Helicobacter

European Helicobacter Study Group

XXVth International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer

Ljubljana, Slovenia
13-15 September 2012

Accepted Abstracts

W1 – Microbiology, Molecular Genetics and Virulence Factors

Abstract no.: W1.1

PREVALENCE AND CLINICAL SIGNIFICANCE OF HOMA AND HOMB, TWO NOVEL HELICOBATER PYLORI VIRULENCE MARKERS, IN SLOVENIAN PAEDIATRIC POPULATION

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Background: Although severe gastroduodenal disease mostly appears in adulthood after long-term *H. pylori* infection, peptic ulcer disease (PUD) may develop in young children, suggesting an involvement of potentially more pathogenic strains. Two novel *H. pylori* outer membrane proteins, *homB* and its paralogue *homA*, were suggested to influence the severity of disease manifestation.
Objective: To determine the prevalence of *homA* and *homB* in Slovenian paediatric population and to evaluate their clinical relevance, previously associated with non-ulcer dyspepsia and PUD, respectively.
Material and Methods: A total of 204 *H. pylori* positive gastric biopsies, obtained from children, were included in the study. The presence of virulence genes was determined by a single polymerase chain reaction (PCR) assay, which generates amplicons of 128-bp and 161-bp for *homA* and *homB*, respectively. Each of the genes was compared with density, activity and chronicity of *H. pylori* infection according to the Updated Sydney Histological Classification.
Results: Strains in which both *homA* and *homB* were detected (13/204) and strains with intermediate PCR product lengths (3/204) were excluded from further analysis. Thus, a total of 121/188 (64%) and 64/188 (34%) strains were positive for *homA* and *homB*, respectively. There was no statistically significant association between the presence of either *homA* and activity ($p = .73$) or chronicity ($p = .13$), while correlation was found between *homA* positivity and density ($p = .02$).
Conclusion: Due to the lack of association between either of genes and severe histological findings, it is unlikely that *homA* or *homB* represent important *H. pylori* virulence markers in children.

Abstract no.: W1.2

IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS IN THE TFS4 TYPE IV SECRETION SYSTEM OF *HELICOBACTER PYLORI*

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Genome sequence data has determined the duodenal ulcer promoting (*dupA*) gene to be encoded within a cluster of *vir* homologous type IV secretion system (T4SS) genes in the plasticity zones of several *H. pylori* strains. Sequence identity and the presence of characteristic sequence motifs indicates that DupA may be the VirB4 ATPase component of the T4SS, however the function of the T4SS, recently termed Tfs4, and the identity of its secretion substrates are unknown.
In this study, we aimed to assess the protein-protein interactions mediated by a *tfs4*-encoded VirD2-like protein using the yeast two-hybrid (Y2H) system. VirD2 proteins are relaxases typically involved in conjugation or interkingdom DNA transfer in association with several other proteins collectively referred to as the relaxosome. A homologue of one other relaxosome protein, VirC1 is also present in *tfs4*.
In a candidate approach, a pairwise Y2H interaction screen determined that VirD2 interacted with itself, VirC1 and an unknown protein encoded adjacent to VirC1. Interactions were generally weak indicating a likely requirement for stabilising factors inherent to a relaxosome complex. An interaction was not observed with a VirD4 coupling protein. In a secondary approach, a high titre genomic library has been constructed from a *tfs3 + /tfs4 + /cag+ H. pylori* clinical strain and is in the process of being screened to define the entire repertoire of T4SS proteins that interact with VirD2 and VirD4.
Our preliminary observations are consistent with known interactions in other T4SSs and suggest that Tfs4 may function in DNA transfer to a host cell.

Abstract no.: W1.3

SCREENING OF PROPHAGE SEQUENCES AMONG *HELICOBACTER PYLORI* ISOLATES

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Until recently, *Helicobacter pylori* was considered a bacterium without prophages. The presence of an incomplete prophage sequence in strain B38 and a complete prophage sequence in strain B45 showed otherwise.
Using a PCR strategy, based on degenerated primers designed after aligning bacteriophage integrase genes from *H. pylori* strains B38 and B45, and *H. acinonychis* prophage II, we found that integrase sequence was present in 21.4% (73/341) of the *H. pylori* clinical strains tested. The phylogenetic analysis of the sequenced region revealed that strains cluster according to their geographic origin, but not to their pathology. We have applied the same methodology to additional 147 European strains and 77 African strains, determining the presence of integrase sequence in 25.2% (37/147) of the former and in 19.5% (15/77) of the latter. Currently, we have a total of 565 strains screened for the presence of integrase gene, with 125 positive for this sequence (22.1%). To understand if these integrase sequences belong to reminiscent or complete prophages we are also screening for the presence of other prophage coding sequences. Among integrase positive strains, we found 19.2% (5/26) positive strains for the primase sequence and 53.3% (8/15) for the presence of the end of the phage. Presently, we are running the sequencing of the PCR amplified products in order to conduct the phylogenetic analysis. The results reinforce the abundance of prophages sequences in *H. pylori* and suggest that the majority of them belong to reminiscent prophages integrated within the bacterium genome. Work supported by FCT (PTDC/EBB-EBI/119860/2010).

Abstract no.: W1.4

GENE POLYMORPHISMS OF MICRORNAS IN *HELICOBACTER PYLORI*-INDUCED HIGH RISK ATROPHIC GASTRITIS AND GASTRIC CANCER

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Background and Aims: Different studies have shown that microRNAs (miRNAs) are deregulated in gastric cancer (GC). Several single nucleotide polymorphisms (SNPs) of genes related to miRNAs were linked with GC and premalignant lesions. The data on the potential association between miRNA SNPs and the risk of GC or *Helicobacter pylori*-induced atrophic gastritis, however, are scarce and partially conflicting.
The aim of our study was to evaluate potential associations between the presence of GC and high risk atrophic gastritis (HRAG) and SNPs of genes related to mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608.
Methods: Gene polymorphisms were analyzed in 538 subjects (GC: n = 106; HRAG: n = 222, controls: n = 210) of Caucasian origin. Mir-146a C>G (rs2910164), mir-149 T>C (rs2292832), mir-196a-2 C>T (rs11614913), mir-379 A>G (rs61991156), mir-499a A>G (rs3746444) and mir-608 C>G (rs4919510) SNPs were genotyped by RT-PCR.
Results: Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. There was a tendency for mir-196a-2 C/C genotype to be associated with lower incidence of HRAG (49.0% in controls vs. 41.4% in HRAG group, $p = .079$). Allele C of mir-196a-2 SNP was more frequent in controls when compared to HRAG group, 67.8% and 60.1% respectively, however it failed to reach significance level ($p = .087$). We did not find any significant associations for all miRNA polymorphisms in relation to GC or HRAG.
Conclusions: Mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608 SNPs are not linked with gastric carcinogenesis, and therefore do not appear as potential biomarkers for identifying individuals with higher risk for GC.

Abstract no.: W1.5

VARIATION IN *HELICOBACTER PYLORI CAGA* PROMOTER REGION IS ASSOCIATED WITH CAGA EXPRESSION

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Background and Aim: *Helicobacter pylori* CagA is associated with peptic ulcer disease and gastric carcinoma. Recently, four motifs that exhibit sequence heterogeneity in the promoter region of *cagA* have been identified. Among them, the AATAAGATA (+59) motif was defined as a determinant of enhanced CagA expression. The aim of this study was to characterize the *H. pylori cagA* promoter region and to assess the relationship between variation in this region and CagA expression levels in Portuguese strains.

Methods: Five-hundred base pairs upstream of the translational start site of *cagA* of 37 clinical isolates and two reference strains (26695 and 60190) were sequenced, and CagA expression levels were evaluated by western blot.

Results: Nucleotide sequences of the *cagA* promoter region showed high heterogeneity among *H. pylori* strains. These differences included variation in sequence and copy number of the -344 and -54 motifs, and variation in sequence of the -10 motif. Additionally, the +59 motif was absent (18.9%), present as one (78.4%) or as two (2.7%) copies. CagA expression levels were associated with the -10 TATAATGA sequence (p = .024) and with the presence of the +59 motif (p = .022). No relationships were found between CagA expression and the number of copies of the -344 or -54 motifs.

