European Helicobacter Study Group

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WS1 Gastric Cancer

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/I 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/I 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 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 form 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/D (rs4646994), NOD1 796G/A (rs3743336), TLR4 3725G>C (rs11536889), FAS 1377G>A (rs2234767), FAS 670G>G (rs1800682) and FASL 844T>C (rs5763110) 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/A 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 polymorphism 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 ± 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 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 DYSPLASIA
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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 (Wilcox 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 patients 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

HELCOBACTER PYLORI-DERIVED Hp2-20 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 mm²) 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-synthese 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 H2-gas clearance technique and luminal NOx 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 NOx 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 NOx 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 FPR receptors and NO/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 D1 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 D3 (1,25D3) 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 D1 exerts considerable influence on the host innate immune response against H. pylori infection acting via the CYP27B1 response and subsequent roles of VDR and 1,25D3, on Cathelicidin production. H. pylori infection elicits a Vitamin D1 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-kB, 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** wild type and ARE-hPAP** 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** and Nrf2** mice for 20 weeks and found H. pylori-induced inflammation was aggravated in Nrf2** 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

CYTOKINE C RELEASE FROM MITOCHONDRIA IN EPITHELIAL GASTRIC CELLS INFECTED 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), Bel-2 protein family and interactions between them are involved in cytochrome release during apoptosis, being unknown the role in H. pylori infection.

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

Methods: AGS cells were cocultured for 24 hour with H. pylori (ATCC 51932) at increasing densities (10⁴–10⁸ CFU/mL). We evaluated: Intracellular (ROS) and mitochondrial (O₂⁻) free radicals; Cytochrome c and mitochondrial fractions; Apoptosis. At 10⁵ 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/vit.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 O₂⁻ proportionally to density. Apoptotic cells were augmented 60%; cytosolic/mitochondrial cytochrome ratio was higher...
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(3.07 ± 1.30 vs 2.03 ± 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. 5S 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.

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**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 Th1 and 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-glutmyltranspeptidase (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 countering the immunosuppressive activity of HPgGT. In infection experiments this vaccinations 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*, *hpA2*, *katA*, *napA* and *tsaA*. Antigenic sequences of these proteins were obtained using Jameson-Wolf, Rothbard-Taylor and AMPH 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 simply the detection of the synthetic protein that resulted from its expression. Three additional protein constructs containing additional sequences that target protein to MHCI pathway were made, namely using apoptosis, secretion or lysosomal target signals.

In vitro transfection efficiency of each one of these constructions was evaluated using A549 cell line and two different chitosan-based nanoparticles with adjuvant properties, as delivery system. Immunocytochemistry and immunoblotting using anti-FLAG antibody revealed that synthetic protein was expressed for every constructs. We are now evaluating constructs ability for target synthetic protein to the MHCI pathway.

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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 where 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 recombiant expression as soluble His-MBP-fusion proteins, they were purified using affinity chromatography and gel filtration. All proteins (*cagA*, *vacA*, *groEL*, *gct*, *babA*, *hepc*, *ureA*, *hisA*, *napA*, *icd*, *omp1*, *omp15*, *omp18*, *hpA2*, *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 infected 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.
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 £ 70 µg/mL and PGI/II ratio £ 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.

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, I² = 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, I² = 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 gastrotoxic 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 14 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 (H. pylori) infected children making susceptibility testing superfluous.

Methods: To evaluate the eradication rate of H. pylori infection with ST in treatment naive children with antibiotic susceptibility results.

Results: 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 at least 1 month results were analyzed (91 female, mean age 12.3 years). Primary resistance was reported for clarithromycin only in 24 (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 2/29 (27%) 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 gastroduodenal 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 2001–2003, 2003–19.3%, 2005–17%, 2008–9.8% and in 2010–8.9%. H. heilmannii infection was documented in 13 subjects in examined period. The highest incidence of H. pylori infection was noted in children form 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)-1B, 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-1B levels were associated with iron deficiency anaemia parameters and H. pylori infection. In 125 children (59.2% girls, mean age 12.2 ± 2.9 years, 4–16 years) excluding 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, ura-PCR and 13C-UBT. Forty-three (37.6%) children were H. pylori-positive. Antral gastric levels of IL-1B (ELISA, Biosource) were significantly higher (p < .001) in H. pylori-positive (333.6±mg/ml of tissue) than in negative (19.67±mg/ml) children. In the H. pylori-positive children, IL-B levels negatively correlated with haemoglobin (r = - .35, p = .004), haematocrit (r = -.40, p = .008) and serum ferritin (r = -.41, p = .007). IL-1B levels positively correlated with the serum pepsinogen II (p = .002) level which is increased with gastric corpus inflammation. Other cytokines were also signifi-
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 infection in children undergoing endoscopy due to gastric complaints from two developing and one developed countries. Two hundred and ninety seven 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 = 0.002*). *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.: W55.1
SEQUENTIAL AND STANDARD LEVOFLOXACIN-BASED H. PYLORI ERADICATION REGIMENS COMPARED TO QUADRUPLE THERAPY: EFFECT OF LEVOFLOXACIN DOSAGE AND WAY OF ADMINISTRATION
L. Xiong, Z. Y. Chen, J. L. Chen
**Abstract no.: WS5.1**

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.

Results: According to the analysis of ITT, the Hp-eradication rate in group A was 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), 83.2% (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 Hp eradication rates than triple therapy; but there are no significant deviation between therapy for 7 days and for 10 days.

Abstract no.: W55.3
ERADICATION THERAPY FOR HELICOBACTER PYLORI INFECTION IN PATIENTS WITH DUODENAL ULCERS BASED ON FURAZOLIDONE TRIPLE AND QUADRUPLE THERAPY: A MULTICENTER RANDOMIZED CONTROLLED TRIAL
N. H. Lv, Y. Cui, Y. Z. Huang, Y. Yang, X. Li, J. C. Liu

Methods: We enrolled 380 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.

Results: According to the analysis of ITT, the Hp-eradication rate in group A was 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), 83.2% (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 Hp eradication rates than triple therapy; but there are no significant deviation between therapy for 7 days and for 10 days.

Abstract no.: W55.4
NICKEL FREE-DIET ENHANCES HELICOBACTER PYLORI ERADICATION RATE
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Methods: Fourty 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 underwent 30 days of nickel free diet and LCA is performed at the 15th day.

Results: Eradication was confirmed by 13C-urea breath test 4 weeks after the end of treatment. According to the analysis of ITT, the Hp-eradication rate in group A was 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), 83.2% (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 Hp eradication rates than triple therapy; but there are no significant deviation between therapy for 7 days and for 10 days.

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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.

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, spiral+coccoids and coccoids, 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 mannos 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 links contained 1,3-mannosyl,1,4-mannosyl and terminal mannosyl linkages. Interestingly, both the spirals+coccoids biofilm and coccoid-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 mannos 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 hypochlorhydria 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 – Minas Gerais, Belo Horizonte, Brazil; §School of Veterinary Medicine, Merelbeke, Belgium; †Laboratory of Pharmaceutical Biotechnology, Ghent, Belgium

Although “Candidate 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 plasmid binding proteins PgbA and PgbB and the secreted serine protease HirA. H. heilmannii encodes several outer membrane proteins (OMPs), such as HpaA, HorB and 2 haemaglutinin-like OMPs, but lacks the important Iab 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 CAG 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*  
HELCOBACTER 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*.

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Abstract no.: P01.01
DOWN-REGULATION OF ACTIVATION-INDUCED CYTIDINE DEAMINASE BY CURCUMIN
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Aberant 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 be of benefit 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. H. pylori strains were co-cultured. Cells were pre-treated or without non-bacterial concentrations of curcumin. Apoptosis was determined by DNA fragmentation assay. Real-time PCR were used to evaluate AID, IL-6, IL-8, and TNF-α mRNA. Immunoblot was performed for the analysis of AID, NF-kB, IkB, and IKK. At the concentration of 10 μmol/L, curcumin did not show any bactericidal activity to H. pylori. Pretreatment of curcumin at ≤10 μmol/L down-regulated the mRNA and protein expression of AID provoked by H. pylori. Similarly, expression of inflammatory cytokines such as TNF-α, IL-6 and IL-8 were also suppressed by curcumin. Moreover, curcumin (≤10 μmol/L) suppressed H. pylori-induced NF-kB activation via inhibition of IKK activation and IkB degradation. Non-bacterial concentration of curcumin down-regulated H. pylori-induced AID expression in gastric epithelial cells, via inhibiting NF-kB pathway. Curcumin might be a potential chemopreventive agent against H. pylori related gastric carcinogenesis.

