: P05.10

#### HELICOBACTER PYLORI AND ATHEROSCLEROSIS IN CHILDHOOD

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**Aim:** The role of inflammation in the pathogenesis and progression of atherosclerosis has been increasingly discussed. Although the seroepidemiological studies have suggested a relationship between *Helicobacter pylori* (*H. pylori*) infection and atherosclerosis; the issue is still controversial. Abnormal lipid profile is related to atherosclerosis and the measurement of carotid-intima media thickness (CIMT) is one of the surrogate marker of atherosclerosis. The aim of this study was to investigate CIMT and serum lipid parameters in *H. pylori* positive children. We report the preliminary results of the study.

**Method:** Children with biopsy proven *H. pylori* gastritis were studied. Intima-media complex thickness was calculated by measuring bilateral carotid arteries' proximal middle and bulbous parts using high resolution gray scale ultrasonography. Sydney classification of gastritis and data collected from ultrasonography were compared.

**Results:** Nineteen children were taken into the study. All children's pathological records and ultrasonographic measurements were correlated using SPSS. There were significant correlation between *H. pylori* density and right common carotid artery, right bulbous part; chronic inflammation and right common carotid artery proximal and middle parts. There was significant negative correlation between left common carotid artery and triglyceride and VLDL levels.

**Conclusion:** *H. pylori* may play atherogenic role in children.

Abstract no.: P05.11

#### HELICOBACTER PYLORI INFECTION IN CHILDREN DIAGNOSED VIA THE UREA BREATH TEST

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**Objective:** To determine the features of *Helicobacter pylori* (H.P) infection in pediatric population seen in our gastroenterology unit.

**Methods:** During the period of January 1st 2010 to December 31st 2010, 159 children underwent an urea breath test (UBT). The following parameters were evaluated: age, gender, first degree relatives with H.P infection, main symptoms, test results, type and duration of treatment, eradication, endoscopic and histological findings and resistances.

**Results:** Hundred and forty-three patients were included with a mean age of 9.28 years and a gender distribution of 56.6% female and 43.4% male. The reason to carry out an urea breath test was epigastralgia 52.4%, functional dyspepsia 34.3% vomiting 5.6% halitosis, first degree relatives H.P infection 2.1% and others 2.8%. H.P infection was confirmed in 23.8% of patients, getting eradication in 25 (78.1%). In 20.6% of positive tests, the main symptom was functional dyspepsia. Triple therapy using as primary treatment was: omeprazole, amoxicillin, clarithromycin 51.5%; esomeprazole, amoxicillin, clarithromycin 42.4%; omeprazole, amoxicillin, metronidazole 6.1%, in all cases during 14 days, without differences among them. 19.7% of patients had familiar history, 50% in those with positive UBT and 10.2% in negative UBT, with statistically significant difference ( $p < .001$ ). In seven patients who did not achieve eradication, five presented chronic gastritis. Three had clarithromycin resistance and in one case double resistance. 73.1% treated patients improve symptomatology after eradication.

**Conclusions:** Familiar transmission in H.P infection. Low rate of positive tests that shows the need to establish indications of UBT. Most treated patients recovered from symptoms after eradication.

## P06 Diagnosis

Abstract no.: P06.01

### MINIMALLY INVASIVE BAYLOR BRUSH SYSTEM FOR NON-ENDOSCOPIC COLLECTION OF *H. PYLORI* CULTURES IN AN OFFICE SETTINGS

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**Background:** A reliable minimally-invasive system for efficiently obtaining cultures of *H. pylori* strains without endoscopy is needed for practice and research.

**Aim:** To assess the acceptability and yield of the Baylor or-gastric extendable brush system for *H. pylori* culture and antimicrobial resistance testing in an office setting.

**Methods:** During the first month of study enrollment in a clinical trial, 32 asymptomatic adults tested positive to both a urine antibody and ti urea breath testing, meeting all selection criteria were invited to the baseline appointment at the office of a gastroenterologist in El Paso, Texas, after fasting for ≥3 hours. After topical oral anesthesia subjects were asked to swallow the brush assembly, which was extended in the stomach 3–4 times and then retracted into the protective sleeve and withdrawn. The brush was placed into transport media, frozen on dry ice, transferred to -70 °C and later shipped to Houston for culture and minimum inhibitory concentration testing.

**Results:** All but one of the eligible subjects (31/32 or 96.9%) accepted the procedure. 87% were middle age women (mean 45 years). Brushing required approximately one minute; the entire interaction with the physician was approximately 11 minutes. Some but not all subjects experienced gagging; no adverse events occurred. *H. pylori* was cultured in 27/28 specimens (96.4%; 95% CI: 83.6-99.8%).

**Conclusion:** This procedure appeared efficient, safe, practical, and accepted by asymptotically infected individuals. Culture specimens can be obtained efficiently without endoscopy with biopsy. This technique would allow culture to be taken for all clinical trials even those in which endoscopy was not done.

Abstract no.: P06.02

### SERUM PEPSINOGEN LEVELS IN DEVELOPING GASTRIC CANCER SCREENING APPROACHES AMONG IRANIAN HIGH RISK POPULATIONS

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Three hundred and eighty-two GC patients (cases), 626 non ulcer dyspeptic patients (NUD) and 179 healthy blood donors were enrolled as hospital and population based controls, respectively. Fasting blood samples were taken for measuring serum PG I, PG II. GC cases were categorized according to tumor subsite and subtype. PMN/neutrophil infiltrations, gastric atrophic and intestinal metaplastic changes were graded according to OLGA staging system.

Serum PG I, II levels, I/II ratio were significantly different among cases and controls ( $p < .05$ ). Logistic regression analyses showed that low PG I/PG II ratio ( $\leq 3.0$ ) increases the risk of GC development by 5.2–7.7, which is mostly owed to cardia than non-cardia GC when compared to population based controls (OR = 4.7; 95% CI = 2.0–10.7 vs OR = 3.6; 95% CI = 1.5–8.5) and hospital based controls (OR = 4.3; 95% CI = 2.2–8.4 vs OR = 3.3; 95% CI = 1.7–6.7).

When cases were stratified according to GC subtypes, low PG I/II ratio also presented a risk for GC development, which was more pronounced for intestinal than diffuse tumor subtypes, when healthy (OR = 5.6; 95% CI = 2.43–12.8 vs OR = 2.9; 95% CI = 1.1–7.5) and NUD (OR = 5.15; 95% CI = 2.7–10.0 vs OR = 2.7; 95% CI = 1.9–6.04) controls were selected as the reference groups.

Microscopic grading of inflammation in gastric biopsies among NUD group demonstrated that PGI/II ratio is significantly ( $p < .001$ ) lower in those who suffer from severe grades of inflammation (grade 3:  $10.6 \pm 15.4$ ; grade 4:  $19 \pm 31.7$ ) vs those with lower grades of inflammation (grade 0:  $15.1 \pm 15.2$ ; grade 1:  $10.5 \pm 5.15$ ; grade 2:  $11.8 \pm 11.43$ ).

The risk impact of low serum PGI/II ratio on GC development in both subsites and subtypes, recommends the application of this non-invasive assay in population screening approaches.

Abstract no.: P06.03

### EVALUATION OF FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) AND REAL-TIME PCR FOR MOLECULAR DETERMINATION OF CLARITHROMYCIN RESISTANCE AND DETECTION OF *HELICOBACTER PYLORI* IN PATIENTS WITH DYSPEPSIA

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**Aim:** To evaluate FISH and Real-time PCR methods to detect *H. pylori* infection and to determine the clarithromycin resistance due to mutations at 23SrRNA gene.

**Methods:** Ninety patients with dyspepsia were referred to upper endoscopy at Gastroenterology. Antrum and corpus biopsy specimens were obtained for RUT, histopathology and culture and *H. pylori* was defined as at least two positivity of these three tests. E-test was applied to isolated *H. pylori* strains from each culture for clarithromycin susceptibility. *H. pylori* and clarithromycin susceptibility (2143 and 2144 positions at 23SrRNA) were detected in paraffin embedded antrum and corpus biopsy specimens by FISH (BactFISH *H. pylori* Combi Kit) method. DNA was extracted from all antrum and corpus biopsy specimens by QIAamp@DNA mini kit (QIAGEN) to detect *H. pylori* and to determine clarithromycin susceptibility in the 23SrRNA gene (the wild-type and A2142G, A2142C, A2143G mutations) by Real-timePCR method (JCM,41,2003).

**Results:** Sixty-two (68.9%) of 90 patients were *H. pylori* positive by gold standard methods. Forty-three (69.4%) out of 62 patients were culture positive, however 58 (93.5%) and 61 (98.4%) patients were positive by FISH and Real-timePCR, respectively (Table 1). The sensitivity, specificity, PPV and NPV of FISH and Real-timePCR to detect *H. pylori* were 93.6%,98.4%; 92.9%,53.6%; 96.7%,82.4%; 86.7%,93.8%, respectively (Kappa = 0.847;0.589). Clarithromycin susceptibility was found in concordance (73.3%) with E-test, FISH and Real-timePCR methods.

**Conclusion:** Clarithromycin resistance was high (23.3%, 13.8%, 32.8%) in clinical strains and biopsies in patients with dyspepsia by three methods and was mostly associated with A2143G mutation confirmed by FISH and Real-timePCR.

Abstract no.: P06.04

### SIMULTANEOUS DETECTION OF *HELICOBACTER PYLORI* INFECTION AND CLARITHROMYCIN RESISTANCE BY FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) IN TURKISH DYSPEPTIC PATIENTS

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**Aim:** Clarithromycin resistance in *H. pylori* is considered a major cause of treatment failure. We aimed to evaluate the efficacy of fluorescence *in situ* hybridization (FISH) method for the simultaneous detection of *H. pylori* and to determine clarithromycin resistance due to mutations in the 2143 and 2144 positions of 23SrRNA gene compared with traditional culture and antimicrobial susceptibility technics. We assessed *cagA* status and correlated *cagA* positivity and clarithromycin resistance.

**Methods:** We studied 179 patients with dyspepsia (45M, 134F;  $43.7 \pm 13.7$  years). Antrum and corpus biopsy specimens were obtained for rapid urease test, histopathology and culture. *H. pylori* status was defined when at least two of three tests were positive. Clarithromycin susceptibility in *H. pylori* strains was assessed by E-test and *H. pylori* and clarithromycin susceptibility were determined by FISH (BactFISH *H. pylori* Combi Kit) using formalin-fixed paraffin-embedded antrum and corpus biopsy specimens. *cagA* status in *H. pylori* strains was also determined by PCR.

**Results:** A total of 119 (66.5%) were *H. pylori* positive. Among those, 84 (70.6%) were culture positive and 100 (84.0%) were FISH positive. The sensitivity, specificity, PPV and NPV of FISH was 84.0%, 95.0%; 97.1%, and 75.0%, respectively (Kappa = 0.741). FISH detected clarithromycin resistance in 22.0% and by E-test 27.4% of the strains. The concordance was 74.8%. Of the 84 *H. pylori* strains, 42 (50.0%) were *cagA* positive.

**Conclusion:** FISH increases the sensitivity to detect *H. pylori* when compared with microbiology routine methods. FISH is a reliable and highly sensitive method, especially when a quick decision is necessary for treating dyspeptic patient with previous treatment failures.

Abstract no.: P06.05

**ATTEMPTS TO IDENTIFY *HELICOBACTER PYLORI* AT THE SPECIES LEVEL AND TO DETERMINE PATHOVARS BY MALDI-TOF MASS SPECTROMETRY****L. Bénéjat**, E. Bessède, P. Lehours, C. Varon and F. Mégraud  
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*Helicobacter pylori* is known as one of the most heterogeneous bacterial species. While easy to identify when it is isolated from gastric biopsies, it can be more difficult when culture is carried out from other specimens. Furthermore, most attempts have been unsuccessful to easily differentiate strains leading to different diseases (pathovars).

**Aim:** To apply MALDI-TOF mass spectrometry for the identification of *H. pylori* at the species level and the determination of pathovars.

**Material and Methods:** Proteins were extracted from 53 *H. pylori* strains including 14 successive strains all positive by a specific PCR and isolated from patients with gastritis, as well as strains from severe diseases: gastric MALT lymphoma (18), gastric adenocarcinoma (7), and peptic ulcer disease (14). A MALDI-TOF mass spectrometer (Ultraflex 3 TOF-TOF, Bruker-Daltonics) was used with the Biotyper 2.0 software (Bruker-Daltonics).

**Results:** The 14 *H. pylori* gastritis strains could not be identified directly with the Biotyper 2.0 containing the profiles of 6 *H. pylori* strains. The spectra obtained from these strains were then used to generate a special database. All the strains studied could be identified at the species level with this database. When a dendrogram was established with all the strains, a cluster grouping the MALT lymphoma strain could be differentiated, while the strains from the other diseases were distributed in the other part of the dendrogram.

**Conclusion:** Due to the heterogeneity of *H. pylori* strains, a large database is required for species identification. This heterogeneity becomes a positive point when pathovar separation is expected. Further studies using this technique may allow us to predict the pathogenic potential of *H. pylori* strains.

Abstract no.: P06.06

**SELECTING PATIENTS FOR RE-GASTROSCOPY****A. Oksanen**,\*<sup>†</sup> L. Paloheimo<sup>‡</sup> and H. Rautelin<sup>§,†</sup>\*Herttoniemi Hospital, City of Helsinki, Helsinki, Finland; <sup>†</sup>Haartman Institute, University of Helsinki, Helsinki, Finland; <sup>‡</sup>Biohit oyj, Helsinki, Finland; <sup>§</sup>Department of Medical Sciences, University of Uppsala, Uppsala, Sweden

**Aim:** We showed earlier that abnormal macroscopical finding at previous gastroscopy was the most significant indicator predicting abnormal findings at re-gastroscopy. Now we wanted to evaluate if serum tests could be used to select patients for re-gastroscopy.

**Methods:** Serum samples were available for 190 patients who had no alarm symptoms and underwent re-gastroscopy. An earlier gastroscopy report was available for 157 patients and 126 of them had had a normal macroscopical finding earlier. Serum samples were analyzed for *H. pylori* antibodies of the IgG class, pepsinogens I and II, and gastrin-17.

**Results:** Only 20 of 190 patients (11%) had an ongoing *H. pylori* infection but 74 further patients (39%) had signs of a previous *H. pylori* infection. If patients with normal earlier gastroscopy had been selected for re-gastroscopy on the basis of positive *H. pylori* serology or low pepsinogen I, 82/126 (65%) gastroscopies would have been saved. However, 4/17 patients with moderate or severe atrophic gastritis and 2/6 patients with severe macroscopical findings (ulcer/severe oesophagitis/Barrett's oesophagus of 10 cm) would have been missed. If low gastrin-17 had been added to the panel, 57/126 (45%) gastroscopies would have been saved but still two patients with moderate to severe atrophic gastritis and two with severe macroscopical findings would have been missed.

**Conclusions:** Macroscopical findings and isolated atrophic changes are not detected by serum tests. In a patient group with a low prevalence of *H. pylori* infection and atrophic gastritis, the usefulness of serum tests to select patients for re-gastroscopy may be limited.

Abstract no.: P06.07

**COMPARISON OF ETEST, GENOTYPE HELICODR AND IN-HOUSE REAL TIME PCR ASSAY FOR THE DETECTION OF CLARITHROMYCIN RESISTANCE****S. Jeverica**, U. Dolinar, P. Camernik and M. Skvarc  
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**Background:** The main reason for failure of clarithromycin based eradication therapy is resistance to clarithromycin. It is mainly caused by three point mutations within peptidyltransferase region of the 23S rRNA. The most frequent are A2146G and A2147G mutations, A2146C is less common. There are different

methods of detection of those mutations. Our aim was to evaluate one of the real-time PCR assays currently in use in the German NRZ fur Helicobacter in Freiburg (courtesy of prof. Kist) in our setting.

**Methods:** The evaluation of the RT PCR assay was done on 107 routine consecutive gastric biopsies. Culture and susceptibility was performed. GenoType HelicoDR (HDR) was done on the culture negative samples. Additionally we performed the RT PCR assay on all samples. Basic test parameters were calculated. Combination of culture and HDR results was used as a "gold standard".

**Results:** RT PCR assay was able to detect *H. pylori* in all culture-positive samples (72/72, 100%), as well as in five additional culture-negative samples. RT PCR assay was able to detect 37/40 resistant strains as determined by Etest/HDR combination. Mutations were not detected in three clarithromycin resistant strains. With GenoType HelicoDR we detected the presence of two strains, wild type and mutant strain in all three cases. Nevertheless, in six other samples RT PCR assay was able to detect the heterogeneous population in the biopsies.

**Conclusion:** RT PCR assay reliably detects HP and clarithromycin resistance from biopsies. GenoType HelicoDR test is better for the detection of heterogeneous populations from stomach.

Abstract no.: P06.08

**CORRELATION OF AGE OF PATIENTS WITH CONCENTRATION OF AMMONIA IN EXPIRED AIR AT USE OF THE UREASE RESPIRATORY TEST****L. G. Bajenov**,\* I. V. Kosnikova,\* and A. N. Aripov,<sup>†</sup> Uzbek Helicobacter Study Group  
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The aim was determination of correlation of age of patients with duodenal ulcer with concentration of ammonia in expired air at use of the modified urease respiratory test.

Forty-eight patients with duodenal ulcer were examined. The quantitative detection of ammonia was fulfilled thereby a biochemical method. At the same time, patients were examined on presence of *Helicobacter pylori* (HP) thereby a bacteriological method. All patients were divided into three groups: 22–34 years old, 35–45 years old, 46–65 years old.

Ammonia concentration in expired air of patients made from 0.05 to 10.15 mmol/L. Its maximum level was registered in the second group. The average concentration of ammonia in this group was 3.8 times more than in the first one ( $p < .02$ ). The lowest level was registered in the third group, 10.5 times less than in the second one ( $p < .01$ ), and more than two times less, than in the first one. The obtained data correlated with results of the bacteriological method. The greatest HP contamination was also revealed in the group of 35–45 years old.

The revealed dependence is probably caused by that fact, that the dystrophic phenomena in a stomach mucous membrane where HP persist are expressed to a greater extent at people of middle age with a long ulcer anamnesis, than at young people. It is necessary to take this fact into consideration at application of the urease respiratory test.

Abstract no.: P06.09

**EVALUATION OF THE "CLARI-RES ASSAY" KIT FOR THE RESEARCH OF THE *HELICOBACTER PYLORI* CLARITHROMYCIN MUTATION RESISTANCE IN GASTRIC BIOPSIES****G. Mucignat**,\* A. M. Baragiotta,<sup>†</sup> G. Benedetti,<sup>†</sup> A. Lagatta\* and M. Crovatto\*\*Biologia Molecolare Ospedale di Pordenone, Pordenone, Italy; <sup>†</sup>Gastroenterology Ospedale Pordenone, Pordenone, Italy

**Background:** The need of sensitivity test on the Helicobacter obtained from gastric biopsies culture is fundamental. However cultural sensitivity test present many difficulties.

**Aim:** The purpose of this study was to evaluate PCR based test performed directly on gastric biopsy specimen to detect mutations indicating CLA resistance, to compare this technique with the histological results and to evaluate the rate of CLA-resistance in our population.

**Methods:** Specimens were derived from 102 patients (44M,58F) presenting upper gastrointestinal symptoms, submitted to endoscopy (EGDS) in the Gastroenterology Unit from December 2008 to February 2011. Gastric biopsies were taken for histopathology examination. Consensually were done molecular analysis in the gastric biopsies. Extraction kit used were QIAamp DNA mini (QIAGEN). We utilized the kit "HP ClariRes Assay" (Ingenetix GmbH, Vienna) for the research of the mutation A2142C, A2142/3G by using RealTime PCR.

**Results:** The 85% of the results were concordant between the two analysis (molecular and histological). 77/102 (75.49%) patients were HP positive. 41 (46.75%) were CLA resistant. In 15/77 patients (19.5%) histology were HP negative whilst were found HP DNA.



**Conclusions:** The molecular method used has been shown sensible in the daily clinical practice and might enable us to identify the presence of Hp directly from biotic material and consensually clarithromycin resistance. High clarithromycin resistance rates were observed in our population suggest the need of this research in order to plan a suitable therapeutic strategy, to better select the population to treat and consequently a better control of the infection.

Abstract no.: P06.10

#### COMPARISON BETWEEN LIOFILCHEM AND ETEST GRADIENT DIFFUSION METHODOLOGY FOR THE MIC DETECTION OF *H. PYLORI*

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**Background:** Increasing resistance mandates susceptibility testing for the selection therapy. One of the methods for resistance testing is gradient diffusion Etest. Other manufacturers of gradient diffusion strips have appeared in the last years with the focus on lowering the price. The aim of the study was to compare Liofilchem strips with Etests.

**Methods:** Sixty-one clinical isolates of *H. pylori* from biopsies collected between march and may 2011 were tested with Etests and Liofilchem strips. MICs for amoxicillin, tetracycline, clarithromycin, levofloxacin and metronidazole were determined. Sensitest agar plates supplemented with 10% horse blood, inoculum of 3–4 McF, microaerophilic incubation at 37 °C, 48–72 hour, was used. The point of interception of the elliptical zone of inhibition and the strips was determined as MIC value.

**Results:** The correlation between Liofilchem and Etest for amoxicillin, clarithromycin, metronidazole and tetracycline was excellent, with 98.4%, 98.4%, 98.4% and 96.7% MIC values within  $\pm 2$  double dilutions and 100% category agreement. Liofilchem results for levofloxacin revealed lower MIC values than as determined by Etests, with 83.6% results being within  $\pm 2$  double dilutions and category agreement of 98.4% (60/61).

**Conclusion:** The correlation between Liofilchem and Etest was excellent for amoxicillin, clarithromycin, metronidazole and tetracycline (96–98%). Liofilchem MIC results for levofloxacin were lower than that obtained by Etests (83.6%). This can be due to the subjective interpretation, heterogeneous populations, different inoculum sizes, or due to some undisclosed reason. Results of our study show that Liofilchem could be cost-effective alternative to Etest for in vitro susceptibility testing of *H. pylori*.

Abstract no.: P06.11

#### CHARACTERIZATION OF JAPANESE STOOL ANTIGEN TESTS USING A MONOCLONAL ANTIBODY TO *HELICOBACTER PYLORI* CATALASE

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**Background and Aim:** Two types of stool antigen tests have been used in the management of *Helicobacter pylori* infection. Testmate® Pylori Antigen EIA (TPAg EIA) is a direct sandwich enzyme immunoassay (EIA) while Testmate® Rapid Pylori Antigen (Rapid TPAg) is performed using immunochromatography. The aim of this study was to study the characterization and usefulness of these tests.

**Methods:** Accuracy of both tests was studied using 111 fecal samples obtained from *H. pylori* positive or negative patients. Cross-reactivity was examined with four other *Helicobacter* spp. and five fecal bacteria in humans. To estimate the sensitivity of both kits, we tested 485 *H. pylori* clinical strains.

**Results:** The accuracy of both Testmate kits was 100% in fecal samples from 111 patients. No cross-reactivity was observed in both Testmate kits in nine tested bacteria. Both TPAg and Rapid TPAg showed positive results in 483 of 485 clinical strains (sensitivity 99.6%). In two strains with negative results, a point mutation (Gly208 → Asp208) of beta-barrel domain was observed.

**Conclusions:** The results of this study may explain, in part, high specificity and sensitivity of TPAg EIA and Rapid TPAg. TPAg EIA would be useful for large-scale screening surveys, and Rapid TPAg would be suitable for out-patients use.

Abstract no.: P06.12

#### EVALUATION OF DIAGNOSTIC TESTS FOR *H. PYLORI* INFECTION IN THE BHUTANESE POPULATION

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**Background:** Bhutan is a small South Asian country locating at the eastern end of the Himalayan Range. The situation of *Helicobacter pylori* infection and the accuracy of diagnostic methods have not been clarified yet.

**Aim:** The aim of this study was to evaluate the diagnostic yields of several methods used to detect *H. pylori* infection in the Bhutanese population.

**Patients and Methods:** A total of 388 subjects (219 females and 169 males) from three Bhutanese cities (Punaka, Thimphu and Wangdue), aged 16–92 (mean age: 39.5) with upper abdominal complaint were recruited. All patients underwent upper gastrointestinal endoscopy in which gastric biopsy specimens were taken. *H. pylori* infection was defined based on the combined results of histology, immunohistochemistry with anti-*H. pylori* antibody, rapid urease test (CLO), serum ELISA and culture. *H. pylori*-positive status required at least one positive test result.

**Results:** Overall infection rate of *H. pylori* in Bhutan was 71.9%. Infection rate differed among the cities; highest in Punaka (82.1%), lowest in Thimphu (65.5%) and intermediate in Wangdue (72.6%). The diagnostic yields of each method were determined as follows: (Table).

**Table 1** Sensitivity, specificity and accuracy of the 4 tests to diagnose *H. pylori* infection

	Accuracy	Sensitivity	Specificity
Culture	89.3	88.5	99.1
CLO-test	82.5	76.0	99.1
Serum Ab	96.7	97.2	95.6
Histology	90.3	86.4	100

**Conclusions:** *H. pylori* infection rate was relatively high in Bhutan and differed among three cities. Each test yielded high accuracy in the diagnosis of *H. pylori* infection in the Bhutanese population with high sensitivity and specificity.

Abstract no.: P06.13

#### COMPARING THE ACCURACY OF STOOL ANTIGEN TESTS FOR THE DETECTION OF *HELICOBACTER PYLORI* INFECTION IN TURKISH DYSPEPTIC PATIENTS

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**Background:** The importance of non-invasive tests has been increased for the evaluation of *H. pylori* infection.

**Aim:** To evaluate and compare three *H. pylori* stool antigen tests with gold standard methods for the diagnosis of *H. pylori* infection in dyspeptic patients.

**Methods:** Fifty-four patients (17 males, 37 females; mean age, 46.4 ± 13 years; range, 21 to 78 years) with dyspepsia who were referred to upper endoscopy were included in this study. Antrum and corpus biopsies were obtained for Rapid urease test (RUT) and histopathology. Stool specimens were examined by Premier Platinum HpSA (Meridian USA) test, one step Simple *H. pylori* antigen cassette test (Linear Chemicals, S.L. Spain) and Femtolab *H. pylori* Cnx (Connex GmbH, Germany) test.

**Results:** Forty-five patients (83.3%) were diagnosed as *H. pylori* infection positive by gold standard methods. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for the Premier Platinum HpSA, one step Simple *H. pylori* stool antigen cassette test and Femtolab *H. pylori* Cnx (Connex GmbH, Germany) test were 54.4%, 100%,

100%, 27.6% and 61.1%; 73.9%, 87.5%, 97.1%, 36.8% and 75.9%; and 97.8%, 100%, 100%, 88.9% and 98.2%, respectively. There was a good correlation with Femtolab *H. pylori* Cnx test ( $p = 1.000$   $\kappa = 0.930$ ) and the gold standard methods. **Conclusions:** The monoclonal stool antigen test Femtolab seems to have better sensitivity and diagnostic accuracy comparing with the other stool antigen tests. So this may be useful in the routine laboratory for the detection of *H. pylori* infection in dyspeptic patients.

Abstract no.: P06.14

#### THE CLINICAL USEFULNESS OF THE NON-INVASIVE RAPID URINE TEST TO *H. PYLORI*: RAPIRUN<sup>®</sup>

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**Background and Aim:** The aim of this study was to evaluate the diagnostic accuracy of RAPIRUN<sup>®</sup> test in clinical practice.

**Methods:** A set of *H. pylori* tests which were composed of endoscopic biopsy, <sup>13</sup>C-urea breath test (<sup>13</sup>C-UBT), serum IgG-ELISA, and urine anti-*H. pylori* IgG test was conducted on 204 patients on the same day. The prevalence of *H. pylori* was calculated using each test independently.

**Results:** The proportion of positive result of *H. pylori* test was 59.3%, 56.4%, 57.4%, and 50.5% with gastric mucosal biopsy, <sup>13</sup>C-UBT, serum IgG-ELISA, and rapid urine RAPIRUN<sup>®</sup> test, respectively. With gastric mucosal biopsy, <sup>13</sup>C-UBT, and serum IgG-ELISA as the gold standard, a patient was considered to be *H. pylori* positive when all three tests were positive, or *H. pylori* negative when all were negative. The sensitivity, specificity, positive and negative predictive value, and accuracy of the rapid urine RAPIRUN<sup>®</sup> test were 83.5%, 98.3%, 98.7%, 79.4%, and 89.3%, respectively.