Conclusion: Variation in the *cagA* promoter region may be useful markers to predict disease risk, and may help to explain why some patients infected with *cagA*-positive *H. pylori* develop disease and others do not.

Acknowledgements: PTDC/SAU-SAP/120024/2010; SFRH/BD/45841/2008.

Abstract no.: W1.6

FUNCTIONAL CHARACTERISATION OF A VIRD2-LIKE RELAXASE COMPONENT OF A NOVEL TYPE IV SECRETION SYSTEM IN *HELICOBACTER PYLORI*

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Genetic factors, often encoded in highly variable ''plasticity zones'' (PZs), are increasingly recognised to contribute to the virulence of *Helicobacter pylori* strains and may lead to severe disease outcomes. The *H. pylori dupA* gene, associated with the development of duodenal ulcer, is located within a cluster of *vir*-like genes in the PZs of several genome sequenced *H. pylori* strains. It likely encodes a VirB4 ATPase component of a fourth *H. pylori* Type IV secretion system, Tfs4. A putative substrate of this secretion system is encoded by a *virD2*-like gene elsewhere in the *tfs4* gene cluster.

VirD2 proteins are relaxases which function as carriers in interbacterial (conjugation) and interkingdom DNA transfer. We have characterised the activity of Tfs4 VirD2, as a candidate secreted protein destined for interaction with a host cell, to provide insight into the functional role of the Tfs4 secretion system.

The VirD2 protein purified from three clinical *H. pylori* strains appears to associate non-specifically with circular ssDNA and dsDNA in vitro. It acts as a relaxase cleaving ssDNA oligonucleotides specifically at the conserved bacterial ''nick site'' (*oriT*) sequence ATCCTG, and covalently attaches to the liberated 5′ DNA end via a conserved catalytic tyrosine residue. The poorly conserved C-terminal domain is not required for relaxase activity but may instead be required for targeting. Collectively, our data suggest that VirD2 functions in the processing and mobilisation of *H. pylori* DNA, indicating a role for Tfs4 in DNA transfer.

W2 – Epidemiology and Pediatrics

Abstract no.: W2.1

INCIDENCE OF HELICOBACTER PYLORI INFECTION IN CHILDREN DURING A 1-YEAR FOLLOW-UP AND THE INFECTION STATUS IN FAMILIES IN A RURAL AREA OF JAPAN

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Objectives: The purpose of this study is to examine the change of infectious status and route of transmission of *H. pylori* infection in a rural area of Japan.
Subjects and Methods: Children aged 0–11 years in 16 schools in Sasayama-city were asked to give stool samples both in 2010 and 2011 (prevalence may be shown by Ueda et al.). Of the participants, 439 gave stool twice with 1-year interval. Their stool samples were examined using Testmate Pylori Antigen EIA (Wakamoto Co. Ltd., Tokyo). According to the stool antigen test in 2011, 15 families (57 members) of positive and 45 families (163 members) of negative index children were asked to give stool samples. To know intra-subjects similarity of *H. pylori* strains, multi locus sequence typing (MLST) is ongoing using DNA from *H. pylori* in stool samples.
Results: Of the children giving samples twice, 431 were negative and eight were positive in 2010, and no change in *H. pylori* antigen status was observed during 1-year interval. Thirteen families of positive and 23 families of negative index children joined the study. Prevalence (positive/subjects) of family members of positive and negative index children was 35.0% (14/40) and 7.4% (4/54), respectively; 42.9% (9/21) and 8.3% (3/36) among the parents, and 14.3% (2/14) and 6.3% (1/16) among the siblings, respectively. Plural children were infected in no families but one with three positive siblings.
Conclusion: Child to child infection seems rare in this population. MLST will make route of transmission clearer.

Abstract no.: W2.2

A POPULATION-BASED ENDOSCOPIC SURVEY OF *HELICOBACTER PYLORI* INFECTION IN MYANMAR

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Aim: Myanmar is located in Southeast Asia, bordering the Andaman Sea and the Bay of Bengal, China, Laos and Thailand. This country has high *H. pylori* infection although gastric cancer prevalence is unknown. The aim of this study was to determine the prevalence of *H. pylori* and the causes of dyspepsia in this population which have never been previously documented.
Method: We conducted a cross-sectional population-based endoscopic survey of volunteers during October 2011. The survey took place in Mandalay and the capital city of Yangon. Consent forms and complete questionnaires were obtained from each patient. Gastroscopy and biopsies were performed for CLO-test, culture, histopathology.
Results: Total of 333 subjects were included (129 men (39%) and 204 women (61%), mean age 43.8 years). Endoscopic findings revealed gastritis 86.8%, GERD 6.9%, gastric ulcer 1.8%, duodenal ulcer 2.1% and gastric cancer 1.5%. Overall *H. pylori* infection rate was 31.2% (104/333 patients). Gastric cancer was found only in Yangon. *H. pylori* infection was significantly higher in the capital city of Yangon than in Mandalay (33.1% vs. 24.3%; $p < .05$). Interestingly, GERD patients was also significantly higher in Yangon than in Mandalay (8.4% vs. 1.4%; $p < .05$).
Conclusion: Prevalence of *H. pylori* infection is not as high as expected in Myanmar from our survey in Yangon and Mandalay. It is important to know the disease burden of PUD, GERD and gastric cancer in relation to *H. pylori* infection in this country to implement screening and reduce the risks of *H. pylori* infection and gastric cancer in Myanmar.

Abstract no.: W2.3

NATIONWIDE MORTALITY IN DUODENAL AND GASTRIC ULCERS; A 40-YEAR FOLLOW-UP

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Background: Bleeding peptic ulcers are known to be the leading cause of *H. pylori*-associated mortality in non-malignant gastric diseases especially in elderly population. Cure of *Helicobacter pylori* infection has been shown to lead to permanent healing of gastritis and peptic ulcer disease.
Aim: To study the mortality in duodenal and gastric ulcers before and during the ''helicobacter era''.
Methods: Age adjusted (World Standard) mortality data from 1971 to 2010 were provided by the Statistics Finland.
Results: In men the mortality in duodenal ulcers was from 0.75 to 2.42 and in women from 0.32 to 1.15. The corresponding values for gastric ulcers were in men from 0.95 to 3.18 and in women from 0.50 to 2.04. The mortality was higher in men than in women for both ulcers. The highest values were seen around 1990. After that the mortality in gastric ulcer decreased clearly and the lowest values were seen during the last 5 years.
Conclusions: The lowest mortality figures for gastric ulcer during the latest observation years may reflect the increasing number of subjects whose helicobacter infection has been treated successfully.

Abstract no.: W2.4

PRE-NEOPLASTIC LESIONS IN GASTRIC BIOPSIES OF CHILDREN ARE NOT DEPENDENT ON INFECTION WITH *CAGA*, *VACA*, *ICEA* AND *BABA2* STRAINS

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Aims: Atrophic gastritis and intestinal metaplasia are frequently observed in gastric biopsy specimens of adults with *Helicobacter pylori* (*H. pylori*). The incidence of the lesions and association between atrophy, intestinal metaplasia and *H. pylori* infection is not well defined in children.
Methods: The gastric antrum was histologically evaluated according to the Updated Sydney Classification in 190 children infected with *H. pylori*. *CagA, vacA and iceA* status was determined as reported previously (*J Pediatr Gastroenterol Nutr* 2009;49:289–96). The presence of *babA2* gene was determined by using two different primer sets targeting 850-bp (*Proc Natl Acad Sci USA* 1999;96:12778–83) and 271-bp (*Gut* 2003;52:927–32) fragments of *babA2*. Each of these genes was compared with atrophy and intestinal metaplasia of gastric mucosa.
Results: Mixed *H. pylori* infection was found in 20/190 (10.5%) children which were excluded from further analysis. Grade 1 and 2 antral atrophy was observed in 52/170 (30.6%) and 2/170 (1.2%) of patients, respectively. No patient had grade 3 antral atrophy. Intestinal metaplasia was detected in 9/170 (5.3%) of children. Except for *VacAs1* genotype ($p = .05$), no significant association was found between antral atrophy and presence of *cagA* ($p = .27$), *iceA1* ($p = .85$), *vacAm1* ($p = .49$), *babA2* (850-bp) ($p = .30$) and *babA2* (271-bp) ($p = .76$) alleles of *H. pylori*. Investigated genes were not associated with detection of intestinal metaplasia, except for *iceA1* allele ($p = .002$).
Conclusion: Our study demonstrated that precancerous lesions are present in a high percentage of children infected with *H. pylori*. The development of lesions is not dependent on infection with more virulent strains.