Abstract no.: P01.02
CAN SERUM GASTRIN, PEPSONIEN 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, and PG I/II ratio in 81 patients with gastric neoplastic lesion before ESD. And intragastric pH in gastric juice was measured. The endoscopic still imaging of AID, NF-κB, and IKK. At the concentration of 10 μmol/L, curcumin did not show any bactericidal activity to H. pylori. Pretreatment of curcumin at ≤10 μmol/L down-regulated the mRNA and protein expression of AID provoked by H. pylori. Similarly, expression of inflammatory cytokines such as TNF-α, IL-6, and IL-8 were also suppressed by curcumin. Moreover, curcumin (≤10 μmol/L) suppressed H. pylori-induced NF-kB activation via inhibition of IKK activation and IkB degradation. Non-bacterial concentration of curcumin down-regulated H. pylori-induced AID expression in gastric epithelial cells, via inhibiting NF-kB pathway. Curcumin might be a potential chemopreventive agent against H. pylori related gastric carcinogenesis.

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-Lipoxigenase (5-LOX) and receptors for LT (Blt1-2, Blt2) and cysteinyl-LT (CysLt1-2) 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-nine patients had gastric cancer of intestinal type, 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 CysLt1 was observed in gastric cancer compared to tumor-free gastric mucosa (p < 0.001 for Blt-1, p < 0.01 for Blt-2 and CysLt1, 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, CysLt1 and CysLt-2 was increased in gastric cancer of intestinal type when compared to the diffuse type (p < 0.05; Mann-Whitney U-test).

Conclusions: LT4 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 among family 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 s1m 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 = 0.006) 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 = 0.04), corpus foveolar hyperplasia (p = 0.05) and corpus atrophy (p = 0.05). 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, suggest 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 LINEGES-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-
visually. In this study, we examined the effects of *Hp* clinical isolates from gastric carcinoma (GC) on gastric epithelial cell lineGES-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) and were co-cultured with GES-1 cells individually. MTX 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.

**Results:** GES-1 cells exhibited a more potent proliferative response to the co-cultivation with *Hp* isolates from GC specimens versus 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 TRFA1 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 TRFA1 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 *Hp* 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 *Hp 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 *Hp pylori* positive (32%) and negative (0%) chronic gastritis patients. Since the infection by *Hp 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 *Hp pylori* infection. Regarding the results from human biopsies we observed an epigenetic inactivation of this gene which significantly correlates with *Hp 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**


**Asan Medical Center, Seoul, Korea**

The effect of *Hp pylori* treatment on recurrence after endoscopic mucosal resection (EMR) of gastric cancer remain uncertain because of contradictory opinions. A total of 2009 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 *Hp pylori*. We investigated group without *Hp pylori* infection (35.4%) and group with *Hp pylori* infection (64.6%) for recurrence rate, recurrence interval. Among group with *Hp pylori* infection, group without *Hp pylori* treatment were 25.8% and group with *Hp 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 homogenously distributed. Recurrence rate of group without *Hp pylori* infection and group with *Hp pylori* infection was 6.1% and 14.8% (*p < .01*). In a subgroup of patients with *Hp pylori* infection, recurrence rate of group without *Hp pylori* treatment and group with *Hp pylori* treatment was 25.9% and 11.2% (*p < .01*). A retrospective study showed that *Hp 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**


**Adelaide and Meath Hospital, Tallaght, Ireland**

**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-duodenooscopies (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 adenocarcinomata that were not in patients identified previous premalignant lesions were T1N0M0 (*p*-value = .002). The patient with a history of IM not under surveillance (local IM) had a synchronous T1N0M0. 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 surveil-lance OGD.

**Abstract no.:** P01.09

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


**Adelaide and Meath Hospital, Tallaght, Ireland**

**Introduction:** Missed and new gastric cancers occurring after oesophago-gastro-duodenooscopies (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 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 our endoscopy department 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-duodenooscopies (OGD) per year. All patients with IM are offered follow-up.

**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 a previous OGD. In four cases the cases were found in asymptomatic patients undergoing surveillance for premalignant lesions. In the other case the patient had been diagnosed with *Hp 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.
Methods:

but cytogenetic criteria of its genotoxic action are not well investigated yet. The

YOUNG AND ELDERLY PATIENTS WITH GASTRIC CANCER

Objectives:

It is known that

N. V. Baryshnikova, Y. P. Uspenskiy, L. N. Belousova, L. Kitaeva and E. I. Tkachenko

INVASION AND CYTOGENETIC

X. Yuan,*

HELICOBACTER PYLORI

EFFECTS OF

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, it is the major cause of young patients with gastric cancer? In this study, we investigated the efficacy of H. pylori on tumorigensis 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 ± 3.11 years (range 18–30). The elderly patients group (E group) consisted of 22 male, 33 female, the mean age was 78.27 ± 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 in more than 58.6% vs 61.5% and 72.7% vs 66.6%), 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 cancerogenetic properties of H. pylori.

Methods: We made g istologic and citologic analysis of stomach antrum and body biopsies of 183 patients (124 patients with H. pylori-positive gastrudodenitis and 59 patients with H. pylori-negative gastrudodenitis). We investigated cytogenetic infringements in epithelial cells of stomach mucosa in chronic gastrudodenitis patients with different level of H. pylori invasion. Cells with true micronuclei and cells with morphologic anomalies (chromatin relations “nucleus-nucleus”, “micronucleus-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 (8.15±) 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 micronuclei and morphologic changes of epithelial cells increased significantly that might be associated with direct action of H. pylori on epithelial cells.

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

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-κB, chemokines; angiogenic growth factors, and free radicals, etc. 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 EHSG, 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 phytoceuticals 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 constructive to stopping inflammatory carcinogenesis that is associated with H. pylori infection, rendering rejuvenation of chronic atrophic gastritis and our novel extract of lichochalcone 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 PS5, CLEAVED CASPASE-3, BCL-2 AND BAX IN GASTRIC BIOPSYs


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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 up regulate caspase-3, Bax and mutated p53, among other proapoptotic factors, and to down regulate 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 (Biogenoma 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 13C-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 p5SMAD1/5/8, 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 RECURRENT 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 = 0.029).

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.
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 oesophagastroduodenoscopy (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 HOMD**

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The genetic diversity and evolution of the homD 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 homD gene at a conserved locus. Phylogenetic reconstruction of homD 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 A1 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 homD 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**

**HIGHS 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 (54 KP) and peptic ulcer disease and the highest number of repeats (57 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 mucus from various animal species and how they colonise the adherent mucin 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 mucus respectively compared to mucus 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 effective 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 Lewisa containing non mucin fractions of E12 mucus. Although the presence of mucin was important for effective infection by both H. pylori and C. jejuni the mechanisms underpinning mucin 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 the 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-cell communication are in progress.

**Abstract no.: P02.05**

**PRODUCTION OF MONOCLONAL ANTIBODY AGAINST ALKYL HYDROPEROXIDE REDUCTASE (AHPR) 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 distinct 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 BALC mice (female, 6–8 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 weeks, 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.

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 RNA-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. pylori (12 cases), C. urealyticum (1 case) and H. cinaedi (2 cases) were detected in 15 19-H pylori Ab staining (-)/Gimsa stain (++) cases. 60 cases (40%) of 150 gastritis specimens were 2-H pylori Ab staining (-)/Gimsa stain (–). In the gastric adenocarcinoma specimens, C. hydrogenis (1 case), C. hominis (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 contained at least one H. pylori strain. H. pylori-associated Gisma staining was detected in the majority of non-H. pylori-related Gisma 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 cultures 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 pylor Special Peptone Agar (HPSPA) with 0.75% agar, 7% horse serum and 0.5% β-cyclodextrin were inoculated with a 10 μl loop-full of fresh H. pylori growth by stabbing into the vials near the bottom portion of the agar medium. Vials were then incubated at 37°C in a 12% CO2 incubator with the tops kept loose. Prior to shipping vials are inoculated and placed in a CO2 incubator for 2–48 hour and then shipped at room temperature. Upon receipt the vials are placed in a 12% CO2 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 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 osmotic shock. 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 differentially abundant (p < 0.05) among clusters. These proteins’ abundance was then compared having the 2-DE-maps of strains isolated from Portuguese patients, presenting different gastric diseases. 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 recruit of SFRE/B/B/386/34/2007 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 OMPLA A gene (iplA) displays phase variation resulting in variants
with high (pldON) or low (pldOFF) OMPLA activity. We have previously reported that pldOFF 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 miCRONNA-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-metabolic, 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* infection status was defined as positive if at least two tests of the 13C-UBT, RUT tests or histology were positive. IL-8 and mir-650 gene expression of IL-8, the housekeeping gene RN7 and the microRNAs mir-204 and mir-650 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 < .001).