**Conclusions:** Urine based RAPIRUN<sup>®</sup> test for detection of anti-*H. pylori* antibody was an accurate test, especially in specificity. With the advantage of easiness, rapidity, and non-invasiveness of RAPIRUN<sup>®</sup> test, we expect that RAPIRUN<sup>®</sup> test would be useful in general practice for *H. pylori* screening.

Abstract no.: P06.15

#### DETECTION OF *HELICOBACTER PYLORI* INFECTION: CLINICAL VALIDATION OF <sup>14</sup>C (37 KBQ) UREA BREATH TEST

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**Objective:** Clinical evaluation of <sup>14</sup>C-urea breath test for diagnosis of *Helicobacter pylori* (Hp) infection, taking the <sup>13</sup>C-urea breath test as reference method.

**Material and Methods:** Prospective study focused on 61 subjects (10 male and 51 female), 46 dyspeptic patients and 15 asymptomatic volunteers, with mean age of 40.8 ± 13.4 years (22 to 77 years) and excluding pregnant or lactating. All individuals were initially subject to the <sup>13</sup>C-urea breath test and, at least two weeks later, to the <sup>14</sup>C-urea breath test, using respectively the infrared spectrophotometry and the Geiger-Müller counters. The <sup>14</sup>C was used in the microdose of 37 kBq (equivalent to 1 µCi).

**Results:** The <sup>14</sup>C-urea breath test correctly discriminated the 32 people infected and 29 uninfected individuals, previously identified by the <sup>13</sup>C-urea breath test. Indeed, the results showed an absolute concordance between both tests (Sensitivity-100%; Specificity-100%; PPV-100%; VP-100%; Observed concordance-100%; Coefficient k-100%).

**Conclusion:** The <sup>14</sup>C-urea breath test proved to be a noninvasive, valid and reproducible tool for diagnosis of Hp infection, being more rapid, easy, cheap and portable than the reference breath test method. However, it presents some safety limitations associated with the long half-life of <sup>14</sup>C, apparently minimized by very low dose of the radioactive isotope used.

Abstract no.: P06.16

#### AMMONIA BREATH TEST – TRANSPORTATION WAY OF AMMONIA

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**Aim:** To determine the transportation way of ammonia and related compounds during the ABT (Ammonia breath test) for *Helicobacter pylori* detection.

Enzyme hydrolysis of urea produces two gases: CO<sub>2</sub> and NH<sub>3</sub>. During the ABT we are measuring NH<sub>3</sub> and amines in mouth cavity. Appearance NH<sub>3</sub> in mouth air is possible by two ways: from lungs through blood and directly from stomach through esophagus.

**Method:** Measuring of ammonia presence in mouth cavity, nasal cavity and stomach after taking portion of urea during ABT by Helic-device and Helic-tubes. HP status of patients is determining by histology.

**Results:** Fifty-one patients with different gastrointestinal diseases – gastritis, duodenitis, ulcer, stomach cancer (age from 7 till 73, 23 HP (+), 28 HP (-) among them) were tested. After taking of urea NH<sub>3</sub> level in mouth cavity (121.0 ± 3.5 conventional unit) is higher than in nasal cavity (59.9 ± 8.6 c.u.) in the same time moment for all tested patients. We also detected ammonia level in stomach and esophagus air during endoscopy. Breath method also plays significant role in generation of measurement signal. Patient during the ABT procedure can breathe by nose as well as by mouth. Nose breath is preferable for testing (85.0 ± 2.7 c.u. comparing with 51.5 ± 0.6 c.u.). It additionally proves way of ammonia transportation from stomach through esophagus not lungs.

**Conclusion:** Way of ammonia transportation from stomach is way through esophagus. During ABT patient has to breathe by nose.

Abstract no.: P06.17

#### AMMONIA BREATH TEST (ABT): HOW TO IMPROVE THE RESULT

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ABT in form of Helic-test is one of the most economic, accessible and fast ways of non-invasive diagnosis of HP. It based on detection the ammonia increase in a mouth after taking portion of normal isotope urea.

**Aim:** To research the factors influencing level of ammonia in a mouth during ABT and to raise sensitivity of method.

**Materials and Methods:** Forty-seven patients (age from 7 till 62 years) were tested by Helic-device with fixing of sampling methods and patient actions during the analysis. Results were processed by statistical methods.

**Results:** The only obstacles in a way of direct penetration of ammonia from a stomach to mouth are bottom and top esophageal sphincters, which protect a top department of digestive system. Therefore, ammonia level in a mouth as a result of urease hydrolysis of carbamide in a stomach depends on motor activity of esophageal sphincters and its internal gleams. We established oscillation frequency of Helic-device target signal and frequency of reflex relaxations of esophageal sphincters (period 40–65 second).

Besides, we stated the statistical correlation between events: (Decrease of ammonia level in a mouth cavity) → (“Dry” swallow by the patient) → (Increase of ammonia level in a mouth cavity). The reason is reflex activity of esophageal sphincters, reacting to a swallow by muscle relaxation and increase of internal gleams.

**Conclusion:** For increase of sensitivity ABT by clearance of low useful signal is necessary to fix the oscillation frequency of Helic-device target signal and provoke “dry” swallow by patient.

Abstract no.: P06.18

#### DIAGNOSIS OF *HELICOBACTER PYLORI* FROM GASTRIC BIOPSIES USING THE FISH TECHNIQUE

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**Background:** *Helicobacter pylori* is the causative agent of gastritis, peptic ulcer disease, MALT lymphoma and is a risk factor in the development of gastric cancer (Blaser et al 1995). However various methods of diagnosis abound, but in Nigeria a suitable method is sought for accurate diagnosis of *H. pylori*. The study was therefore aimed at using the FISH technique for diagnosis of *H. pylori* from biopsy and comparing with known standard methods of diagnosis such as histology and CLO test.

**Methods:** A total of 57 biopsies from 19 patients (three biopsies each) was obtained after informed consent and screened for CLO test, histology and FISH. The FISH technique also went further to screen for clarithromycin resistance amongst the positive biopsies.

**Results:** The results show that CLO test was positive in 11/19 (57.9%) of the samples while histology and FISH results showed that 9/19 (47.4%) were positive each. ClAWT accounted for 8/9 (88.9%) of the cases with ClAR1 genotype accounting for 1/9 (11.1%) of cases.

**Discussion:** From this study although a pilot study, *H. pylori* can be diagnosed using the FISH technique, which was comparable to known methods of *H. pylori*

diagnosis. It also shows that clarithromycin is a useful antibiotic for part of *H. pylori* regimen in Nigeria as only one was resistant to clarithromycin. This is the first report in Nigeria using the FISH technique for *H. pylori* diagnosis. The study was supported by a grant from ICGEB no NIG-07 to SIS

Abstract no.: P06.19

**HELICOBACTER PYLORI: DIAGNOSIS OF INFECTION IN ADULTS IN ALGERIA**

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**Background:** *Helicobacter pylori* infection is an important risk to development of the gastroduodenal disease. Several methods, are actually used in our laboratory "Laboratoire Algérien de Recherche sur Helicobacter", some of them raise problems by their invasive character which make the control of the eradication more difficult.

**Aim:** To evaluate performances of different methods used by our laboratory to diagnose *Helicobacter pylori* infection and to control the eradication.

**Material and Method:** It is a prospective study made between March 2000 and December 2010. To make this study, between 337 and 693 samples were used

before treatment (biopsy samples, sera, stool samples...) and between 150 and 278 samples after eradication treatment. Subjects were between 17 and 70 years old, 67% of them were women.

**Methods:** Urea Breath test, Histology, Culture, Rapid Urease test and stool antigen test.

**Results:** The Urea Breath test is the best test to use even to diagnose the infection than to control the eradication it has an excellent sensitivity (99% before and 98% after treatment) and an excellent specificity (79%). The association between Rapid Urease Test and histology gives good results (SE: 96% before treatment, 65% after treatment), (specificity: 98%). Culture is also a reference test (specificity: 100%); it offers the possibility to evaluate the *Helicobacter pylori* sensitivity to the antibiotics. About the stool antigen tests were used: the first one "HpSA, Miridian" (SE: 64% before treatment versus 56% after treatment), the second one "HpStAr, Oxoid" (SE: 91% before treatment, 85% after), these results were different to the literature ones. The serological test is available but not very specific; it can't be used as a diagnostic test.

**Conclusion:** To make a decision about the choice of the diagnostic method, specificity and sensitivity are very important, but it is also important to consider the Availability and the cost of the test.

# P07 Drug Resistance

Abstract no.: P07.01

## NATIONWIDE SURVEY OF ANTIBIOTIC RESISTANT *HELICOBACTER PYLORI* IN THAILAND

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**Objective:** The aim of this study was to survey the antibiotic resistant pattern of *H. pylori* in different geographical locations in Thailand and to determine factors associated with antibiotic resistance.

**Methods:** A total of 3837 dyspeptic patients who underwent upper endoscopy from different regions (North, Northeastern, Central and Southern) of Thailand during January 2005 – March 2011 were enrolled in this study. Two antral gastric biopsies were obtained for culture and susceptibility tests were performed using E-test.

**Results:** Thousand three hundred and twenty-seven patients (34.6%) were infected with *H. pylori* identified by rapid urease test. E-test for all four antibiotics was successful in 374 isolates (152 male, 222 female, mean age 48.7 years). The endoscopic findings demonstrated 301 gastritis patients and 73 peptic ulcer patients. The prevalence of antibiotic-resistant *H. pylori* was amoxicillin 5.6%, tetracycline 1.9%, clarithromycin 3%, metronidazole 47.1%, and multi-drugs 5%. In amoxicillin, clarithromycin and metronidazole resistant strains, age > 40 years was significantly higher than age < 40 years (90% vs 10%; *p*-value = .04, 100% vs 0%; *p*-value = .03 and 65% vs 35%; *p* = .02 respectively).

**Conclusion:** Prevalence of *H. pylori* infection has decreased in all regions of Thailand. The prevalence of metronidazole resistant strain was high and remains the most common antibiotic resistant strains in Thailand whereas clarithromycin resistance has markedly declined in recent years. The reason for such a decline is likely due to the wide use of other newer antibiotics in place of clarithromycin. Age > 40 years might be a predictor for amoxicillin, clarithromycin and metronidazole resistant strain in Thailand.

Abstract no.: P07.02

## HIGH PREVALENCE OF METRONIDAZOLE RESISTANT *H. PYLORI* IN BHUTAN

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**Objective:** Data on antibiotics sensitivity of *H. pylori* infection in Asia varies and has not been adequately described in many countries. The aim of this study was to survey the antibiotic resistant pattern of *H. pylori* infection in different geographical locations in Bhutan.

**Methods:** Total of 375 volunteers who underwent upper GI endoscopy from different regions (Thimphu, Punakha and Wangdue) of Bhutan during December 2010 was enrolled in this study. Two antral gastric biopsies were obtained for culture and susceptibility tests performed using E-test.

**Results:** Two hundred and seven patients (55.2%) were infected with *H. pylori* identified by rapid urease test. E-test for all six antibiotics was successfully in 76 isolations (34 male, 42 female, mean age 37.1 years). The endoscopic findings demonstrated 70 gastritis patients and six peptic ulcer patients. The prevalence of antibiotic-resistant *H. pylori* was amoxicillin 0%, tetracycline 0%, clarithromycin 0%, metronidazole 84.2%, ciprofloxacin 5.3%, levofloxacin 5.3% and multi-drugs 5.3%. However, age, gender and endoscopic findings were not statistical different between patients with resistant and sensitive strains.

**Conclusion:** The prevalence of metronidazole resistant strain was extremely high whereas clarithromycin resistant strain was not demonstrable in Bhutan. Our findings imply that standard triple therapy that includes metronidazole is not suitable for *H. pylori* infection in Bhutan and a careful consideration is required in formulating the national therapeutic guidelines for the first-line and second-line therapies of *H. pylori* infection taking into consideration disease prevalence, health care access, diagnostics facilities and the burden of health care cost.

Abstract no.: P07.03

## DETECTION OF CLARITHROMYCIN RESISTANCE IN *H. PYLORI* FOLLOWING NONCRYOGENIC STORAGE OF RAPID UREASE TESTS FOR 30 DAYS

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**Objective:** Traditional *H. pylori* eradication therapy has been undermined by increasing antimicrobial, especially clarithromycin, resistance. Susceptibility testing in most areas is difficult or unavailable. We assessed whether gastric biopsies stored at room temperature in a rapid urease test get were suitable for *H. pylori* clarithromycin susceptibility testing.

**Methods:** After 30 days of storage at room temperature, DNA was extracted from a gastric biopsy present within a rapid urease test (Hpfast). *H. pylori* status and clarithromycin susceptibility were evaluated using *H. pylori*-specific PCR for ureA, vacA, and allele-specific primer-polymerase chain reaction of the 23S rRNA genes. The PCR results were compared with histology, RUT, and culture. *H. pylori* positive was defined as RUT and either culture or histology positive; *H. pylori* negative as RUT, culture and histology negative.

**Results:** Samples from 31 subjects were evaluated; 11 were *H. pylori* positive including nine by culture; eight of which had allele-specific primer-PCR results from the RUT specimen for the detection of mutations of the 23S rRNA gene. When both tests were available, culture and PCR results were concordant in 8/8 (100%). Fifteen of the 20 histology, RUT and culture negative cases had all three PCR's negative. In one all three were positive; in three only the 23S rRNA was positive and in 1 only ureA was positive.

**Conclusion:** Gastric biopsy specimens stored within the gel of an RUT for 30 days can be used to for molecular testing confirm the diagnosis of *H. pylori* infection and test for clarithromycin susceptibility.

Abstract no.: P07.04

## RESISTANCE OF *HELICOBACTER PYLORI* TO CLARITHROMYCIN AND LEVOFLOXACIN FROM 2009 TO 2011 IN BOGOTÁ – COLOMBIA

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**Objective:** To determine the prevalence of primary resistance of *Helicobacter pylori* against clarithromycin and levofloxacin from 2009 to 2011 in Bogotá – Colombia.

**Methods:** A total of 259 clinical isolates of *H. pylori* were collected from January 2009 to April 2011 from patients in Bogotá, Colombia. Antimicrobial susceptibility to clarithromycin and levofloxacin were tested by agar dilution. The isolates were considered resistant when the MIC values were  $\geq 1$   $\mu$ g/mL for both antibiotics. DNA was extracted from strains and regions involved in clarithromycin (23S rRNA gene) and fluoroquinolone (gyrA gene) resistance were amplified by PCR and DNA sequencing.

**Results:** The prevalence of *H. pylori* resistance to clarithromycin (3.2%, 5.8% and 14%) and levofloxacin (9.1%, 13.9% and 20%) increased from 2009 to 2011 in Bogotá – Colombia. Fifteen strains revealed mutations in 23S rRNA gene, 12 with A2143G mutations and three with A2142G mutations. 39 resistant strains had mutations in the gyrA gene; mutations in Asn 87 position were observed; 15 with N87L, 6 with N87K, 1 with N87H and 2 with N87Y. 15 mutations in Asp 91 position were found; 12 of them with D91G mutation, 2 with D91N and 1 with D91Y. The MIC distribution in clarithromycin resistant strains ranged from 1 to 32  $\mu$ g/mL, and the MIC distribution in levofloxacin resistant strains ranged from 1 to 8  $\mu$ g/mL.

**Conclusions:** Resistance to clarithromycin and levofloxacin in *H. pylori* increased significantly in Colombia. The continuous surveillance of macrolide and quinolone resistance among *H. pylori* is important in this country.

Abstract no.: P07.05

## A STUDY TO EXPLORE HP ANTIBIOTIC RESISTANCE AND EFFICACY OF ERADICATION THERAPY IN CHINA (MULTI-CENTER, NATION-WIDE, RANDOMIZED, CONTROL STUDY)

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**Aims:** To explore Hp antibiotic resistance and efficacy of eradication therapy in patients with RE, chronic superficial gastritis (CG), FD and gastric injuries secondary to NSAID.

**Methods:** Patients were recruited to this study from 2008–5 to 2010–12. Hp strain culture and drug resistance E test were performed if RUT positive. The patients with positive Hp culture were suggested to eradicate Hp with randomization to EAC or sequential (EACM) regimens for 10 day. Gastroscopy and UBT were repeated two months later.

**Results:** In 562 Hp strains, the resistance rates (%) were Amoxicillin (AC 4.9), Clarithromycin (CH 37.8), Metronidazole (MZ 69.7), Levofloxacin (LE 36.4), Tetracycline (TC 2.3), Azithromycin (AZ 49.8), Moxifloxacin (MX 38.2), Gentamycin (GM 2.5) and Rifampicin (RI 6.6). The 0–9 multi-resistance rates were 12.2, 22.8, 15.0, 21.0, 10.4, 14.4, 2.8, 1.2, 0 and 0.2, which was mainly caused by MZ, AZ, CH, LE and MX. Significant differences (SD) were found in resistance rate of CH and TC among RE, CG, FD and NSAID; CH between success and fail in EAC regimen; CH and MZ between success and fail in sequential regimen. No SD was found in Hp eradication rates between EAC and sequential regimen in RE, CG, FD and NSAID.

**Conclusions:** The rates of Hp antibiotic resistance and multi-resistance were high, which was mainly related to antibiotics of nitroimidazoles, macrolides and quinolones. Hp resistance rates were different in various diseases. Hp eradication rates were similar in EAC and sequential regimen.

Abstract no.: P07.06

#### EFFECTS OF EFFLUX PUMP SYSTEM ON THE MULTIPLE ANTIBIOTIC RESISTANCE CHARACTERISTICS OF *H. PYLORI* STRAINS

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**Aim:** It was reported efflux pump system might play an important role in development of multiple antibiotics resistance characteristics of *H. pylori* strains (multidrug resistance, MDR). The purpose of this study is to observe the effects of efflux pump on the development of dual or multiple antibiotics resistance in clinical isolated *H. pylori* strains.

**Materials and Methods:** Eight clinical isolated *H. pylori* strains which were resistant to dual or multiple antibiotics were selected, including two strains with no QRDR mutations of *gyrA* gene in dual antibiotics resistance strains. The *hefA*, *hefB* and *hefC* mRNA expression in three efflux pumps were detected by Real-time Quantitative Polymerase chain Reaction (Real-time PCR). *H. pylori* strain 26695 was treated as control.

**Results:** Compared with the standard strains, the over-expression of *hefC* gene were detected in two of the eight selected antibiotics resistant strains. The over-expression of *hefB* gene was detected in one strain, the over-expression of *hefC* and *hefB* were detected in two strains. Over-expression of *hefA* was not found in any strain.

**Conclusions:** There were not the same in the expression of efflux pump system genes in different *H. pylori* antibiotics resistant strains. Further study on the role of efflux pump system in development of antibiotics resistance characteristics of *H. pylori* strains should be performed.

Abstract no.: P07.07

#### PRIMARY ANTIBIOTIC RESISTANCE AND *HELICOBACTER PYLORI* VIRULENCE FACTORS – IS THERE AN ASSOCIATION?

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Our aim was to study any potential association between the presence of *H. pylori* virulence factors and primary *H. pylori* antibiotic resistance to amoxicillin (AMO), clarithromycin (CLA), tetracycline (TET), metronidazole (MET) and levofloxacin (LEV) in Greek adult and children patients. A total of 133 clinical *H. pylori* strains were isolated from 69 adults (age 53.8 ± 14) and 64 children (age 10.7 ± 2.8) following gastroscopy. None of the patients had received any previous eradication therapy or PPIs. Antibiotic susceptibility was determined by E-test. MIC breakpoints adopted were >0.5 mg/L for AMO, CLA and LEV, >1 mg/L for TET and >8 mg/L for MET (3rd European Multicentre Study on *H. pylori* antibiotic susceptibility). *VacA* genotypes (*s*, *i* and *m*) as well as *cagA* presence and EPIYA status were determined by polymerase chain reaction. Eighty eight (66.2%) strains exhibited resistance to one or more antimicrobial agents, mainly to CLA (adults: 18/69, 26.1%; children 29/64, 45.3%), MET (adults: 28/69, 40.6%;

children 18/64, 28.1%) and LEV (adults: 11/69, 15.9%; children 1/64, 0.2%). No association was detected between *vacA* genotypes and antibiotic resistance. However, a significant association between *cagA*-negative status and the presence of antibiotic resistance to at least one antimicrobial agent was observed within our population ( $p = .0219$ , OR: 1.346, 95% CI: 1.07–1.69). This was evident in the adult ( $p = .0297$ , OR: 1.469, 95% CI: 1.09–1.97) rather than the children group ( $p = .2907$ ). High primary resistance rates to clarithromycin and metronidazole were observed. Absence of *cagA* gene might be a risk factor in the development of antimicrobial resistance.

Abstract no.: P07.08

#### MONITORING OF RESISTANCE TO ANTIBIOTICS OF *HELICOBACTER PYLORI* STRAINS IN JIANGXI PROVINCE OF CHINA

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**Aim:** To monitor the resistance to metronidazole, clarithromycin, Levofloxacin, furazolidone and amoxicillin of *H. pylori* strains in Jiangxi Province of China.

**Method:** The tissue samples were collected by gastroscopy biopsy from the outpatients and inpatients with gastric diseases. 121 tissue samples cultured in microaerobic condition were identified as typical *H. pylori* strains by biochemical and slice checking methods. E-test method was used to measure the minimum inhibitory concentration (MIC) of these identified *H. pylori* strains resistant to metronidazole, clarithromycin, Levofloxacin and amoxicillin. Drug sensitivity tests of furazolidone was performed by means of Kirby-Bane.

**Result:** Among 121 *H. pylori* strains, the resistance rate to metronidazole was 72.70% (88/121), and the MIC ranged from 0.016 mg/L to beyond 256 mg/L; to clarithromycin, 14.88% (18/121), MIC ranged from 0.016 mg/L to beyond 256 mg/L; to Levofloxacin, 14.05% (17/121), MIC from 0.02 mg/L to beyond 256 mg/L; amoxicillin 0.83% (1/121), MIC from 0.016 mg/L to 2 mg/L; furazolidone 0% (0/121).

**Conclusion:** In Jiangxi Province, the resistance rate of *H. pylori* to metronidazole was the highest (72.70%), and the second was to clarithromycin and Levofloxacin (14.88%, 14.05% respectively). It is interesting that the *H. pylori* strain resistant to amoxicillin appeared. There have been no *H. pylori* strains resistant to furazolidone up to now.

Abstract no.: P07.09

#### DRUG RESISTANCE OF *HELICOBACTER PYLORI* TO ANTIBIOTIC AMONG CHRONIC GASTRITIS AND DUODENAL ULCER

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**Objective:** To evaluate the sensitivity and resistance of *Helicobacter pylori* (*H. pylori*) strains isolated from chronic gastritis and duodenal ulcer patients to metronidazole, amoxicillin, clarithromycin in vitro. In order to give suggestion for drug select in clinic.

**Methods:** Biopsy specimens were taken from the patients merely diagnosed chronic gastritis or duodenal ulcer without eradication of *H. pylori* before. E-test method was used to measure the minimum inhibitory concentration (MIC) of these identified *H. pylori* strains resistant to metronidazole, clarithromycin and amoxicillin.

**Result:** Thirty-three and 73 samples identified as typical *H. pylori* strains were obtained from chronic gastritis and duodenal ulcer patients respectively. The resistance rate of *H. pylori* to metronidazole was 78.8% (26/33) and 21.9% (16/73), to amoxicillin was 12.1% (4/33) and 0% (0/73), to clarithromycin was 54.5% (18/33) and 17.8% (13/73). There was significance of difference between the two groups ( $p < .05$ ).

**Conclusions:** The resistance rate of *H. pylori* strains isolated from chronic gastritis is high than those from duodenal ulcer to metronidazole, clarithromycin and amoxicillin. This maybe one of the reason that eradication rate of *H. pylori* among duodenal ulcer is high than chronic gastritis. Eradication of *H. pylori* depending on drug sensitivity tests is the optimal treatment selection in clinic.

Abstract no.: P07.10

**HELICOBACTER PYLORI IN A SOUTH-EUROPEAN COUNTRY – PRIMARY AND SECONDARY RESISTANCES TO ANTIMICROBIALS (FIRST RESULTS)**J. Romãozinho,\*<sup>†</sup> N. Almeida,\* M. M. Donato,<sup>†</sup> C. Luxo,<sup>‡</sup> O. Cardoso,<sup>‡</sup> M. A. Cipriano,\* C. Marinho,\* R. Figueiredo<sup>†</sup> and C. Sofia\*<sup>†</sup>\*Coimbra University Hospital, Coimbra, Portugal; <sup>†</sup>Gastroenterology Center, Faculty of Medicine, Coimbra University, Coimbra, Portugal; <sup>‡</sup>Laboratory of Microbiology, Faculty of Pharmacy, Coimbra University, Coimbra, Portugal**Aims:** To determine the prevalence and mechanisms of primary and secondary resistance of *Helicobacter pylori* (Hp) to antimicrobial agents in a South-European country.**Patients and Methods:** Prospective study involving anemic/dyspeptic adult patients with positive 13C Urea Breath Test (UBT), which were divided in two groups: A-no previous Hp treatment (primary resistance); B-previous, failed, Hp treatment (secondary resistance). All patients were submitted to upper digestive endoscopy with biopsies for isolation of Hp. Genotyping and antibiotic susceptibility were determined. Patients received standard treatment protocol (Group A-Pantoprazol + Amoxicillin + Clarithromycin, 14 days; Group B-Pantoprazol + Amoxicillin + Levofloxacin, 10 days). Hp eradication was assessed with UBT after 8–12 weeks. Statistical analysis was performed with SPSS v17.0.**Results:** Ninety eight patients (Male/Female-28/70; mean age-42 ± 14 years; Groups A/B-59/39) completed the protocol. Eradication was successful in 62.2% (Group A-71.2%; Group B-48.7%;  $p = .025$ ). Significant differences ( $p < .05$ ) between both groups (Group A-Group B) for: tobacco use (11.9%–28.2%), alcohol use (22%–41%), 23S rRNA gene mutation A2143G (15.3%–79.5%) and genotype VacA s1b (39%–17.9%). All Hp isolates were susceptible to tetracycline and amoxicillin but 49% were resistant to clarithromycin (22%–89.7%;  $p < .0001$ ), 42.9% to metronidazole (30.5%–61.5%;  $p = .002$ ) and 29.6% to levofloxacin (25.4%–35.9%; ns).**Conclusions:** High prevalence of primary and secondary resistance of Hp to clarithromycin, metronidazole and levofloxacin were observed in our country. Rates of eradication for empirical treatments were lower than the usually accepted. Suggestion that 23S rRNA gene mutation A2143G, tobacco and alcohol use are associated with failure of empiric initial treatments; on the contrary genotype VacA s1b can determine a more favorable outcome.

Abstract no.: P07.11

**PREVALENCE OF A2143G, A2142G AND T2717C MUTATIONS OF H. PYLORI-23S RRNA IN KAZAN (RUSSIA)**E. R. Abuzarova,\* R. A. Abdulkhakov,<sup>†</sup> V. M. Chernov,\* O. A. Chernova\* and S. R. Abdulkhakov<sup>†</sup>\*Kazan Institute of Biochemistry and Biophysics of Russian Academy of Sciences, Kazan, Russia; <sup>†</sup>Kazan State Medical University, Kazan, Russia**Introduction:** In *Helicobacter pylori* (*H. pylori*) infection resistance to clarythromycin is mostly due to the presence of A2143G, A2142G and T2717C point mutations of the 23S rRNA gene. The aim of our work was to investigate the prevalence of clarythromycin-related mutations of *H. pylori* strains among patients with gastroduodenal diseases in Kazan (Russia).**Materials and Methods:** Gastric biopsies obtained from 86 patients with peptic ulcer disease, chronic gastritis and GERD were examined. *H. pylori* was revealed by cytology, rapid urease test and ureC PCR analysis (“Lytech”, Russia). *H. pylori*-positive biopsies were taken for further evaluation. To detect A2142G, A2143G and T2717C mutations of the 23S rRNA gene MboII-, Bso3II (BsaI)- and AspLE (HhaI)-restriction PCR-RFLP assays were conducted (“SibEnzim”, Russia).**Results:** As a result of cytology, rapid urease tests and PCR analysis, *H. pylori* was revealed in 70 samples. A2143G mutations determining clarythromycin resistance were revealed in 8 out of 70 (11.4%) examined gastric biopsies. A2142G and T2717C mutations weren’t found in any biopsies.**Conclusions:** It was found that the prevalence of clarythromycin-resistant *H. pylori* strains is 11.4% (8/70) among patients with gastroduodenal pathology in Kazan (Russia). These numbers (11.4%) of clarythromycin resistance allow to start eradication with standard first-line therapy consisting of PPI, amoxicillin and clarythromycin.