Abstract no.: W2.5

DYSPEPSIA AND *H. PYLORI* INFECECTION: A 15 YEARS STUDY

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Background: Dyspepsia is claimed to be correlate with Hp infection and the majority of the literature proves improvement in dyspeptic symptoms after eradication. The majority of studies nevertheless are based on short follow-up.
Aim: To verify the changement in dyspeptic symptoms in a cohort of patients cured by Hp infection 15 years follow-up.
Material and Methods: One hundred and forty-one patients (82 F, mean age: 61 years) were considered, according the following criteria: eradication Hp

successfully proved by at least two test; cure administered before 1999; availability of symptomatological assessment before the eradication therapy; proved persistence of negative Hp between after January 2011; availability of new assessment of dyspeptic symptoms by means of a structurated interview by using a visual analogic scale based on six scored symptoms namely: nausea, bloating, postprandial fullness, epigastric pain, regurgitation, heartburn; no previous diagnosis of peptic ulcer; age <50 years at the time of the Hp eradication.

Results: One hundred and nine patients experienced same symptoms after the 15 years period of follow-up, while a group of 32 remained asymptomatic. In the group of the symptomatic patients, 45 referred two symptoms or more; it's noteworthy that 24 patients experienced typical GERD symptoms like hearthburn or regurgitation.

Conclusions: Dyspepsia is a multifactorial long-life clinical condition; over 75% of patients 15 years after Hp cure experienced currently dyspeptic symptoms. Further studies might be encouraged in better define the profile of asymptomatic patients after a very long follow-up from the Hp eradication, to try to identify factor involved in a more favourable prognostic outcome.

Abstract no.: W2.6

NOVEL IMMUNO-LINE SYSTEM TO DETECT INFECTIONS 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 associated diseases. To date, it is not possible to identify patients with 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. Genes were amplified from *H. pylori* strains G27 and 26995 and cloned into the expression plasmid pQETri2. After expression as soluble 6xHis-fusion proteins, they were purified using affinity chromatography and gel filtration. All proteins (CagA, VacA, GroEL, gGT, HcpC, UreA) were bound to nitrocellulose membranes to detect serologic immune responses using HRP-conjugated antibodies. For validation of the assay a cohort of 1350 patients was established. The mean age of the cohort was 51.7 ± 16.5 years and the female/male distribution was 54% and 46% respectively. Of the 1350 patients, 646 (47.9%) were negative and 265 (19.6%) were *H. pylori* positive in histology. 439 (32.5%) patients who had previously undergone *H. pylori* treatment, were excluded from the study. The assay showed a sensitivity and specificity of >95% compared to histology and ELISA. In direct comparison to the previous lysate blot, the immuno-line assay had increased discriminatory power. We also could show that the assay score correlates significantly with the degree of inflammation and the degree of activity in infected patients.

Abstract no.: W3.1

W3 – Inflammation and Host Response

Abstract no.: W3.1

IL-8 RESPONSE OF DENDRITIC CELLS TO HELICOBACTER PYLORI IS DETERMINED BY STRAIN SPECIFIC LPS

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The aim of our study was to test the in vitro stimulatory effect of different *Helicobacter pylori* isolates on dendritic cells (DCs) maturation and antigen presenting function in order to elucidate the differences between *Helicobacter pylori* strains, susceptible/resistant to antibiotic eradication therapy. DCs maturation and antigen presentation were monitored by flow cytometry analysis of the MHC-II, Toll-like receptor (TLR) and costimulatory molecules expression, and by determining cytokine secretion. DCs stimulated with *Helicobacter pylori* isolated from patients with previous eradication failure expressed less HLA-DR, CD86, TLR-2 and interlevkin-8 (IL-8) compared to *Helicobacter pylori* strains susceptible to antibiotic therapy; the latter expressed lower production of IL-10. Polymyxin B inhibition of lipopolysaccharide reduce IL-8 secretion in the group of *Helicobacter pylori* strains susceptible to antibiotic therapy. The differences in IL-8 secretion between both groups are lipopolysaccharide dependent, while the differences in secretion of IL-10 remain unchanged after lipopolysaccharide inhibition. Cathepsin X inhibitor Mab 2F12 reduced the secretion of IL-6 and the secretion was significantly lower in the group of *Helicobacter pylori* strains isolated from patients with previous eradication failure. In conclusion, *Helicobacter pylori* strains, susceptible/resistant to antibiotic eradication therapy differ in their capability to induce DCs maturation and antigen presenting function.

Abstract no.: W3.2

INVOLVEMENT AND SOURCE OF B-CELLS ACTIVATING FACTOR(S) IN *H. PYLORI*-ASSOCIATED DISEASES

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Chronic inflammation during *Helicobacter pylori* (*HP*) infection can give rise to organized lymphoid tissue in the gastric mucosa. This so-called mucosa-associated lymphoid tissue (MALT) may progress to B-cell lymphoma of MALT type. In this context, it remains unclear which cytokine(s) or factor(s) promote lymphomagenesis. We demonstrate that gastric MALT lymphoma express high levels of the proliferation inducing ligand (APRIL), a cytokine crucial in sustaining B cell proliferation. We found that APRIL is produced in vivo almost exclusively by tumour-infiltrating macrophages located in proximity to neoplastic B-cells. Accordingly, macrophages produce APRIL in vitro upon *HP*-infection or stimulation with *HP*-specific T cells. Collectively, our results represent the first evidence for an involvement of APRIL in gastric MALT lymphoma development in *HP*-infected patients.
The cytokine BLyS has been reported to be crucial in promoting the expansion of Th17 cells and IL-17 was indicated as a crucial cytokine for BLyS-mediated pro-inflammatory effects in two models of autoimmune diseases. Given that *HP*-driven chronic gastritis is the typical inflammatory condition that leads to autoimmune gastritis (AIG) in susceptible patients, we hypothesized that the axis *HP*/BLyS/Th17 could be crucial for the development of AIG. We got preliminary evidence that in *HP*-gastritis there is an increased expression of BLyS and IL-17. We found that in vitro *HP*-infected neutrophils and macrophages secrete high amount of BLyS. Moreover, we found that BLyS-treated monocytes secrete Th17 polarizing cytokines, IL-6, IL-23, TGF-β, IL-1β. Taken together these data suggest the involvement of BLyS/Th17 axis in the development of AIG in *HP* induced gastritis.

Abstract no.: W3.3

MUCIN EXPRESSION IN *HELICOBACTER PYLORI* ASSOCIATED GASTRIC ULCER DISEASE

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Introduction: *H. pylori* is the main cause for peptic ulcer disease in addition to NSAID treatment. By changing mucin expression in the host, as well as by adapting mechanisms for mucin manipulation, *H. pylori* is overcoming the protection of the mucin unstirred layer.
Aim: To describe mucin expression in *H. pylori* associated peptic ulcer in the stomach.
Method: We randomly selected 92 patients with *H. pylori* positive (group 1, n = 54), or negative (group 2, n = 38). Immunohistochemistry for T-cell CD/CD8, MUC1, MUC4, MUC5AC, MUC6 and MUC17 staining was performed on sections of the mucosa from the ulcer margins. Inflammation score was assessed according to the Sidney system. Specific lectin staining was also performed: ECA (for Neu5Acα2-6gal/GalNAc), SNA (Galβ1-4GlcNAc, type 2 backbone structure exposed after removal of sialic acid), VVA (Tn Ag), and PNA (T Ag).
Results: Inflammation score and CD4/CD8 ratio was higher in *H. pylori* positive patients ($p = .009$). In *H. pylori* infected patients foveolar MUC1 and glands MUC4 expression and SNA, ECA and PNA staining intensity were significantly higher than in negative patients. No statistically significant difference was demonstrated for the other mucins or sugar side chains.
Conclusion: Increased expression of the membrane bound mucins MUC1 and MUC4, and of specific sugar side chains may reflect the influence of *H. pylori* on the mucin secretion by the gastric epithelium, and enable disruption of the mucus unstirred layer by the bug. This observation might be important, since the protection efficiency against acid and pepsin provided by different mucins is probably not equal.