**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.

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

<table>
<thead>
<tr>
<th>(n)</th>
<th>(n)</th>
<th>IL-8</th>
<th>mir-204</th>
<th>mir-650</th>
<th>mir-204/mir-650</th>
<th>RN7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>4.86E + 02</td>
<td>2.54E + 04</td>
<td>5.21E + 02</td>
<td>27.2</td>
<td>6.54E + 05</td>
<td>6.54E + 05</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>9.97E + 03*</td>
<td>3.24E + 03*</td>
<td>2.69E + 03ns</td>
<td>1.54*</td>
<td>9.37E + 05ns</td>
</tr>
<tr>
<td>Gastritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal stomach</td>
<td>8</td>
<td>3.26E + 02</td>
<td>2.56E + 04</td>
<td>2.13E + 02</td>
<td>137</td>
<td>4.00E + 05</td>
</tr>
<tr>
<td>Hp-negative</td>
<td>11</td>
<td>5.15E + 02ns</td>
<td>2.54E + 04ns</td>
<td>3.75E + 02</td>
<td>16.2*</td>
<td>7.10E + 05ns</td>
</tr>
<tr>
<td>Hp-positive</td>
<td>16</td>
<td>9.97E + 03$</td>
<td>3.24E + 03$</td>
<td>2.69E + 03§</td>
<td>1.54§</td>
<td>9.37E + 05ns</td>
</tr>
</tbody>
</table>

Units are Medians of Arbitrary Florence 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.

fp < .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* < 0.05). Akt expression showed no significant difference among all the groups (*p* > 0.05). 2. After the introduction of 1:4 dilution of culture filtrate of *H. pylori* to GES-1 cells, Akt changed little (*p* > 0.05). 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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Microbiology Department, Madrid, Spain

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% CO2 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 VacA genes was: 21 CagA (26.25%) and 21 vacA (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

<table>
<thead>
<tr>
<th>dupA</th>
<th>cagA positive</th>
<th>cagA negative</th>
<th>vacA s1</th>
<th>vacA s2</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>42.86%</td>
<td>32.20%</td>
<td>47.61%</td>
<td>39.53%</td>
</tr>
</tbody>
</table>

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 doble of prevalence 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 vesicles 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 (ABFFFF). 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 motif (ABFFFF) failed to fully activate IL-8 gene transcription. Moreover, the ABFFFF 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-kB 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 chemosattractive 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 TNFα, via a pathway independent of PI3K, Src and MAPK but dependent on Nuclear Factor-kappa B (NF-κB). 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 MKN 28) 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 β catenin (at S641) and dissociation of α/β 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 β-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-γ AND SRC ARE INVOLVED IN HELICOBACTER PYLORI-MEDIATED HOST CELL INVASION IN A C-MET-DEPENDENT MANNER**

A. M. Costa,* M. J. Oliveira,† R. Y. Churin,‡ R. M. Ferreira,* M. Leite,* T. F. Meyer‡ and F. Figueredo‡∗

– Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; †Medical Faculty of the University of Porto, Porto, Portugal; ‡Department Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany

*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 TASS 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-uml, Nck, Gab1, and Src, and also of other known downstream targets of the c-Met pathway, c-Src, FAK, PLC-γ, 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-γ 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-γ, 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, while no phosphorylation was observed in c-Met-silenced AGS cells, suggesting that activation of Nck, PLC-γ 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. **Acknowledgements:** ERA-PTG/0002/2006; SFRH/BD/36013/2007; SFRH/ BD/33420/2008.

**Abstract no.: P03.05**

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

B. Dolan,* J. Naughton,* N. Tegtmeyer,† S. Backert† and M. Clyne†

*UCD School of Medicine and Medical Science, Dubin, Ireland; †UCD School of Biomolecular & Biomedical Science, Dublin, Ireland

*Helicobacter pylori* colonisation 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 mucis, is mediated by *H. pylori* lipopolysaccharide and promotes mucus colonization. Polarisated 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
Our results show that HtrA is highly conserved among clinical isolates, reinforcing its essentiality for H. pylori survival.

**Acknowledgements:** SFRH/BD/45841/2008; SFRH/BPD/33420/2008.

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**Abstract no.: P03.09**

**REGULATION OF MDM2 ONCogene BY HELICOBACTER PYLORI LIPopolysaccharide IN GASTRIC epithelial cells X. Part* and G. Zhang**

*First Affiliated Hospital of Nanjing Medical University, Nanjing, China; First Affiliated Hospital of Nanjing Medical University, Nanjing, China

**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, pAKK-S473 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 LPS/29402 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.

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**Abstract no.: P03.10**

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

**A. S. H. Wee,* , A. S. H. Wee,** A. S. H. Wee***, Y. Toyama*** and B. Ho***

*National University of Singapore, Singapore, Singapore; **Mechanobiology Institute, Singapore, Singapore; ***Department of Biological Sciences, Singapore, Singapore; **Temasek Life Sciences Laboratory, Singapore, Singapore

**Helicobacter pylori cytotaxin-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 ΔcagA separately.**

**Results:** 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 ΔcagA-infected host cells began at 4 hours post-infection. Once the process of tight junction disruption is 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.

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**Abstract no.: P03.11**

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

I. Vitoriano,* K. D. Saravia-Pava,* A. Rocha-Gonçalves,* A. Santos,* A. I. Lopes,* M. Oleastro* and M. Roxo-Rosa**

*Faculty of Engineering, Rio de Mouro, Portugal; **Chymiotachnèn, Coimbra, Portugal; *Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr. Ricardo Jorge, Lisboa, Portugal; **Department of Pediatrics, Hospital Universitário de Santa Maria/ Faculdade de Medicina de Lisboa, Lisboa, Portugal

**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 of secretion of mucus 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, vacA, nfaC, nipA ‘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 mucus. Work supported by PPCDT/SAL-IMI/57297/2004 research-grant. IV and KDSP are recipients of SFRI/B/38634/2007 and SFRI/B/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
*Medical Research Council Laboratories, Fajara, Gambia; †Newcastle University, Newcastle upon Tyne, UK; ‡Washington University, St Louis, MO, USA; ∥London School of Hygiene and Tropical Medicine, London, UK; †Bill and Melinda Gates Foundation, Seattle, WA, USA

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 toxicogenic 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 C-GLUTAMYL TRANSEPTIDASE ACTIVITY
S. S. M. Ling,* T. H. B. Khoi,* L. A. Hwang, and B. Ho*
*Inflammatory University of Singapore, Singapore; Singapore, Singapore, †Institute of Molecular and Cell Biology, Singapore, Singapore

Helicobacter pylori C-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 are 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 DYSPEPTIC PATIENTS FROM A POPULATION AT HIGH RISK OF GASTRIC CANCER IN COSTA RICA
S. E. Molina-Castro,* I. Gaviria-Cambromero,* C. Ure,* W. Malepsin-Benaida,* R. Sierra,* P. Golcher,* F. Mégraud and V. Ramirez*
*Instituto for Health Research, University of Costa Rica, San José, Costa Rica; †University Victor Segalen Bordeaux 2, Bordeaux, France

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 s1/m1 genes with atrophic gastritis

<table>
<thead>
<tr>
<th>Gene (n)</th>
<th>OR (AG vs NAG)</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>.dupA* (59)</td>
<td>0.74</td>
<td>(0.27–2.06)</td>
<td>.425</td>
</tr>
<tr>
<td>oipa* (77)</td>
<td>1.40</td>
<td>(0.15–13.31)</td>
<td>.584</td>
</tr>
<tr>
<td>vacA s1/m1 (57)</td>
<td>2.04</td>
<td>(0.66–6.24)</td>
<td>.296</td>
</tr>
</tbody>
</table>

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

Conclusion: Infection with Helicobacter pylori strains carrying the dupA, oipa, vacA s1/m1 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 (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 taking drugs for a month in extreme cold weather (temperature <10°C). PH values of gastric juice were obtained on site by precise PH dipstick. H.p infection were determined by modified Giensa staining. Tregs, macrophages infiltrating and Occludin, HSP70, NOS, 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 EGFR 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.

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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 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 radioimmunoassy.

Results: 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). The results of radioimmunoassy showed that Serum Gastrin and somatostatin concentration of Hp+ group were not significantly different from Hp- group (76.0 ± 64.1 vs 82.0 ± 66.1 pg/mL, t = .34, p > .05; 564.0 ± 1437.0 vs 776.2 ± 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.

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

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Objectives: cag-group genes are genes coding synthesis of factors pathogenecity 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 gastroduodenitis and healthy microbial carriers.