Abstract no.: P07.12

**DETECTION OF A2142G AND A2143G MUTATIONS IN 23S RRNA GENE OF HELICOBACTER PYLORI IN BIOPSIES FROM MULTIPLE GASTRIC SITES**  
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In *Helicobacter pylori*, A2142G and A2143G mutations of 23SRNA gene confer clarithromycin resistance. Many studies reported coexistence of resistance andsensitive *H. pylori* strains in gastric mucosa. However, distribution of these mutations in various gastric sites i.e., antrum, corpus and fundus has not been extensively investigated. Biopsies from eighteen subjects were obtained to diagnose *H. pylori* infection using PCR amplification of either *ureC* gene or a 1.9 kb chromosomal DNA sequence or both. Samples positive for *H. pylori* were used for 23SRNA gene amplification followed by PCR-RFLP for mutations A2142G and A2143G, identified using *MboII* and *BsaI* endonucleases, respectively. PCR-RFLP on fourteen amplicons from antrum samples showed five A2142G and two A2143G. In fifteen amplicons from corpus samples nine A2142G and four A2143G were found. In fourteen fundus amplicons seven A2142G and three A2143G were present. Moreover, one subject showed presence of A2142G in both the corpus and fundus samples whereas no mutation from antrum samples was found. Only one subject showed A2142G in antrum samples but neither in corpus nor in fundus samples. Furthermore, one sample showed A2142G in corpus samples but no mutation in antrum and fundus samples. Similarly, one subject showed A2143G in both corpus and fundus samples but not in antrum samples. One subject showed A2143G in antrum samples while no mutations in corpus and fundus samples. These results show that distribution of mutations varies with gastric sites. Certain new PCR-RFLP patterns were also observed which had not been reported previously and need to be further investigated.

Abstract no.: P07.13

**ANTIBIOTIC RESISTANCE AND ERADICATION RATE OF HELICOBACTER PYLORI STRAINS ISOLATED IN KOREAN PATIENTS**B. S. Moon,\* B. An,<sup>†</sup> H. Kim,<sup>‡</sup> H. C. Lim,\* Y. C. Lee,\* G. Lee,<sup>§</sup> S. Kim,<sup>§</sup> M. Park<sup>§</sup> and J. B. Kim<sup>§</sup>\*Department of Internal Med., Yonsei University, Seoul, Korea; <sup>†</sup>Yongin Severance Hospital, Yonsei University, Seoul, Korea; <sup>‡</sup>Department of Lab. Med., Yonsei University, Seoul, Korea; <sup>§</sup>Department of Biomedical Lab. Science, Yonsei University, Wonju, Korea**Background:** The antibiotics commonly used for eradication of *Helicobacter pylori* (HP) infection were amoxicillin, clarithromycin, metronidazole, tetracycline, and quinolone. Recently, primary antibiotic resistance is increasing worldwide and it has been regarded main factors reducing the efficacy of HP eradication therapy. This study aimed to evaluate the prevalence of antibiotic resistance in Korea and the role of culture in assessing the antibiotic resistance in terms of therapeutic outcomes.**Methods:** From August 2005 to April 2011, 102 HP infected patients were enrolled. Specimens obtained from antrum and corpus by endoscopic biopsy were cultivated. Susceptibility to antibiotics was assessed using agar dilution method. Eradication rate of HP was assessed by urea breath test 4 weeks after 7-day standard triple therapy.**Results:** Among the prevalence of antibiotic resistance to each drug was as following: 10.8% (11/102) for amoxicillin, 8.8% (9/102) for clarithromycin, 45.1% (46/102) for metronidazole, 0% (0/102) for tetracycline, and 32.4% (33/102) for levofloxacin. MIC levels for five antibiotics were increased in 2009 and 2010 isolates than that of 2005 isolates. Among cultured specimens, 31 cases were used to assess the success rates of the eradication treatment. HP eradication was achieved in 77.4% (24/31). The infection was cured in 84.6% (22/26) with clarithromycin susceptible + amoxicillin susceptible strains. HP eradication was not achieved with clarithromycin resistant + amoxicillin susceptible strains in 0% (0/3). But HP eradication rate was achieved in 100% (2/2) in clarithromycin susceptible + amoxicillin resistant strains.**Conclusions:** This study shows that clarithromycin resistance markedly reduces HP eradication, but amoxicillin resistance seems to have no significant effect on HP eradication.

Abstract no.: P07.14

**SUSCEPTIBILITY OF METRONIDAZOLE-RESISTANT H. PYLORI ISOLATES TO ANTIFUNGAL DRUGS; KETOCONAZOLE AND FLUCONAZOLE**E. Siavoshi,\* S. Eyvazi,\* S. Nasser Moghaddam<sup>†</sup> and P. Saniee\*\*Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran; <sup>†</sup>Digestive Disease Research Institute, Shariati Hospital, Medical Sciences, University of Tehran, Tehran, Iran**Introduction:** Metronidazole-containing regimens for *H. pylori* infection treatment, limit effectiveness because of increasing resistance to this drug. Accordingly, search for alternative drugs is necessary. We investigated two antifungal drugs (ketoconazole and fluconazole) against metronidazole-resistant *H. pylori* isolates.**Methods:** Thirty-five *H. pylori* strains were isolated from gastric biopsies. For determination of susceptibility to metronidazole (turbidity: 2 MacFarland), serial dilutions of this antibiotic (32, 16, 8, 4 µg/mL) were inoculated in to blank discs

deposited on the surface of brucella agar containing 5% blood. The diameter of inhibition zones was recorded after 3 days microaerobic incubation. The 12 metronidazole-resistant strains were recruited to examine their susceptibility to ketoconazole and fluconazole, using serial dilutions of 64, 32, 16, 8 µg/mL according to the method mentioned above.

**Results:** Of 35 *H. pylori* isolates, 65.71% showed resistance to metronidazole. Ten out of 12 metronidazole-resistant strains (75%) were susceptible to ketoconazole (inhibition zones >17 mm within MIC 8 µg/mL), but only one was susceptible to fluconazole (MIC 32 µg/mL).

**Conclusion:** Ketoconazole and fluconazole are considered as effective antifungal drugs. They inhibit biosynthesis of fatty acids of fungal membranes. Since fatty acids, namely cholesteryl glucosides, have been found in the cell membrane of *H. pylori* species, it is tempting to speculate that imidazole antifungals such as ketoconazole might interfere with the biosynthesis of these fatty acids from cholesterol in this bacterium. In this study 75% and 2.58% metronidazole-resistant strains were susceptible to ketoconazole and fluconazole, respectively. Accordingly, ketoconazole can be considered as a likely substitute for metronidazole, especially for treatment of *H. pylori* strains which exhibit resistance to this drug.

Abstract no.: P07.15

#### THE PRIMARY RESISTANCE OF *H. PYLORI* STRAINS IN ADULTS

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Methods of culture and drug sensitivity of *H. pylori* strains are not used routinely in the diagnosis of *H. pylori* infection in adults.

**Aim:** To estimate the prevalence of antimicrobial resistance of *H. pylori* strains isolated from adult symptomatic patients with primary infection.

**Material and Methods:** Hundred and seventy-eight adults aged 19–89 years with dyspeptic symptoms suggesting gastroduodenal pathology were enrolled in the study. The study was performed in years 2009–2011. Fifty *H. pylori* strains were isolated from biopsy samples of examined patients. Antimicrobial susceptibility to six drugs (metronidazole, clarithromycin, levofloxacin, rifampin, tetracycline and amoxicillin) was tested by the E-test method.

**Results:** The prevalence of *H. pylori* infection among examined patients was 28% (n = 50). From 50 *H. pylori* strains isolated from adults, 24% (n = 12) showed resistance to CH, 68% (n = 34) to MZ and 8% (n = 4) to LE alone. The combined resistance to more than one drug was detected in 26% (n = 13) strains, whereas 20% (n = 10) of isolates were resistant to MZ and CH. Resistance to RB, TC as well as to AC was not observed.

**Conclusions:** It is necessary to continuously monitor *H. pylori* strain resistance in adult patients. The high incidence of primary infections with multi-drug resistance strains in adults is a cause for concern and indicates the necessity of microbiological tests before treatment.

Abstract no.: P07.16

#### INCREASED RESISTANCE NEEDS TO CHANGE THE FIRST LINE TREATMENT STRATEGY FOR ERADICATION OF *HELICOBACTER PYLORI* IN TURKEY

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The clarithromycin resistance has been increasing in Turkey, so the efficacy of the triple therapy has decreased and the success of the *H. pylori* eradication has dropped down under 60%. Therefore, we have planned this study aiming to investigate the resistance to levofloxacin, which has been suggested as a powerful alternative drug, and its effectiveness in treatment. Biopsy samples were taken from 116 patients during endoscopy between June 2010 and February 2011. Rapid Urease Tests were performed on all biopsy samples. Sensitivity tests for amoxicillin, clarithromycin and levofloxacin were conducted with the E-test method. The MIC values used for amoxicillin, clarithromycin, and levofloxacin were >0.5 µg/mL, ≥1 µg/mL, and >1 µg/mL, respectively. Cultures were grown from biopsy samples taken from 52 patients. Amoxicillin, levofloxacin and clarithromycin resistances, determined with the E-test method, were found to be 15.4%, 26.9% and 25.5% respectively. In this study, amoxicillin resistance was

found to be 15.4% (If the two samples with intermediate sensitivity are not considered to be “resistant”, amoxicillin resistance would be 11.5%). This resistance prevalence rate is much higher than the worldwide resistance prevalence of 0–3%. Clarithromycin resistance was found to be 26.9%, which is higher than the resistance upper limit of 20%, determined by EHPG for clarithromycin treatment. Levofloxacin resistance was found to be 25.5%. Hence, high success rates may not be possible with levofloxacin. So, we can conclude that a regimen with levofloxacin is not an ideal treatment in Turkey.

Abstract no.: P07.17

#### STABLE HIGH RATE OF PRIMARY CLARITHROMYCIN RESISTANCE OF *HELICOBACTER PYLORI* BETWEEN 2005 AND 2009 IN A CENTRAL DISTRICT OF BUDAPEST, HUNGARY

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**Background:** Antimicrobial susceptibility is a major determinant of eradication treatment outcome.

**Aims:** The purpose of this study was to assess of the prevalence of primary clarithromycin resistance in our district.

**Methods:** Between 2005 and 2009, 454 patients were randomly selected by the pathologists to determine clarithromycin resistance. *Helicobacter pylori* was assessed by the modified Giemsa stain and rapid urease test. Previous use of macrolides was excluded with a detailed history. The endoscopist was unaware of the patient's selection for susceptibility analysis. Clarithromycin resistance was determined by fluorescent *in-situ* hybridization.

**Results:** The patients selected were residents of Ferencváros, a central district of Budapest with an adult population of about 50,000. The annual distribution of primary clarithromycin resistance rates is given in Table 1.

**Discussion:** The prevalence of primary clarithromycin resistance in the period studied was stable high, with minor fluctuations from year to year. The results suggest a more cautious use of macrolides for *Helicobacter pylori* eradication in this region.

**Table 1** Prevalence of primary clarithromycin resistance between 2005 and 2009 in Ferencváros, Budapest

Year	No. of cases	C-sensitive (%)	C-resistant (total) (%)	Complete resistance (%)	Partial resistance (%)
2005	28	78.5	21.4	33.3	66.6
2006	241	82.8	16.1	43.6	60.0
2007	46	78.3	21.7	60.0	40.0
2008	57	66.2	22.8	46.1	53.8
2009	81	82.7	18.0	28.5	71.4
Total	454	81.9	18.0	42.7	57.3

Abstract no.: P07.18

#### ANTIMICROBIAL SUSCEPTIBILITY OF *HELICOBACTER PYLORI* ISOLATED FROM PAKISTAN

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**Objectives:** To determine the antimicrobial susceptibility profile of *Helicobacter pylori* isolated from biopsy samples of dyspeptic patients in Islamabad, Pakistan.

**Materials and Methods:** Forty nine *H. pylori* strains isolated from gastric biopsy samples of dyspeptic patients were used. Primary isolation of *H. pylori* was done using Columbia blood agar plates supplemented with Dent antibiotic supplement and 5% defibrinated sheep blood. Isolates were identified on the basis of colony morphology, Gram's staining, biochemical characteristics and PCR for 16S rRNA gene. Antimicrobial susceptibility profile of isolates was determined against metronidazole, clarithromycin, amoxicillin, tetracycline and levofloxacin by using disc diffusion and agar gel dilution methods.

**Results:** Isolated were identified as *H. pylori* being Gram negative spiral bacteria with positive oxidase, catalase and urease activity. All isolates were confirmed by PCR amplification of 138 bp fragment from 16S rRNA gene. Antimicrobial susceptibility profile reflected highest resistance rate against metronidazole (>90%), higher against clarithromycin (38 & 40%) and amoxicillin (40 & 49%), and comparatively lower against tetracycline (24 & 28%) and levofloxacin (20%) by disc diffusion and agar dilution methods, respectively.



**Conclusions:** *H. pylori* isolates from Pakistan are highly resistant to antibiotics used in eradication regimens. Slightly higher resistance was observed using agar dilution as compared to disc diffusion method.

Abstract no.: P07.19

#### HELICOBACTER PYLORI RESISTANCE TO CLARITROMYCIN IN DUODENAL ULCER PATIENTS IN SAINT-PETERSBURG, RUSSIA

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**Objective:** *Helicobacter pylori* is recognized as one of the basic causal factors in development of stomach ulcer and duodenal ulcer. Steady growth of *H. pylori* resistance to Metronidazole and/or Clarithromycin sharply reduces efficiency of eradication therapy from 80–90% to 30–60%.

**Aim:** To define frequency of occurrence resistance to Clarithromycin *H. pylori* strains at duodenal ulcer patients in St-Petersburg.

**Methods:** Under supervision there were 150 duodenal ulcer patients, associated with *H. pylori* infection. These patients were from five gastroenterological centers of St-Petersburg. By all patients gastroduodenoscopy diagnostics procedures with biopsies from stomach antrum were done for investigate of *H. pylori* genes by polymerase chain reaction: gene *ureC* (detector of *H. pylori* presence) and mutations of a gene *23S rRNA*, connected with resistance to Clarithromycin, such as A2144G, A2143G, A2143C, A2115C, A2142G, C2182T, T2717C.

**Results:** It has been established that at duodenal ulcer patients, associated with *H. pylori*, mutations of a gene *23S rRNA* were found out in 40% of cases with prevalence of A2143C mutation (66.7%).

**Conclusions:** According to the received data, in St-Petersburg use of eradication therapy schemes on a base of Clarithromycin is inexpedient. It is necessary to use antibiotics with the proved high sensitivity to them *H. pylori* or absence that, for example, Amoxicillin, Nitrofurans and bismuth medicine. Definition of *H. pylori* resistance to Clarithromycin before treatment is additional way of increase of eradication therapy efficiency.

Abstract no.: P07.20

#### COMPARISON OF SEQUENTIAL AND CLASSICAL THERAPIES FOR HELICOBACTER PYLORI ERADICATION IN CHILDREN AND INVESTIGATION OF CLARITHROMYCIN RESISTANCE

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**Objectives:** The aim of this study was to compare the efficacy of sequential and standard triple-drug regimen for *Helicobacter pylori* (*H. pylori*) eradication in children and to determine the clarithromycin resistance rate.

**Patients and Methods:** Children with *H. pylori* infection randomized to receive either standard regimen consisting of lansoprazole, amoxicillin and clarithromycin for 14 days or sequential regimen consisting of lansoprazole, amoxicillin for 7 days, followed by clarithromycin and metronidazole for the next 7 days. Clarithromycin susceptibility of *H. pylori* was assessed with fluorescence in-situ hybridization technique.

**Results:** Twenty-eight children in the standard therapy group and 16 children in the sequential therapy group between 4 and 17 years of age were included in the study. *Helicobacter pylori* eradication rate was higher in the sequential therapy group (93.7%), compared to the standard therapy group (46.4%) ( $p = .002$ ). There was no difference in adverse drug reactions and in compliance to the treatment between the groups. Primary clarithromycin resistance rate for *H. pylori* was found as 25.7% ( $n = 9$ ).

**Conclusion:** Sequential therapy can be suggested to improve the eradication rate in *H. pylori* eradication. Our country needs to reassess the effectiveness of standard triple therapy regimen for *H. pylori* eradication.

Abstract no.: P07.21

#### FREQUENCY OF SITE-SPECIFIC MUTATIONS IN THE 23S RRNA GENE OF HELICOBACTER PYLORI IN MOSCOW

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Efficacy of *Helicobacter pylori* (HP) eradication by triple treatment (PPI – clarithromycin – amoxicillin) depends on prevalence of clarithromycin resistance strains of HP. Previous studies have revealed that mutations responsible for alteration in the 23S rRNA gene are the mechanism of clarithromycin resistance.

**Aim:** To determine frequency of site-specific mutations in the 23S rRNA gene of HP in Moscow.

**Methods:** Nineteen patients with HP-associated chronic gastritis were investigated. Upper gastrointestinal endoscopy was performed. Morphology and urea test were used to detect presence of HP. A series of point mutations A2143G, A2143C or A2144G of the HP 23S rRNA gene were generated by sequential PCR method.

**Results:** Point mutations A2143G were founded in 3 (15.8%) patients. Two of them were treated with clarithromycin in former times. Therefore, in those (10.5%) cases we deal with secondary clarithromycin resistance. Primary clarithromycin resistance revealed in 5.3%.

**Conclusion:** Our preliminary data suggested about low prevalence of clarithromycin resistance strains of HP in Moscow. Nevertheless further research in this field is to be carried out.

Abstract no.: P07.22

#### ANTIBIOTIC RESISTANCE PROFILES IN HELICOBACTER PYLORI STRAINS ISOLATED IN SICILY (ITALY)

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*Helicobacter pylori*, relived as a major risk factor for the development of gastric adenocarcinoma, is responsible for different gastric mucosa-associated diseases that can be cured after eradication.

Although triple therapy with proton pump inhibitor, clarithromycin and amoxicillin or metronidazole is still recommended, it should only be used when the local prevalence of resistance of *H. pylori* to antibiotics is below a certain level. Resistance of microorganism to antibiotics, particularly to clarithromycin is the main reason for the failure of therapies for *H. pylori*-associated diseases. Since the initial eradication of *H. pylori* can no longer be achieved due to its increasing resistance to antibiotics, it is necessary to investigate the local resistance of *H. pylori* to antibiotics for choosing effective therapy.

In our study the resistance to seven commonly used antibiotics has been evaluated in one hundred *H. pylori* strains isolated in Sicily, from patients with gastric pathology.

In vitro the antibiotic susceptibilities were determined by Kirby-Bauer test and clarithromycin resistance was confirmed by molecular techniques.

Moreover, two loci, *vaca* and *cagA*, were analysed in the isolated strains in order to identify virulence-associated genotypes.

We found that 25% of the analysed strains were resistant to clarithromycin, 20% to metronidazole, 17% to amoxicillin, 5% to cephalothin and 1% to tetracycline. No *H. pylori* strain was resistant to gentamicin and chloramphenicol.

Our isolated strains showed a clarithromycin resistance percentage higher than that reported by European Guidelines (Maastricht III) as threshold (15–20%) for not to use it first line treatment.

Abstract no.: P07.23

#### SUSCEPTIBILITY OF H. PYLORI ISOLATES TO COMMONLY USED ANTI-BIOTICS AND FLUROQUINOLONES

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**Introduction:** Resistance of *H. pylori* to antibiotics has been reduced the efficiency of eradication therapy. The aim of present study was to assess the susceptibility of *H. pylori* isolates from dyspeptic patients to antibiotics commonly used in *H. pylori* treatment and the three antibiotics from fluoroquinolones family.

**Methods:** Thirty-five *H. pylori* strains were isolated. Suspensions of *H. pylori* isolates (turbidity:2 MacFarland) were inoculated on Brucella agar containing 5% blood. serial dilutions of metronidazole (32, 16, 8, 4 µg/mL), amoxicillin (2, 1, 0.5, 0.25 µg/mL), clarithromycin, tetracycline, furazolidone, ciprofloxacin, ofloxacin and Levofloxacin (4, 2, 1, 0.5 µg/mL) were inoculated in to blank disks deposited on the agar plates. Results were recorded after 3 days of incubation under microaerobic condition at 37 °C.

**Result:** The rate of resistance to metronidazole (MIC 8 µg/mL) was higher than other antibiotics (65.71%). Resistance to amoxicillin (MIC 1 µg/mL) clarithromycin (MIC 2 µg/mL), tetracycline and furazolidone (MIC 0.5 µg/mL) was 8.57%, 14.28%, 28.57%, 5.71%, respectively. Resistance to fluoroquinolones



nolones; ofloxacin, ciprofloxacin and levofloxacin (MIC 1 µg/mL) was observed in 37, 14%, 34.28% and 34.28% of isolates, respectively. A considerable number of metronidazole-resistant strains (25%) exhibited resistance to all of three fluoroquinolones. Six drug resistant (metronidazole, tetracyclin, furazolidone, ciprofloxacin, ofloxacin and levofloxacin) was also observed in 5.71% of isolates.

**Conclusion:** The resistance rate of *H. pylori* isolates to metronidazole is common in our country and resistance to other antibiotics also is increasing. Although, fluoroquinolones have been used as effective drugs in alternative therapy against *H. pylori* infection, high rate of resistance was observed among *H. pylori* isolates in our study. Accordingly they are not recommended for first-line therapy in Iran.

# P08 Inflammation and Host Response

Abstract no.: P08.01

## POLYMORPHISMS OF SIGNALING RECEPTORS GENES OF *H. PYLORI* AND GASTRIC CANCER RISK IN THE EPIC-EURGAST STUDY

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*H. pylori* is the most relevant causal factor for the noncardia localization of gastric cancer (GC). LPS of *H. pylori* is recognized by TLR4 and CD14 cell surface host proteins of the immune system, while NOD2 participates in signal transduction to activate NFκB1 for transcription of inflammatory cytokines. SNPs in these genes have associated with GC in different populations. As part of a wider study using the Illumina GoldenGate technology we genotyped 32 tagging (selected from HapMap information for Caucasians) and functional SNPs of these genes in 365 gastric adenocarcinomas and 1284 matched controls from the EPIC cohort, carried-out in ten European countries. Association analysis by unconditional logistic regression for the whole sample, and stratified by histological and anatomical subtypes was performed.

One SNP in *CD14*, two in *NOD2*, one in *TLR4* and two in *NFKB1* were found significantly associated with noncardia GC, and for some SNPs the association was significant only in CagA positive individuals. Three SNPs in *CD14* and *NOD2* were significantly associated with the intestinal subtype of GC. One of the *NOD2* SNPs and five SNPs in *NFKB1* were associated with diffuse type. After Bonferroni correction, significance was maintained for two SNPs: one in *NFKB1*, associated with the diffuse-type GC (OR 0.44; 95% CI 0.25–0.77,  $p = .0015$ ; recessive model) and one in *NOD2*, associated with the noncardias localization (OR 0.62; 95% CI 0.47–0.82,  $p = .0005$ ; log-additive model). We conclude that SNPs in genes involved in *H. pylori* recognition and signaling pathways are associated with GC in the EPIC cohort.

Abstract no.: P08.02

## PD1 EXPRESSION IN REGULATORY T CELLS OF *HELICOBACTER PYLORI* INFECTED PATIENTS

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**Introduction:** Regulatory T cells (Treg; CD4+CD25++FoxP3+) play a fundamental role in balance between inflammation and immune tolerance and are identified as a factor that contributes to bacterial persistence and to infection chronicity. Treg can be subclassified based on the expression of programmed death receptor 1 (PD1), a negative co-stimulatory molecule of immune system.

**Aim:** To evaluate Treg cells respect to CD25 and PD1 expression in *H. pylori* (+) and (-) biopsies from patients suffering gastritis.

**Methods:** *H. pylori* infection was diagnosed with histological techniques, rapid urease test and confirmed by RT-PCR (ureC gen). Twelve cases of *H. pylori* (+)-gastritis and four of gastritis where *H. pylori* was not detected were studied. Lymphocytes were isolated by incubation with collagenase-DNase solution at 37 °C for 2 hour. The following antibodies were used: anti-PD1-FITC, anti-CD4-PECy5 and anti-CD25-APC. For intracellular labeling with anti-Foxp3-PE, cells were previously permeabilized using Cytotfix/Cytoperm solution. After staining, flow cytometric analysis was performed. CD4(+) cells were classified in CD25+ and CD25++ (cut-off was determined with CD8(+) fluorescence).

**Results:** The percentage of CD4+CD25++Foxp3+ was elevated 5-fold in *H. pylori* (+) compared to *H. pylori* (-) samples; CD4+CD25++Foxp3+ number was similar in both cases. CD4+CD25++Foxp3+PD1+ cells number in biopsies *H. pylori* (+) were increased 1.7-fold compared to those found in biopsies *H. pylori* (-).

**Conclusions:** In gastric mucosa, a marked increase of Treg expressing PD1 is associated with *H. pylori* infection. The use of antibodies anti-PD1 inhibiting only such cells subpopulation should be investigated as a potential new therapy to reduce gastric inflammation associated with *H. pylori* infection.

Abstract no.: P08.03

## IN VIVO ANALYSIS OF *HELICOBACTER PYLORI* CAGC AND CAGL DELETION MUTANTS IN THE MONGOLIAN GERBIL

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CagC and CagL are proteins in the *Helicobacter pylori* type IV secretion system. To evaluate their role in *H. pylori* induced pathology *cagC* and *cagL* mutants were constructed in the gerbil-adapted *cagPAI+* strain 36.9. Mongolian gerbils were inoculated with either 36.9, 36.9Δ*cagC*, or 36.9Δ*cagL* of similar passage number. Gerbils were killed at 30 weeks to investigate gastric pathology, epithelial cell proliferation (Ki67 immunolabelling), bacterial density (Immunohistochemistry), cytokine transcripts (qRT-PCR) and to recover output strains. Output 36.9 strains were tested for their ability to induce IL-8 transcription in gastric epithelial cells (IL-8 reporter assay).

Seven of nineteen gerbils (37%) were colonized by 36.9, and 14 of 20 gerbils (70%) by 36.9Δ*cagC*. None of 19 animals inoculated by 36.9Δ*cagL* were colonized. There was no significant difference in density of 36.9 and 36.9Δ*cagC* colonization. Chronic inflammation, gastric *IFN-γ* and *TNF-α* transcripts were similarly increased in 36.9 and 36.9Δ*cagC* infected gerbils relative to controls. Corpus pathology was greater in 36.9Δ*cagC* than 36.9 infected gerbils. Additionally, 36.9Δ*cagC* infected gerbils had significantly increased epithelial cells proliferation ( $p < .03$ ) in both antrum and corpus compared to 36.9 infected gerbils. Thirty week 36.9 output strains had a marked decreased ability to induce IL-8 transcription in L5F11 cells in vitro compared to 36.9 input strain, indicating a loss of *cagPAI* function in vivo. These studies demonstrate in vivo loss of *cagPAI* function in a third generation gerbil-passaged strain. Enhanced epithelial cell proliferative responses induced by 36.9Δ*cagC* compared to 36.9 in the absence of differences in inflammation requires further investigation.

Abstract no.: P08.04

## ROLE OF GASTRIC EPITHELIAL CELL IN THE IMMUNE RESPONSE ACTIVATION DURING *HELICOBACTER PYLORI* INFECTION

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**Introduction:** *H. pylori* infection triggers mechanisms of innate and adaptive immunity requiring antigen-presenting cells (APC) to express co-stimulatory molecules that activate specific T lymphocytes. However, the infection persists for throughout the life of the host, suggesting that there is some mechanism that switches off immune response.

**Aims:** To assess if *H. pylori* causes overexpression of co-stimulatory and co-inhibitory signals of immune system in epithelial cells, and its relationship with density and bacterial genotype.

**Methods:** AGS epithelial cells were coinfectd (24 hour) with three *H. pylori* strains:(HP1):cagA (-); (HP3):urease (-);and (HP4):cagA (+), to different densities. From healthy volunteers, lymphocytes were isolated and incubated (2 hour) in a 4 : 1 (lymphocytes:AGS) ratio with the cocultives' supernatants. We evaluated by Flow Cytometry: -in AGS: HLA-DR: marker of HLA-II molecules expression on non-professional APC; ICAM-1: costimulatory molecule; PDL-1: co-inhibitory molecule; -in T lymphocytes: CD11b molecule whose co-receptor is ICAM-1.