Abstract no.: W3.4

MYELOID DIFFERENTIATION PRIMARY RESPONSE GENE 88 (MYD88) SIGNALING IS PROTECTIVE DURING HELICOBACTER-INDUCED GASTRIC CANCER

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The chronic inflammation induced during infection with *Helicobacter pylori* is widely accepted to be the trigger for gastric carcinogenesis. The central objective of our study was to investigate the role of a key signaling molecule, myeloid differentiation primary response gene 88 (MyD88) in *Helicobacter*-induced gastric cancer. MyD88 is an adaptor molecule in the inflammatory pathways involved in interleukin (IL) -1/IL-18 and Toll-like receptor signaling and is considered to be responsible for the chronic inflammation attributed to *Helicobacter* infection. To determine the role of MyD88 in *Helicobacter*-induced gastric cancer, we used a well-characterized gastric cancer mouse model that involves infection of C57BL/6 mice with *H. felis*. Wild type (WT) and mice deficient in MyD88 (*Myd88*$^{-/-}$) in the C57BL/6 background were infected with *H. felis* for 25 or 47 weeks and mouse stomachs processed for histology, immunohistochemistry, gene expression analysis, and apoptosis. *Myd88*$^{-/-}$ mice were more heavily colonized with *H. felis* and had a higher apoptotic index than WT mice. At 25 weeks, *Myd88*$^{-/-}$ mice showed evidence of dysplasia, which was absent in WT mice. By 47 weeks, *Myd88*$^{-/-}$ mice had developed severe dysplasia, which is considered gastric cancer in situ while WT mice only showed early evidence of dysplasia. MyD88 deficiency therefore resulted in rapid progression to gastric dysplasia. In conclusion, our data show that in the absence of MyD88, infection with *Helicobacter* resulted in accelerated development of gastric cancer. These data suggest that MyD88 signaling may protect the gastric epithelium from damage associated with *Helicobacter*-induced inflammation.

Abstract no.: W3.5

THE HYPOXIA INDUCED FACTOR 1 IS INVOLVED IN *HELICOBACTER PYLORI*-INDUCED PRO-INFLAMMATORY RESPONSE OF GASTRIC EPITHELIAL CELLS IN VITRO

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The pathogenicity of *H. pylori* has been associated with the production of numerous virulence factors. The proinflammatory effect is mediated mainly by the type IV secretion system encoded by the *cag* pathogenicity island (*cag*PAI). Recent literature suggests that the hypoxia inducible factor (HIF) could be involved in response to pathogens.
The aim of this study was to determine if HIF is involved in the pro-inflammatory response induced by *H. pylori* in gastric epithelial cells in vitro.

To achieve this goal, we used different *H. pylori* strains for coculture experiments with AGS gastric epithelial cells. The expression and activation of HIF1alpha and HIF2alpha was evaluated by western blot on coculture cell lysates. The secretion of IL-8 proinflammatory cytokines and VEGF in coculture supernatants was evaluated by ELISA. Transduction experiments of AGS cells with lentiviral vectors were performed in order to overexpress HIF1alpha or HIF2alpha or to inhibit their expression by shRNA.

Infection with *H. pylori* strains induced and increased IL-8 and VEGF expression, *cag*PAI dependent manner for IL-8 and to a lesser extent for VEGF. In AGS cells, HIF1alpha but not HIF2alpha is expressed. *H. pylori* infection only stimulated HIF1alpha expression.

Overexpression of either HIF1alpha or HIF2alpha did not stimulate *H. pylori*-induced IL-8 and VEGF secretion. However, inhibition of HIF1alpha by shRNA significantly inhibited *H. pylori*-induced VEGF and IL-8 secretion, confirming the role of HIF1alpha in *H. pylori*-induced cell signaling. Our results suggest that HIF1alpha plays a significant role in *H. pylori* induced proinflammatory response in gastric epithelial cells.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
CD44	None	2	51,3750	,88388	,62500	43,4336	59,3164	50,75	52,00
	Little	15	45,8000	28,16504	7,27218	30,2027	61,3973	12,00	98,75
	Mild	8	82,9062	29,87621	10,56284	57,9291	107,8834	31,75	138,00
	Dense	2	87,3750	44,01740	31,12500	-308,1056	482,8556	56,25	118,50
	Total	27	60,2870	32,90997	6,33353	47,2683	73,3058	12,00	138,00
CD105	None	2	,0000	,00000	,00000	,0000	,0000	,00	,00
	Little	15	11,8833	22,87854	5,90721	-,7864	24,5530	,00	83,75
	Mild	8	,0000	,00000	,00000	,0000	,0000	,00	,00
	Dense	2	27,1250	38,36054	27,12500	-317,5308	371,7808	,00	54,25
	Total	27	8,6111	19,98766	3,84663	,7043	16,5180	,00	83,75
Msi	None	2	15,5000	2,12132	1,50000	-3,5593	34,5593	14,00	17,00
	Little	15	34,2333	29,29125	7,56297	18,0124	50,4543	,00	92,75
	Mild	8	28,1250	27,14182	9,59608	5,4339	50,8161	,00	59,25
	Dense	2	89,7500	33,23402	23,50000	-208,8458	388,3458	66,25	113,25
	Total	27	35,1481	31,26771	6,01747	22,7791	47,5172	,00	113,25

Abstract no.: W3.6

THE EFFECT OF *H. PYLORI* DENSITY ON GASTRIC STEM CELLS AND GASTRIC MESENCHYMAL STEM CELLS

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H. pylori is wellknown as a gastric class 1 carcinogen, therefore we aimed to search the effect of *H. pylori* on gastric stem cells and gastric mesenchymal stem cells.

The gastric biopsy materials of *H. pylori* positive children were retrospectively studied. *H. pylori* gastritis was graded according to Sydney classification Stem cell evaluation was made immunohistochemically.

There was a positive correlation between *H. pylori* density both CD44 positive mesenchymal stem cells and Msi positive gastric stem cells. CD105 positive cell counts were also increased among *H. pylori* density not being statistically significant.

As *H. pylori* density increased both mesenchymal and gastric stem cells are increased. The increase in CD44 cells may have an effect on the chronicity and premalignant capacity of the infection. On the other side the increase in CD105 and gastric stem cells are helpful for reperation and regeneration of the lesions. CD44 and Msi may be helpful on the prognosis of patients having *H. pylori* gastritis.

W4 – Clinical Trials and Drug Resistance

Abstract no.: W4.1

TRIPLETHERAPY GUIDED BY MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCES VERSUS STANDARD TRIPLETHERAPY FOR HELICOBACTER PYLORI INFECTION: A RANDOMISED TRIAL

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Introduction: Efficacy of clarithromycin (Cl) or levofloxacin (Levo) based triple therapies is decreasing due to high level of resistance. A new test, Geno Type HelicoDR, for molecular detection of resistance to Cl and to Levo is now available. The aim of the study was to evaluate the eradication rate of *H. pylori* with a triple-therapy guided by HelicoDR vs. the standard triple-therapy

Patients and Methods: A prospective randomised open-label multi-center study (13 sites in France). Patients selected on the basis a *H. pylori* positive HelicoDR on gastric biopsies. Randomisation in two arms: standard triple therapy with IPP-amoxicillin – Cl for 7 days or treatment guided by the results of molecular sensitivity testing (ClS strains: standard therapy; ClR-LevoS strains: IPP-amoxicillin- Levo for 10 days; ClR-LevoR strains: IPP-amoxicillin- metronidazole for 14 days. *H. pylori* eradication was assesssed by C13 urea breath test (UBT).

Results: Five hundred and thirty out of 1.386 included patients (38%) were *H. pylori* positive. Two hundred and sixty-two were randomised in the control arm and 268 in the test arm; H/F: 52%; mean age: 47 year; peptic ulcer: 20%; born in France: 35%. Results of UBT were available in 200 control patients and 198 test patients. Eradication rates were respectively 72.5% and 85.6% ($p = .001$).

Conclusion : This study shows that triple therapy guided by HelicoDR is a better option to eradicate *H. pylori* than probabilist standard triple therapy in a country with a high rate of clarithromycin resistance. It could be proposed as an alternative to sequential therapy or bismuth-containing quadruple therapy.