Methods: It has been surveyed 91 person: 38 patients with duodenal ulcer, 39 – with chronic gastroduodenitis and 14 – healthy microbial carriers. Gastroscopy with antrum biopsy was made for each patient. Polymerase chain reaction was made with all biotlates 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 gastroduodenitis 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 gastroduodenitis 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 gastroduodenitis 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 gastroduodenitis 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 gastroduodenitis patients and healthy microbial carriers. Chronic gastroduodenitis 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 symptoms 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, gastritis, 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 diagnostic symptoms using a validated questionnaire. Specific symptoms were grouped into none/any and categorized by the highest severity mentioned. Hundred and eight-nine participants (10–80 years, 57% female, 91% Aboriginal) 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>
<thead>
<tr>
<th>Symptom severity and medical attention by diagnostic category</th>
<th>None (%)</th>
<th>Mild-mod (%)</th>
<th>Severe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoscopy (few duodenal lesions: 7 duodenitis, 1 duodenal erosions, 0 duodenal ulcers)</td>
<td>164</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>Gastritis</td>
<td>25</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>Gastric erosions</td>
<td>12</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>6</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>Histopathology (normal = chronic inflammation = 0)</td>
<td>0</td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td>H. pylori density (negative = 0)</td>
<td>64</td>
<td>42</td>
<td>39</td>
</tr>
<tr>
<td>Density = 1</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>Acute inflammation = 1</td>
<td>74</td>
<td>35</td>
<td>54</td>
</tr>
<tr>
<td>2–3</td>
<td>45</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Chronic inflammation = 1</td>
<td>13</td>
<td>23</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>36</td>
<td>61</td>
</tr>
<tr>
<td>Atrophy = 1–3</td>
<td>27</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>Metaplasia = 1–3</td>
<td>16</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

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 χ² 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*

<table>
<thead>
<tr>
<th>H. pylori density score</th>
<th>Acute inflammation</th>
<th>Chronic inflammation</th>
<th>Glandular atrophy</th>
<th>Intestinal metaplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (none)</td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td>n = 65</td>
<td>1 (mild)</td>
<td>81%</td>
<td>100%</td>
<td>6%</td>
</tr>
<tr>
<td>n = 32</td>
<td>3 (marked)</td>
<td>100%</td>
<td>100%</td>
<td>35%</td>
</tr>
<tr>
<td>n = 47</td>
<td>χ² for trend p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Table percentages represent the prevalence of each histopathological diagnosis within an H. pylori density score.
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), \(p = .001\)). 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 subite 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 (1.179–27.138), \(p = .031\)) 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 non-cardia subite 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 Helicobacter 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>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual household income (in CAD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$25,000</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$25,000–$49,999</td>
<td>0.67 (0.26–1.7)</td>
<td>0.74 (0.30–1.8)</td>
</tr>
<tr>
<td>$50,000–$74,999</td>
<td>0.41 (0.16–1.0)</td>
<td>0.50 (0.21–1.2)</td>
</tr>
<tr>
<td>≥$75,000</td>
<td>0.26 (0.12–0.56)</td>
<td>0.33 (0.16–0.69)</td>
</tr>
<tr>
<td>Highest educational attainment by a household member</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Grade 12</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Grade 12</td>
<td>0.82 (0.40–1.7)</td>
<td>0.86 (0.42–1.7)</td>
</tr>
<tr>
<td>≥Grade 12</td>
<td>0.42 (0.19–0.91)</td>
<td>0.60 (0.26–1.4)</td>
</tr>
<tr>
<td>Number of children in the house</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>0.82 (0.41–1.6)</td>
<td>0.76 (0.36–1.6)</td>
</tr>
<tr>
<td>2</td>
<td>0.98 (0.42–2.3)</td>
<td>0.98 (0.38–2.5)</td>
</tr>
<tr>
<td>3–6</td>
<td>4.6 (1.4–15)</td>
<td>4.2 (1.2–15)</td>
</tr>
<tr>
<td>Number of people per bedroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1.01–2</td>
<td>1.5 (0.84–2.8)</td>
<td>1.4 (0.72–2.8)</td>
</tr>
<tr>
<td>2.01–3</td>
<td>4.0 (0.85–19)</td>
<td>3.1 (0.63–15)</td>
</tr>
</tbody>
</table>

*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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University Hospital Merkur, Zagreb, Croatia


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, –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 25.7%.(\(p < .0001\)).

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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*Department of Physiology Jagiellonian University Medical College, Krakow, Poland; †Department of Anatomy, University School of Physical Education of Cracow, Krakow, Poland; ‡Gastroenterology Clinic, Eva Center, Krakow, Poland

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 2011 in Poland.

Methods: A retrospective population-based study was performed on 11,104 adults inhabitants of Krakow municipal area (aged 18–78, mean 47.2 years: 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 urea breath test (UBT) with capulated 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 30 year group of 46–55 year. From 1996 to 2005 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 < .001, R2 = 0.926). Endoscopy revealed an increase of “idiopathic” peptic ulcers (PU) which correlated also significantly (p < .001) with GDP per capita (R2 = 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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*Biotechnology Department Universitat Politècnica de Valencia, Valencia, Spain; †Research Institute of Water Engineering and Environment, Universitat Politècnica de Valencia, Spain

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 VaaA DNA PCR, for investigating the presence of H. pylori in water samples from a secondary wastewater treatment plant with a UV final efficient 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 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 VaaA 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 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. 12-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

SERORELOGICAL PREVALENCE OF HELICOBACTER PYLORI-INFECTION IN SAXONY-ANHALT, GERMANY

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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 ELISA. 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 52.4% 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

A. G. Salinas Ibáñez,† T. I. Cortiñas,‡ C. S. Lucero Estrada,§ P. E. Gómez,* P. Vallejos Bianchi,† M. Celiz,* T. Alarcón Caveró† and A. E. Vega†

*Universidad Nacional de San Luis, San Luis, Argentina; †Hospital Universitario La Princesa, Madrid, Spain

Helicobacter pylori is the main etiologic agent of chronic gastritis, peptic ulcer, gastric cancer and 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 neoplasms 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 urea 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 celiacs 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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1St-Petersburg State Medical Academy n.a. I.I. Mechnikov, St-Petersburg, Russia

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 neoplasms 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 urea 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 celiacs patients. The delay in diagnosis of people infected with H. pylori and CD can increase risk of lymphoproliferative lesions.
Abstract no.: P04.12

FOOD-BORNE SACCHAROMYCES CEREVISIAE HARBORS H. PYLORI SPECIFIC GENE

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Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran

Introduction: The routes of transmission of Helicobacter pylori are unknown. Saccharomyces cerevisiae is the most useful yeast in baking and brewing since the date. Bakery leaven and standard H. pylori-16s rDNA according to green colonies 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 (m) genes 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 plates. Gastrointestinal tract and when released to the environment.

Discussion: 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 rRNA 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 yeast 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 Helicobacter pylori-specific genes 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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*Department of Plant Molecular Biology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; †Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran; ‡School of Public Health, Medical Sciences, University of Tehran, Tehran, Iran

Introduction: Uninoculated 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 plates 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 & vacA (m) genes were compared with their oral Candida isolates for having the H. pylori species-specific genes.
COMMUNITY-BASED, PARTICIPATORY RESEARCH ON H. PYLORI: MAKING MICROBIOLOGY RESULTS MEANINGFUL TO PARTICIPANTS

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*University of Alberta, Edmonton, AB, Canada; †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, housekeeping 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.

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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Objective: 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 kindergarten, 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, 0.6% (0/146) in those aged 0–3, 0.6% (1/120) in 4, 3.7% (5/134) in 5 years, 2.2% (2/90) 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 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

Results: 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 strong association between CagA+ genotype (t2-test, p = .0039) and BbA2 + genotype (p = .0062) and eradication of H. pylori. No associations with eradication were found for lca alleles (p = .423) and VacAs1/2p (p = .055) and VacA m1/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.: P05.04

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 presence of vac gene 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 MICRA PCR was used for detection of the following H. pylori genotypes: 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 substrate (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/VacA2 = in 18 (43.9%) 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%) of 41 children. Positive BabA2 status was revealed in 18 (45.9%) patients.

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 ± 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 on biopsy urease test or histology) and 214-negative. Children that 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. pylori infection in children for several countries, available in.
pylori infection even after adjustment for age and gender \( (p = 0.02) \). In the regression logistic model, iron deficiency, detected in 16 (4.4%) children, was associated with female sex \( (p = 0.04) \), increasing age \( (p = 0.03) \) and antral nodularity \( (p = 0.02) \). 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, upper gastrointestinal endoscopy macroscopical findings and histopathological evaluation with Sydney classification were made.

Results: Three hundred and fifty-seven children underwent upper gastrointestinal 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 HP 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

HELCOBACTER PYLORI GENOTYPES IN ROMANIAN CHILDREN WITH CHRONIC GASTRITIS: A SINGLE CENTER STUDY
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Aim: 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 cagA, vacA (alleles s1a, s1b, s2, m1 and m2), gene ureA 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 cagA, vacA and its allele types. DNA was extracted from cultured strains. PCR for cagA and vacA alleles were performed using primers previously described (a 165-235R DNA and ure markers for species identification).

Results: Out of 111 children, 55 (49.54%) were rapid urease positive, and 45 of them (81.81%) were culture positive. The presence of the gene vacA was detected in 84% H. pylori infected children. The predominant combination found for gene vacA was m1s1, without s2/m1. The H. pylori strains, vacA s1b/m1/cagA A-positive, were associated with an increased antral nodularity and higher degrees of activity of gastritis \( (p < 0.001) \).