**Results:** HLA-DR (control =  $12.5 \pm 4.9$  and (HP10<sup>8</sup>) =  $20.2 \pm 7.5^*$ ) and PD-L1 (control =  $12.0 \pm 5.1$  and (HP2x10<sup>8</sup>) =  $22.3 \pm 9.5^*$ ) (mean values of all *H. pylori* strains) were infection density-dependent, but strain-independent. ICAM-1 and CD11b are both dose and strain-dependent. T lymphocytes incubated with HP4(cagA+), showed the highest values for CD11b (control =  $219.3 \pm 54.5$  and (HP410<sup>8</sup>) =  $287.1 \pm 67.2^*$ ) and for ICAM-1 (control =  $407.8 \pm 207.5$  and (HP410<sup>8</sup>) =  $711.2 \pm 218.1^*$ ).

**Conclusions:** Our data suggest that gastric epithelial cells may acquire features of APC and therefore, capacity to deliver antigen-specific co-stimulatory signals to T lymphocytes. However, simultaneously, they overexpress negative co-stimulatory molecules that would be undermining the effectiveness of immune system to eliminate bacteria. These molecules could be targeted for the design of future strategies to eradicate *H. pylori*.

Abstract no.: P08.05

**INFLAMMATORY RESPONSE OF PRIMARY HUMAN GASTRIC EPITHELIAL CELLS TO *HELICOBACTER PYLORI***P. Mustapha, C. Bodet, J. Cremniter, M. Garnier, J. Lecron and C. Burucoa  
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*Helicobacter pylori* infection systematically causes a chronic gastric inflammation that can persist asymptotically or evolve towards more severe gastroduodenal pathologies such as ulcer, MALT lymphoma or gastric cancer. Many inflammatory mediators such as cytokines and chemokines are involved in this inflammatory response whose diversity likely reflects complex interactions between bacterial virulence factors and host genetic polymorphisms. An experimental protocol for isolating and culturing human primary gastric epithelial cells was established using pieces of stomach from patients who underwent gastric sleeve surgery. These cells were stimulated with HP B128 and HP B128 ΔcagM strains of *Helicobacter pylori* and the induction of inflammatory mediators was analyzed by RT-PCR and ELISA assays. The production of inflammatory mediators was bacterial dose-dependent but independent of the presence of the cagM bacterial virulence factor. Using a relevant cellular model, this study may provide a better understanding of the host-bacterial relationship involved in the modulation of the immune inflammatory response induced by *Helicobacter pylori*.

Abstract no.: P08.06

**PROTEASE-ACTIVATED RECEPTOR-2 (PAR2) IN HUMAN GASTRIC MUCOSA AS MEDIATOR OF PROINFLAMMATORY EFFECTS IN *H. PYLORI*-INFECTION**

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**Introduction:** Protease-activated receptors (PAR) are seven transmembrane receptors that are expressed throughout the gastrointestinal tract. In vitro experiments using gastric tumor cell lines, murine models and one clinical study provided evidence for a potential role of PAR2 in *H. pylori*-induced gastritis.

**Aim:** To investigate PAR-2 expression in *H. pylori*-infected patients and correlation with proinflammatory IL-8, IL-1β as well as histological changes of the mucosa. Furthermore, PAR2 expression was studied in context to mucosal amounts of secretory leukocyte protease inhibitor (SLPI), a putative regulator of PAR2.

**Methods:** Twenty-two *H. pylori*-infected patients and 72 *H. pylori*-negative subjects underwent upper GI endoscopy. In antrum-derived mucosal biopsies, PAR2, IL-1β, IL-8 and SLPI expression were analyzed by quantitative RT-PCR, and in part by ELISA and immunohistochemistry. Histopathological evaluation of gastritis was performed according to the updated Sydney classification.

**Results:** IL-8 gene expression was 5-fold increased in the mucosa of *H. pylori*-infected patients compared to non-infected ( $p < .0001$ ), whereas no differences for PAR2 and IL-1β mRNA amounts were observed between both groups. PAR2 gene expression correlated positively with transcript levels of IL-8, IL-1β as well as mucosal SLPI levels in *H. pylori*-infected patients ( $r: .47-0.84$ ;  $p < .0001$ ), whereas no correlation was found with the degree of gastritis.

**Conclusions:** PAR2 represents an additive pathway of IL-8 secretion and pro-inflammatory effects in *H. pylori*-induced gastritis. Reduced SLPI levels leading to higher serine protease activities in the mucosa of infected subjects might regulate PAR2 activation.

Abstract no.: P08.07

**THE POLYMORPHISM IN GENES AND PATHOMORPHOSIS OF THE MUCOUS MEMBRANE OF THE STOMACH AT *HELICOBACTER PYLORI*-ASSOCIATED GASTRITIS**

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**Aim of Investigation:** The purpose of the research is to estimate polymorphism genes (IL-1β, IL-1Ra, IL-8) with morphological changes the mucous membrane of the stomach at the *H. pylori*-associated gastritis.

The objects of the research were gastric biopsy materials from 66 patients with diagnosis *H. pylori*-associated chronic gastritis (CG) which were divided into two groups: the 1st group – patients with atrophy; the 2nd – without atrophy. All patients Aborigines Republics Khakassias (khakases).

**Methods:** endoscopy, gastric biopsy samples were investigated according to Sydney classification. Genomic DNA was typed for polymorphisms at position C +3953 T in the IL-1B gene using RFLP analysis (Tag I), -251 A/T IL-8 (Mfe I) and IL-1Ra VNTR. Analysis was performed by PCR and agarose gel electrophoresis.

**Results:** There was registered more activity of inflammation from patients without atrophy CG. The most widespread genotypes are TT +3953 IL-1β, R2R2 IL-1Ra and AA -251 IL-8 from patients CG with atrophy; CT +3953 IL-1β, R3R4 IL-1Ra and TT -251 IL-8 from patients CG without atrophy. Genotypes R2R2 IL-1Ra, AA -251 IL-8 and allele T +3953 IL-1β associated with atrophy and determines its degree.

Abstract no.: P08.08

**ASSOCIATION BETWEEN POLYMORPHISMS OF APE1 AND *HELICOBACTER PYLORI*-RELATED GASTRODUODENAL DISEASES IN THE CHINESE POPULATION**

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**Background and Aims:** *Helicobacter pylori* (*H. pylori*) infection is known to cause non-cardia gastric cancer and duodenal ulcer. But those with duodenal ulcers are associated with a decreased risk of developing gastric cancer. The host genetic factors may be relevant in the different clinical outcomes of *Helicobacter pylori*-infected individuals, and APE1 gene has been reported to be involved in pathogenesis of cancer and inflammation. So this study was to elucidate the risk of APE1 polymorphisms and *H. pylori*-related gastric cancer and duodenal ulcer.

**Methods:** In this study, the Asp148Glu and Ile64Val polymorphism in the APE1 gene was investigated in 282 patients with *H. pylori*-related gastroduodenal diseases. (126 non-cardia gastric cancer and 156 duodenal ulcer). Genotypes were determined by matrix assisted laser desorption ionization time of flight mass spectrometry.

**Results:** In the site of Asp148Glu in APE1 gene, the frequency of genotype of TT, TG, GG were 21%, 51%, 29% respectively in gastric cancer group, which were 36%, 45%, 19% in the duodenal ulcer group accordingly. There was a significant difference between the two groups ( $p = .013$ ). Patients carrying APE1-148 G showed an increased risk of gastric cancer compared with duodenal ulcer (OR = 2.154, 95% CI, 1.253–3.701,  $p = .005$ ). However, for the polymorphism in the site of APE1 Ile64Val, no significant difference was observed between two groups.

**Conclusion:** The Asp148Glu polymorphism in APE1 gene was a susceptible factor of *H. pylori*-related gastric cancer compared with *H. pylori*-related duodenal ulcer, but Ile64Val polymorphism was not.

Abstract no.: P08.09

***H. PYLORI* LPS – DRIVEN INHIBITION OF IFN-γ, IL-2 AND IL-10 PRODUCTION IN PERIPHERAL BLOOD LEUKOCYTE CULTURES**

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**Introduction:** *Helicobacter pylori* (*H. pylori*) is a pathogen that colonizes the gastric mucus layer of at least half of the world population, causing gastric inflammation, ulcers or cancers. The modulatory effects of *H. pylori* LPS, on the cytokine activity of peripheral blood mononuclear leukocytes (PBML), are still too little understood.

**Aim:** We evaluated the influence of *H. pylori* LPS on the PBML from *H. pylori* (+) and *H. pylori* (-) donors to secrete major pro-, and anti-inflammatory cytokines: IFN-γ, IL-2 and IL-10.

**Methods:** PBML from 44 donors classified as a Hp (+) or Hp (-) were incubated for 24 hour, in 24 well plates ( $5 \times 10^6$ /well) in the medium with or without *H. pylori* LPS or standard *E. coli* LPS, in a final concentration of 25 ng/mL. The level of cytokines: IFN-γ, IL-2 and IL-10 in the supernatants, was estimated using commercial immunoenzymatic assays (R&D Systems).

**Results:** The concentration of IFN-γ in the PBML cultures stimulated with *H. pylori* LPS was significantly lower than in the cultures of PBML unstimulated ((Hp+( $p = .00003$ ), Hp-( $p = .000006$ )) or treated with the LPS of *E. coli* (Hp+( $p = .0002$ ), Hp-( $p = .003$ )). Similarly, the IL-10 concentration was significantly lower in the PBML cultures stimulated with *H. pylori* LPS as compared to IL-10 secretion by unstimulated PBML (Hp+( $p = .0002$ ), Hp-( $p = .003$ )) or cultured in the presence of *E. coli* LPS (Hp+( $p = .0002$ ), Hp-( $p = .003$ )). The production of IL-2 was totally inhibited in the cultures with Hp LPS, whereas the concentration of this cytokine was higher in the PBML cultures stimulated with LPS of *E. coli* (Hp (+):270 pg/mL, Hp (-):230 pg/mL).

**Conclusions:** The Hp LPS may modulate negatively the cytokine secretion by the inflammatory cells and by this may influence the course of Hp infection. (grantNN401015136)

Abstract no.: P08.10

**APRIL, MACROPHAGES AND T-CELLS IN GASTRIC MALT LYMPHOMA**

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*Helicobacter pylori* (HP) infection represents a pre-neoplastic condition of the mucosa associated lymphoid tissue (MALT) which may evolve to a B cell lymphoma. While it is well established that the initial neoplastic proliferation of B cells is antigen-driven and dependent on the helper activity of HP-specific T cells, it needs to be elucidated which cytokine or soluble factor(s) promote B cell activation and lymphomagenesis. We report that gastric MALT lymphoma express high level of proliferation inducing ligand (APRIL), a crucial cytokine to sustain B cell proliferation and survival. APRIL production is induced in macrophages by *Helicobacter pylori* itself or by *H. pylori*-activated T helper cells. APRIL is expressed almost exclusively by gastric macrophages in the context of MALT lymphoma. Collectively our results represents the first evidence for the involvement of APRIL in gastric MALT lymphoma development.

Abstract no.: P08.11

**IMPROVEMENT IN PEPINOGENS LONG-TERM MONITORING AFTER ERADICATION OF *HELICOBACTER PYLORI***

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**Introduction:** *H. pylori* infection induces active inflammatory process and causes upregulation of serum pepsinogens (PG).

**Aim:** To evaluate the long term dynamic of serum pepsinogens after *H. pylori* eradication.

**Patients and Methods:** Altogether 77 *H. pylori*-positive patients (33 from Latvia, 44 from Lithuania) who underwent *H. pylori* eradication according to Mastricht guidelines. Patients with gastric cancer, peptic ulcer, having undergone gastric surgery or having received eradication therapy were excluded. Patients were evaluated for fasting serum PGI and PGII before *H. pylori* eradication and after 30 months.

PGI and PGII were determined by ELISA method (Biohit, Plc., Finland). Gastric atrophy was determined by histology. Biopsies were sampled and read according to the modified Sydney classification by two expert pathologists.

**Results:** Atrophy in gastric mucosa was detected in 32 (41.6%) patients.

**Conclusions:** The mean level of PGII was significantly lower 30 months after successful *H. pylori* eradication compared to the mean level of PGII before treatment. The correlation was more expressed in non-atrophic patient group. This indicates steady decrease of inflammation after cure of *H. pylori* infection and approves the use of PGII as a diagnostic tool in the verification of successful *H. pylori* eradication.

**Table 1** Mean values of PG I and PG II levels before and after *H. pylori* eradication

Groups of patients	PGI <sup>a</sup>	PGI <sup>b</sup>	p value	PGII <sup>a</sup>	PGII <sup>b</sup>	p value
Atrophy (n = 32), SD	62.89±/65.90	74.65±/82.49	p = .36	15.99±/14.73	9.52±/10.60	.17
No atrophy (n = 45), SD	93.11±/50.40	96.22±/62.79	p = .75	15.47±/7.69	8.32±/4.91	<.01
All patients (n = 77), SD	80.55±/58.88	87.25±/71.52	p = .53	15.69±/11.08	8.82±/7.75	<.01

<sup>a</sup>Before eradication; <sup>b</sup>after eradication.

Abstract no.: P08.12

**HISTOLOGICAL CHARACTERISTIC OF *H. PYLORI* INFECTED GASTRITIS IN NEPAL**

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**Background:** Atrophy and intestinal metaplasia of gastric mucosa caused by *H. pylori* is considered to be precancerous lesion, especially for intestinal type gastric cancer. In Nepal, gastric cancers are rare in spite of high prevalence of *H. pylori* infection.

**Methods:** Patients clinically necessary for gastroduodenal endoscopy at Tribhuvan University Teaching Hospital in Nepal were accessed in the period of May to December 2010. Patients who were taking PPIs or NSAIDs, or had a history of eradication were excluded. Eligible patients underwent endoscopy and three biopsy specimens (antrum, incisura, and corpus) were taken for the evaluation of histological scores (0–3) based on updated Sydney system. *H. pylori* infection was diagnosed by immunohistochemistry with anti-*H. pylori* antibody.

**Results:** Total of 92 patients was included. Patient's characteristics and median histological scores with IQR are shown as tables.

**Conclusion:** Activity and chronic inflammation were mild and atrophy in corpus and intestinal metaplasia were rarely developed among the patients with *H. pylori* in Nepal.

**Acknowledgement:** This work was supported by the Grant for National Center for Global Health and Medicine (21–108 and 22–202).

**Table 1** Patient's characteristics

	Hp positive (n = 52)	Hp negative (n = 40)	p-value
Median age (year-old)	46	52	.05
Gender (male: female)	30 : 22	20 : 20	N.S.
Peptic Ulcer (GU/DU)	17 (3/14)	5(2/3)	.05

**Table 2** Histological scores of activity and chronic inflammation

	Hp positive (n = 52)	Hp negative (n = 40)	p-value
<b>Activity</b>			
Antrum	1(1–1)	0(0–0)	.05
Incisura	1(1–1.5)	0(0–0)	.05
Corpus	1(0–1)	0(0–0)	.05
<b>Chronic inflammation</b>			
Antrum	1(1–1)	1(0–1)	.05
Incisura	1(1–2)	0(0–0.5)	.05
Corpus	1(0–1)	0(0–0)	.05

**Table 3** Histological scores of atrophy and intestinal metaplasia

	Hp positive (n = 52)	Hp negative (n = 40)	p-value
<b>Atrophy</b>			
Antrum	1(0–1)	0(0–1)	<.05
Incisura	1(0–1)	0(0–0)	<.05
Corpus	0(0–0)	0(0–0)	N.S.
<b>Intestinal metaplasia</b>			
antrum	0(0–0)	0(0–0)	N.S.
incisura	0(0–0)	0(0–0)	N.S.
corpus	0(0–0)	0(0–0)	N.S.



Abstract no.: P08.13

**THE INFLUENCE OF BLOCKING CD25 ON IMMUNE PATHOPOIESIS OF *H. PYLORI* INFECTION AND TLR4 SIGNAL PATHWAY AND IGA**Y. Xie,\* Y. F. Gong,\* N. J. Zhou,<sup>†</sup> W. Liu,\* X. J. Zhou,\* D. S. Liu\* and N. H. Lu,\*\*Digestive Disease Institute, the First Affiliated Hospital of Nanchang University, Nanchang, China; <sup>†</sup>Jiangxi Medical Science Institute, Nanchang, China**Objective:** To observe the influence of blocking CD25 on immune pathopoiesis of Hp infection and TLR4 signal.**Methods:** BALB/c mice, 10 as control and the other 20 as Hp infected model, were allocated into tow groups: non-pretreatment; Anti-CD25 antibody pretreatment. And mice were inoculated by Hp. Twelve weeks after inoculation, Hp, the expression of TLR4, MyD88, NF- $\kappa$ Bp65 in gastric mucosa and anti-HpIgA in saliva were determined.**Results:** 1, Hp colonized in mice infected with Hp was significantly higher than those in control ( $p < .01$ ), and in group with anti-CD25 antibody pretreatment were significantly lower than group without pretreatment ( $p < .05$ ). 2, Inflammatory degree in mice infected with Hp were significantly higher than those incontrol ( $p < .01$ ), and in group with anti-CD25 antibody pretreatment were significantly higher than group without pretreatment ( $p < .05$ ). 3, The expression of TLR4, MyD88 and NF- $\kappa$ Bp65 in mice infected with Hp were significantly higher than those in control ( $p < .05$ ); and in group with anti-CD25 antibody pretreatment were significantly higher than group without pretreatment ( $p < .05$ ). 4, The level of anti-HpIgA in saliva of mice infected with Hp were significantly higher than those in control ( $p < .01$ ); and in group with anti-CD25 antibody pretreatment were significantly higher than group without pretreatment ( $p < .05$ ).**Conclusion:** Blocking CD25 can reduce the Hp colonization density, exacerbate the inflammatory degree in mice infected with Hp, and can up-regulate TLR4, MyD88 expression, promote the NF- $\kappa$ B activation and secretion of anti-HpIgA, this could be the mechanism that it reduce the Hp colonization density of mice infected with Hp.

# P09 Extragastric and Hepatobiliary Diseases

Abstract no.: P09.01

## THE POSSIBLE ROLE OF ANTI-HSP60 ANTIBODIES IN THE CORONARY HEART DISEASE (CHD) DEVELOPMENT AND MAINTENANCE

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**Introduction:** Bacterial heat shock proteins (Hsp) may provoke pathologies in the host due to a generation of autoantibodies crossreacting with human Hsp homologues. The antibodies (Ab) to Hsp proteins of gastric pathogen *Helicobacter pylori* (Hp) are considered to play a role in the development of coronary heart disease (CHD).

**Aim:** We estimated the prevalence and the levels of serum IgG to human and bacterial Hsp60 proteins: standard Hsp 65 *Mycobacterium bovis* (MbHsp65), *H. pylori* HspB (HpHsbB) and human recombinant Hsp60 (rhHsp60).

**Methods:** The study group consisted of 58 healthy donors and 170 CHD patients. The enzyme linked immunosorbent assay (ELISA) was conducted with MbHsp65 and rhHsp60. The anti-HpHspB IgG were detected by commercial Western blot.

**Results:** The anti-rhHsp60 IgG were present in all analyzed sera, whereas the IgG to MbHsp65 and HpHsbB were detected more frequently in the CHD patients than in the healthy donors: 89% versus 65% and 86% versus 68%, respectively ( $p < .05$ ). Similarly, the levels of anti-MbHsp65 and anti-rhHsp60 IgG were higher in the CHD patients than in the healthy group,  $p < .05$ . Absorption of serum samples with inactivated *H. pylori* cells caused a decrease in antibody levels reacting in ELISA both with MbHsp65 and rhHsp60.

**Conclusions:** It is possible that in *H. pylori* infected CHD patients the *H. pylori* HspB may induce a production of crossreacting antibodies which might be engaged in the development of deleterious inflammatory response during atherogenesis. Grant no. NN 303 451 738.

Abstract no.: P09.02

## THE ASSOCIATION OF *HELICOBACTER PYLORI* INFECTION AND MICROALBUMINURIA

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**Backgrounds and Aims:** The role of *Helicobacter pylori* (*H. pylori*) infection in the pathogenesis of atherosclerosis has been identified. Microalbuminuria is known to be an early marker for renal and cardiovascular diseases. The aim of this study was to evaluate the effect of *H. pylori* infection on microalbuminuria, as an early marker of a diffuse microvascular injury.

**Methods:** Between December 2003 and February 2010, persons presenting for health checkups who examined both microalbuminuria and *H. pylori* status were included. Microalbuminuria was measured using spot urine microalbumin/creatinine ratio. Current *H. pylori* infection was determined by measuring IgG antibody.

**Results:** A total of 2392 patients (male, 72.1%; mean age, 55.2 year) were included. Old age, high body mass index, high serum levels of glucose and triglyceride and *H. pylori* infection had significant effects on microalbuminuria. Multivariate analysis showed that the relative risk ratio of *H. pylori* infection was 1.419 (95% confidence interval, 1.031–1.951,  $p = .032$ ). The percentage of positive *H. pylori* infection gradually increased in accordance with microalbuminuria quartiles: 23.6%, 24.7%, 25.3% and 26.4%, respectively ( $p = .026$ ), suggesting that *H. pylori* infection is positively associated with microalbuminuria.

**Conclusions:** *H. pylori* infection independently increased the risk of microalbuminuria. These findings suggest that *H. pylori* infection might be involved in the pathogenesis of early atherosclerosis.

Abstract no.: P09.03

## INFLUENCE OF *HELICOBACTER PYLORI* INFECTION ON THE LEVELS OF GHRELIN AND OBESTATIN IN HUMAN SEMEN

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**Background:** We recently observed that *H. pylori* (HP) infection may decrease the semen quality in men with reproductive disorders and that ghrelin and obestatin are present at high concentrations in human semen. Since these hormones are also involved in reproduction and are mainly produced in the stomach, we verified whether HP infection can influence the systemic and semen concentrations of ghrelin and obestatin in a group of 78 consecutive individuals.

**Methods:** We determined HP infection and CagA status by ELISA and Western blotting, the semen quality following WHO guidelines and ghrelin and obestatin levels by radioimmunoassay.

**Results:** Twenty-seven men (34.62%) were infected (HP+) and 11 infected men (40.74%) were seropositive for CagA (CagA+). Sperm motility in HP+CagA+ men was significantly poorer than that observed in CagA-men ( $p < .01$ ). Although obestatin levels were not influenced by HP infection, ghrelin levels in semen of HP+ men were significantly lower than those observed in uninfected subjects ( $p < .05$ ). CagA+ men showed values of semen ghrelin significantly higher than those measured in the semen of infected CagA-men ( $p < .01$ ). Ghrelin concentrations in semen of CagA-infected men were significantly decreased compared to those of uninfected subjects ( $p < .001$ ).

**Conclusions:** HP infection may influence the concentration of ghrelin in seminal plasma, presumably as a response to a negative effect of this infection on semen quality. These findings need being confirmed in further studies in which a greater number of individuals infected by CagA+HP strains are examined.

Abstract no.: P09.04

## RELATIONSHIP OF ACID SUPPRESSIVE AGENTS AND GUT HORMONES IN RAT

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Although the uses of acid suppressive agents are increased, the relationships between appetite hormones and long-term treatment of antacid drugs are not well known. The equilibrium of energy expenditure and the intake of food are achieved by the exquisite relationship between the peripheral tissue and the central nervous system. Leptin, Ghrelin, PYY, and Insulin are understood as the major regulators of this complexity. The purpose of this study was to determine the effects of acid suppressive agents on gut hormones and body weight. 50 Sprague-Dawley male rats were randomly subjected to five groups ( $N = 10$ , each) and were received drugs once daily: A) placebo, 1 mL/day of distilled water; B) Cimetidine 300 mg/kg; C) Famotidine 20 mg/kg; D) Omeprazole 30 mg/kg; E) Lansoprazole 30 mg/kg for four weeks. The serum concentrations of hormones (Leptin, Ghrelin, PYY, Adiponectin) and body weights were measured every two weeks. Body weights are not significantly different in all groups, except Famotidine group. Body weights of Famotidine group were significantly increased after 4 weeks. Mean serum Adiponectin concentrations in Cimetidine, Famotidine, and Lansoprazole group were significantly lower than control group. There were no significant differences in serum concentrations of Ghrelin, Leptin, and PYY in all groups. The results suggested that long-term use of antacids decreases the level of serum Adiponectin and this leads to the increase of appetite and body weight. However, the efficacy is not significant. Therefore further study on patients and with greater power in near future.

Abstract no.: P09.05

## ASSOCIATION OF *HELICOBACTER PYLORI* INFECTION AND GASTRIC MUCOSAL ATROPHY TO SERUM LEVEL OF ZINC AND COPPER IN HEALTHY ADULTS

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**Backgrounds:** Trace elements are essential components for wound healing and maintenance of immune systems. However, only a few previous studies have shown the association between *Helicobacter pylori* infection and trace elements in

developing countries. We examined the association between *H. pylori* infection and serum level of zinc and copper in healthy subjects with or without gastric mucosal atrophy.

**Methods:** Subjects were 330 males and 541 females aged 26–83 years old who attended mass survey. Serum level of zinc, copper, pepsinogens (PG), antibodies to *H. pylori*. *H. pylori* stool antigen test was also performed. *H. pylori* status was defined positive or negative when the results of both serology and stool antigen were concordant. Gastric mucosal atrophy was defined as PG I < 70 µg/L and PG I/II < 3.

**Results:** Serum level of zinc in *H. pylori*-infected subjects with gastric mucosal atrophy ( $973.7 \pm 153.5$  µg/L) was lower than that of non-infected subjects ( $1008.0 \pm 159.4$ ) ( $p < .01$ ). In subjects who were born in 1950s, serum level of copper in *H. pylori*-infected subjects without gastric mucosal atrophy was  $919.9 \pm 136.9$  µg/L, and it was lower than that of non-infected subjects ( $986.8 \pm 141.3$ ) ( $p < .05$ ). Serum level of copper was also lower in *H. pylori*-infected subjects with gastric mucosal atrophy ( $875.6 \pm 111.9$ ) comparing with non-infected subjects ( $955.5 \pm 141.6$ ) who were born in 1960s ( $p < .05$ ).

**Conclusion:** *H. pylori* infection and gastric mucosal atrophy may associate with lower serum zinc concentration. Lower level of serum copper was observed in *H. pylori* infected subjects among middle-aged subjects.

Abstract no.: P09.06

#### HELICOBACTER AND SMALL INTESTINAL BACTERIAL OVERGROWTH: CONSECUTIVE PLAYERS IN THE PATHOGENESIS OF IDIOPATHIC PARKINSONISM

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**Background:** Hypokinesia in idiopathic parkinsonism (IP) improved following *Helicobacter pylori* eradication, flexor-rigidity worsened (*Helicobacter* 2010;15:279–95), whereas with failed eradication hypokinesia worsened, rigidity was unchanged.

**Methods:** We survey outcome of all antimicrobial-interventions at a clinic, where parkinsonism was objectively-quantified; *Helicobacter* screened for (urea-breath-test (INFAI) and/or stool-antigen ELISA (DakoCytomation)); and serial 4h-lactulose-hydrogen-breath-tests (LHBT, using 25G lactulose) performed.

**Results:** Of 66 IP-probands, 11 were *Helicobacter*-positive, 25 had been previously (median 3.4 (interquartile range 2.8, 5.9) years). Seventy-seven percent were LHBT-positive (>20 ppm increment at two consecutive 15-minutes readings within 2 hour) once or more during surveillance (343 LHBT, over 1.8 (0.4, 3.5) years). Hydrogen-breath-test-positivity was associated inversely with *Helicobacter*-positivity (OR 0.20 (95% CI 0.04, 0.99),  $p < .05$  after adjustment for personal covariates). Peak-hydrogen (breath-hydrogen/time curves not bimodally-distributed) was reduced following a 1st ( $n = 42$ ), 2nd (26) and 3rd (11) antimicrobial-intervention (by 23 (11, 36), 27 (12, 42) & 31 (12, 50) ppm/year, all  $p = .001$ , adjusted for *Helicobacter*-status, time-lapse). In the 41 on stable-background, long- $t_{1/2}$  medication or untreated (298 objective-assessments), *Helicobacter*-eradication (in 7) was associated with improved stride-length (by 15 (10, 19) cm,  $p = .001$ , age, height adjusted), and failed-eradication (2) with deterioration (12 (3, 22) cm,  $p = .01$ ), other antimicrobial-intervention (67) having no effect. In contrast, anti-*Helicobacter*-treatment had no effect on flexor-rigidity, other antimicrobial-interventions increasing it (11 (1, 22) %,  $p = .03$ , time-since-diagnosis adjusted).