Abstract no.: W4.2

PHASE IV, PROSPECTIVE, RANDOMIZED AND COMPARATIVE STUDY BETWEEN SEQUENTIAL AND CONCOMITANT THERAPY FOR *HELICOBACTER PYLORI* ERADICATION IN ROUTINE CLINICAL PRACTICE. PRELIMINARY RESULTS

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Background: *H. pylori* eradication rate with standard triple therapy is lower than 80%. Non-bismuth quadruple ''sequential'' and ''concomitant'' regimens have demonstrated higher effectiveness. Few trials had compared both strategies.

Aim: To compare the effectiveness and safety of ''sequential'' and ''concomitant'' therapies for *H. pylori* treatment.

Methods: Design: We conducted a prospective randomized multicenter clinical trial in 11 Spanish hospitals. Patients naïve to eradication therapy with dyspepsia or peptic ulcer disease were included and randomized (1:1) to sequential (omeprazole [20 m/12 hours] and amoxicillin [1 g/12 hours] for 5 days, followed by 5 days of omeprazole [20 mg/12 hours], clarithromycin [500 mg/12 hours] and metronidazole [500 mg/12 hours]) or concomitant treatment (same four drugs taken concomitantly for 10 days). Eradication was confirmed with ^{13}C-UBT or histology 8 weeks after treatment: Adverse events (AEs) and treatment compliance were evaluated with questionnaires and pill count.

Results: Two hundred and seventeen consecutive patients have finished follow-up. Mean age was 47 years, 61% were females and 18% had peptic-ulcer; 24% were smokers. Intention-to-treat eradication was 89.2% (95% CI = 85–93%) for the ''concomitant'', and 84.9% (80–89%) for the ''sequential'' regimen ($p = .23$). Respective per-protocol rates: 91.4% (87–95%) and 84.9% (80–90%) ($p = .128$). Logistic regression: no association found (sex, age, smoking habit, type of disease, and type of treatment). Compliance was similar for both strategies. AEs were reported in 56% of patients (Sequential = 51% vs. Concomitant = 61%; $p = .12$) Most common AEs were metallic taste (36%) and diarrhea (28%). AEs were all mild or moderate.

Conclusion: ''Concomitant'' and ''sequential'' non-bismuth quadruple therapies seem similarly effective for first-line *H. pylori* eradication treatment, and both achieve >80% cure rates. The rate of adverse events was high but their intensity was mild.

Abstract no.: W4.3

SEQUENTIAL AND QUADRUPLE THERAPIES FOR *HELICOBACTER PYLORI* ERADICATION COMPARED WITH TRIPLE THERAPY IN SLOVENIA: A MULTICENTER, PROSPECTIVE, RANDOMIZED, CONTROLLED TRIAL

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Background: Lower *H. pylori* eradication rates with triple regimen (<80%) in Slovenia are due to clarithromycin resistance. Whether sequential and quadruple therapies give better results was unknown.

Methods: Multicenter prospective randomised trial comparing esomeprazole 20 mg, amoxicillin 1000 mg, clarithromycin 500 mg bid for 7 days (EAC) with esomeprazole 20 mg, amoxicillin 1000 mg bid for 5 days and esomeprazole 20 mg, clarithromycin 500 mg, metronidazole 400 mg bid for another 5 days (sequential) and esomeprazole 20 mg, amoxicillin 1000 mg, clarithromycin 500 mg and metronidazole 400 mg bid for 10 days (quadruple) were compared. *H. pylori* was diagnosed with RUT, histology, UBT and culture. Eradication was confirmed 1 month after therapy with UBT. We included 199 *H. pylori* treatment naive patients.

Results: Eradication rates (ITT) were 80% in EAC; 94.3% in seguential; 95.3% in quadruple. Both sequential and quadruple therapies were superior to EAC ($p = .005$). *H. pylori* resistance rates were: metronidazole 22.7%, clarithromycin 11% and amoxicillin 0.6% . More clarithromycin and metronidazole resistance strains were eradicated with sequential and quadruple therapies than with EAC ($p < .05$). There were no differences in indications for therapy, smoking or alcohol consumption between patient groups.

Conclusions: *H. pylori* eradication rate is significantly better with sequential and quadruple therapies compared to EAC. There were no differences in side effects or patients' drop out rate.

Abstract no.: W4.4

COMPARISON OF THREE THIRD-LINE RESCUE TRIPLE REGIMENS FOR *HELICOBACTER PYLORI* INFECTION IN JAPAN

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Objectives: We compared the efficacy of quinolone- and ecabet sodium-based *Helicobacter pylori* eradication regimens as rescue therapies for the eradication of *H. pylori*.

Methods: We attempted to eradicate *H. pylori* in 132 Japanese patients who had ever failed in eradication of *H. pylori* with the triple PPI/amoxicillin/claithromycin therapy (1st line) and then the triple PPI/amoxicillin/metronidazole therapy (2nd line). They were assigned to either the triple therapy with rabeprazole, amoxicillin 500 mg qid and sitafloxacin therapy 100 mg bid (RAS) for 1 or 2 weeks, the triple therapy with rabeprazole, metronidazole 250 mg bid and sitafloxacin 100 mg bid (RMS) for 1 or 2 weeks, or the triple therapy with rabeprazole, amoxicillin 500 mg qid and ecabet sodium 1 g qid (RAE) for 2 weeks. Rabeprazole 10 mg was dosed twice daily (bid) for poor metabolizers of CYP2C19 and four times daily

(qid) for intermediate or rapid metabolizers of CYP2C19. Eradication was assessed via the 13C-urea breath test and RUT.
Results: Per-protocol analyses of eradication rates were 84.4% (27/32) by RAS for 1 week, 94.1% (16/17) by RAS for 2 weeks, 93.3% (28/30) by RMS for 1 week, 92.9% (13/14) by RMS for 2 weeks and 94.7% (36/38) by RAE for 2 weeks.
Conclusion: The eradication rate with the rabeprazole/sitafloxacin/amoxicillin therapy for 2 weeks appeared better than that for 1 week, whereas rabeprazole/sitafloxacin/metronidazole therapy could attain sufficient eradication rate for 1 week. The triple rabeprazole/amoxicillin/ecabet sodium therapy is also an excellent rescue regimen.

Abstract no.: W4.5

ANTIMICROBIAL RESISTANCE OF *HELICOBACTER PYLORI* ISOLATES IN ALASKA NATIVE PERSONS FROM 2000 TO 2011: RESULTS FROM THE ALASKA SENTINEL SURVEILLANCE PROJECT

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Introduction: *Helicobacter pylori* (*H. pylori*) infection is more common in Alaska Native (AN) people than the general US population, with seroprevalence approaching 75%.
Methods: AN persons undergoing endoscopy between January 2000 and December 2011 were identified by the Centers for Disease Control and Prevention's sentinel surveillance system and biopsy specimens were cultured to isolate *H. pylori*. Susceptibility testing (agar dilution) for metronidazole (minimum inhibitory concentration [MIC] of >8 μg metronidazole/mL), clarithromycin (MIC ≥ 1), amoxicillin (MIC ≥ 1), and tetracycline (MIC ≥ 2) was performed on *H. pylori* isolates from 593 persons and levofloxacin testing (MIC ≥ 2) was performed on isolates from 586 persons.
Results: *H. pylori* was isolated from 593/1373 (43%) persons undergoing upper endoscopy. Metronidazole resistance was demonstrated in isolates from 294 (46%) persons, clarithromycin resistance from 180 (30%), amoxicillin resistance from 10 (2%), and levofloxacin resistance from 76/586 (13%) persons. Dual resistance to clarithromycin and metronidazole was observed in 14% (85/593) of persons. Of those levofloxacin-resistant, 17% (13/76) were also resistant to metronidazole and clarithromycin. Antibiotic resistance varied by region. Female patients were more likely than males to demonstrate metronidazole, and clarithromycin resistance ($p < .01$ both). No statistically significant change in the proportion of persons with resistant isolates was observed over time.
Conclusion: Resistance to metronidazole, clarithromycin, and levofloxacin is more common among *H. pylori* isolates from AN persons residing in Alaska compared to those from elsewhere in the US. Resistance varied by region and no increase in the proportion of resistant isolates was observed over the time period of the study.