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 cagA vacA 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 esophagogastroduodenoscopy (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%), hiatosis (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 stil 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 for 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 PSEPSINOGEN 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 OLEGA staging system.

Serum PG I, II levels were significantly different among cases and controls (p < .05). Logistic regression analyses showed that low PG I/PG II ratio (≥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 PG/I/II ratio is significantly (p < .001) lower in those who suffer from severe grades of inflammation (grade 3: 10.6 ± 15.4; grade 4: 19 ± 3.7) vs those with lower grades of inflammation (grade 0: 15.1 ± 15.2; grade 1: 10.5 ± 5.15; grade 2: 11.8 ± 11.43).

The risk impact of low serum PG/I/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 DYSPESPIA
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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 for Molecular Determination of Clarithromycin Resistance and Detection of Helicobacter pylori in Patients with Dyspepsia.

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%, 93.6%, 96.7%, 82.4%, 86.7%, 93.8%, respectively (Kappa = 0.847.0.589). Clarithromycin susceptibility was found in concordance (73.5%) with E-test, FISH and Real-timePCR methods.

Conclusion: Clarithromycin resistance was high (23.3%, 13.6%, 32.8%) in clinical samples and biopsies in patients with dyspepsia by three methods and was mostly associated with A2143G mutation confirmed by FISH and Real-timePCR.
ATEMMPTS TO IDENTIFY HELICOBACTER PYLORI AT THE SPECIES LEVEL AND TO DETERMINE PATHOVARSS BY MALDI-TOF MASS SPECTROMETRY

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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 dendogram.

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.

SELECTING PATIENTS FOR RE-GASTROSCOPY

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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 197 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, 57/1126 (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: Macrogenoscoical 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.

COMPARISON OF ETEST, GENOTYPE HELICODR AND IN-HOUSE REAL TIME PCR ASSAY FOR THE DETECTION OF CLARITHROMYCIN RESISTANCE

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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 RNA. The most frequent are A2142G and A2147G mutations, A2146G 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.

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.

CORRELATION OF AGE OF PATIENTS WITH CONCENTRATION OF AMMONIA IN EXPIRED AIR AT USE OF THE UREASE RESPIRATORY TEST

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Background: 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.

Methods: Fourty-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.

Results: 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.

EVALUATION OF THE "CLARI-RES ASSAY" KIT FOR THE RESEARCH OF THE HELICOBACTER PYLORI CLARITHROMYCIN MUTATION RESISTANCE IN GASTRIC BIOPSIES

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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), GenoType HelicoDR 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 (46.73%) were CLA resistant. In 15/77 patients (19.5%) histology were HP negative whilst were found HP DNA.

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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 bioplastic material and consensually clarithromycin resistance. High clarithromycin resistance rates were observed in our population suggest the need for 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, metronidazole and levofloxacin were determined. Sensitit 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 ±2 double dilutions and 100% category agreement. Liofilchem results for levofloxacin revealed lower MIC values than determined by Etests, with 83.6% results being within ±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 CATALASAE

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††Department of Environmental and Preventive Medicine, Oita University, Oita, Japan

Background: MONOCLONAL ANTIBODY TO HELICOBACTER PYLORI CHARACTERIZATION OF JAPANESE STOOL ANTIGEN TESTS USING A MONOCLONAL ANTIBODY TO HELICOBACTER PYLORI CATALASAE

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 complaints 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 histological and nohistochemistry 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 (63.5%) 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

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>89.3</td>
<td>88.5</td>
<td>99.1</td>
</tr>
<tr>
<td>CLO-test</td>
<td>82.5</td>
<td>76.0</td>
<td>99.1</td>
</tr>
<tr>
<td>Serum Ab</td>
<td>96.7</td>
<td>97.2</td>
<td>95.6</td>
</tr>
<tr>
<td>Histology</td>
<td>90.3</td>
<td>96.4</td>
<td>100</td>
</tr>
</tbody>
</table>

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.
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 ($p = 1.000 \times 0.993$) 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.

**Background and Aim:** The aim of this study was to evaluate the diagnostic accuracy of RAPiRUN® test in clinical practice.

**Methods:** A set of H. pylori tests which were composed of endoscopic biopsy, $^{13}$C-urea breath test ($^{13}$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, $^{13}$C-UBT, serum IgG-ELISA, and rapid urine RAPiRUN® test, respectively. With gastric mucosal biopsy, $^{13}$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 specificity, sensitivity, positive and negative predictive value, and accuracy of the rapid urine RAPiRUN® test were 83.5%, 98.3%, 98.7%, 79.4%, and 89.3%, respectively.

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

**Abstract no.: P06.15**

**DETECTION OF HELICOBACTER PYLORI INFECTION: CLINICAL VALIDATION OF $^{13}$C (37 KBQ) UREA BREATH TEST**

Centro de Gastrenterologia da Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal

**Objective:** Clinical evaluation of $^{13}$C-urea breath test for diagnosis of Helicobacter pylori (Hp) infection, taking the $^{13}$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 $^{13}$C-UBT, serum IgG-ELISA, and rapid urine RAPiRUN® test, respectively. With gastric mucosal biopsy, $^{13}$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 specificity, sensitivity, positive and negative predictive value, and accuracy of the rapid urine RAPiRUN® test were 83.5%, 98.3%, 98.7%, 79.4%, and 89.3%, respectively.

**Conclusion:** Urine based RAPiRUN® test for detection of anti-H. pylori antibody was an accurate test, especially in specificity. With the advantage of caseness, rapidity, and non-invasiveness of RAPiRUN® test, we expect that RAPiRUN® test would be useful in general practice for H. pylori screening.

**Abstract no.: P06.16**

**AMMONIA BREATH TEST – TRANSPORTATION WAY OF AMMONIA**

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**Background and Aim:** The aim of this study was to evaluate the diagnostic accuracy of RAPiRUN® test in clinical practice.

**Methods:** A set of H. pylori tests which were composed of endoscopic biopsy, $^{13}$C-urea breath test ($^{13}$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, $^{13}$C-UBT, serum IgG-ELISA, and rapid urine RAPiRUN® test, respectively. With gastric mucosal biopsy, $^{13}$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 specificity, sensitivity, positive and negative predictive value, and accuracy of the rapid urine RAPiRUN® test were 83.5%, 98.3%, 98.7%, 79.4%, and 89.3%, respectively.

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

**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).

**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 BIOPSY USING THE FISH TECHNIQUE**

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*Institute of Medical Research, Yaba, Lagos, Nigeria; †College of Medicine, University of Lagos, Lagos, Nigeria; ‡Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; †Max von Pettenkofer Institut, Munich, Germany

**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.

**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.

**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 NH3 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 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.
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

**HELCOBACTER 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 diagnosticate 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 diagnosticate the infection than to control the eradication it has an excellent sensibility (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 sensibility to the antibiotics. About the stool antigen tow 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 sensibility 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%, tetracyclin 1.9%, clarithromycin 3%, and multi-drugs 5%. In amoxicillin, clarithromycin and metronidazole resistant strains, ages 40 years was significantly higher than age <40 years (90% vs 10%; p-value = 0.04, 100% vs 0%; p-value = 0.3 and 65% vs 35%; p = 0.02 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 strain 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: To determine the prevalence of primary resistance of Helicobacter pylori against clarithromycin and levofloxacin from 2009 to 2011 in Bhutan – Colombia.

Methods: A total of 259 clinical isolates of H. pylori were collected from January 2009 to April 2011 from patients in Bogotá, Colombia. Antibiotic susceptibility to clarithromycin and levofloxacin were tested by agar dilution. The isolates were considered resistant when the MIC values were ≥1 μg/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 and fluoroquinolone (gyrA gene) resistance were amplified by PCR and DNA sequencing.

Conclusion: 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.: P07.03
DETECTION OF CLARITHROMYCIN RESISTANCE IN H. PYLORI FOLLOWING NONCRYOGENIC STORAGE OF RAPID UREASE TESTS FOR 30 DAYS
Baylor College of Medicine, Houston, TX, USA

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 biopsy samples 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 used 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 8/10 (80%). 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 rDNA 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 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
A. Trespalacios1, W. Otero1, M. Mercado1, E. Caminos1, J. Avila1, L. Rosero1 and A. Arévalo1
1Porfiria University Javeriana, Bogotá, DC, Colombia; 2Universidad Nacional de Colombia, Bogotá, DC, Colombia

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. Antibiotic susceptibility to clarithromycin and levofloxacin were tested by agar dilution. The isolates were considered resistant when the MIC values were ≥1 μg/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 and fluoroquinolone (gyrA gene) resistance were amplified by PCR and DNA sequencing.

Conclusion: 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 ERADIATION 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. H. pylori 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) regimen 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), Gentamicin (GM 2.5) and Rifampicin (RI 6.6). The 9-9 multi-resistance rates were 12.2, 22.8, 15.0, 21.0, 10.4, 14.4, 2.8, 1.2, 0.2, which was mainly caused by CH, 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 nitromidazoles, 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 266895 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 = 0.0219, OR: 1.346, 95% CI: 1.07–1.69). This was evident in the adult (p = 0.0297, OR: 1.469, 95% CI: 1.09–1.97) rather than the children group (p = 2.907). 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 gastroscopic 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-Bailey.