**Conclusion:** In IP, improvement in hypokinesia follows *Helicobacter*-eradication, but not antimicrobials given for other indications. *Helicobacter* protects against small-intestinal-bacterial-overgrowth. Increased rigidity following antimicrobials points to consequent alteration in intestinal microbiota as a player in pathogenesis.

Abstract no.: P09.07

#### CYSTIC FIBROSIS, HELICOBACTER PYLORI INFECTION AND GASTRODUODENAL ABNORMALITIES

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Cystic fibrosis (CF) is a common inherited fatal disease. Duodenal impaired bicarbonate secretion and unbuffered gastric acid are always described. However, the development of duodenal ulceration is considered an uncommon event ("CF paradox", Kaunitz & Akiba, 2001). There are scarce studies on HP infection in CF.

**Aim:** To evaluate the prevalence of HP infection and morphologic alterations on gastroduodenal mucosa in adult CF patients.

**Patients and Methods:** Thirty-two patients (53% female, mean age 29 years) were included. All patients performed serological test (Helicoblot 2.1, Genelabs, Singapore) and 13C-urea breath test (UBT) after withdrawal of oral and/or parenteral antibiotics and proton pump inhibitor for, at least 30 and 10 days, respectively. Gastroscopy with measurement of fasting gastric pH was performed in 20 patients (nine refused to perform the exam and it was contraindicated in three due to pulmonary insufficiency) and biopsies from corpus, antrum and duodenum in 18 patients (two had coagulation disorders).

**Results:** 19/32 (68%) patients showed HP infection, being active (histology or UBT) in seven (22%). Gastroscopy showed erosive esophagitis in 4/20 (20%), and duodenal ulcer scar in 2/20 (10%) patients. Mean fast pH was 1.89 (SD 0.51). Histology showed gastric metaplasia, mostly mild, in the duodenum of 12/18 (67%) patients and chronic gastritis in 6/18 (33%) patients.

**Conclusions:** Adult CF patients have 68% prevalence of HP infection and all the spectrum of HP-induced gastroduodenal abnormalities, including duodenal ulcer. Lifetime use of antibiotics reduces active HP infection and severity of histological and clinical manifestations. CF paradox may not exist.

Abstract no.: P09.08

#### CHARACTERISTICS OF ATROPHIC GASTRITIS IN PATIENTS WITH EROSIIVE REFLUX DISEASE (ERD) AND NON-EROSIVE REFLUX DISEASE (NERD)

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**Objectives:** Non-erosive reflux disease (NERD) and erosive reflux disease (ERD) have different characteristics, including the prevalence of *Helicobacter pylori* (*H. pylori*) infection. The aim was to investigate relations between atrophic gastritis and gastroesophageal reflux disease.

**Methods:** A total of 97 patients who underwent esophagogastroduodenoscopy were enrolled and grouped as NERD, ERD or control. We compared severity of atrophic gastritis and biomarkers such as PG I (pepsinogen I), PG II, gastrin-17 and total ghrelin. Biopsies were also performed for the determination of *H. pylori* infection and measurement of gastric ghrelin mRNA.

**Results:** *H. pylori* infection rate was lower in ERD group than NERD, but there was no statistical difference. In patients with atrophy, PG I/II ratio was low and gastric ghrelin mRNA was increased. In patients with histological moderate or marked corpus atrophy, PG I/II ratio and serum ghrelin were low. Endoscopic and histological atrophy was milder in ERD group than NERD. There were no significant differences between two groups in PG I, PG II, PG I/II ratio and gastrin-17. Total ghrelin level was low in ERD, but there was no difference after adjusting gender. There was no difference between two groups in gastric ghrelin mRNA.

**Conclusion:** Gastric mucosal atrophy is associated with low PG I/II ratio and high gastric ghrelin mRNA. And, corpus atrophy is associated with low serum ghrelin. Endoscopic atrophy is milder in ERD group than NERD, however there are no differences in biomarkers such as PG I, PG II, PG I/II ratio, and ghrelin.

Abstract no.: P09.09

#### RESIDUAL DENTAL NUMBER IS NOT ASSOCIATED WITH HELICOBACTER PYLORI INFECTION AND THE DEGREE OF ATROPHIC GASTRITIS IN MIDDLE-AGED AND ELDERLY JAPANESE SUBJECTS

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**Background and Aim:** The association between *H. pylori* infection and residual dental number has been examined in some Western populations. Gastric acid reflux has also been associated with dental erosions. In Japan, prevalence of *H. pylori* infection is higher and severe atrophic gastritis, which reduces gastric acidity, is frequently seen in *H. pylori*-infected elderly subjects. We examined whether both *H. pylori* infection and the degree of atrophic gastritis associate with residual dental number in healthy elderly subjects.

**Methods:** Subjects were 236 males and 412 females aged over 50 years old who attended mass survey. We measured both *H. pylori* stool antigen and serum anti-*H. pylori* IgG antibodies. *H. pylori* status was defined as positive or negative when the results of both tests were concordant. We counted residual dental number, and measured serum level of pepsinogen (PG) I and II. Atrophic gastritis was defined as PGI <70 ng/mL and PGI/II <3, and severe atrophy was PGI <50 and PGI/II <2.0.

**Results:** Positivity of *H. pylori* infection was 66.9%. In *H. pylori* infected subjects, prevalence of atrophic gastritis and severe atrophic gastritis was 70.7% and 49.1%, respectively. The residual dental number was not significantly different between *H. pylori*-positive and negative patients in any age groups and gender. Degree of atrophic gastritis was not associated with residual dental number in *H. pylori* infected subjects.

**Conclusions:** *H. pylori* infection and the development of gastric mucosal atrophy would not be associated with residual dental number in elderly Japanese subjects.

Abstract no.: P09.10

#### ERADICATION OF *HELICOBACTER PYLORI* DID NOT HAVE SIGNIFICANT INFLUENCE ON SERUM LIPID LEVEL IN JAPANESE HEALTHY ADULTS

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**Introduction:** Infection of *H. pylori* is associated with serum pectin levels and increase of body weight is often seen after eradication. The aim of this study was to investigate whether eradication of *H. pylori* modulates serum lipid level and BMI in healthy subjects with high incidence of atrophic gastritis.

**Methods:** Four hundred and seven healthy adults (age 40<) who received mass survey in both April 2005 and May 2009 were studied. In the studied subjects, 69.0% were defined to have atrophic gastritis by pepsinogen I and II. *H. pylori* stool antigen, IgG antibody to *H. pylori* and level of total cholesterol (TC), HDL-C, triglyceride (TG) were measured. Subjects were defined as infected or non-infected if both tests showed concordant results. Subjects using anti-hyperlipidemia agents were excluded. In 2005, 224 subjects were defined as infected and *H. pylori* was eradicated in 68 of the subjects who agreed eradication therapy.

**Results:** In *H. pylori* eradicated male, level of TC was 198.4 ± 38.0 mg/dL in 2005 and 189.1 ± 36.7 in 2009 ( $p = .09$ ) while it was 195.0 ± 26.4 and 194.7 ± 29.5 in 2005 and 2009, respectively in male who had persistent infection (NS). In *H. pylori* eradicated female, level of TG tended to increase 74.1 ± 31.3 to 84.4 ± 36.1 mg/dL ( $p = .08$ ). No significant difference was seen in HDL and BMI between 2005 and 2009 in both *H. pylori* eradicated and non-eradicated male subjects.

**Conclusion:** Although level of TC and TG was changed slightly, no remarkable influence of *H. pylori* eradication was found on serum lipid level and BMI in this series of subjects.

Abstract no.: P09.11

#### THE EFFECT OF *HELICOBACTER PYLORI* INFECTION ON THE RESULT OF REFLUX ESOPHAGITIS THERAPY

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**Aims:** To explore the effect of *H. pylori* infection and *H. pylori* eradication on the result of reflux esophagitis therapy.

**Methods:** Patients with reflux symptoms and diagnosed as reflux esophagitis by endoscopy were enrolled. The patients were divided into *H. pylori* positive and *H. pylori* negative group. *H. pylori* positive group were randomly divided into *H. pylori* eradication group and *H. pylori* non-eradication group. Patients of *H. pylori* eradication group underwent *H. pylori* eradication therapy for ten days, then Esomeprazole 20 mg bid for 46 days. Two eradication regimens were used in this study: EAC and sequential therapy. Patients of *H. pylori* non-eradication group and *H. pylori* negative group underwent Esomeprazole 20 mg bid therapy for 8 weeks. Before and after therapy, the symptoms of reflux esophagitis were scored and compared. After 8 weeks of treatment, gastroscopy was performed in all the patients again, and the healing rate of each group was compared.

**Results:** 1. Three hundred and fifty-six patients were enrolled with 178 *H. pylori* positive cases. For *H. pylori* positive group, 123 patients underwent *H. pylori* eradication. 2. The healing rate of EAC, sequential therapy and non-eradication group was 81.8%, 78.9%, 78.2% respectively ( $p = .869$ ). The scores of reflux symptoms were 0.19, 0.11, 0.26 ( $p = .657$ ) respectively. 3. The healing rate of esophagitis in *H. pylori* non-eradication group and *H. pylori* negative group was 78.2% and 82.6% respectively ( $p = .462$ ); The scores of reflux symptoms were 0.26 and 0.20 respectively ( $p = .653$ ).

**Conclusions:** *H. pylori* infection and *H. pylori* eradication have not significant effect on the result of reflux esophagitis therapy.

Abstract no.: P09.12

#### RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* INFECTIONS, DIABETES MELLITUS AND GALLSTONES: FACTS OR FICTION?

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**Aim:** Of our study was to evaluate any relationship between *Helicobacter pylori* infection, diabetes mellitus and gallstones.

**Methods and Patients:** In this study have participated 66 dyspeptic patients with diabetes mellitus 39 F/27 M mean age 63.6 years. The patients divided into two groups. Group A: 38 dyspeptic patients with gallstones and Group B: 36 dyspeptic patients without gallstones. The both group of patients were comparable in sex, age and body mass index. All patients had echosonography of upper stomach and upper gastro-intestinal endoscopy. The gastroduodenal pathology was identified. H.P. infection was confirmed by gastric histology. As metabolic control were measured fasting glucose and glycated hemoglobin (HbA1c), and was also observed duration of diabetes mellitus (>1 year, 1–3 year, >3 year).

**Results:** A higher HP infection was found in group of patients with gallstones (76%) versus 46% in patients without gallstones <0.001. Its correlated with fasting glucose and HbA1c values and was directly related to the duration of diabetes mellitus /<1 year 8%, 1–3 year 26%, and >3 year 56%. In Group A patients with gallstones, eradication of Hp infection was 63%, versus in non diabetic patients 82% ( $p > .001$ ). In patients with duration of DM >3 year was eradication rate 47%. For data analysis Chi Square test, Friedman test and T-tests were used.

**Conclusion:** According to our results we found that HP infection was higher in group A than in Group B. The rate of infection increases with DM duration. The eradication rate was significantly lower in patients with gallstones than in Group without gallstones. Further studies are needed to clarify the possible role of HP in the development of gallstones.

Abstract no.: P09.13

#### PREVALENCE OF *HELICOBACTER PYLORI* IN THE ORAL CAVITY, PHARYNX AND LARYNX IN PATIENTS WITH PROVEN GASTRIC COLONISATION

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*Helicobacter pylori* (Hp) is known to play an important role in the development of gastric disease. However its colonisation and possible effect on diseases concerning the oral cavity, pharynx and larynx (OPL) is controversial.

**Aim:** The main aim of this study was to show that people with a proven gastric Hp infection could also have a colonisation of the OPL site.

**Methods:** In this retrospective study Patients were included who had a proven gastric Hp colonisation and of whom a biopsy of the OPL site was taken before the gastric biopsy. DNA was extracted and PCR was performed. The DNA from the gastric biopsy was used as positive control.

**Results:** 36 Patients qualified for the study; in 27 Patients PCR was possible to perform. 26 Gastric biopsies were positive for Hp; 38% (10/26) of the OPL biopsies were positive for Hp; 70% (7/10) showed histopathologically a malignant neoplasia, 30% an infection.

**Discussion:** With this study we could show that there is a possibility of colonisation of Hp in the stomach and the OPL-site. In 70% (7/10) of the Patients a carcinoma was present with Hp colonisation in the OPL-Biopsies which was not significantly more than in the Hp negative group (65%; 11/17). The numbers of this study are very small but it proves that the OPL site can also be colonized by Hp.

Abstract no.: P09.14

#### IGG-ANTIBODIES TO *HELICOBACTER PYLORI* IN BLOOD SERUM AND SALIVA OF PATIENTS WITH ISCHEMIC HEART DISEASE

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The aim of the study is to determine the frequency of *Helicobacter pylori* (HP) contamination of patients with ischemic heart disease (IHD).

Thirty patients with IHD were examined (males) at the age from 39 to 77 years (the basic group). The first control group was formed from 17 practically healthy men, the second control group was composed from 17 practically healthy women of the similar age. IgG-antibodies to HP in their blood serum and their saliva were detected by means of immunochromatographic method.



In control groups the frequency of detection of antihelicobacter antibodies in blood serum of men and women was equal (15.4%), the antibodies to HP were detected more frequently at patients with IHD, than in control groups (40.0%,  $p < .05$ ).

Under simultaneous examination of the saliva and blood serum of patients and healthy persons on presence of IgG-antibodies to HP it was found out, that antibodies were detected more frequently in saliva both in the main and control groups. The IgG-antibodies to HP were detected more frequently in saliva of patients with IHD, than in saliva of practically healthy persons, at men more frequently, than at women (58.8%, 41.7%, 31.6%, correspondingly,  $p > .05$ ). The presence of IgG to HP in blood and saliva of patients examined by us is indicative of earlier HP-infection or its carriage. The presented data confirm the information about the certain HP participation in genesis of atherosclerosis and IHD.

Abstract no.: P09.15

#### EFFICIENCY EVALUATION OF DIFFERENT *HELICOBACTER PYLORI* ERADICATION AND CONCOMITANT IRON REPLACEMENT THERAPY IN PATIENTS WITH GASTRITIS B AND IRON DEFICIENCY ANEMIA

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In study investigated 318 adult persons with dyspepsia. Included to study all *Helicobacter pylori*-infected patients (Group 1) by isolating persons with following two criteria: 1, a lengthy history of iron deficiency anemia: defined as hemoglobin concentration (13–11 g/L for men and 12–10 g/L for women – in group with mild iron deficiency anemia (Group 2a and 2b), and 10.9–9 g/L for men, 9.9–8 g/L for women – in group with moderate iron deficiency anemia (Group2c)), a mean corpuscular volume <80 fL, and a serum ferritin level <30 µg/L). 2, *Helicobacter pylori*-associated gastritis.

Age matched patients were randomized into different therapeutic schemes for eradication of *Helicobacter pylori* : 1, Standard triple therapy: 2, Sequential eradication: lansoprazole 15 mg bid plus amoxicillin 1000 mg bid for 5 days, followed by lansoprazole 15 mg bid, clarithromycin 500 mg bid and tinidazole 500 mg bid for 5 days. Patients in Group 2a also received oral iron therapy. An isolated Group 2b with mild iron deficiency anemia was not received iron therapy, because of they hypersensitivity to oral iron medications in history.

Group 2c was also received intravenous iron therapy concordantly of they individual iron deficit.

Results of the study shows, that prevalence of *Helicobacter pylori* in Ukraine is still very high. Treating *Helicobacter pylori* infection in patients with iron deficiency anemia and *Helicobacter pylori*-related gastritis is associated with reversal of iron treatment and dependence, in patients with mild iron deficiency – even without simultaneous use of iron replacement therapy.

Abstract no.: P09.16

#### PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN PATIENTS WITH LIVER CIRRHOSIS

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**Background:** *Helicobacter pylori* (HP) infection plays a crucial role in the pathogenesis of a variety of gastric diseases ranging from dyspepsia and peptic ulcer to gastric adenocarcinoma and gastric MALT lymphoma. The role of HP in liver cirrhosis is still conflicting.

**Aim:** To investigate the prevalence of HP infection in patients with liver cirrhosis and to correlate it with gastric pathology.

**Methods:** Data from 72 patients with cirrhosis, who had been investigated with upper GI endoscopy for a variety of symptoms and signs were collected and *Helicobacter pylori* infection was confirmed either with a rapid urease test (RUT) and/or histology specimens and Wright-Giemsa staining.

**Results:** The global prevalence of *H. pylori* infection in cirrhotic patients was 31.9%, less than what is generally recorded in patients with non-ulcer dyspepsia or peptic ulcer. The prevalence of HP infection in patients with Child-Pugh class A, B and C liver cirrhosis was 35.7%, 28.5%, and 31.5%, respectively. The prevalence of peptic ulcer disease in patients with cirrhosis was 20.6%. The prevalence of *H. pylori* infection did not differ significantly between patients with or without peptic ulcer (32.9% vs 30.9%).

**Conclusions:** *Helicobacter pylori* does not seem to play the main role in the pathogenesis of peptic ulcer disease in patients with liver cirrhosis.

# P10 Clinical trials & NSAIDs

Abstract no.: P10.01

## META-ANALYSIS OF SEQUENTIAL VERSUS STANDARD TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION

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**Background:** Sequential regimen therapy has been recently suggested as a new first-line treatment option to replace the standard triple therapy, where eradication rates have declined to unacceptable levels.

**Aim:** To conduct a meta-analysis of studies comparing the sequential therapy versus the standard triple therapy for *H. pylori* eradication.

**Methods:** Selection of studies: randomized controlled trials comparing sequential (10 days) and standard triple therapies (at least 7 days).

**Search strategy:** bibliographical and manual searches were conducted up to May 2011.

**Data synthesis:** intention-to-treat eradication rate.

**Results:** We updated previous meta-analyses including 28 randomized controlled studies that, up to now, have compared these two regimens with a total of 8146 patients. The overall analysis showed that sequential therapy was significantly more effective than standard triple therapy (84% vs 77% in the intention-to-treat analysis; OR = 1.60; 95% CI = 1.43–1.79;  $p < .001$ ). Results were highly heterogeneous ( $I^2 = 85\%$ ), and nine studies were unable to demonstrate differences between sequential and standard triple therapy. So far, almost all the studies analyzing sequential therapy have been performed in Italy. Although, overall, mean eradication rate with sequential regimen was nearly 90%, a tendency towards lower efficacy with this regimen was observed in the more recent studies performed outside Italy.

**Conclusion:** The meta-analysis demonstrated that sequential regimen is more effective than standard triple therapy. Nevertheless, the apparent advantages of sequential treatment over standard triple therapy should be further and continuously assessed in different countries before a generalized change in all settings is recommended in first line *H. pylori* treatment.

Abstract no.: P10.02

## NON-BISMUTH QUADRUPLE (CONCOMITANT) THERAPY FOR ERADICATION OF *HELICOBACTER PYLORI*: A SYSTEMATIC REVIEW

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**Background:** Traditional standard triple therapy for *Helicobacter pylori* infection (PPI-clarithromycin-amoxicillin) can easily be converted to non-bismuth quadruple (concomitant) therapy by the addition of a nitroimidazole twice daily.

**Aim:** To review evidence on the role of non-bismuth quadruple (concomitant) therapy in the treatment of *H. pylori* infection.

**Methods:** Bibliographical searches were performed in MEDLINE and relevant congresses up to April 2011. We performed a meta-analysis of the studies evaluating the concomitant therapy, and of the randomized controlled studies comparing the concomitant and the standard triple therapy.

**Results:** Fifteen studies (including 1723 patients) evaluated the concomitant therapy: mean *H. pylori* cure rate (intention-to-treat) was 90% (95% CI = 86–93%). We then performed a meta-analysis of the randomized controlled studies comparing the concomitant (428 patients) and the standard triple therapy (418 patients). The former was more effective than the latter: 91.1% vs 80.6% (intention-to-treat analysis). Results were homogeneous ( $p = .45$ ;  $I^2 = 0\%$ ). The odds ratio for this comparison was 2.4 (95% CI = 1.63–3.55). A tendency toward better results with longer concomitant treatments (7–10 days vs 3–5 days) was observed. Clarithromycin resistance may reduce the efficacy of concomitant therapy, although the decrease in eradication rates seemed to be far lower than in standard triple therapy. The first randomized comparison of the sequential and the concomitant regimens (Wu 2010) recently concluded that both were similar in terms of efficacy and safety and that the sequential administration protocol may be unnecessarily complex.

**Conclusions:** Non-bismuth quadruple concomitant therapy appears to be an effective, safe, and well-tolerated alternative to triple therapy and is less complex than sequential therapy. Therefore, this regimen appears well suited for use in settings where the efficacy of triple therapy is unacceptably low.

Abstract no.: P10.03

## SECOND-LINE RESCUE THERAPY WITH LEVOFLOXACIN AFTER *H. PYLORI* TREATMENT FAILURE. TIME TRENDS OF ERADICATION IN A SPANISH MULTICENTER STUDY OF 936 PATIENTS

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**Background:** Second-line quadruple therapy is complex and can induce frequent adverse effects. A rescue levofloxacin-containing regimen may represent an alternative; however, resistance to quinolones is rapidly increasing.

**Aim:** To evaluate the efficacy and tolerability of a triple second-line levofloxacin-containing regimen, extending the experience of an ongoing multicenter study, and to assess whether its efficacy decreases with time.

**Methods:** Design: Prospective multicenter study.

**Patients:** In whom a treatment with PPI-clarithromycin-amoxicillin had failed. Intervention: levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.) and PPI (standard dose b.i.d.) for 10 days.

**Outcome:** Eradication was confirmed with <sup>13</sup>C-urea breath test 4–8 weeks after therapy.

**Compliance and tolerance:** Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

**Results:** 936 consecutive patients were included (mean age 49 years, 42% males, 34% peptic ulcer and 65% dyspepsia). 96% patients took all medications correctly. Per-protocol and intention-to-treat eradication rates were 76% (95% CI = 73–78%) and 74% (71–77%). Efficacy (intention-to-treat) was 77% in the year 2006, 68% in 2007, 72% in 2008, 76% in 2009, 75% in 2010, and 92% in 2011 (only 26 patients included). In the multivariate analysis, none of the studied variables (including diagnosis and year of treatment) was associated with eradication success. Adverse effects were reported in 19% of patients, most commonly nausea (8%), metallic taste (5%), myalgias/arthralgias (3.5%), and abdominal pain (3%), none of which were severe.

**Conclusion:** Ten-day levofloxacin-containing rescue therapy constitutes an encouraging second-line strategy representing a safe and simple alternative to quadruple therapy in patients with previous PPI-clarithromycin-amoxicillin failure. Efficacy of this regimen remains stable with time.

Abstract no.: P10.04

## EFFICACY OF CONCOMITANT NONBISMUTH-BASED QUADRUPLE THERAPY AS FIRST-LINE TREATMENT FOR ERADICATION OF *HELICOBACTER PYLORI*

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**Introduction:** The eradication rate of first-line *Helicobacter pylori* (HP) treatment has decreased due to the increasing rate of antibiotic resistance. Concomitant nonbismuth-based quadruple therapy is recently used in order to increase the successful HP eradication.

**Methods:** The aim of this study was to evaluate the efficacy of concomitant nonbismuth-based quadruple therapy for seven days in Korea. From October 2009 to April 2011, 138 patients who were diagnosed with HP infections by endoscopy were enrolled in Korea. Standard triple therapy group (85 patients) received proton pump inhibitor (PPI) standard dose bid, amoxicillin 500 mg tid, and clarithromycin 500 mg bid for 1 week. Concomitant therapy group (53 patients) received PPI standard dose bid, amoxicillin 500 mg tid, clarithromycin 500 mg bid and metronidazole 500 mg tid for 1 week. After 4 weeks, the success

rate of HP eradication was assessed by urea breathing test (UBT) and the side effects were assessed by questionnaire.

**Results:** Intention to treatment (ITT) eradication rate was higher in the concomitant therapy group than that of the standard therapy group (81.1% (43/53) vs 64.7% (55/85),  $p = .04$ ). The side effects including taste alteration and epigastric discomfort were more frequent in the concomitant group than in standard triple therapy group (37.7% (20/53) vs 9.4% (8/85),  $p < .01$ ), but treatment failure due to side effects showed no significant differences between two groups (2/85 vs 0/53).

**Conclusion:** Concomitant nonbismuth-based quadruple therapy for seven days was effective in eradicating HP infection as a standard triple therapy with mild side effects in Korea.

Abstract no.: P10.05

#### META-ANALYSIS OF LEVOFLOXACIN-CONTAINING TRIPLE THERAPY VERSUS BISMUTH-CONTAINING QUADRUPLE THERAPY AS SECOND-LINE TREATMENT IN THE ERADICATION OF *HELICOBACTER PYLORI*

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**Background:** After one *Helicobacter pylori* eradication failure, the most recommended rescue option has been the bismuth-containing quadruple therapy (BQT). Levofloxacin-containing triple therapy (LTT) has been presented as an alternative option.

**Aim:** To conduct a meta-analysis of studies comparing the efficacy and safety of LTT versus BQT in the eradication of *H. pylori* after one treatment failure.

**Methods:** Selection of studies: randomized controlled trials comparing LTT and BQT after one *H. pylori* eradication failure.

**Search strategy:** electronic and manual bibliographical searches.

**Data synthesis:** intention-to-treat eradication rate and adverse events rate.

**Results:** Thirteen studies were included, with a total of 1709 patients (1011 in the LTT and 698 in the BQT). The overall analysis showed a tendency towards better eradication results for LTT (79% vs 70%; OR = 1.43; 95% CI = 0.88–2.31;  $p = .15$ ;  $I^2 = 72%$ ) with a significantly lower rate of adverse effects (14% vs 32%; OR = 0.30; 95% CI = 0.19–0.50;  $p < .001$ ;  $I^2 = 46%$ ) and serious adverse effects (0.7% vs 7.8%; OR = 0.15; 95% CI = 0.04–0.59;  $p = .007$ ;  $I^2 = 0%$ ). There were two outlying studies showing better results for BQT, what may be explained because both studies used a 7 (instead of 10) day LTT. Excluding these studies, heterogeneity was reduced and results improved for LTT (81% vs 68%; OR = 1.88; 95% CI = 1.27–2.79;  $p = .002$ ;  $I^2 = 52%$ ). As LTT showed better efficacy in ten days courses than in seven days (89% vs 70%), a subanalysis including only 10 day LTT with levofloxacin, amoxicillin and PPI studies showed an even better efficacy for LTT compared with BQT (89% vs 66%; OR = 4.22; 95% CI = 2.84–6.26;  $p < .001$ ;  $I^2 = 0%$ ; NNT = 4).

**Conclusion:** The meta-analysis performed demonstrates that ten day LTT is more effective and better tolerated than BQT, as a second-line rescue option for *H. pylori* eradication.

Abstract no.: P10.06

#### GENIPIN CROSSLINKED CLARITHROMYCIN LOADED CHITOSAN MICROSPHERES FOR ERADICATION OF *HELICOBACTER PYLORI*

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**Aim:** Controlled drug delivery provides the optimum therapeutic drug concentration in blood, elimination of side effects, frequent dosing and better patient compliance. The aim of this study was to produce clarithromycin loaded chitosan microspheres, to examine clarithromycin release from uncrosslinked and genipin (*Gardenia Jasminoides* extract)-crosslinked microspheres and to determine the antibacterial activity of clarithromycin released from microspheres on *Helicobacter pylori*.

**Method:** 1% chitosan solution was prepared by dissolving chitosan in 2% acetic acid. Clarithromycin was incorporated into chitosan solution yielding 0.1% final concentration. 1 mmol/L and 5 mmol/L genipin solution was used for cross-linking. Control and clarithromycin loaded microspheres were obtained by drying solutions at 140 °C and 180 °C in Spray-Dryer (Buchi®-B290), respectively. Clarithromycin release from microspheres in phosphate buffer (pH5) was performed at 37 °C and 150 rpm. Clarithromycin concentration was determined by HPLC. The antibacterial activity of UV-sterilized control, clarithromycin and genipin-crosslinked microspheres were determined by adding 0.0022 g microspheres into *H. pylori* NCTC 11637 standard strain suspension (McFarland 2) of

40 mL 5%FBS incorporated Brucella Broth and incubated at 37 °C in microaerophilic environment. 100 µL sample taken in different times from this suspension was inoculated onto Columbia Blood Agar (7% horseblood, DENT), incubated in same condition and viable colonies were counted.