Abstract no.: W4.6

TIME TRENDS IN THE CHOICE OF *H. PYLORI* RESCUE TREATMENTS AFTER ONE OR TWO FAILURES OF STANDARD PPI-CLARITHROMYCIN/METRONIDAZOLE CONTAINING THERAPY IN REFERRAL CENTRE FOR *H. PYLORI* IN ZAGREB, CROATIA

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Introduction: Almost 10–30% of our patients failed to eradicate *H. pylori* infection with one or two, 7–14 days, standard triple-therapy regimes. Standard rescue regimen was quadruple bismuth-containing regimen till year 2010. Last 2 years we started with testing 10-day PPI-levofloxacin-amoxicillin regimen.
Aim: Aim was to evaluate the efficacy of two different rescue therapies: prior quadruple bismuth-containing and today's 10-day PPI-levofloxacin-amoxicillin regimen.
Methods: In first time period (till year 2010.) 143 consecutive claritromycine resistant patients (nonulcer dyspepsia-NUD-63, or peptic ulcer-PUD-80 patients) were treated with 10-day quadruple bismuth-containing regimen (Group A), and in last 2 years 57 patients with similar diagnoses (NUD-30, and PUD-27 levofloxacin-susceptible patients) were treated with PPI-levofloxacin-amoxicillin regimen (Group B). All patients underwent endoscopy with histology and culture (dilution agar method) at the beginning and endoscopy or 13C-urea breath test 4–8 weeks after the end of the treatment.
Results: All strains were sensitive to amoxicillin and tetracycline and in Group B to levofloxacin. Eradication rate with levofloxacin-containing therapy was higher in both, the intention-to-treat (91.2% vs. 79.0%; 95% CI 1.01–7.52) and per protocol (94.5% vs. 83.1%; 95% CI 1.05–12.58) analysis.
Conclusion: Both, quadruple and levofloxacin containing therapy, improves the *H. pylori* eradication rate after failed multiple standard triple therapies, but the protocol containing levofloxacin was significantly better (ITT $p = .028$, PP $p = .021$). The main problem with both rescue treatment in Croatia is that all three drugs (bismuth, tetracycline, and levofloxacin) today are not available, and patients have to pay therapy for themselves, despite fully health insurance.

W5 – Gastric Cancer and Preneoplastic Lesions

Abstract no.: W5.1

THE ANGULUS BIOPSY IS CRITICAL IN THE HISTOLOGICAL ASSESSMENT OF PREMALIGNANT GASTRIC CONDITIONS IN THE STOMACH

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Background: OLGA (operative link on gastritis assessment using atrophy) and OLGIM (using intestinal metaplasia [IM]) are histological staging systems for gastritis. A biopsy from the Incisura angularis (angulus) is optional in the biopsy protocol as an alternative to antrum biopsy.
Aim: To determine the value of an additional angulus biopsy for the assessment of OLGA and OLGIM stages prospectively.
Methods: Gastric biopsies from antrum (2), Incisura angularis (1–2) and corpus (2) were obtained from 157 patients (age 19–94, median 55 years, f:m 101:56) with normal gastric mucosa (54), chronic gastritis (37), atrophy or IM (51) and ulcer (15). Histopathological assessment according to the Sydney System, OLGA and OLGIM staging was performed by one expert pathologist. Statistical analysis was performed using the Mann–Whitney *U*-Test.
Results: The overall scores for activity, chronicity, atrophy and intestinal metaplasia did not differ significantly in the angulus biopsy vs. antrum. Sixty-one of 157 patients (39%) were classified as stage I–IV according to OLGA or OLGIM. In this group of patients the angulus biopsy was the only manifestation of atrophy or IM in 12/61 cases (19%) or increased the OLGA/OLGIM score in 9/61 cases (15%). Thus, OLGA scores dropped significantly when recalculated without the angulus biopsy in this subgroup ($p < .00001$), but OLGIM scores did not ($p > .05$).
Conclusions: Biopsies from the Incisura angularis are crucial for the histopathological assessment of gastric atrophy in particular. Correct staging and follow-up of premalignant gastric conditions requires at least five biopsies including one from Incisura angularis.

Abstract no.: W5.2

THE GENE EXPRESSION OF THE WNT-MODULATOR RACGAP1 IS DOWNREGULATED IN *H. PYLORI* POSITIVE PATIENTS WITH GASTRIC CANCER

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Objective: RACGAP1 is a multifunctional kinase involved in the regulation of cell growth and differentiation by downstream modulation of the Wnt-signaling pathway, relevant in gastric carcinogenesis. Aim of the study was to evaluate RACGAP1 gene expression in gastric cancer (GC).
Methods: We prospectively included 49 patients with GC (intestinal n = 29; diffuse n = 20; 56% male, mean age 65.6 ± 14.2 years). Two biopsies were taken each from the tumor, tumor-adjacent and distant tumor-free mucosa. Biopsies from healthy controls were taken from antrum and corpus (n = 30; 28.8 ± 7.4 years). Transcript levels of RACGAP1, β-catenin and DKK2 were quantified by real-time PCR, group comparison was performed by non-parametric tests (significance: $p < .05$).
Results: Pairwise comparison revealed no difference in the RACGAP1 gene expression between tumor, tumor-adjacent and tumor-distant mucosa. Expression of RACGAP1 was significantly lower compared to mucosa from the corpus ($p = .008$) and antrum of controls ($p < .001$; table); the expression was highest in the healthy antrum. Gene expression of RACGAP1 correlated positively with β-catenin and DKK2 in tumor tissue (β-Catenin: $r = 0.300$; $p = .036$; DKK2: $r = 0.373$; $p = .008$). Individuals positive for *H. pylori* infection presented with significantly lower RACGAP1 and β-catenin transcript levels than *H. pylori* negative subjects ($p = .004$).
Conclusion: In patients with GC there is a dysregulation of RACGAP1 gene expression compared to healthy controls. RACGAP1 expression is impaired in *H. pylori*-positive individuals. The correlation of RACGAP1 with β-catenin and DKK2 indicates involvement in Wnt-dependent signaling. Gene expression of RACGAP1 in arbitrary units [a.u.] (mean ± standard deviation)

	Overall	*H. pylori* positive	*H. pylori* negative
Tumor	7.82e-03 + 1.98e-02	7.7e-03 + 2.0e-02	8.2e-03 + 1.9e-02
Control-Antrum	2.55e-02 + 4.87e-02	1.4e-02 + 1.5e-02	3.1e-02 + 4.87e-02
Control-Corpus	8.40e-03 + 1.16e-02	6.9e-03 + 3.5e-03	9.2e-03 + 1.4e-02

Abstract no.: W5.3

ATTENUATED *HELICOBACTER PYLORI*-ASSOCIATED GASTRIC CANCER IN *FAT*-1 TRANSGENIC MICE PRODUCING Ω-3 PUFA

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ω3-polyunsaturated fatty acids, based on anti-inflammatory and anti-oxidative actions, had been prescribed in diverse clinical conditions, but there has been no conclusion whether the ω3-PUFAs can prevent *Helicobacter pylori*-associated cancer. Using *fat-1* transgenic mice, generating ω3-PUFA through overexpression of 6-desaturase, the influence of ω3-PUFA on *H. pylori*-induced gastritis or cancer was investigated. Wild-type C57BL/6 and *fat-1* transgenic mice were deprived of food 24 hours before inoculation of *H. pylori* and administered salt containing pellet diets to promote cancer. The mice were sacrificed after 16, 24, 36, and 44 weeks serially. At 16 weeks, the inflammatory cytokines as well as angiogenic factors were significantly increased in control mice, whereas these expressions were significantly attenuated in *fat*-1 transgenic mice. Huge tumorous and nodular changes simulating intestinal metaplasia were noted in control mice at 24 and 36 weeks, whereas no apparent changes were noted in *fat*-1 transgenic mice. Molecular changes relevant to *H. pylori*-associated carcinogenesis were significantly decreased in *fat*-1 transgenic mice. Preserved lipid rafts were responsible for these protections of *fat*-1 transgenic mice against *H. pylori* infection. Our result provided the rationale to apply ω-3 PUFAs as strategy to protect from *H. pylori*-associated cancer in high risk patients.