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 < 0.05).

Conclusions: The resistance rate of H. pylori strains isolated from chronic gastritis is high than those from duodenal ulcer to metronidazole, clarithromycin and amoxicillin. Eradication of H. pylori depending on drug sensitivity tests is the optimal treatment selection in clinic.
Abstract no.: P07.10

HELCOBACTER PYLORI IN A SOUTH-EUROPEAN COUNTRY – PRIMARY AND SECONDARY RESISTANCES TO ANTIMICROBIALS (FIRST RESULTS)

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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 = 0.25). Significant differences (p < 0.05) 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%–49.7%; p < .0001), 42.9% to metronidazole (30%–63.5%; p = 0.002) 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 empirical 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 RNA IN KAZAN (RUSSIA)

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Introduction: In Helicobacter pylori (Hp) infection resistance to clarithromycin 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 clarithromycin-related mutations of H. pylori strains among patients with gastrointestinal 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). Hp-positive biopsies were taken for further evaluation. To detect A2142G, A2143G and T2717C mutations of the 23S rRNA gene MboII, BsoI11 (BsaI)- and ApoLE (HbaI)-restriction PCR-RFLP assays were conducted (“SibEnzim”, Russia).

Results: As a result of cytology, rapid urease tests and PCR analysis, Hp was revealed in 70 samples. A2143G mutations determining clarithromycin 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 clarithromycin-resistant H. pylori strains is 11.4% (8/70) among patients with gastrointestinal pathology in Kazan (Russia). These numbers (11.4%) of clarithromycin resistance allow to reveal 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 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

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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 follows: 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 (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

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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 µg/mL) were inoculated 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 metronidazol-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 metronidazol-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.

**Aims:** 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.

**Conclusion:** It is necessary to continuously monitor H. pylori strain resistance in adult patients. The high incidence of primary infections with multi-drug-resistant 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 an powerful alternative drug, and its effectiveness in treatment. Biopsy samples were taken from 116 patients during endoscopy between June 2010 and February 2011. Rapid Urea Test was 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, 2 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%, which is higher than the resistance upper limit of 20%, determined by EBPSPG for clarithromycin treatment. Levofloxacin resistance was found to be 25%. 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 the prevalence of primary clarithromycin resistance in our district.

**Methods:** Between 2005 and 2009, 454 patients were randomly selected by the pathologist to determine clarithromycin resistance. Helicobacter pylori was assessed by the modified Giensa 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.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases</th>
<th>C-sensitive (%)</th>
<th>C-resistant (total) (%)</th>
<th>Complete resistance (%)</th>
<th>Partial resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>28</td>
<td>78.5</td>
<td>21.4</td>
<td>33.3</td>
<td>66.6</td>
</tr>
<tr>
<td>2006</td>
<td>241</td>
<td>82.8</td>
<td>16.1</td>
<td>43.6</td>
<td>56.0</td>
</tr>
<tr>
<td>2007</td>
<td>46</td>
<td>78.3</td>
<td>21.7</td>
<td>60.0</td>
<td>40.0</td>
</tr>
<tr>
<td>2008</td>
<td>57</td>
<td>66.2</td>
<td>22.8</td>
<td>46.1</td>
<td>53.8</td>
</tr>
<tr>
<td>2009</td>
<td>81</td>
<td>82.7</td>
<td>18.0</td>
<td>28.5</td>
<td>71.4</td>
</tr>
<tr>
<td>Total</td>
<td>454</td>
<td>81.9</td>
<td>18.0</td>
<td>42.7</td>
<td>57.3</td>
</tr>
</tbody>
</table>

**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.
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 CLARITHROMYCIN 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, 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 or absence that, for example, Amoxicillin, Nytrofurans and bismuth medicine. Definition of prevalence of A2143C mutation (66.7%).

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 found 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, tetrA 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 cefalothin and 1% to tetracycline. No H. pylori strain was resistant to gentamicin and chloramphenicol.

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

Abstract no.: P07.23
SUCEPTIBILITY 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 in 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, tetracycin, 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%, respectively. Resistance to fluoroqui-
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 corrected, significance was maintained for two SNPs: one in NFKB1, one in CD14, two in NOD2 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
PDI 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.

Aims: 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 pathological techniques, rapid urease test and confirmed by RT-PCR (ureC gen). Twelve cases of H. pylori (+) gastritis and four of gastritis where (-) biopsies from patients suffering gastritis.

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.

P08 Inflammation and Host Response

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 cag-PAI strain 36.9. Mongolian gerbils were inoculated with either 36.9, 36.9ΔcagC, or 36.9ΔcagL 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 36.9ΔcagC. None of 19 animals inoculated by 36.9ΔcagL were colonized. There was no significant difference in density of 36.9 and 36.9ΔcagC colonization. Chronic inflammation, gastric IFN-γ and TNF-α transcripts were similarly increased in 36.9 and 36.9ΔcagC infected gerbils relative to controls. Corpus pathology was greater in 36.9ΔcagC than 36.9 infected gerbils. Additionally, 36.9ΔcagC infected gerbils had significantly increased epithelial cells proliferation (p < 0.05) 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 36.9ΔcagC 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 coinoculated (24 hour) with three H. pylori strains (HP1(cagA+); HP3(ureC+);(and HP4(cagA+), to different densities. From healthy voluntaries, lymphocytes were isolated and incubated (2 hour) in a 4:1 lymphocytes:AGS ratio with the cocultivates' 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 ± 4.9 and (HP1) = 20.2 ± 7.5* and PDL-1 (control = 12.0 ± 5.1 and HP2(105) = 22.3 ± 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 = 219.5 ± 54.5 and (HP4(105) = 287.1 ± 67.2* and for ICAM-1 (control = 407.8 ± 207.5 and (HP4(105) = 711.2 ± 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:

**INFLAMMATORY RESPONSE OF PRIMARY HUMAN GASTRIC EPITHELIAL CELLS TO HELICOBACTER PYLORI**

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Helicobacter pylori infection systemically causes a chronic gastric inflammation that can persist asymptomatically 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 AcgM 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.

**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 Helicobacter pylori-induced gastric inflammation. The aim of this study was to evaluate the PAR2 expression in human gastric biopsy materials and its correlation with the degree of gastritis.

**Aim:** To investigate PAR2 expression in H. pylori-infected patients and correlation with proinflammatory IL-8, IL-1ß as well as histological changes of the mucosa. Furthermore, PAR2 expression was studied in context to mucosal SLPI levels in H. pylori-infected patients and the induction of IL-2 associated with atrophy.

**Methods:** Twenty-two H. pylori-infected patients and 72 H. pylori-negative subjects underwent upper GI endoscopy. Intratumor derived mucosal biopsies, PAR2, IL-8, -1ß 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-1ß mRNA amounts were observed between both groups. PAR2 gene expression correlated positively with transcript levels of IL-8, IL-1ß as well mucosal SLPI levels in H. pylori-infected patients (r = 0.70; p < .0001), whereas no correlation was found with the degree of gastritis.

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

**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-1b, IL-1Ra, IL-8) with morphological changes in stomach at Helicobacter 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 Aboriginals Repubbles Khakassias (khakases).

**Methods:** endoscopic, gastric biopsy samples were investigated according to Sydney classification. Genomic DNA was typed for polymorphisms at position C+3953 T in the IL-1ß gene using RFLP analysis (Ban I), -251 A/T IL-8 (Mle 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-1ß, B2R2 IL-1Ra and AA -251 IL-8 from patients CG with atrophy; CT +3953 IL-1ß, B3R4 IL-1Ra and TT -251 IL-8 from patients CG without atrophy. Genotypes B2R2 IL-1Ra, AA -251 IL-8 and allele T +3953 IL-1ß associated with atrophy and determines its degree.

**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 Helicobacter pylori-related gastric cancer and duodenal ulcer.

**Methods:** In this study, the Asp1486Glu and Ile64Val polymorphism in the APE1 gene was investigated in 282 patients with Helicobacter 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 cases of Asp1486Glu in APE1 gene, the frequency of genotype of TT, TG, GG were 21%, 51%, 28% 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 Ile64Val, there was no significant difference was observed between two groups.

**Conclusion:** The Asp1486Glu polymorphism in APE1 gene was a susceptible factor of Helicobacter pylori-related gastric cancer compared with Helicobacter pylori-related duodenal ulcer, but Ile64Val polymorphism was not.
Conclusions: The Hp LPS may modulate negatively the cytokine secretion by the inflammatory cells and by this may influence the course of Hp infection.

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 PEPSSINOGENS 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 Multidisciplinary 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 (Biosit, 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 eradication compared to the mean level of PGII before treatment.