**Results:** Microspheres were wrinkled and spherical with size of 1–5 µm. As the genipin concentration increased, clarithromycin release rate decreased whereas extent of release increased. Except control microspheres, clarithromycin loaded uncrosslinked and genipin-crosslinked microspheres inhibited the *H. pylori* growth.

**Conclusion:** Clarithromycin loaded genipin-crosslinked chitosan microspheres have a great potential to be used as a control release system in treatment of *H. pylori* infection.

Abstract no.: P10.07

#### FOURTH-LINE RESCUE THERAPY WITH RIFABUTIN IN PATIENTS WITH THREE *H. PYLORI* ERADICATION FAILURES

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**Background:** In some cases, *Helicobacter pylori* infection persists even after three eradication treatments.

**Aim:** To evaluate the efficacy of an empirical fourth-line rescue regimen with rifabutin in patients with three eradication failures, extending the experience of an ongoing multicenter study.

**Methods:** Design: Multicenter, prospective study.

**Patients:** Patients in whom the following three eradication treatments had consecutively failed: 1st treatment: PPI + clarithromycin + amoxicillin; 2nd treatment: quadruple therapy (PPI + bismuth + tetracycline + metronidazole); 3rd treatment: PPI + amoxicillin + levofloxacin.

**Intervention:** In patients failing these three regimens, a 4th regimen with rifabutin (150 mg b.i.d.), amoxicillin (1 g b.i.d.) and a PPI (standard dose b.i.d.) was prescribed for 10 days. Compliance with therapy was determined from interrogatory and recovery of empty envelopes of medications.

**Outcome variable:** *H. pylori* eradication was confirmed with <sup>13</sup>C-urea breath test.

**Results:** Eighty-seven patients (mean age 51 years, 35% males, 37% peptic ulcer/63% functional dyspepsia) were included. Compliance: seven patients did not take correctly the medication (in six cases due to adverse effects): vomiting (three patients), fever/myalgia/abdominal pain/diarrhoea (two patients) and abdominal pain (one patient). Per-protocol and intention-to-treat eradication rates were 53% (95% CI = 41–64%) and 52% (41–63%). Adverse effects were reported in 29 (34%) patients (none severe): nausea/vomiting (12 patients), fever (5), abdominal pain (5), diarrhoea (4), myalgia (3), hypertransaminasemia (2), leucopenia (<1500 neutrophils)(2), and thrombopenia (<150,000 platelets)(2). Myelotoxicity resolved spontaneously in all cases.

**Conclusion:** Even after three previous *H. pylori* eradication failures, an empirical fourth-line rescue treatment with rifabutin may be effective in approximately 50% of the cases. Therefore, rifabutin-based rescue therapy constitutes a valid strategy after multiple previous eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin.

Abstract no.: P10.08

#### EFFECT OF THE *HELICOBACTER PYLORI* ERADICATION IN PATIENTS WITH DIFFERENT SUBTYPE OF FUNCTIONAL DYSPESPIA: A RANDOMIZED MULTICENTER TRIAL

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**Objectives:** To evaluate the effect of *H. pylori* eradication therapy in patients with epigastric pain syndrome (PDS) and epigastric pain syndrome (EPS).

**Methods:** This randomized, multicenter trial enrolled 210 patients with FD and *H. pylori* infection, 106 in PDS group and 104 in EPS group, according to Rome III criteria. Each group was randomized to receive EAC regimen (esomeprazole, amoxicillin, clarithromycin for 10 days), sequential regimen (esomeprazole, amoxicillin for the first 5 days, followed by esomeprazole, clarithromycin, tinidazole for the remaining 5 days) or traditional therapy (PDS patients: domperidone for 2 weeks; EPS patients: Talcid for 2 weeks). Patients were followed up for 52 weeks, symptoms were assessed with dyspepsia score, which was derived by

grading four dyspeptic symptoms (fullness, early satiation, epigastric pain and epigastric burning).

**Results:** Of the 106 PDS patients, 37 received EAC regimen, 34 received sequential regimen, and 35 received traditional therapy. *H. pylori* eradication rates of EAC and sequential regimen was 80% and 69.2% respectively. There was no significant difference in dyspepsia score at baseline ( $\chi^2 = 2.199, p = .333$ ) and at 52 weeks ( $\chi^2 = 5.583, p = .061$ ). Of the 104 EPS patients, 36 received EAC regimen, 34 received sequential regimen, and 34 received traditional therapy. *H. pylori* eradication rates of EAC and sequential regimen was 83.3% and 69.2% respectively. There was no significant difference in dyspepsia score at baseline ( $\chi^2 = 2.241, p = .326$ ) but dyspepsia score was significant lower in EAC and sequential therapy group than control at 52 weeks ( $\chi^2 = 12.576, p = .002$ ).

**Conclusion:** Eradication of *Helicobacter pylori* in patients with EPS is more effective improving symptoms than traditional therapy.

Abstract no.: P10.09

#### EFFICACY OF 10-DAY NON-BISMUTH QUADRUPLE "CONCOMITANT" REGIMEN AS FIRST-LINE THERAPY FOR *HELICOBACTER PYLORI* INFECTION IN A SETTING WITH HIGH RATES OF CLARITHROMYCIN AND DUAL CLARITHROMYCIN AND METRONIDAZOLE RESISTANCE

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**Background:** Recently, several studies have reported unacceptable eradication rates of *H. pylori* infection (<80%) with 10-day "sequential" therapy.

**Aim:** To assess in vitro antibiotic susceptibility of *H. pylori* and to evaluate the efficacy of empiric 10-day non-bismuth quadruple "concomitant" therapy in a geographical area where "sequential" therapy is inefficient (76% cure rate in a previous study).

**Methods:** *H. pylori* culture (E-test) was performed in 235 dyspeptic patients undergoing upper endoscopy, with no previous eradication treatment. Simultaneously, 155 naive *H. pylori*-positive patients (mean age 49 years, 53% males, 65% non-ulcer dyspepsia) without microbiological study were treated with 10-day "concomitant" therapy (PPI at standard dose, amoxicillin 1 g, clarithromycin 500 mg and metronidazole 500 mg, all drugs prescribed b.i.d. for 10 days). Eradication was confirmed with 13C-urea breath test or histology 8 weeks after completion of treatment.

**Results:** Culture was positive in 75% (85/114) of *H. pylori*-positive patients. Antibiotic resistance rates were: clarithromycin (20%, 17/85), metronidazole (34%, 29/85), dual resistance (clarithromycin and metronidazole) (11%, 9/85) and levofloxacin (28%, 24/85). Eradication rates for "concomitant" therapy were 88% (95% CI:82–93%) by per protocol and 85% (95% CI:80–91%) by intention-to-treat. In the multivariate analysis, ulcer disease was a predictor of eradication success (OR:11; 95% CI:1.4–83). On follow-up, all excepting three patients strictly completed therapy. Adverse effects, all of them mild, were reported in 39% of the patients.

**Conclusion:** In settings with high clarithromycin resistance (20%) and dual resistance to clarithromycin and metronidazole (11%), and documented failure of "sequential" therapy, non-bismuth quadruple "concomitant" therapy achieves acceptable eradication rates.

Abstract no.: P10.10

#### TIME TRENDS OF ERADICATION RATES OF STANDARD TRIPLE THERAPY FOR *H. PYLORI* INFECTION FOR THE LAST 12 YEARS: ARE CURE RATES REALLY DECREASING?

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**Background:** It has been reported that resistance to clarithromycin is rapidly increasing and that, consequently, efficacy of standard triple therapy is progressively declining to unacceptable levels.

**Aim:** To evaluate the trend of *H. pylori* eradication rates with standard clarithromycin-containing triple therapy in a single center for the last 12 years.

**Methods:** From January 1998 through December 2010, *H. pylori* eradication rates in consecutive patients who received one-week triple regimen with a PPI (standard dose b.i.d.), amoxicillin (1 g b.i.d.) and clarithromycin (500 mg b.i.d.) were retrospectively evaluated according to years. Patients having received a previous eradication treatment were excluded.

**Results:** Four hundred and nine patients were included (mean age 53 years, 62% males, 64% ulcer disease). The overall *H. pylori* eradication rate was

83.4% (95% CI = 80–87%) by intention-to-treat, and 84.5% (81–88%) by per-protocol. Yearly eradication rates from the year 1998 to 2010 were 78.6%, 82.6%, 80.6%, 83.8%, 81.5%, 88.6%, 88.1%, 87.5%, 87.5%, 81.0%, 88.2%, 90.0%, and 83.3%. Almost all patients (97.6%) were compliant with treatment. Adverse events were reported by 11.5% of the patients. No evidence of decreasing tendency of eradication rate was seen during the past 12 years. In the multivariate analysis (including age, sex, smoking, diagnosis, PPI type, and year of treatment), the only variable associated with the eradication success was the diagnosis (peptic ulcer vs non-ulcer disease; OR = 2.01; 95% CI = 1.19–3.57). In particular, there was no significant difference in the eradication rates according to the year of treatment.

**Conclusion:** *H. pylori* eradication rates of standard triple therapy have not changed at our center for the last 12 years. Even nowadays, higher than 80% cure rate may be obtained with one-week clarithromycin-containing triple treatment.

Abstract no.: P10.11

#### META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS (RCT) ON THE EFFECTIVENESS OF *H. PYLORI* ERADICATION THERAPY VERSUS ANTI-SECRETORY NON-ERADICATION THERAPY AFTER SIMPLE CLOSURE OF PERFORATED DUODENAL ULCER

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**Background:** *H. pylori* causes duodenal ulcers which in complicated cases, resulted in perforation. Because *H. pylori* eradication is currently a standard treatment in peptic ulcer patients whom infected with this organism, the use of bacterial treatment in ulcer with complications seems reasonable. In the year 2000, the first RCT from Hong Kong reported the effectiveness of *H. pylori* eradication therapy versus antisecretory non-eradication therapy after simple closure of perforated duodenal ulcer. Nevertheless, very small numbers of RCTs focused on the same topic were published during 2000 to 2010. This study aims to perform meta-analysis comparing the effectiveness of *H. pylori* eradication therapy versus maintenance antisecretory non-eradication therapy for prevention of ulcer recurrence after simple closure in perforated duodenal ulcer patients.

**Materials and Methods:** A search on the Cochrane Controlled Trials Register, Medline, Embase were made for controlled trials of duodenal ulcer perforation patients using simple closure method plus postoperative *H. pylori* eradication therapy versus simple closure plus antisecretory non-eradication therapy. The long-term results for prevention of ulcer recurrence were compared.

**Results:** Mean percentage of one-year ulcer recurrence in *H. pylori* eradication group was 5.3% which is significantly lower than that of the control group (35.2%, RR, 0.15; 95% confidence interval (CI), 0.06–0.37).

**Conclusions:** *H. pylori* eradication after simple closure of duodenal ulcer perforation gives better result than the operation plus maintenance antisecretory non-eradication therapy for prevention of ulcer recurrence. All duodenal ulcer perforation patients should be tested for *H. pylori* infection and eradication therapy is required in all infected patients.

Abstract no.: P10.12

#### PHOTODYNAMIC THERAPY FOR *HELICOBACTER PYLORI* ERADICATION

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**Introduction:** The decrease in the effectiveness of conventional antibiotic treatments to eradicate *H. pylori*, suggests the search for alternative strategies. Photodynamic therapy, which generates singlet oxygen, is commonly used for treatment of localized infectious diseases.

**Aims:** To evaluate in vitro effect of a photosensitising material for *H. pylori* inactivation.

**Methods:** Two *H. pylori* strains (CagA (-) and (+)) isolated from biopsies, were cultured. Assays were performed at  $5 \times 10^4$  and  $10^5$  CFU/mL. Photosensitising material (P) was a ruthenium complex supported over glass beads (S). Each strain was placed in three wells: A:bacteria, B:bacteria + S (1–3 mg), and C:bacte-



ria + P + S (1–3 mg) and was incubated in the dark or illuminated (blue LED, 20–25 mW). Aliquots were taken every 5' until 30', cultured, and colonies were counted. On the other hand, DNA was isolated and damage was evaluated by RT-PCR (ureC and cagA genes) and by incubation with endonuclease III (excises oxidized pyrimidines causing DNA fragments) following by alkaline gel electrophoresis.

**Results:** C wells exposed to light showed a 90–95% decrease in the colonies number compared to the other wells. In samples irradiated, Ct mean values (cycle threshold, inversely proportional to the amplified target gene) obtained by RT-PCR were compared: ureC 37.9 and 43.9; cagA 38.1 and 39.7 in B and C, respectively. In gel electrophoresis DNA from irradiated C wells showed a fluorescence background that did not appear in the other wells. All data were strain independent.

**Conclusion:** *H. pylori* incubated with the photosensitising material were susceptible to the singlet oxygen action. A novel phototherapy approach could be applied to cure *H. pylori* infection.

Abstract no.: P10.13

#### INFLUENCE OF CLINICAL DEMOGRAPHIC FACTORS IN SUCCESSFUL ERADICATION OF *HELICOBACTER PYLORI*

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**Introduction:** There have been few studies which examined the cause of *Helicobacter pylori* (HP) treatment failures among the general clinical factors. The aim of this study is to determine the general risk factors that affect the success rate of first line therapy for patients with HP infection in Korea.

**Methods:** From January 2007 to December 2010, patients who were treated by the first line therapy of HP were enrolled. They were assigned to receive a seven-day eradication therapy with proton pump inhibitor (PPI: lansoprazole, rebaprazole, esomeprazole strontium, or esomeprazole magnesium), amoxicillin and clarithromycin. Urea breath test (UBT) was performed 4 weeks after the end of treatment in order to evaluate the response of therapy.

**Results:** Seven hundred and seventy patients were enrolled, including 416 male and 354 female patients. The overall eradication rate was 70.9% (546/770). The eradication rates for male and female were 73.3% (305/416) and 68.1% (241/354), respectively. The eradication rates for patients who are <30 years-old, 30–39 years-old, 40–49 years-old, 50–59 years-old, 60–69 years-old, and more than 70 years old were 55.3%, 79.4%, 76.9%, 70.7%, 67.8%, and 61.4%, respectively. Young age (<30 years) and old age (more than 60 years) groups were significantly associated with the poor response to HP eradication. Esomeprazole magnesium and rebaprazole group showed higher eradication rate (76.0% (225/297), 73.2% (127/220)) than those of other PPI treatment groups (lansoprazole: 65.5% (131/200), esomeprazole strontium: 66.7% (96/144)).

**Conclusion:** The poor responses to HP eradication were significantly associated to age (<30 years or more than 60 years) and types of PPI.

Abstract no.: P10.14

#### META-ANALYSIS: RABEPRAZOLE AND ESOMEPRAZOLE IN THE ERADICATION OF *HELICOBACTER PYLORI*

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**Aim:** To conduct a meta-analysis of studies comparing rabeprazole and esomeprazole with other PPI regimens or with each other in *H. pylori* eradication treatment.

**Methods:** Selection of studies: Randomized controlled trials comparing esomeprazole or rabeprazole with “old” generation PPIs (omeprazole-lansoprazole-pantoprazole) or with each other. Analysis was done using studies comparing dual antibiotic regimens differing only on the PPI used, not on treatment’s duration or number of medication intakes per day. Search strategy: electronic and manual. Study quality: independently assessed by two reviewers. Data synthesis: Meta-analysis combining the Odds Ratios (OR) (by Intention-To-Treat). Number-Needed-to-Treat (NNT) were calculated.

**Results:** Meta-analysis (including 40 studies, 2167 esomeprazole, 2446 rabeprazole and 3436 “old” PPI treated patients) showed better results for esomeprazole and rabeprazole (overall and separately) than for “old” PPIs (overall vs “old”: 81.4% vs 77.9%; OR = 1.23; 95% CI = 1.09–1.39; NNT = 29 / esomeprazole vs “old”: 83.2% vs 78.6%; OR = 1.27; 95% CI = 1.06–1.52; NNT = 22 / rabeprazole vs “old”: 79.9% vs 75.6%; OR = 1.20; 95% CI = 1.02–1.40; NNT = 23). Subanalyses based on the PPI dose were performed: only esomeprazole 40 mg improved

results (83.5% esomeprazole vs 72.4% “old” PPIs; OR = 1.68; 95% CI = 1.21–2.34; NNT = 9), while rabeprazole 20 mg b.i.d. maintained results. Lower PPI doses (esomeprazole 20 mg b.i.d and rabeprazole 10 mg b.i.d) reduced the efficacy of the new PPIs. Esomeprazole and rabeprazole were compared in a sub-analysis including five studies (811 esomeprazole and 769 rabeprazole patients), with no statistically significant differences (77.3% vs 76.5%; OR = 1.06; 95% CI = 0.84–1.35).

**Conclusion:** Esomeprazole and rabeprazole have similar eradication success, but both show better overall *H. pylori* eradication rates than “old” generation PPIs. However, this clinical benefit is more pronounced in esomeprazole 40 mg b.i.d regimens.

Abstract no.: P10.15

#### AMOXICILLIN GASTRIC RETENTION SYSTEMS FOR *HELICOBACTER PYLORI* TREATMENT

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**Objective:** To study the behaviour of amoxicillin poly (DL-lactide acid)(PL) gastroretentive polyionic complexes for *H. pylori* treatment.

**Methods:** Hydrogels were prepared with chitosan (CS), sodium carboxymethylcellulose (CMC) and poly (DL-lactide acid)(PL). The ratios (w/w) of amoxicillin (A) dried hydrogels were: CS:PL:A (3 : 2 : 3), CS:CMC:A (3 : 2 : 3) and CS:CMC:PL:A (3 : 1 : 1 : 3).

**Characterization:** SEM study of hydrogels before and after being immersed in an acetate buffer (pH = 5.0, USP = 29). In-vitro drug release studies were carried out in USP-Apparatus-2. For swelling and erosion studies, each hydrogel formulation was weighed ( $W_i$ ) and then immersed in buffer. The remaining system was weighed at time  $t$  ( $W_w$ ). The system was then dried to constant weight ( $W_d$ ). We calculated: normalized swelling degree ( $S = (W_w - W_i)/W_i$ ), and apparent polymer erosion rate constant  $K_e$  ( $\text{min}^{-1}$ ):  $[(a - K_e t = (W_d/W_i)^{1/3})]$ .

**Results:** CS and CMC hydrogels presented a porous surface. Pores were unevenly shaped (diameter: 50–300  $\mu\text{m}$ ). CS:CMC:PL:A formulation was structurally similar to CS:CMC:A, but with less pores. All formulations released the total amount of amoxicillin within 2 hour. CS:CMC:A and CS:CMC:PL:A showed a lower burst effect than CS:PL:A. Hydrogels containing CS and CMC complex obtained a fast S values (9 and 14 at 10 minutes). PL addition modulated  $K_e$  due to the PL steric hindrance and hydrophobicity. CS:CMC:PL:A presented a suitable swelling and eroding profile prevailing erosion after 120 minutes ( $K_e$  0.672  $10^{-3} \text{ min}^{-1}$ ).

**Conclusions:** Controlled-release gastro-retentive formulations were successfully obtained by dispersion under acidic conditions and vacuum drying process. Interpolymer complexes (CS:CMC:A and CS:CMC:PL:A) have demonstrated suitable swelling properties and drug release profiles at pH 5.0, providing a controlled amoxicillin release for 2 hour. PL addition decreases electrostatic interactions providing suitable eroding characteristics, which enable evacuation following drug release.

Abstract no.: P10.16

#### CLINICAL EVALUATION OF A 10 DAY REGIMEN WITH ESOMEPRAZOLE, METRONIDAZOLE, AMOXICILLIN, AND CLARITHROMYCIN FOR THE ERADICATION OF *H. PYLORI* (E-MACH STUDY)

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**Aims:** We aimed to assess effectiveness and safety of a 10 day quadruple non bismuth containing therapy for *H. pylori* in a population with relatively high resistance to metronidazole (M) and clarithromycin (C).

**Patients and Methods:** We included 96 consecutive patients who had upper GI endoscopy. Excluded patients had: eradicated *H. pylori*, recent use of antibiotics, bismouth, NSAID or aspirin, allergy, gastrectomy, pregnant women. All eligible patients were CLO-test and either histology or culture positive and were prescribed: Esomeprazole 40 mg, Metronidazole 500 mg, Amoxicillin 1000 mg, and Clarithromycin 500 mg, twice daily, for 10 days. Compliance to treatment and

adverse effects were recorded. Eradication was tested 4–6 weeks later by means of histology and/or <sup>13</sup>C-UBT and/or stool test.

**Results:** Ninety three patients (41F/52M, aged 18–81, mean: 51.8 years) were evaluated for eradication (35.5% smokers, 21.5% with ulcer disease). Adherence to treatment was 97.7% (95% CI 95.9–99.6). Six (6.2%) patients experienced severe side effects. Overall PP and ITT eradication rates were 90.3% (95% CI 84.2–96.4) and 87.5% (95% CI 80.7–94.2) but were significantly higher when the regimen was prescribed as a first line therapy (92.6% PP, 90.4% ITT) than in the remaining cases (63.6% PP, 58.3% ITT) ( $p < .001$ ). Positive cultures and antibiotic sensitivity tests were carried out in 40/47 (85.2%) patients. Eradication rates were significantly higher in sensitive and single resistance strains (12/12, 100% and 18/19, 94%) than in those with double resistance (5/9, 55%) ( $p < .0001$ ).

**Conclusions:** The 10 days concomitant regimen is effective and safe as first line *H. pylori* eradication therapy although double (M and C) resistance may compromise its effectiveness.

Abstract no.: P10.17

#### EFFECT OF FURAZOLIDONE QUADRUPLE REGIMEN PLUS DENTAL PLAQUE REMOVAL PROCEDURES AS RESCUE TREATMENT OF REFRACTORY *HELICOBACTER PYLORI* INFECTION

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**Objective:** To observe the effect of furazolidone quadruple regimen plus dental plaque removal procedures as rescue treatment of refractory *H. pylori* infection.

**Methods:** Hundred and four patients with *H. pylori* positive (<sup>13</sup>C-UBT or RUT positive) failed in previous treatment three times or more were enrolled and divided into two groups. One group (64 patients) were given quadruple regimen (PPI + Bismuth + amoxicillin + furazolidone, 10 days) treatment and dental plaque removal treatment, the others (40 patients) accepted only quadruple regimen treatment. To detect *H. pylori* by <sup>13</sup>C-UBT 4 weeks after the therapy and to compare the eradication rates of the two groups.

**Results:** The eradication rate of the quadruple regimen + dental treatment group was 85.9% (55/64), while that of the other group was 72.5% (29/40) ( $p = .091$ ).

**Conclusion:** PPI + Bismuth quadruple regimen plus dental plaque removal procedures as rescue treatment may increase the eradication rate of refractory *H. pylori* infection patients. Furazolidone quadruple therapy could be chosen for the treatment of refractory *H. pylori* infection. Oral *H. pylori* infection might play a role in the failure of *H. pylori* infection treatment.

Abstract no.: P10.18

#### EFFECTIVENESS OF 5-DAY AND 7-DAY QUADRUPLE “CONCOMITANT” THERAPY REGIMEN FOR *HELICOBACTER PYLORI* INFECTION IN KOREA

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**Objectives:** Concomitant therapy containing three antibiotics showed higher eradication rate over 90%, compared with standard triple therapy for *H. pylori* eradication in several studies. The aim of this study was to assess the efficacy of quadruple concomitant regimen as the first line therapy for *H. pylori* infection in Korea and test whether prolonging treatment duration from 5 days to 7 days could increase the eradication rate.

**Methods:** A total of 110 patients with proven *H. pylori* infection were randomly assigned to one of two regimens: amoxicillin 1000 mg with clarithromycin 500 mg, metronidazole 500 mg and lansoprazole 30 mg twice daily for 5 days (5-day therapy group) or 7-days (7-day therapy group). The success of *H. pylori* eradication was evaluated 4–5 weeks after completing treatment.

**Results:** A total of 97 patients completed the study. Eradication rates were 87.8% in the 5-day therapy group and 89.6% in the 7-day therapy group by per protocol analysis; there was no statistically significant difference. There were also no significant differences in compliance and mild adverse events between two groups.

**Conclusion:** Although 5-day or 7-day quadruple concomitant therapy is found to achieve better eradication rate than the standard triple therapy of recent studies, the success rate was <90%, and there are no benefit in improving the treatment outcome by extending duration from 5 days to 7 days.

Abstract no.: P10.19

#### SECOND-LINE RESCUE TRIPLE THERAPY WITH LEVOFLOXACIN AFTER QUADRUPLE NON-BISMUTH “SEQUENTIAL” OR “CONCOMITANT” TREATMENT FAILURE

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**Background:** Quadruple non-bismuth containing “sequential” and “concomitant” regimens, including amoxicillin, clarithromycin and a nitroimidazole, are increasingly used as first-line treatments. *H. pylori* eradication is a challenge in patients failing these eradication regimens including key antibiotics such as clarithromycin and nitroimidazoles.

**Aim:** To evaluate the efficacy and tolerability of a second-line levofloxacin-containing triple regimen (PPI-amoxicillin-levofloxacin) in the eradication of *H. pylori* after “sequential” or “concomitant” treatment failure.

**Methods:** Design: Prospective multicenter study.

**Patients:** In whom a “sequential” regimen (PPI + amoxicillin for 5 days followed by PPI + clarithromycin + metronidazole for five more days) or a “concomitant” regimen (PPI + amoxicillin + clarithromycin + metronidazole for 10 days) had previously failed.

**Intervention:** levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.) and PPI (standard dose b.i.d.) for 10 days.

**Outcome:** Eradication was confirmed with <sup>13</sup>C-urea breath test 4–8 weeks after therapy.

**Compliance and tolerance:** Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

**Results:** Thirty-five consecutive patients have been included up to now (mean age 52 years, 37% males, 19% smokers, 15% peptic ulcer and 85% dyspepsia): 15 after “sequential”, and 20 after “concomitant” treatment failure. All patients took all medications correctly. Overall, per-protocol and intention-to-treat *H. pylori* eradication rates were both 80% (95% CI = 65–95%). Respective cure rates for “sequential” and “concomitant” failure regimens were 67% (10/15) and 90% (18/20). Adverse effects were reported in one (3%) patient: mild metallic taste and heartburn.

**Conclusion:** Ten-day levofloxacin-containing rescue triple therapy constitutes an encouraging second-line strategy in patients with previous quadruple “sequential” or “concomitant” treatment failure.

Abstract no.: P10.20

#### NON-BISMUTH QUADRUPLE “CONCOMITANT” THERAPY VERSUS STANDARD TRIPLE THERAPY FOR CLARITHROMYCIN-SUSCEPTIBLE *H. PYLORI* AND VERSUS QUADRUPLE “SEQUENTIAL” THERAPY FOR CLARITHROMYCIN-RESISTANT *H. PYLORI*

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**Background:** Information of antimicrobial susceptibility may enhance the efficacy of standard triple therapy (STT), but this should be evaluated and compared with quadruple therapies.

**Aims:** 1. To compare quadruple “concomitant” therapy versus STT for clarithromycin-susceptible (CLA-S) *H. pylori* and 2, to compare quadruple “concomitant” versus “sequential” therapies for clarithromycin-resistant (CLA-R) and dual clarithromycin and metronidazole (MET) resistant (DUAL-R) strains.

**Methods:** Prospective study including 83 patients. Antibiotic resistance (E-test): CLA-R (MIC >1 mg/mL) and MET-R (MIC >8 mg/mL). Randomization: Patients with CLA-S strains received either 10-day “concomitant” therapy (n = 25) (STT adding metronidazole 500 mg b.i.d.) or 10-day STT (n = 22). Patients with CLA-R (n = 9) or DUAL-R (n = 7) received either 10-day “concomitant” therapy or “sequential” therapy (PPI and amoxicillin 5 days plus PPI, clarithromycin and metronidazole 5 days, all drugs b.i.d.). Eradication was confirmed with <sup>13</sup>C-urea breath test or histology 8 weeks after completion of treatment.

**Results:** Indications for eradication were functional dyspepsia (75%) and ulcer disease (25%). Regarding CLA-S *H. pylori*, a statistically non-significant tendency ( $p = .2$ ) to better results was observed with “concomitant” therapy compared with STT both by PP (88%; 95% CI:81–100% vs 78%; 95% CI:56–99%) and by ITT (88%; 95% CI:79–100% vs 73%; 95% CI:51–95%). PP and ITT eradication rates for CLA-R strains with “concomitant” and “sequential” treatments were 100% (4/4) and 80% (4/5), and for DUAL-R strains 66% (2/3) and 75% (3/4).