Abstract no.: W5.4

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

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Objectives: *Helicobacter pylori* infection induces chronic inflammation in the gastric mucosa and promotes the development of gastric cancer. The effect of *H. pylori* eradication on recurrence after endoscopic resection (ER) of early gastric cancer (EGC) remains uncertain.
Methods: We retrospectively assessed outcomes in 2089 adult patients, aged 28–88 years, who underwent ER of gastric adenoma and EGC from November 2004 to December 2008 at Asan Medical Center. We excluded 602 patients, including those followed-up <1 year, those with recurrence at ≤3 months, and patients not tested for active *H. pylori* infection, including by UBT, RUT or histology. We investigated incidence of recurrence among three groups, those without active *H. pylori* infection (Hp- group, n = 530, 35.6%), those with successful *H. pylori* eradication (eradicated group, n = 669, 44.9%), and those with failed or no *H. pylori* eradication (non-eradicated group, n = 288, 19.3%).
Results: Among 1487 enrolled patients, 1149 (77.7%) were male. Median age was 62 years old (range, 28–88), median duration of follow-up was 49 months (range, 29–147) and median time to recurrence was 16 months (range, 5–66). There were no significant differences in recurrence rate (6.0% vs. 5.1%, $p = .508$) and recurrence-free survival time between eradicated group and Hp- groups. Recurrence rate (17.0% vs. 5.1%; hazard ratio 3.6) was greater and recurrence-free survival was shorter in non-eradicated group than in Hp- group.
Conclusion: Successful *H. pylori* eradication may reduce the recurrence of gastric cancer following ER of gastric tumors.

Abstract no.: W5.5

VALIDATON OF GASTRITIS OLGA-STAGING SYSTEM FOR GASTRIC CANCER RISK IN A REGION OF HIGH PREVALENCE: A CASE-CONTROL STUDY

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Background: Operative Link on Gastritis Assessment (OLGA) staging system has been proposed for gastric cancer risk estimation using gastric atrophy. The aim of our study was to validate the OLGA staging system in a high risk region of the disease.
Methods: This study was performed as a case-control study. Age and sex matched 483 patients with gastric cancer and 483 control subjects were enrolled in National Cancer Center, Korea. Gastric cancer was diagnosed according to the Vienna classification and tumors classified 5.1 or more were included. OLGA stage (0–IV) was obtained by combining antral and body atrophy scores using the updated Sydney system. Gastric cancer risk according to OLGA stage was evaluated using logistic regression analysis.
Results: Mean age was 53 years and male patients were 65%. Gastric cancer group had more patients with stages III-IV (223/483, 46.2%) than control group (127/483, 26.3%, $p < .001$; Table 1), which was prominent in patients with intestinal-type gastric adenocarcinoma (122/195, 62.6%). OLGA stages III and IV were associated with the increased risk of gastric cancer (OR, 2.24; 95% CI, 1.31–3.82, and OR, 2.31, 95% CI, 1.32–4.04, respectively) even after adjusting risk factors for gastric cancer.
Conclusions: High OLGA stages are independent risk factor for gastric cancer in a region where incidence of gastric cancer is high.

Table 1. OLGA stage distribution and gastric cancer risk

	Gastric cancer (n (%)) n = 483	Control (n (%)) n = 483	Crude OR (95% CI)	Adjusted OR* (95% CI)
Stage 0	37 (7.7)	92 (19.0)	1.00	1.00
Stage I	84 (17.4)	135 (28.0)	1.55 (0.97-2.47)	1.25 (0.75-2.09)*
Stage II	139 (28.8)	129 (26.7)	2.68 (1.71-4.22)	1.65(1.00-2.73)*
Stage III	115 (23.8)	70 (14.5)	4.14 (2.53-6.79)	2.24 (1.31-3.82)*
Stage IV	108 (22.4)	57 (11.8)	4.49 (2.77-7.29)	2.31 (1.32-4.04)*

Abstract no.: W5.6

ONLY MINORITY OF GASTRIC ADENOCARCINOMAS PRESENT WITH DECREASED PEPSINOGEN LEVELS

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Background: Pepsinogen (Pg) levels in plasma or serum are suggested for gastric cancer screening, in particular, for intestinal type cancer. To achieve high sensitivity of the screening, most of the cancers would be expected to present with low pepsinogen levels.
Methods: We studied Pg levels in Caucasian patients presenting with gastric adenocarcinoma. PgI and PgII were measured in plasma by ELISA test (Biohit, Plc., Finland). PgI/Pg II <3 was considered a cut-off for decreased pepsinogen level.
Results: Plasma samples from 109 patients (72 men, 37 women, median age 66, range: 31–88) with confirmed gastric adenocarcinoma were analyzed. Three patients had early gastric cancer, 24 – Stage I, 29 – Stage II, 52 – Stage III, but 1 – Stage IV gastric cancer. Fifty patients were found to have intestinal type, 23 – diffuse type, 29 – mixed type cancer according to Lauren classification, but seven cases were not classified. PgI/PgII≥3 was found in 75 cases (68.8%), but PgI/PgII<3 – in 34 cases (31.2%). The mean PgI/PgII was 4.49 (95% CI: 3.44–5.62) in the group with intestinal type cancer, 6.04 (95% CI: 4.69–7.24) with diffuse, and 5.51 (95% CI: 4.19–6.57) in the group with mixed type cancer.
Conclusions: The majority of gastric adenocarcinoma cases in Caucasians present with normal pepsinogen levels, also for intestinal-type cancer. This may be limiting the value of Pg testing for gastric cancer screening, at least with the currently accepted cut-off values.

W6 – Extra-Digestive Diseases and Other Helicobacters

Abstract no.: W6.1

A LENTIVIRUS BASED STRATEGY FOR EXPRESSING THE CYTOLETHAL DISTENDING TOXIN B SUBUNIT OF *HELICOBACTER PULLORUM* – A PROMISING ALTERNATIVE TO STUDY THE EFFECTS OF THIS PARTICULAR GENOTOXIN

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Helicobacter pullorum is associated to human digestive disorders. As several enteric pathogens, *H. pullorum* secretes the cytolethal distending toxin (CDT). This genotoxin is active in the form of a three protein complex: CdtA, CdtB and CdtC, CdtB being the active subunit. Studies on the CDT are faced with the difficulty of purifying or producing the CdtB subunit of the toxin. To bypass these problems, we have developed a lentivirus-based strategy allowing to study activity of the CdtB of *H. pullorum* on epithelial cells. The *H. pullorum* CdtB fused at its 3′ end to three repeats of the human influenza hemagglutinin epitope was directly expressed in intestinal epithelial cells (Caco-2, HCA-7, and HT-29). Preliminary experiments revealed expression of the toxin in the target cells; a nuclear and perinuclear localisation was observed. A cellular distending phenotype with enlarged nuclei was observed for all the epithelial cells as well as an increase of the percentage of cells in G2/M phase. Immunofluorescence analyses revealed profound remodelling of the actin cytoskeleton, focal adhesions and microtubule network. These effects were attributed to the CdtB since they were not observed when the experiments were performed with a control lentivirus expressing the GFP. A CdtB dependent upregulation of the proinflammatory cytokine interleukin8 secretion was also observed. In conclusion, this study confirms the cytopathogenic effects of *H. pullorum* CDT previously reported by co-culture and underlines the importance of developing alternative strategies that allow a fast and simple study of the effects of this toxin.

Abstract no.: W6.2

ANTIBODIES ANTI-CAGA CROSS REACT WITH TROPHOBLAST CELLS: A RISK FACTOR FOR PRE-ECLAMPSIA?

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Background: Previous studies reported an epidemiological association between CagA-positive *H. pylori* strains and pre-eclampsia. Since antibodies anti-CagA cross-react with endothelial cells and trophoblast cells show an endothelial phenotypic profile, we hypothesized that anti-CagA antibodies may recognize antigens of cytotrophoblast cells, thus impairing their function.

Materials and Methods: Placenta samples were obtained from healthy women. Cytotrophoblast cells were cultured in a medium containing increasing concentration of polyclonal anti-CagA antibodies. Binding of anti-CagA antibodies to cytotrophoblast cells was evaluated by cell ELISA and immunoflurescence assay. Invasive potential of those cells was assessed by an invasion culture system and by measuring of MMP-2. Protein sequencing was performed on antigens precipitated by anti-CagA antibodies. Measurement of phosphorylated ERK expression and NF-kB DNA binding activity in trophoblast cells incubated with anti-CagA or irrelevant antibodies was also performed.