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>PGIa</th>
<th>PGIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy (n = 32), SD</td>
<td>62.89±/46.90</td>
<td>74.65±/46.90</td>
</tr>
<tr>
<td>No atrophy (n = 45), SD</td>
<td>93.11±/50.40</td>
<td>96.22±/62.79</td>
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<tr>
<td>All patients (n = 77), SD</td>
<td>80.55±/58.88</td>
<td>87.25±/71.52</td>
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*p value

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Abstract no.: P08.13

THE INFLUENCE OF BLOCKING CD25 ON IMMUNE PATHOPOIESIS OF H. PYLORI INFECTION AND TLR4 SIGNAL PATHWAY AND IGA

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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 pre-treatment. And mice were inoculated by Hp. Twelve weeks after inoculation, Hp, the expression of TLR4, MyD88, NF-κBp65 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 in control (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-κBp65 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-κB 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* Hp68 (HpHsp68) 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-HpHsp68 IgG were detected by commercial Western blot.

**Results:** The anti-rHsp60 IgG were present in all alanyzed sera, whereas the IgG to MbHsp65 and HpHsp68 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-rHsp60 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* HpHsp68 may induce a production of crossreacting antibodies which might be engaged in the development of deleterious inflammatory response during atherosclerosis. Grant no. NN 303 451 738.

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Abstract no.: P09.02

THE ASSOCIATION OF HELICOBACTER PYLORI INFECTION AND MICROALBUMINURIA

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**Background 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 triglycerides 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.

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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 radiomunnoassay.

**Results:** Twenty-seven men (34.62%) were infected (Hp+) and 11 infected men (40.74%) were seropositive for CagA (CagA+). Sperm motility in Hp+ and CagA+ men was significantly poorer than that observed in Hp− and Cag−A men (p < .01). Although semen levels were not influenced by Hp infection, ghrelin levels in men of Hp− men were significantly lower than those observed in infected 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.

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Abstract no.: P09.04

RELATIONSHIP OF ACID SUPPRESSIVE AGENTS AND GUT HORMONES IN RAT

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Although the use of acid suppressive agents is 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.

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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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**Background:** 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...
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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 < 1.3.

**Results:** Serum level of zinc in *H. pylori*-infected subjects with gastric mucosal atrophy (973.7 ± 153.5 µg/L) was lower than that of non-infected subjects (1008.0 ± 159.4 µg/L) (p < .01). Subjects who were born in 1950s, serum level of copper in *H. pylori*-infected subjects without gastric mucosal atrophy was 919.9 ± 136.9 µg/L, and it was lower than that of non-infected subjects (986.8 ± 141.3) (p < .05). Serum level of copper was also lower in *H. pylori*-infected subjects with gastric mucosal atrophy (875.6 ± 111.9) comparing with non-infected subjects (955.5 ± 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.

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**Abstract no.:** P09.06

**HELICOBACTER AND SMALL INTESTINAL BACTERIAL OVERGROWTH: CONSECUTIVE PLAYERS IN THE PATHOGENESIS OF IDIOPATHIC PAROXYSMAL DYSPEPSIA**

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**Background:** Hypokinesia in idiopathic Parkinsonism (IP) improved following *Helicobacter pylori* eradication, flexor-rigidity worsened (*Helicobacter pylori* 2010;15:279–95), whereas with failed eradication hypokinesia worsened, rigidity was unchanged.

**Methods:** We survey outcome of all anti-microbial-interventions at a clinic, where parkinsonism was objectively-quantified; *Helicobacter* screened for (urease-breath-test (INFAl) and/or stool-antigen ELISA (DakoCytomation)) and senal 4h-lactulose-hydrogen-breath-tests (LHBT, using 25G lactulose) performed.

**Results:** Of 66 IP-probands, 11 were *Helicobacter* positive, 25 had been previously treated (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) anti-microbial-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-term 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 anti-microbial-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 psychiatric-motor-overgrowth. Increased rigidity following antimicrobials points to consequent alteration in intestinal microbiota as a player in pathogenesis.

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**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 withdrawn 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 (60%) 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.

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**Abstract no.:** P09.08

**CHARACTERISTICS OF ATROPHIC GASTRITIS IN PATIENTS WITH EROSIIVE REFUX DISEASE (ERD) AND NON-EROISIVE REFUX 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 relationships 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/I ratio was low and gastric ghrelin mRNA was increased. In patients with histological moderate or marked corpus atrophy, PG I/I 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/I 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/I 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/I ratio, and ghrelin.

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**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 acid secretion, is frequently seen in *H. pylori*-infected elderly subjects. We examined whether both *H. pylori* infection and the degree of atrophic gastritis are associated 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 PG I <70 ng/mL and PG I/II < 3, and severe atrophy was PG I <50 and PG I/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
ERADIATION 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-<70) 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 infected 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 REFUX 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 Esomaprazole 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 compared. 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 had not significant effect on the result of reflux esophagitis therapy.
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 lgg/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 lansoprazole15 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 trials.

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 ($\chi^2 = 45.3$; $p = 0.00$). 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.

Conclusion: 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 unacceptable low.

Abstract no.: P10.03
SECOND-LINE RESCUE TREATMENT 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 13C-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-treatment 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 emerging 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
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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 64.2% (35/55), p = 0.04). 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 < 0.01), but treatment failure due to side effects showed no significant differences between two groups (2/85 vs 0/53).

Conclusion: Concomitant nonbithum-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 failure treatment.

Methods: Selection of studies: randomized controlled trials comparing LTT and BQT after one H. pylori eradication failure.


Data synthesis: intention-to-treat eradication rate and adverse events rate.

Results: Thirty-three 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; I² = 72%) with a significantly lower rate of adverse effects (14% vs 32%; OR = 0.30; 95% CI = 0.19–0.50; p < 0.001; I² = 46%) and serious adverse effects (0.3% vs 7.8%; OR = 0.15; 95% CI = 0.04–0.59; p = 0.007; I² = 0%). There were two outlying studies showing better results for BQT, which 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 = 0.002; I² = 52%). As LTT showed better efficacy in ten days compared with 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 < 0.001; I² = 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 Jasmoides 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 crosslinking. 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 (pH 7.4) 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. 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
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: 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.

Patient: 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 13C-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/µl), and thrombopenia (<150,000 platelets/µl).

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 DYSPESIA: 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, tini-dazol for the remaining 5 days) or traditional therapy (PDS patients: domperidone for 2 weeks; EPS patients: talidom for 2 weeks). Patients were followed up for 52 weeks, symptoms were assessed with dyspepsia score, which was derived by

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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 ($q_2 = 2.199$, $p = .333$) and at 52 weeks ($q_2 = 5.583$, $p = .061$). 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 ($q_2 = 2.241$, $p = .326$) but dyspepsia score was significant lower in EAC and sequential therapy group than control at 52 weeks ($q_2 = 12.576$, $p = .002$).

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 INFECION 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 empirical 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 clarithromycin-based therapy (PPI at standard dose b.i.d., 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:92-93%) by per protocol and 85% (95% CI:89-91%) by intention-to-treat. In the multivariate analysis, ulcer disease was a predictor of eradication success (OR:4.5; 95% CI:1.8–11). 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%, 78.7%, 85.2%, 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:bacteria + S (10 mg).


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 infection with endonucleases 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, rabeprazole, esomeprazole, or omeprazole) 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% (308/416) 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 rabeprazole group showed higher eradication rate (76.0% (225/297), 70.8% (172/243)) than those of other PPI treatment groups (lansoprazole: 65.5% (131/200), esomeprazole strontium: 66.7% (76/114)).

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). Subanalysis 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) gastrointestinal polymeric 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:PLA = (3 : 2 : 3), CSM:CMC:PLA = (3 : 2 : 3) and CSM:CMC:PLA = (3 : 1 : 1 : 3).

Characterisation: 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 (W0) and then immersed in buffer. The remaining system was weighted at time (Wn).

Results: CS and CMC hydrogels presented a porous surface. Pores were unevenly shaped (diameter:50–300 μm). CS:CMC:PLA formulation was structurally similar to CS:CMC, but with less pores. All formulations released the total amount of amoxicillin within 2 h. CS:CMC:CS:CMC:PLA showed a lower burst effect than CS:PLA. Hydrogels containing CS and CMC complex obtained a fast S values (9 and 14 at 10 minutes). PL addition modulated K, due to the PL steric hindrance and hydrophobicity. CS:CMC:PLA presented a suitable swelling and eroding profile prevailing erosion after 120 minutes (K, 0.672 10-1 min-1).

Conclusions: Controlled-release gastro-rettentive formulations were successfully obtained by dispersion under acidic conditions and vacuum drying process. Interpolymer complexes (CS:CMC:CS and CS:CMC:PLA) have demonstrated suitably swelling properties and drug release profiles at pH 5.0, providing a controlled amoxicillin release for 2 h. 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 efﬁciency 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, bismuth or NSAID or aspirin, allergy, gastronomy, pregnant women. All eligible patients were CLO-test and either histology or culture positive and were precribed: 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 13C-UBT and/or stool test.