**Conclusion:** Quadruple “concomitant” therapy may be more effective than STT for CLA-S *H. pylori*, whereas both quadruple “concomitant” and “sequential” regimens maintain acceptable eradication rates for CLA-R and DUAL-R strains.

Abstract no.: P10.21

**IMPORTANCE OF DETERMINING THE PATTERN OF *H. PYLORI* RESISTANCE IN COUNTRIES WITH A HIGH PREVALENCE OF GASTRIC CANCER SUCH AS NICARAGUA**

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**Background:** Successful anti-*H. pylori* therapy depends on the appropriate antibiotic-containing regimen. The major barrier to high cure rates is antimicrobial resistance. The pattern of resistance is unknown in much of Central and South America where the burden of *H. pylori*-related diseases is high.

**Aim:** To assess the *H. pylori* antimicrobial resistance pattern in Nicaragua.

**Methods:** Consenting, symptomatic patients presenting to the Hospital Escuela Antonio Lenin Fonseca, Universidad Nacional Autónoma de Nicaragua-Managua underwent upper endoscopy with biopsy (n = 140). One antral and one corpus biopsy was placed into transport media (Brucella broth with 20% glycerol), frozen, and shipped to Houston for culture. The specimens were stored at -70 °C until cultured. Frozen specimens were thawed and cultured using two types of selective media, Brain Heart Infusion (BHI) blood agar and *Helicobacter pylori* Special Peptone Agar (HPSPA) plates. Mueller Hinton agar plates with 5% sheep blood were used to perform Etest (AB Biodisk) to determine minimum inhibitory concentrations (MIC) of clarithromycin (>1 µg/mL), metronidazole >8 µg/mL, amoxicillin (>0.5 µg/mL) and tetracycline (>4 µg/mL).

**Results:** To date results on 43 culture positive are available. The proportions resistant were: 91% to metronidazole and 16.3% to clarithromycin (all were dual clarithromycin and metronidazole resistance). All isolates were amoxicillin and tetracycline susceptible.

**Conclusion:** In Nicaragua, metronidazole resistance is almost universal and clarithromycin and dual resistance was sufficiently high that triple or sequential therapy would be poor recommendations. This study points out the importance of determining the local susceptibility pattern before deciding on a likely best therapy.

Abstract no.: P10.22

**CONCOMITANT THERAPY WAS MORE EFFECTIVE THAN PPI-BASE TRIPLE THERAPY IN KOREA: A PRELIMINARY REPORT**

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**Background and Aims:** The eradication rate of proton-pump inhibitor (PPI) – based triple therapy for *Helicobacter pylori* has decreased due to increasing antibiotic resistance, especially clarithromycin. Recently, the concomitant therapy is tried to overcome antibiotic resistance and has produced good outcomes in many countries. The aim of this study was to assess the efficacy of concomitant therapy in Korea.

**Materials and Methods:** A total 38 patients with proven *H. pylori* infection received concomitant therapy (20 mg of rabeprazole, 1 g of amoxicillin, 500 mg of clarithromycin, and 500 mg of metronidazole, twice a day for 14 days) and the other 38 patients took PPI-based triple therapy. Eradication was evaluated by the <sup>13</sup>C-urea breath test at least 4 weeks later after end of treatment.

**Result:** The eradication rate of concomitant therapy (63.2%, 24/38) was higher than PPI-based triple therapy (39.5%, 15/38) but not statistically significant ( $p = .066$ ). However, the adverse effect was higher in concomitant therapy group (62.2%, 23/38) than PPI-based triple therapy group (28.9%, 11/38) ( $p = .005$ ) although the treatment was well tolerable.

**Conclusion:** Concomitant therapy as a first-line therapy against *H. pylori* was more effective than PPI-based triple therapy although it was not statistically significant in Korea but the adverse effect of it was higher.

Abstract no.: P10.23

**INCREASING THE DURATION OF DUAL AMOXICILLIN PLUS OMEPRAZOLE TO 6 WEEKS FOR CURE OF *HELICOBACTER PYLORI* INFECTIONS**

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**Background:** *Helicobacter pylori* infections have become increasingly difficult to treat as antimicrobial resistance has increased. Prolonging therapy has been suggested to overcome the persist state of the bacteria.

**Aim:** To test the hypothesis that a 6 week dual regimen of amoxicillin 1 g and omeprazole 20 g therapy BID would cure at least 90% of treatment naive *H. pylori* infections.

**Methods:** This was an open label prospective pilot study in which treatment naive subjects with active *H. pylori* infection (positive by two tests) received dual amoxicillin 1 g and omeprazole 20 mg, B.I.D. daily for 6 weeks. Success was assessed by UBT 4 to 6 weeks later. A tentatively effective therapy was defined as a per-protocol (PP) treatment success of 90% or greater; treatment success of 80% or less was prespecified as unacceptable.

**Results:** Sixteen patients were entered (14 men 2 woman) average age 49 before achieving the prespecified stopping rule of six treatment failures which excluded a 90% success rate if 50 patients had been entered. and enrollment was stopped. Sixteen completed the final follow up. PP treatment success was 62.5%; 95% CI = 35–84%, Intention to treat success was the same. Compliance has been >99%, 5 (31%) reported side effects, all mild and none that interrupted therapy.

**Conclusion:** Despite the theory and preexisting data from Japan, in the US it does not appear that prolonging the duration of dual amoxicillin-PPI therapy greatly improves the outcome compared to 14 day therapy.

Abstract no.: P10.24

**THE NEW APPROACH TO THE TREATMENT OF RESISTANT FORMS OF *HELICOBACTER PYLORI* IN PATIENTS WITH *HELICOBACTER PYLORI* ASSOCIATED GASTRIC PATHOLOGY**

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**Introduction:** In recent years, Russia has registered growth of *Helicobacter pylori* resistance to the components of triple eradication therapy: metronidazole and clarithromycin, which necessitates the search for and testing new options for eradication of *Helicobacter pylori* (Hp).

**Aim:** Determine the effectiveness of a new method of sequential eradication with use of rifaximin and amoxicillin in patients with Hp associated gastric pathology.

**Materials:** Open noncomparative prospective study involved 36 patients with erosive gastritis associated with Hp which was not achieved eradication after a full course of conventional triple therapy. Patients received pantoprazole 20 mg for two times a day for 4 weeks, against which he was appointed a serial receive rifaximin 400 mg two times daily for 5 days and amoxicillin 1000 mg × 2 times a day. Control of eradication was carried out in 6 weeks using the rapid urease test, histology and urea breath test.

**Results:** The effectiveness of eradication in rapid urease test was 29 (80.6%), histology and urea breath test 31 (86.1%), respectively. Adverse events and intolerance to treatment were not registered in any case.

**Conclusions:** Results of an open prospective study of course consistent eradication therapy rifaximin, amoxicillin against four weeks receiving pantoprazole showed its high efficacy and safety, it can be used as an alternative system of treatment-resistant forms of Hp associated diseases of the stomach.

Abstract no.: P10.25

**THE EFFECTS OF PREBIOTICS AND PREBIOTICS DURING THE ANTI-*HELICOBACTER PYLORI* TRIPLE THERAPY**

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**Aim:** Triple eradication therapy, consisting of two antibiotics and PPI is often accompanied by adverse events (AE) caused by inhibition of obligate enteric flora. To assess the effect of pre- and probiotics evaluated in an open comparative study of 63 patients with gastroduodenal pathology associated with *Helicobacter pylori* (Hp).

**Methods:** Sixty-three Hp positive patients were randomized into three groups: 1 (n = 23) – triple therapy, 2 (n = 18) – triple therapy + prebiotic and 3 (n = 22) – triple therapy + prebiotic and probiotic. The classic triple therapy consisted of PPI bid, clarithromycin 500 mg bid, and amoxicillin 1 g bid for 7 days. Patients two



groups received 4 weeks prebiotic zakofalk NMX (Dr. Falk Pharma) 1 tabl. three times a day. Zakofalk NMX contains 307 mg of calcium butyrate and 250 mg of inulin. Three groups received probiotic normoflorin D, contains a combination of bifidobacteria and lactobacilli at least 10 bil./mL, 60 mL per day for 4 weeks. AE were assessed using tests at 2 and 4 weeks after the beginning of eradication. Small intestine bacterial overgrowth (SIBO) was assessed at week 4 using hydrogen breath test.

**Results:** AE were recorded at 1, 2 and 3 groups of 6 (26.1%), 2 (11.1%) and 1 (4.5%) respectively. SIBO of 2 degrees was registered only in groups 1 and 2, and was 10 (43.5%) and 4 (22.2%) respectively.

**Conclusions:** The combination of prebiotics and probiotics on the background of triple therapy reduces the incidence of AE in the treatment of Hp associated gastrointestinal pathology.

Abstract no.: P10.26

#### PATIENTS WHO TAKE ERADICATION FOR *HELICOBACTER PYLORI* IN MEDITERRANEAN AREA

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**Objective:** In this study, we aimed to evaluate the effects of nutrition and lifestyle changes in *H. pylori* eradication in Turkey as a Mediterranean country.

**Methods:** The study included patients who admitted to Dokuz Eylul University Faculty of Medicine, Department of Gastroenterology between January and April 2010 and who have taken eradication treatment for *H. pylori* infection. Hundred and twenty-four patients that were suitable for the study were interviewed either face to face or by phone call.

**Results:** Hundred and twenty-four patients (66 female, 58 male), were involved in the study. The average age of the patients was 50.8 ± 13.7 We found that the increase in consumption of yoghurt, cheese, onion-garlic, green vegetables and fruits affected the success of *H. pylori* eradication in a positive way and this finding was compatible with the literature (respectively  $p = .002$ ,  $p = .003$ ,  $p = .019$ ,  $p = .035$ , and  $p = .01$ ). When effect of alcohol consumption was examined, we noticed that the success of eradication increased in patients who has drunk "Turkish alcoholic beverage-raki" 9 cc/day for at least 15 days ( $p = .001$ ). There was not any relation between *H. pylori* eradication and the consumption of beer, wine and any other alcoholic beverages as well as tea, coffee and fruit juices. We discovered that the consumption of cigarette and red meat affected *H. pylori* eradication negatively ( $p = .044$  and  $p = .027$  respectively).

**Conclusion:** Consumption of nutrients which are related to Mediterranean diet such as yoghurt, cheese, onion-garlic, green vegetables, fruits and raki increases the success rate of *H. pylori* eradication.

Abstract no.: P10.27

#### IMPORTANCE OF BRAIN-GUT AXIS AND SENSORY NEUROPEPTIDES IN GASTRIC ADAPTATION TO ASPIRIN IN *HELICOBACTER PYLORI* (*H. PYLORI*)-INFECTED MONGOLIAN GERBILS

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*H. pylori* and aspirin (ASA) are considered as independent risk factors for the peptic ulcerogenesis but their interaction in the stomach is controversial. ASA induces gastric lesions but with its prolonged ingestion, the gastric adaptation to its ulcerogenic action develops. We determined the effect of capsaicin-induced functional ablation of sensory nerves on the adaptation to ASA in *H. pylori*-infected gerbils. ASA was given once or five times to *H. pylori* (cagA+ vacA+, 5 × 10<sup>6</sup> CFU/mL i.g.)-infected Mongolian gerbils with intact or capsaicin-deactivated sensory nerves. At day 5 upon ASA treatment, the histology, *H. pylori*-culture, mucosal PGE2 generation, MPO activity, MDA and gastric blood flow (GBF) were assessed. The gastric *H. pylori* infection was detected in all animals at 8 weeks by histology, culture of gastric biopsy samples. ASA-induced gastric lesions were exacerbated in *H. pylori*-infected gerbils with capsaicin-sensory denervation followed by dramatic fall in the GBF, PGE2 generation, and the rise in MPO activity and MDA content. ASA given five times significantly reduced gastric lesions in gerbils with intact sensory nerves, decreased MPO and MDA content. This adaptation was significantly attenuated in *H. pylori*-infected gerbils and completely lost in those with capsaicin-denervation accompanied by the rise in MDA content, MPO activity and plasma IL-1beta and TNF-alpha levels. CGRP (10 µg/kg s.c.) added to ASA restored gastric adaptation.

**Conclusions:** 1, ASA damage is aggravated in *H. pylori*-infected stomach; 2, ASA-adaptation is impaired by sensory denervation suggesting that brain-gut axis and sensory nerves releasing vasodilatory neuropeptides such as CGRP may contribute to this phenomenon.

Abstract no.: P10.28

#### ERADICATION OF *HELICOBACTER PYLORI* FOR THE PREVENTION OF PEPTIC ULCER REBLEEDING. LONG-TERM FOLLOW-UP STUDY OF 1000 PATIENTS

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**Aim:** To evaluate the effect of *H. pylori* eradication on ulcer bleeding recurrence in a prospective, long-term study including 1000 patients.

**Methods:** Patients with peptic ulcer bleeding were prospectively included. Prior NSAID use was not considered an exclusion criteria. *H. pylori* infection was confirmed by rapid urease test, histology or <sup>13</sup>C-urea breath test. Several therapies were used. Afterwards, ranitidine 150 mg o.d. was administered until eradication was confirmed by <sup>13</sup>C-urea breath test 8 weeks after completing eradication therapy. Patients with therapy failure received a second or third course of therapy. Patients with eradication success did not receive maintenance anti-ulcer therapy, and were controlled yearly with a repeated breath test. NSAID use was not permitted during follow-up.

**Results:** Thousand patients were followed up for at least 12 months, with a total of 3263 patient-years of follow-up. Mean age 56 years, 75% males, 41% previous NSAID users. 69% had duodenal ulcer, 27% gastric ulcer, and 4% pyloric ulcer. Recurrence of bleeding was demonstrated in three patients at 1 year (which occurred after NSAID use in two cases, and after *H. pylori* reinfection in another one), and in two more patients at 2 years (one occurred after NSAID use and another after *H. pylori* reinfection). The cumulative incidence of rebleeding was 0.5% (95% CI = 0.16–1.16%), and the incidence rate of rebleeding was 0.15% (0.05–0.36%) per patient-year of follow up.

**Conclusion:** Peptic ulcer rebleeding does not occur in patients with complicated ulcers after *H. pylori* eradication. Maintenance anti-ulcer (antisecretory) therapy is not necessary if eradication is achieved. However, NSAID intake or *H. pylori* reinfection may cause rebleeding in *H. pylori*-eradicated patients.

Abstract no.: P10.29

#### THE TREND OF ERADICATION RATES OF FIRST LINE THERAPY AND SECOND LINE QUADRUPLE THERAPY CONTAINING METRONIDAZOLE FOR *HELICOBACTER PYLORI* INFECTION IN KOREA

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**Background and Aims:** Increasing tendency of antibiotic resistance in *Helicobacter pylori* infection has been reported. In the case of failure in first line therapy, quadruple therapy are recommended. But there is a difference of duration in second line therapy between the countries. Therefore, we evaluated the trend of eradication rate for recent 5 years and analyzed the eradication rate according to duration of treatment in second line therapy.

**Methods:** From January 2006 to December 2010, 782 patients who received triple regimens for two weeks were enrolled. Eradication regimens consisted of proton pump inhibitor, metronidazole, clarithromycin. Fourty seven patients who failed to the first line therapy, received quadruple therapy consisting of proton pump inhibitor, bismuth, metronidazole and tetracycline. Four to six weeks after completion of eradication treatment, <sup>13</sup>C-urea breath test and biopsies were performed to diagnose *H. pylori*.

**Results:** The eradication rate of first line therapy from the year 2006 to 2010 were 87.4%, 89.1%, 81.9%, 76.6%, and 70.5%, respectively. There was decreasing tendency of eradication rates of first line therapy in recent 5 years in Korea ( $p < .001$ ). The eradication rate of PBMT therapy was 86.7%. The eradication rate of one week PBMT treatment was 70.6% and that of two week PBMT therapy was 86.7%.

**Conclusion:** Our data suggested that the eradication rate of *H. pylori* had decreased in recent 5 years. New first line regimen will be needed in Korea for *H. pylori* eradication.



Abstract no.: P10.30

CLINICAL CHARACTERISTICS OF *HELICOBACTER PYLORI*-NEGATIVE BLEEDING ULCER

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**Objectives:** *Helicobacter pylori* (*H. pylori*, HP) infection was the most important cause of peptic ulcer. Recently, the proportion of patients with HP-negative (-) is increasing, that is considered to be a clinical significance. Our aims were to investigate proportion of HP (-) ulcer and compare to clinical characteristics between HP positive (+) and HP (-) groups in the bleeding gastroduodenal ulcer.

**Method:** We recruited consecutive patients with gastroduodenal bleeding ulcer who underwent HP diagnostic test (rapid urease test, urea breath test, tissue biopsy and serologic test) within 48 hours of admission (at least two tests were done). From June 2006 to October 2010, one hundred sixty three were enrolled. We examined recent use of NSAIDs, aspirin and clopidogrel within 4 weeks and defined as a drug user. Exclusion criteria were exposure to antibiotics or proton pump inhibitor within 4 weeks, previous gastrectomy, variceal or malignant ulcer bleeding. HP (-) ulcer was defined as all negative diagnostic test.

**Results:** Among 163 ulcer patients, drug nonusers with HP (+) was 36% (59/163), drug users with HP (+) was 35% (57/163). Drug nonusers with HP (-) was 15% (24/163), drug users HP (-) was 14% (43/163). Multiple ulcer was more frequent in HP (-) group ( $p = .035$ ). HP (-) group was older than HP (+) group ( $p = .06$ ). Ulcer size, transfusion blood volume, Galsgow-Blanchford score, comorbidity, drug history were not different between two groups.

**Conclusion:** About one third of patients with gastroduodenal bleeding ulcer showed HP negative infection. Multiple ulcers and old age were more prevalent in HP negative group.

Abstract no.: P10.31

RELATIONSHIP BETWEEN *H. PYLORI* INFECTION AND LOW-DOSE ASPIRIN DAMAGE IN UPPER GASTROINTESTINAL TRACT

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**Introduction:** It is well-known that low-dose aspirin (ASA) induces gastrointestinal toxicity. *H. pylori* infection (HP) also damages the GI tract. In our study we focused on the synergic effect between ASA and *H. pylori* infection on GI damage.

**Methods:** The subjects were 120 cardiovascular department outpatients with ASA (91) and without ASA (29). All subjects underwent endoscopy without ceasing their antiplatelet or anticoagulant therapy. Endoscopic gastric mucosal injury was determined in three gastric areas, the antrum, body and fornix. Gastric mucosal injuries detected in the endoscopy were evaluated by the Modified Lanza score (Ono S et al JCBN 2009). *H. pylori* infection was investigated using UBT.

**Results:** Lanza score was  $1.0 \pm 1.3$ ,  $0.7 \pm 1.2$  and  $0.3 \pm 0.7$  in the antrum, body and fornix respectively. Moreover the Lanza score in the antrum was  $0.5 \pm 1.0$ ,  $0.3 \pm 0.7$ ,  $1.8 \pm 1.5$  and  $0.2 \pm 1.6$  in HP (+)ASA (+), HP (+)ASA (-), HP (-)ASA (+) and HP (-)ASA (-) respectively. The Lanza score in the body was  $1.0 \pm 1.5$ ,  $0.4 \pm 1.1$ ,  $0.8 \pm 0.9$  and  $0.0 \pm 0.0$  respectively. The Lanza score was  $0.1 \pm 0.3$ ,  $0.1 \pm 0.3$ ,  $0.5 \pm 0.9$  and  $0.1 \pm 0.3$  respectively. Statistically *H. pylori* infection has a defensive effect and low-dose aspirin has an offensive effect with reference to gastric mucosal injury in the antrum (significant) and fornix (not significant). On the other hand, *H. pylori* infection and low-dose aspirin had an offensive effect with reference to gastric mucosal in the body (significantly).

**Conclusion:** There are synergic effects and negative synergic effects between *H. pylori* infection and ASA with reference to GI mucosal damage.

Abstract no.: P10.32

THE FREQUENCY OF *HELICOBACTER PYLORI* CONTAMINATION AND THE STRUCTURE OF GASTROINTESTINAL TRACT'S MUCOSAL'S LESIONS IN PATIENTS OF RHEUMATOLOGICAL PROFILE INDUCED BY ADMINISTRATION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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**Purpose:** To evaluate the frequency of contamination of the gastric mucosa (GM) *Helicobacter pylori* (Hp) and the structure of the digestive tract lesions in patients with rheumatological profile (RP) during therapy with nonselective nonsteroidal anti-inflammatory drugs (n-NSAID).

**Material and Methods:** A retrospective analysis of histories 844 patients receiving more than 8 weeks of n-NSAI. The structure of patients: 246 (29.1%) patients with rheumatoid arthritis and 598 (70.9%) patients with osteoarthritis. Were rated 830 (98.3%) endoscopic protocols, 640 (75.8%) the results of urease test and morphological study of gastric and duodenal biopsy materials.

**Results:** Erosive and ulcerative lesions of the gastric mucosa and duodenum were recorded in 716 (86.3%) patients. The structure of the NSAID lesions: typical ulcerative defects in the stomach were detected in 33 (3.9%) patients, erosive gastritis associated with Hp in 221 (26.2%), erosive lesions duodenum in 462 (55.7%) patients, respectively. In 219 (47.4%) cases were localized erosion in the distal duodenum.

**Conclusions:** The high percentage of erosive and ulcerative lesions of GM and the distal duodenum determines the necessity of inclusion in the examination protocol of rheumatological patients duodenoscopy, urease and morphological tests.

Abstract no.: P10.33

## OPTIMIZATION OF THE EXAMINATION PROTOCOL OF RHEUMATOLOGICAL PATIENTS RECEIVING LONG-TERM NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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**Purpose:** To estimate efficacy of the instrumental examination protocol of rheumatological patients receiving long-term non-steroidal anti-inflammatory drugs (NSAID) with chronic anemia.

**Material and Methods:** In order to optimize the diagnosis of NSAID-induced lesions of the gastrointestinal tract and clarify the causes of anemia were examined 18 patients with osteoarthritis (mean age  $52.6 \pm 9.7$  years) receiving NSAIDs for more than 12 weeks. The examination program included: video endoscopy (VE) gastroduodenal, videocapsular endoscopy (VCE) Given M2A Plus and urea breath test. When VEs erosive gastropathy diagnosed in 8 (44.4%) cases, erosive and hemorrhagic duodenopathy in 6 (33.3%) cases. At VCE in 11 (61.1%) cases revealed erosive-haemorrhagic enteropathy segmental localization, 7 (63.6%) cases in the ileum. Contamination of the gastric mucosa in *Helicobacter pylori* stated in the 7 (38.9%) cases.

**Results:** The suggested examination protocol of rheumatological patients increase the diagnosis of erosive NSAID enteropathy, explains the cause of chronic anemia and improves treatment.

# P11 Immunity, Animal Models, Vaccines, Probiotics, and Other Helicobacters

Abstract no.: P11.01

## HIGH PREVALENCE OF ENTEROHEPATIC *HELICOBACTER* SPP. IN AGED MACAQUES WITH INTESTINAL ADENOCARCINOMA

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Intestinal adenocarcinoma is the most frequently diagnosed neoplasm in aged macaques. Fecal samples of 34 (aged 25–32 years) Rhesus monkeys and tumor tissues from a single monkey diagnosed with intestinal adenocarcinoma were analyzed by a combination of PCR and microaerobic culture for the presence of *Helicobacter* species. Nine (8F,1M) of the macaques had a history of successful surgical resection of intestinal tumors. Using *Helicobacter*-species specific primers, C05 and C97, which amplify a portion of 16S rRNA gene, 32 of 34 (94%) fecal samples were tested positive for *Helicobacter* spp. Isolates of *Helicobacter* spp. were obtained from 27 (79%) of the fecal samples by microaerobic culture. Of the nine Rhesus with a history of intestinal tumor resection, seven of nine (78%) were *Helicobacter* spp. positive by PCR. An analyses using a combination of restriction fragment length polymorphism (RFLP), 16S rRNA gene sequencing, and BLAST search revealed that the isolates obtained from these samples represent two previously identified *Helicobacter* species -*Helicobacter* sp. MIT 03-7674C and *Helicobacter macacae* MIT 99-10773 (99% sequence homology). *Helicobacter* sp. MIT 03-7674C represents the species identified in 67% (18 of 34 samples) of the culture positive samples, while *Helicobacter macacae* MIT 99-10733 represents 30% (8 of 34) of the positive samples. A single fecal sample as well tumor samples from a monkey diagnosed with adenocarcinoma were identified as containing a mixture of the two *Helicobacter* species. Studies should be undertaken to ascertain whether *Helicobacter* spp. associated inflammation promotes intestinal carcinogenesis in macaques.

Abstract no.: P11.02

## *HELICOBACTER BILIS* STRAIN ATCC 43879 INDUCED TYPHLOCOLITIS IN C57BL/6 IL-10<sup>-/-</sup> MICE

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*Helicobacter bilis* strain ATCC 43879 was originally isolated in 1988 from a human with diarrhea and was designated as “*Flexispira rappini*”. In 2000, it was classified as *Helicobacter Flexispira* taxon 8 and grouped with *H. bilis* taxa in 2005. *H. bilis* strains have been linked to a wide spectrum of diseases in various hosts with zoonotic implications. However, the role of *H. bilis* in the pathogenesis of gastrointestinal diseases is unknown. To evaluate the pathogenic potential of *H. bilis* strain ATCC 43879, an infection study was performed using *Helicobacter* species free C57BL/6 IL10<sup>-/-</sup> mice. Mice were infected orally with *H. bilis* strain ATCC 43879 and control mice were sham dosed, with equal proportions of either sex per group. At 12 weeks post infection, all mice were evaluated for *H. bilis* colonization and pathology. *H. bilis* ATCC 43879 colonized the cecum, colon, and stomach but not the liver as assessed by bacterial culture and quantitative PCR analysis. *H. bilis* ATCC 43879 induced severe typhlocolitis in IL10<sup>-/-</sup> mice. The cecum had the highest *H. bilis* colonization levels which correlated with the most severe pathological lesions. The histopathology was characterized by severe inflammation, epithelial defects, edema, hyperplasia, and high grade dysplasia with progression to carcinoma. These results demonstrate that the human *H. bilis* strain can be successfully adapted in an IL-10<sup>-/-</sup> mouse model to study the pathogenic potential of this enterohepatic *Helicobacter* spp and its possible association with IBD and hepatobiliary diseases in humans.

Abstract no.: P11.03

## SYSTEM FOR HETEROLOGOUS ANTIGENS DISPLAY ON SURFACE OF BACILLUS SUBTILIS SPORES

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Bacterial spores have been used to expose heterologous proteins. It provides a powerful biological tool with variety of applications, including the development of bioadsorbents and biocatalysts, the identification of new antibiotics and antigens and the delivery of vaccines and drugs.

We used system based on *Bacillus subtilis* spores to expose two subunits of urease (UreA and UreB) of *Helicobacter acinonychis*. This bacterium is recognized as a useful model to study the mechanism of virulence of closely related human pathogen – *Helicobacter pylori*.

To create recombinant spores which express urease subunits, we used spore coat proteins named CotB, CotC and CotG, and compared the efficiency of display obtained with those carriers.

It occurred that UreA was efficiently expressed when fused with CotC and CotG but was not displayed on the spore surface. In the case of CotB, it was expressed less efficiently but was surface exposed. In different manner behaved UreB, which was efficiently expressed and displayed only when fused with CotC.

So we can conclude that the efficiency of surface expression and display mainly depends on the heterologous protein. What is more, different coat proteins should have been tested to define the most appropriate carrier.

Immunological experiments are now in progress to check whether the surface display of an antigen is essential requirement for inducing an immune response. Besides, we consider if a more efficient approach is to put possible high number of recombinant molecules on the spores coat even though these are not exposed on the spore surface.

Abstract no.: P11.04

## NOVEL VACCINE AGAINST *HELICOBACTER PYLORI*: THE EFFECT OF THE DELIVERY SYSTEM

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DNA vaccines provide several important advantages over current vaccine strategies such as live or attenuated vaccines, because they mimic the effects of natural infection in their ability to endogenously express foreign protein and also due to its unique ability to induce humoral as well as cellular immune responses. With that respect, we have developed multigenic *Helicobacter pylori* DNA-vaccines based on pathogenic relevance. Three antigens were chosen for the DNA-vaccine construction: the chaperonin GroEL; the external membrane protein HombB; and the highly virulent marker VacA protein. The plasmid backbone of our constructions contains the nucleotide sequence coding for 50 amino-acid residues long fragments, each being representative of the most conserved and immunogenic region of each of the three target proteins.