**Results:** Ninety three patients (41F/52M, aged 18–81, mean: 51.8 years) were evaluated for eradication (39.5% smokers, 21.5% with ulcer disease). Adherence to treatment was 97.7% (95% CI 96.9–99.6). 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 (66.6% PP, 58.3% ITT) (p < .0001). 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 FUROXALIDONE 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 furoxalidone quadruple regimen plus dental plaque removal procedures as rescue treatment of refractory H. pylori infection.

**Methods:** Hundred and four patients with H. pylori positive 13C-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 + furoxalidone, 10 days) treatment and dental plaque removal treatment, the others (40 patients) accepted only quadruple regimen treatment. To detect H. pylori by 13C-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. Furoxalidone 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 ranitidine 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 is 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 13C-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, 15% 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).

**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-RESISTANT 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 13C-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-H. pylori, whereas both quadruple “concomitant” and “sequential” regimens maintain acceptable eradication rates for CLA-R and DUAL-R strains.

Abstract no.: P10.21

IMPORTANT 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 the appropriate antibiotic-containing regimen. The major barrier to high cure rates is antimicrobial resistance. The pattern of resistance in 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 Autonoma 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 cultured using two types of selective media, Brain Heart Infusion (BHI) blood agar and Helicobacter pylori Special Pepsine 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-BASED 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 antibiotics 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 13C-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 tolerated.

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 persistor state of the bacteria.

Aim: To test the hypothesis that a 6 week dual regimen of amoxicillin 1 g and omeprazole 20 g therapy combination daily for 6 weeks. Success was achieved 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 predefined as unacceptable.

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 accessed 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 predefined 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 was beyond 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 (H.p).

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 ANTIHELICOBACTER 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 + probiotic 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 probiotic 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 probiotics 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 Eylül 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 = 0.02, p = 0.03, p = 0.19, p = 0.35, and p = 0.31). When effect of alcohol consumption was examined, we noticed that the success of eradication increased in patients who have drunk “Turkish alcoholic beverage-raki” 9 cc/day for at least 15 days (p = 0.01). 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 = 0.044 and p = 0.027 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-INFECTED MONGOLIAN GERBILS


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Objective: 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 13C-urea breath test. Several therapies were used. Afterwards, ranitidine 150 mg o.d. was administered until eradication was confirmed by 13C-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.

Recurrent 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: Pectin ulcer bleeding 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 eradication rate in the first 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. Forty 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, 13C-urea breath test and biopsies were performed to diagnose H. pylori infection.

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 a decreasing tendency of eradication rates of first line therapy in recent 5 years in Korea (p < 0.01). The eradication rate of PBMTP therapy was 86.7%. The eradication rate of one week PBMTP treatment was 70.6% and that of two week PBMTP 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 the 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. Exclusional 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, transudion blood volume, Galsgow-Blachford 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 antplatelet 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: 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, transudion blood volume, Galsgow-Blachford 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.
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 2 single monkeys diagnosed with intestinal adenocarcinoma were analyzed by a combination of PCR and microaerobic culture for the presence of Helicobacter species. Nine (8.1%) of the macaques had a history of successful surgical resection of intestinal tumors. Using Helicobacter-specific species-specific primers, CO5 and OS7, 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 shown to be positive by culture and PCR for Helicobacter macacae. 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−/− 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 tappini”. In 2000, it was classified as Helicobacter flexispira taxon 8 and grouped with H. bilis taxon 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 IL-10−/− 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 IL-10−/− 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−/− mouse model to study the pathogenic potential of this enteropathic 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 broadsorbs 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 HOLE, and the highly virulent marker VacA protein. The plasmid backbone of our constructs 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 of 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-5 and IFNgamma, the IL-2 was measured as TH1 cytokine. 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.

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

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 26c/A (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/NSI-MS/MS. In this study, we detected eleven immunoreactive spots with the sera from three groups of patients. S5S ribosomal protein L7/L12 was the only protein 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 H.p vaccine and Th respond.

Methods: BALB/c mice were divided into three groups and immunized by: 1. Control group: 2. H.p vaccine; 3. Anti-Tim-3 antibody pretreatment + H.p vaccine; At 4 weeks after the last immunization, the mice from 1 and 3 groups were challenged by H.p gastric. At 4 weeks after the last challenge, mice were sacrificed and sample were collected. Hp, the level of cytokine, Foxp3+ 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 < 0.05). 2. Immunofluorescence of Hp was higher than that in control (p < 0.01), and in group with anti-Tim-3 antibody pretreatment were higher than group without pretreatment (p < 0.05). 3. The level of foxp3+ Tcell in mice of Hp vaccine were significantly higher than that in control (p < 0.01), and in group with anti-Tim-3 antibody pretreatment were significantly higher than those in groups without pretreatment (p < 0.01).

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 the last 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 < 0.05). 2. Immunofluorescence of Hp was higher than that in control (p < 0.01), and in group with anti-CD25 antibody pretreatment were higher than group without pretreatment (p < 0.05). 3. The level of Foxp3+ Tcell in mice of Hp vaccine were significantly higher than that in control (p < 0.01), and in group with anti-CD25 antibody pretreatment were significantly higher than those in groups without pretreatment (p < 0.01).

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 H. pylori-infected mucosa.

Materials and Methods: Eighteen C57BL/6 mice were inoculated with Hp SS1 by gavage three times with 3 x 107 viable cells. Mice were then treated with either PBS, curcumin (10 mg/mouse) or Symbiotic 2000® (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 (immunoresponsive and immunity pathway) analysis (Sabiosciences, Quagen).

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 1.6–1192). Similarly, the expression of the proinflammatory cytokines IL-1β, IL6, IL9, IL10, IL23, TNFα and INFγ was significantly augmented (range of fold-change 1338–8251). The treatment with either curcumin or sibutramine dramatically 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 Symbiotics 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 mice were separated in three groups: Control group (CG) (n = 5). Infected group (IG) (n = 10 and Symbiotic 2000® (SG) n = 10. CG received PBS by gavage; IG and SG were inoculated intragastrically with H. pylori SS1 cell susension (109 CFU/mL). Then, mice were treated either with PBS (CG and IG) or Symbiotic 2000® (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 Hp 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® may influence the outcome of Hp gastric inflammation.
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 Lactobacilli 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.: P11.11

DETECTION OF ENTEROHEPATIC AND GASTRIC HELICOBACTER SPP. IN WILD CHIMPANZES (Pan troglodytes) AND WESTERN LOWLAND GORILLAS (Gorilla gorilla)

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Little is known about the prevalence of gastric and enterohelobacterial 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 habituated 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. trogontum, H. typhlonius, H. gansniti 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. fennelliae/ 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 enterohelobacterial helicobacters should be considered.

Abstract no.: P11.14

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF HELICOBACTER SUI S 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-
Abstracts

Detection of Viable Helicobacter Suis in Pork by a Combination of Ethidium Monoazide (EMA) and Real-Time-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 findings suggest that pork is a source of H. suis infections for humans.

HISTONE-LIKE DNA BINDING PROTEIN (HH-15) FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION

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Background and Aim: We developed a monoclonal antibody HRII-51 with high specificity for H. pylori. MAB HRII-51-immunoreactant was molecular weight of 15 kDa, and then named as HHI-15 (H. hepaticus 15 kDa antigen). We purified HHI-15 and examined the possibility of HHI-15 to diagnose H. hepaticus infection. We also studied the structure of HHI-15 and analyzed the epitope to HRII-51.

Methods: HHI-15 antigen was highly purified by immunochromatography. HHI-15 direct sandwich ELISA (HHI-15-ELISA) was prepared in which HHI-15 was immobilized on ELISA plates. Accuracy of HHI-15-ELISA was examined using sera obtained from mice inoculated with Helicobacter spp. To identify the epitope to HRIII-51, HHI-15 mimotope peptides were synthesized and analyzed by ELISA and Western blot using HRIII-51 as the first antibody.

Results: By using Helicobacter spp. inoculated mouse sera, specificity and sensitivity of HHI-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 HRII-51. The 34 amino acids peptide of C-terminal sequence (Gly61-Lys94) was also reacted with HRII-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 HRII-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.

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. Helicobacteria detection in bile and tissue is based on two broad-spectrum and three species-specific (2 bilis and 1 hepatitis) 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.
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 Tapiurus terrestris associated with Helicobacter Like Organisms. A 12-year-old male Tapiurus 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 mucose 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 Warnering-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 Tapiurus terrestris in Venezuela.

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 Hematoxilin & Eosin (H&E) for light microscopy. Additionally the special staining procedure of Warnering-Starry was also carried out. Urease Test: the rapid urease test (commercial kit) was performed of gastric tissue. The necropsy showed mucosa cyanotic, abdominal distension severed. Gastric dilatation 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.