The constructs were first evaluated in vitro by transfection efficacy assays using the AGS human gastric cell line.

In vivo evaluation the multigenic construct was performed either as recombinant protein as DNA based vaccine. In order to protect the antigens of proteolysis degradation was developed a delivery system based on chitosan nanoparticles.

Th1 or Th2 responses were measured by cytokine profiles produced by antigen-stimulated cells. As markers of Th2 were evaluated the production of IL-4 and IL-6 and IFN $\gamma$  and IL-2 was measured as Th1 cytokines. It was also measured the ratio of antigen-specific IgG2a-IgG1 in the serum samples. The specific IgA was also evaluated in order to determine the mucosal immunity.

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Abstract no.: P11.05

## IMMUNOPROTEOMICS OF *HELICOBACTER PYLORI* RELATED TO DIFFERENT GASTRIC PATHOLOGIES

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*H. pylori* infection is linked to development of ulcer, atrophic gastritis and adenocarcinoma. Immunoproteomics has been used to detect *H. pylori* antigens,

which may act as potential markers for neoplastic diseases a may be used in specific serological tests. Immunoproteome assay was used to identify *H. pylori* antigens, recognized by sera from patients with peptic ulcer, bleeding peptic ulcers, gastric cancer, and dyspepsia. We performed proteomic maps of *H. pylori* strain 23Ca3 (patient with gastric cancer), probed against single sera from three groups of *H. pylori* -positive patients (peptic ulcer, gastric cancer, and dyspepsia). Immunoreactive spots were identified by LC/MSI-MS/MS. In this study, we detected eleven immunoreactive spots with the sera from three groups of patients. 50S ribosomal protein L7/L12 was the only proteina recognized by the three groups of sera, which highlights it as a protein useful in the diagnosis of *H. pylori* infection regardless of the pathology in the stomach. Additionally, we found proteins that share recognition in sera from patients with gastric cancer and dyspepsia. These immunoreactive spots may be promising for developing specific serological tests to differentiate patients with gastritis at high risk for gastric cancer, to be evaluated in prospective investigations.

Abstract no.: P11.06

#### THE INFLUENCE OF BLOCKING TIM-3 SIGNAL PATHWAY ON IMMUNE PROTECTION OF *H. PYLORI* VACCINE AND TH IMMUNE RESPOND

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**Objective:** To observe the influence of blocking Tim-3 signal on immune protection of Hp vaccine and Th respond.

**Methods:** BALB/c mice were divided into three groups and immunized by: 1. Control group; 2. Hp vaccine; 3. Anti-Tim-3 antibody pretreatment + Hp vaccine; At 4 weeks after the last immunization, the mice from 1 and 3 groups were challenged by Hp quartic. At 4 weeks after the last challenge, mice were sacrificed and sample were collected. Hp, the level of cytokine, Foxp3<sup>+</sup>Treg in gastric mucosa were determined.

**Results:** 1, Hp colonized was significantly lower in group with anti-Tim-3 antibody pretreatment than that in group without pretreatment ( $p < .05$ ). 2, Inflammatory of mice of Hp vaccine was higher than that in control ( $p < .01$ ), and in group with anti-Tim-3 antibody pretreatment were higher than group without pretreatment ( $p < .05$ ). 3, The level of Th1 cytokine in mice of Hp vaccine were significantly higher than that in control ( $p < .05$ ), and in group with anti-Tim-3 antibody pretreatment were significantly higher than those in groups without pretreatment ( $p < .05$ ); The level of Th2 cytokine in mice, there were no significant difference among all groups ( $p > .05$ ). 4, The Foxp3<sup>+</sup>Treg in mice of Hp vaccine were significantly higher than that in control ( $p < .01$ ), and in group with anti-Tim-3 antibody pretreatment were significantly lower than those in groups without pretreatment ( $p < .01$ ).

**Conclusion:** Blocking Tim-3 signal pathway can improve the Hp vaccine protection and promote Th1 immune respond and reduced the numbers of CD4+CD25+Foxp3+Treg, this could be the mechanism that it enhanced Hp vaccine immune protection.

Abstract no.: P11.07

#### THE INFLUENCE OF BLOCKING CD25 ON IMMUNE PROTECTION OF *H. PYLORI* VACCINE AND TH IMMUNE RESPOND

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**Objective:** To observe the influence of blocking CD25 on immune protection of Hp vaccine and Th respond.

**Methods:** BALB/c mice were randomly divided into three groups: 1. Control; 2. Hp vaccine; 3. Anti-CD25 antibody pretreatment + Hp vaccine. At 4 weeks after immunization, mice from 2 and 3 groups were challenged by Hp. At 4 weeks after challenge, Hp, the level of cytokine, Foxp3+Treg in gastric mucosa were determined.

**Results:** 1, Hp colonized was significantly lower in group with anti-CD25 antibody pretreatment than that in group without pretreatment ( $p < .05$ ). 2, Inflammatory degree in mice of Hp vaccine was higher than that in control ( $p < .01$ ), and in group with anti-CD25 antibody pretreatment were higher than group without pretreatment ( $p < .05$ ). 3, Level of Th1 and Th17 cytokine in mice of Hp vaccine were significantly higher than that in control ( $p < .05$ ), and in group with anti-CD25 antibody pretreatment were significantly higher than those in groups without pretreatment ( $p < .05$ ); Level of Th2 cytokine, there were no significant difference between in control and vaccine ( $p > .05$ ), and between in group with anti-CD25 antibody pretreatment and without pretreatment ( $p > .05$ ). 4, Foxp3+Treg in mice of Hp vaccine were significantly higher than that in control

( $p < .01$ ), and in group with anti-CD25 antibody pretreatment were significantly lower than those in groups without pretreatment ( $p < .05$ ).

**Conclusion:** Blocking CD25 can improve the Hp vaccine protection and exacerbate the inflammation in mice of Hp vaccine; and can promote Th1 and Th17 respond and reduced Foxp3+Treg, this could be mechanism that it enhanced Hp vaccine immune protection.

Abstract no.: P11.08

#### NUTRACEUTICALS: A NEW THERAPEUTIC APPROACH AGAINST *HELICOBACTER PYLORI* INFECTION?

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**Background and Aim:** *H. pylori* induces severe gastric chronic inflammation and is the cause of gastritis, peptic ulcer and a major risk factor for gastric cancer. The aim of the study was to investigate the anti-inflammatory effect of two nutraceuticals in Hp-infected mucosa.

**Materials and Methods:** Eighteen C57BL/6 mice were inoculated with Hp SS1 by gavage three times with  $3 \times 10^9$  viable cells. Mice were then treated with either PBS, curcumin (10 mg/mouse) or Symbiotic 2000<sup>®</sup> (50 mg/mouse), three times per week. Half of the infected and three non-infected mice were euthanized at week 6, the remaining at week 18. Gastric samples were removed for immunohistochemistry and PCR array (inflammatory response and immunity pathway) analysis (Sabiosciences, Qiagen).

**Results:** All the 18 mice were Hp positive by immunohistochemistry. The production of the chemokines CCL2, CCL5, CCL20, CCL25, CXCL1 and CXCL11 was significantly up-regulated at both week 6 (range of fold-change 4.3–718) and week 18 (range of fold-change 16–1192). Similarly, the expression of the proinflammatory cytokines IL-1 $\beta$ , IL6, IL9, IL10, IL23, TNF $\alpha$  and INF $\gamma$  was significantly augmented (range of fold-change 1338–8251). The treatment with either curcumin or symbiotic drastically decreased the expression of all these mediators, restoring their levels to those similar to the non-infected mice.

**Conclusions:** The present study confirmed that Hp infection induces a strong inflammatory response. Curcumin and Symbiotic treatments exerted a significant anti-inflammatory effect in Hp-infected mucosa.

The supplementation of diet with these nutraceuticals may be a novel clinical approach against gastric inflammation induced by Hp infection.

Abstract no.: P11.09

#### *HELICOBACTER PYLORI* INFECTION: THE ROLE OF INTESTINAL MICROBIOTA MODULATION

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Intestinal microbiota may influence inflammation in the host. The aim of the present study was to explore the role of modulation of intestinal microbiota in the outcome in the *Helicobacter pylori* (Hp) gastric inflammation.

Twenty five C57BL/6 male mice were separated in three groups: Control group (CG) n = 5 Infected group (IG) n = 10 and Symbiotic 2000<sup>TM</sup> (SG) n = 10. CG received PBS by gavage; IG and SG were inoculated intragastrically with *H. pylori* SS1 cell suspension ( $10^9$  CFU/mL). Then, mice were treated either with PBS (CG and IG) or Symbiotic 2000<sup>TM</sup> (SG). Five mice from each group were sacrificed at week 6 and the other at week 18. At each time samples were collected from: gastric tissue to immunohistochemistry and histological evaluation (HE) and faeces to evaluate intestinal microbiota composition by FISH, targeting 14 bacterial groups.

IG and SG groups were *H. pylori* positive by immunohistochemistry. Microbiota analysis: In IG there were significant changes in the microbiota composition, comparing to CG. At week 6 there were changes in 12 of 14 (85.7%) bacterial groups, while at week 18 there was a change in 6/14 (42.9%). In SG, there were changes in 7/14 (50.0%) at week 6, and in 4/14 (28.6%) at week 18, comparing to CG. Histology: IG at weeks 6 and 18 has 40% (2/5) of intramucosal inflammation and SG at the same end points has 0% (0/5).

These results suggest that modulation of the intestinal microbiota by Symbiotic 2000<sup>TM</sup> may influence the outcome of Hp gastric inflammation.



Abstract no.: P11.10

DOCOSAHEXAENOIC ACID INHIBITS *HELICOBACTER PYLORI* MICE GASTRIC COLONIZATIONJ. C. Machado,\* M. Correia,\* V. Michel,<sup>†</sup> M. Heurre,<sup>†</sup> R. Seruca,\* C. Figueiredo\* and E. Touati<sup>†</sup>\*IPATIMUP, Porto, Portugal; <sup>†</sup>Institut Pasteur, Paris, France

**Background:** Drug-resistant strains of *H. pylori* and non-compliance to therapy are the major causes of *H. pylori* eradication failure. We have already demonstrated that docosahexaenoic acid (DHA), a polyunsaturated fatty acid, inhibit in a dose-dependent way *H. pylori* growth.

**Aims:** Our main aim was to assess the efficacy of DHA to inhibit *H. pylori* gastric colonization in a mouse model over time. We also compared the effectiveness of standard therapy (ST) and DHA in *H. pylori* eradication and recurrence prevention success.

**Results:** We performed an in vivo gastric colonization assay in which mice were infected with *H. pylori* and given DHA orally, ST or a combination of both. Our data demonstrate that DHA decreases *H. pylori* gastric colonization and gastric mucosa inflammation,  $p < .05$ . Furthermore, the addition of DHA to ST results in lower recurrence of *H. pylori* infection in a mouse model.

**Conclusions:** In conclusion, DHA inhibits *H. pylori* mice gastric colonization, and associates with lower recurrence of *H. pylori* infection. These observations may pave the way to evaluate DHA as a co-adjuvant agent in *H. pylori* eradication treatment.

**Relevance:** Although the established therapeutical regimens for *H. pylori* eradication have been proved effective, treatment failure still occurs and is increasingly common. It should be emphasized that DHA is not only less toxic than standard therapeutic agents, but also well tolerated, and therefore could be administered for longer periods of time.

Abstract no.: P11.11

ANTAGONISTIC ACTIVITY OF LACTOBACILLUS LBO-2 AGAINST *HELICOBACTER PYLORI* AND SALMONELLAB. Mandkhaj,\* J. Sarantuya,<sup>†</sup> N. Bira,<sup>†</sup> S. Demberel,<sup>‡</sup> J. Dugersuren<sup>‡</sup> and D. Tselmen<sup>§</sup>\*Ach Medical Institute, Ulaanbaatar, Mongolia; <sup>†</sup>HSUM, Ulaanbaatar, Mongolia;<sup>‡</sup>Mongolian Veterinary Institute, Ulaanbaatar, Mongolia; <sup>§</sup>Institute of medical sciences, Ulaanbaatar, Mongolia

**Background and Aims:** Prevalence of *H. pylori* antibiotic resistance is increasing worldwide and it is the main factor affecting efficacy of current therapeutic regimens. However, national probiotic therapeutic treatment for *H. pylori* eradication has not been studied in Mongolia. Therefore aim of study was in vitro testing of the potential inhibitory effect of Lactobacillus LBO-2 strain on *H. pylori* and Salmonella strains.

**Method:** Lactobacillus LBO-2 strain isolated from Mongolians traditional milk products. We recovered *H. pylori* from gastric biopsies were obtained gastrointestinal endoscopy from four patients with gastric ulcer disease; the isolates were then cultured in selective pylori agar and antibiotic sensitivity was examined using Etests. Parallel, we examined five Salmonella strains causing human enteric disease. An in vitro disk diffusion assay was employed to assess the Lactobacillus LBO-2 and cell free supernatant LBO-2 were anti-*H. pylori* and Salmonella activity. The diameters of inhibition zones around the disk measured, inhibition zones 8 mm or more were scored as positive.

**Result:** Antibiotic resistance of *H. pylori* were as following: 1 strain for clarithromycin and tetracycline, two strains for erythromycin, three strains for metronidazole. LBO-2 strain and its cell free supernatant did not inhibit *H. pylori* growth. However LBO-2 strain had low sensitivity inhibition zone against *Sal. typhimurium*, *Sal. enteritidis*, *Sal. glostry* and medium sensitivity against *Sal. bovismorbificans*, *Sal. spp.* The cell free supernatant was assessed medium and good sensitivity inhibition zone.

**Conclusion:** Lactobacillus LBO-2 strain from our traditional milk products had inhibitor activity against *Salmonella spp.*, but not inhibit *H. pylori*. Furthermore the microorganisms should be investigated in combination with other national probiotic species.

Abstract no.: P11.12

THE EVOLUTIONARY IMPACT OF LACTOBACILLI ON *H. PYLORI* AND GASTRIC ACID SECRETION: DID A CENTURY OF DIETARY CHANGE ALTER THE GASTRIC MICROBIOTA?

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Since the mid 1980s, it has been accepted that *Helicobacter* species are the only organisms capable of surviving the hostile mammalian gastric environment. Here

we present the evidence that *Helicobacter* is not the only bacterial genus able to colonize the gastric mucosa. *Lactobacillus* species, which have recently been shown to colonize the human stomach, are probably indigenous to the stomach and commonly co-existed with *Helicobacter pylori* for millennia. Lactic acid produced by *Lactobacilli* can impact the survival of *Helicobacter pylori* as well as modulate gastric physiology as a natural antisecretory agent. *Helicobacter* species with its potential detrimental effects and the *Lactobacillus* species with potential beneficial effects acting as a natural gastric antisecretory probiotic, have co-existed in the stomach throughout human evolution, serving as a good example of self-regulating bacterial co-existence. Changes in the gastric microbiota with the emergence of the industrial evolution have subsequently lead to an increasing gastric secretory capacity, dominance and acquisition of pathogenic genes by *Helicobacter pylori* in humans, resulting in the sequential emergence of the "modern" acid related diseases. We propose that the diminished prevalence and loss of *Lactobacillus spp.* over the past century as a result of the modernization of our diet and environment have contributed to a dominance of *H. pylori*-induced inflammation, hyperchlorhydria and subsequently the increase of peptic ulcer disease and GERD over this time. Thus, *Lactobacilli* should be explored as a normal organism of the gastric microbiota and as such positively impact *Helicobacter pylori*-induced inflammation and potentially acid-related diseases.

Abstract no.: P11.13

DETECTION OF ENTEROHEPATIC AND GASTRIC *HELICOBACTER SPP.* IN WILD CHIMPANZEES (*PAN TROGLODYTES*) AND WESTERN LOWLAND GORILLAS (*GORILLA GORILLA*)B. Flahou,\* D. Modry,<sup>†,‡</sup> K. Pomajbíková,<sup>†</sup> K. Petzelková,<sup>§,¶</sup> A. Smet,\* R. Ducatelle,\* F. Pasmans,\* R. Sá\*\*<sup>††</sup> and F. Haesebrouck\*\*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; <sup>†</sup>Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Brno, Brno, Czech Republic; <sup>‡</sup>Biology Center, Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; <sup>§</sup>Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic; <sup>¶</sup>Liberec Zoo, Liberec, Czech Republic; \*\*Biodiversity and Ecological Processes Group, School of Biosciences, Cardiff University, Cardiff, UK; <sup>††</sup>Anthropology Department, Human and Social Sciences Faculty, Universidade Nova de Lisboa, Lisboa, Portugal

Little is known about the prevalence of gastric and enterohepatic *Helicobacter (H.)* species in endangered wild primates. Fresh faecal samples from 68 chimpanzees (*Pan troglodytes*) and 21 gorillas (*Gorilla gorilla*) were screened for the presence of *Helicobacter spp.* After DNA extraction, a genus-specific PCR was performed, amplifying part of the 16S rRNA gene. In wild gorillas of the Central African Republic, *Helicobacter* DNA was detected both in unhabituated and human-habituated wild animals. In wild, unhabituated chimpanzees from Guinea Bissau and wild but human-habituated chimpanzees from Uganda, *Helicobacter* DNA could be detected in the majority of animals. Also chimpanzees housed in sanctuaries in both Cameroon and Kenya were often *Helicobacter*-positive. A selection of *Helicobacter*-positive samples from all groups was used for species identification by cloning and sequencing. 16S rRNA gene sequences with high similarity to that of *H. troglodytes*, *H. typhlonicus*, *H. ganmani* and *H. rodentium* were observed in gorillas. For all groups of chimpanzees, the vast majority of obtained 16S rRNA gene sequences showed 99% similarity with *H. fenelliae/H. cinaedi*. Finally, a number of gorillas and chimpanzees also tested positive using a primer set designed to amplify part of the urease A and B genes of gastric helicobacters. Sequence analysis of all PCR products revealed a similarity of 86% or less with urease gene sequences of known gastric helicobacters, suggesting these bacteria constitute a new *Helicobacter* taxon/species. Besides a possible risk for the endangered species themselves, a possible zoonotic role of these gastric and enterohepatic helicobacters should be considered.

Abstract no.: P11.14

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *HELICOBACTER SUIIS* STRAINS

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*Helicobacter suis* is a very fastidious porcine gastric pathogen, which is also considered to be of zoonotic importance. In vitro antimicrobial susceptibility can not be determined using standard assays, as this agent only grows in a biphasic medium with an acidic pH. Therefore, a combined agar and broth dilution method was used to analyse the activity of nine antimicrobial agents against nine *H. suis* isolates. After 48 hour microaerobic incubation, minimal inhibitory concentra-



tions (MICs) were determined by software-assisted calculation of bacterial growth. Only for enrofloxacin a bimodal distribution of MICs was demonstrated, indicating acquired resistance in one strain, which showed an AGT → AGG (Ser → Arg) substitution at codon 99 of *gyrA*. In conclusion, the assay developed here is suitable for determination of the antimicrobial susceptibility of *H. suis* isolates, although activity of acid sensitive antimicrobial agents may be higher than predicted from MIC endpoints.

Abstract no.: P11.15

#### DETECTION OF VIABLE *HELICOBACTER SUI*S IN PORK BY A COMBINATION OF ETHIDIUM MONOAZIDE (EMA) AND REALTIME-PCR

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The prevalence of gastric infections in humans with non-*H. pylori* helicobacters (NHPH), also referred to as *H. heilmannii sensu lato*, is probably underestimated. Although human infections with these micro-organisms most likely originate from animals, the exact transmission routes remain largely unknown. Since it has been shown that direct contact with dogs, cats and pigs is a significant risk factor for contracting these infections, it is remarkable that the pig-related species *H. suis* is the most prevalent gastric NHPH in humans. This might indicate that consumption or manipulation of contaminated pork is a source of infection. The presence of viable *H. suis* bacteria in pork is a prerequisite for foodborne infections. However, cultivation of *H. suis* bacteria from samples is highly laborious and insensitive. In order to determine whether or not *H. suis* can act as a foodborne pathogen we first developed a quantitative detection technique which differentiates viable from dead *H. suis* bacteria. This approach combines the viable/dead stain ethidium monoazide (EMA) and real-time PCR. Using the EMA real-time PCR, we demonstrated the presence of viable *H. suis* bacteria in pork samples. This finding suggests that pork is a source of *H. suis* infections for humans.

Abstract no.: P11.16

#### HISTONE-LIKE DNA BINDING PROTEIN (HH-15) FOR THE DIAGNOSIS OF *HELICOBACTER HEPATICUS* INFECTION

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**Background and Aim:** We developed a monoclonal antibody HR11-51 with high specificity for *H. hepaticus*. MAb HR11-51-immunoreactant was molecular weight of 15 kDa, and then named as HH-15 (*H. hepaticus* 15 kDa antigen). We purified HH-15 and examined the possibility of HH-15 to diagnose *H. hepaticus* infection. We also studied the structure of HH-15 and analyzed the epitope to HR11-51.

**Methods:** HH-15 antigen was highly purified by immunochromatography. HH-15-direct sandwich ELISA (HH-15-ELISA) was prepared in which HH-15 was immobilized on ELISA plates. Accuracy of HH-15-ELISA was examined using sera obtained from mice inoculated with *Helicobacter* spp. To identify the epitope to HR11-51, HH-15 mimotope peptides were synthesized and analyzed by ELISA and Western blot using HR11-51 as the first antibody.

**Results:** By using *Helicobacter* spp. inoculated mouse sera, specificity and sensitivity of HH-15-ELISA were estimated as 95.2% (20 of 21) and 95.7% (22 of 23). The 30 amino acid residues of N-terminal sequence corresponded to that of histone-like DNA binding protein of *H. hepaticus*. In direct sandwich ELISA, a mimotope peptide (Lys35-Lys94) was strongly reacted with HR11-51. The 34 amino acids peptide of C-terminal sequence (Gly61-Lys94) was also reacted with HR11-51 by Western blot whereas the 60 amino acids peptide of N-terminal sequence (Met1-Thr60) showed no reactivity.

**Conclusion:** The epitope domain recognized by MAb HR11-51 presents in C-terminal of histone-like DNA binding protein of *H. hepaticus*. This protein could be a potential indicator for *H. hepaticus* infection.

Abstract no.: P11.17

#### *HELICOBACTER* SPECIES AND PRECANCEROUS LESIONS OF THE GALLBLADDER: PRELIMINARY RESULTS FROM FRANCE

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**Background:** Experimental animal studies and human case series suggest that a causal association between *Helicobacter* species and gallbladder diseases, including cancer, is plausible.

**Objective:** To assess the association between *Helicobacter* species detection by PCR and severity of precancerous lesions of the gallbladder in France.

**Methods:** Since 2010, a cross-sectional epidemiological study is ongoing within two surgery units of a university hospital in Lyon, France. It is expected to collect specimens from 250 consecutive patients undergoing scheduled surgery requiring cholecystectomy, regardless of indication. Bile samples and biopsies from three gallbladder anatomical areas (neck, body, and fundus) are collected under strict sterile conditions to undergo independent diagnostic testing. *Helicobacter* detection in bile and tissue is based on two broad-spectrum and three species-specific (2 *bilis* and 1 *hepaticus*) 16s rDNA PCR tests. All generated amplicons are sequenced for species identification.

**Results:** So far, 210 patients have been recruited and specimens from 32 selected patients have been analysed for the presence of *Helicobacter* DNA. Histopathological diagnoses included dysplasia (n = 1), intestinal metaplasia (n = 4) and pyloric metaplasia (n = 13). To date, no *Helicobacter*-like DNA sequences were detected in bile samples.

**Conclusion:** Our preliminary results suggest that *Helicobacter* species are not detected in the gallbladder of patients undergoing cholecystectomy in France. Although France is a low-risk country for gallbladder cancer, evidence of precancerous lesions (dysplasia and intestinal metaplasia) is found in a minority of patients. Further testing of bile samples and frozen biopsies is ongoing. We plan to conduct a companion study in a high-risk country.

Abstract no.: P11.18

#### IS *HELICOBACTER PYLORI* INFECTION RELATED WITH THE DISEASE ACTIVITY OF ULCERATIVE COLITIS?

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**Objectives:** Epidemiologic studies have shown the lower prevalence of *Helicobacter pylori* (*H. pylori*) infection in patients with ulcerative colitis (UC), and suggested a protective effect of *H. pylori* on the immunologic disease. However, it is unclear whether *H. pylori* infection is related with the UC. The aim of this study was to investigate the effect of *H. pylori* infection on the severity of UC.

**Methods:** Among 334 patients diagnosed and treated with UC in our institution, 158 patients were eligible for this study. Their medical records were retrospectively reviewed and the severity of UC was assessed by endoscopic findings, clinical symptoms, treatment regimen, and Mayo score.

**Results:** The prevalence of *H. pylori* infection was 23.4% (37/158) in patients with UC. Endoscopic severity of UC was not influenced by the status of *H. pylori* infection. Symptom severity assessed by stool frequency, rectal bleeding severity, and admission history due to UC aggravation was not related with *H. pylori* infection. The steroid use for the induction therapy was not different between patients with and without *H. pylori* infection (45.9% vs 54.5%,  $p = .359$ ). When UC patients were classified by Mayo score, there was no relationship between the *H. pylori* infection and UC severity.

**Conclusions:** The prevalence of *H. pylori* infection in patients with UC is lower than general population in Korea. However, the status of *H. pylori* infection did not affect the UC severity, viewed in endoscopic, clinical severity, treatment regimen and Mayo Score.

Abstract no.: P11.19

**GASTRIC ULCERATION SYNDROME IN *TAPIRUS TERRESTRIS* ASSOCIATED *HELICOBACTER* LIKE ORGANISMS**

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Gastric ulceration syndrome is a common pathology in domestic animals and wild life in captive. Infection with *Helicobacter* genus plays a predominant role in this syndrome, although this syndrome has received poorly study in wild animals under captive conditions. We describe a multidisciplinary study of gastritis syndrome and ulceration in *Tapirus terrestris* associated with *Helicobacter Like Organisms*. A 12-year-old male *Tapirus terrestris* died after prolonged anorexia, chronic emesis and weight loss in the Zoological Park "El Pinar", Caracas, Venezuela. On necropsy, the stomachs showed dilated gastric and ulcerations of the gastric mucosa (squamous mucosa and squamous glandular). Others organs did not have any significant alterations. Urease Test: the rapid urease test (commercial kit) was performing of gastric tissue. Microscopically, there was gastritis erosive and ulcerative gastric and duodenal enteritis with plasma cells and severe lymphocyte infiltration. The stomach samples were positive for Warthing-starry stain, and showed spiral shape similar a *Helicobacter Like Organisms*. Urease activity was then demonstrated in gastric tissue. Our results show that *Helicobacter Like Organisms* can cause infection in wild species in captivity, although it is possible that the infection was accidental in this case. This is the first report on *Helicobacter Like Organisms* infection in *Tapirus terrestris* in Venezuela.

Abstract no.: P11.20

**DETECTION OF *HELICOBACTER* LIKE ORGANISMS IN GASTRIC MUCOSA OF A ZEBRA (*EQUUS QUAGGA*) REPORT OF CASE**

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The aim of this study was to detection of *Helicobacter* Like Organisms in gastric mucosa of a zebra (*Equus quagga*). A six old zebra (*Equus quagga*), in captive with history of sudden death was brought into the postmortem room of the "Las Delicias" Zoological, Maracay, Aragua State-Venezuela. Samples of gastric tissues were collected. Tissue sections were prepared and stained with Hematoxylin & Eosin (H&E) for light microscopy. Additionally the special staining procedure of Wharting-Starry was also carried out. Urease Test: the rapid urease test (commercial kit) was perform of gastric tissue. The necropsy showed mucosa cyanotic, abdominal distension severed. Gastric dilation and rupture in the stomach funds by strangling torsion of small intestine (jejunum segment). The histological sections of gastric mucosa showed coagulation necrosis of epithelial cells in the glandular and squamous acute erosive gastritis, with abundant neutrophilic infiltrate and few polymorphonuclear cells. Using the Wharting Starry special stain, were observed spiral shaped bacteria was found in gastric mucosa. Urease activity was then demonstrated in gastric tissue. In conclusion were reported a syndrome of gastric dilatation and detection *Helicobacter* Like Organisms in gastric mucosa of a zebra (*Equus quagga*). Future studies will be necessary to achieve the culture of this bacterium in order to identify its species and relation to lesions and pathogenesis of Gastric Ulcer Syndrome.