Results: Anti-CagA antibodies recognized β-actin of cytotrophoblast cells, showing a dose-dependent binding. Incubation of cytotrophoblast cells with increasing doses of anti-CagA antibodies significantly reduced their invasiveness and determined a significant decrease in phosphorylated ERK expression and a reduced NF-kB translocation activity.

Conclusions: This study shows that anti-CagA antibodies recognize β-actin of cytotrophoblast cells, reducing their invasiveness ability, possibly giving a biological explanation for the epidemiological association.

Abstract no.: W6.3

COLONIZATION CAPACITY OF *HELICOBACTER HEILMANNII* SENSU STRICTO STRAINS IN A MONGOLIAN GERBIL MODEL

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Helicobacter (*H.*) *heilmannii* sensu stricto (s.s.) is a zoonotic bacterium that naturally colonizes the stomach of dogs and cats. In humans, this microorganism has been associated with gastritis, peptic ulcer disease and mucosa associated lymphoid tissue (MALT) lymphoma. For determination of the colonization capacity of nine *H. heilmannii* s.s. strains, all originally isolated from the gastric mucosa of cats, Mongolian gerbils were intragastrically inoculated with these bacteria. At nine weeks after inoculation, the animals were euthanized and samples from the fundus and antrum of the stomach and from the duodenum were taken for histological examination and quantitative PCR analysis. Histology showed induction of a chronic active gastritis with formation of lymphocytic aggregates for seven *H. heilmannii* isolates. The lymphocytic aggregates were predominantly located in the antrum of the stomach. Two *H. heilmannii* strains (ASB 7.1 and ASB 9.4) did not cause inflammation. Detection of *H. heilmannii* with quantitative PCR revealed high-level colonization of strains ASB 1.4, ASB 2.1, ASB 3.2 and ASB 6.3, predominantly in the antrum. In contrast, colonization of strains ASB 11.2, ASB 13.1 and ASB 14.1 was more restricted while ASB 7.1 and ASB 9.4 were not detected in the stomach. These results indicate that *H. heilmannii* s.s. strains may differ in virulence. In an ongoing study, the host response to the nine *H. heilmannii* s.s. strains is further characterized by measuring the expression of several cytokines in the stomach of the experimentally infected gerbils.

Abstract no.: W6.4

COULD GASTRIC *HELICOBACTERS* OTHER THAN *H. PYLORI* BE OF SIGNIFICANCE IN IDIOPATHIC PARKINSONISM?

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Background: *Helicobacter pylori* is an arbiter for progression of hypokinesia in idiopathic parkinsonism (IP) (*Helicobacter* 2010;15:279–95). Since rural-living and farm-experience are associated with IP, might zoonotic-transmission of animal-associated gastric helicobacters have a role?

Methods: Sixty sets (antral/corporal) of archived DNA-extracts from gastric-biopsies in IP-probands (20 had been treated for *H. pylori*) were examined for *H. suis, H. heilmannii* s.s., *H. bizzozeronii* and *H. felis,* using species-specific PCRs and qPCRs. No spiral helicobacters had been found using cresyl-fast violet staining. Antral/corporal-biopsy immunohistochemical staining for non-*H. pylori* helicobacters (NHPH) (validated in NHPH-infected animal mucosae) was performed where *H. pylori* had not previously been detected (culture and, if negative, molecular-microbiology).

Results: Prevalences, in the 60 IP-probands, were:- for *H. suis* 52 (binomial exact 95% CI 38–65) %, *heilmannii* s.s. 18 (10, 30) %, *bizzozeronii* 32 (20, 45) %, *felis* 0 (one-sided 97.5% CI 6) %, compared with an *H. pylori* prevalence of 42 (29, 55) % on culture or, if culture-negative, PCR-positivity. No *Helicobacter* species was found in seven probands, one species in 29, two in 16, three in seven, four in one. There was no association between statuses for different species. Presence of *H. suis* was associated with a lower serum B12 concentration (-25 [95% CI -38 to -9] %, $p = .005$), *pylori* was not. Both were associated with lower folate ($p = .03$ & 0.02). No NHPH was detected by immunohistochemistry.

Conclusion: Molecular-microbiology and haematinic-association suggest that NHPH-infection is common in IP. Failure to identify NHPH on histopathology might reflect sparseness and poor mucosal-adhesion.

Abstract no.: W6.5

HELICOBACTER PULLORUM – A POTENTIAL GASTROINTESTINAL PATHOGEN?

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Background: *Helicobacter pullorum* has been isolated from the liver, duodenum and caecum of chickens and detected in humans with gastroenteritis, chronic hepatic diseases and Crohn's disease. While *H. pullorum* has been demonstrated to initiate an inflammatory response on human epithelial cells in vitro and to have cytolethal distending toxin (CDT) activity, currently there is limited information on the pathogenesis of this bacterium.
Aim: To determine the ability of *H. pullorum* to attach to and invade intestinal epithelial cells and to elucidate the *H. pullorum* secretome.
Methods: Adherence and gentamicin protection (invasion) assays and scanning electron microscopy (SEM) were employed to elucidate the interaction of *H. pullorum* with the human intestinal cell line Caco-2 and proteomic analysis coupled with mass spectrometry to characterise the *H. pullorum* secretome.
Results: Adherence and invasio assays showed that *H. pullorum* could attach to Caco-2 cells (mean attachment value = 1.98 ± 0.16%) and invade Caco-2 cells (mean invasion value = 0.25 ± 0.02%. SEM studies confirmed these results and further elucidated the mechanism by which *H. pullorum* attached to and invaded Caco-2 cells. Of 137 proteins detected, 34 were secretory proteins and 103 non-secretory proteins. Further functional classification revealed putative virulence and colonisation factors, including flagellin, secreted protein Hcp, a type VI secretion protein, an S-layer RTX protein and a protease.
Conclusion: *H. pullorum* has the ability to attach to and invade intestinal epithelial cells and to secrete both colonisation and virulence factors that may play a role in the development of gastrointestinal disease.

Abstract no.: W6.6

GENOMICS AND MICROEVOLUTION DYNAMICS OF HUMAN-DERIVED *HELICOBACTER BIZZOZERONII*

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We present a comprehensive study on the genomics and short-term evolution of a human-derived non-*H. pylori* gastric *Helicobacter* species.
H. bizzozeronii (Hbz) was isolated in March 2008 from a patient with severe gastric symptoms and in November of the same year after a failed treatment. The genome of a corpus-derived isolate was sequenced, annotated and compared to those of *H. pylori* (Hpy). Hbz differs from Hpy by having a wider metabolic flexibility, allowing it to respond to a wider spectrum of environmental signals and therefore to move between different hosts.
To investigate the microevolution dynamics of Hbz in the human stomach, the genomes of antrum-derived strains isolated before (T0) and after (T1) the treatment were sequenced and mapped against the isogenic reference genome. Polymorphic sites (PS) were identified as positions in which the mutation occurred in more than 5% of the reads. The total number of PSs detected in the Hbz population at T0 and T1 were 135 and 227, affecting 100 and 163 coding sequences, respectively. At T0 in 86.7% of the PSs the mutation appeared at a frequency of 50% or less, while in the majority of the PSs observed in T1 (71.8%) the mutation had a frequency of 75% or more. We observed slight overrepresentation of non-synonymous mutations, indicating a moderate sign of positive selection. Overall, these data indicated that the patient was colonized by a highly heterogenic population of Hbz. An increased accumulation of sequence diversity was observed during the infection, suggesting an adaptive short-term evolution.

Posters
P1 – Microbiology, Molecular Genetics and Virulence Factors

Abstract no.: P1.01

MOLECULAR DETECTION OF *HELICOBACTER PYLORI* AND ANTIMICROBIAL RESISTANCE DETERMINANTS FROM BIOPSY SPECIMENS

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The aim of this study was to establish whether molecular methods would be a suitable alternative to culture for the detection and susceptibility testing of *Helicobacter pylori* in the clinical microbiology laboratory in St. James's Hospital.
Template DNA was extracted from 74 biopsy specimens using NucliSens EasyMag platform (bioMerieux, Marcy-l'Etoile, France). The commercial assay Genotype® HelicoDR (Hain; Diagnostika, Nehren, Germany) was used for detection of *H. pylori* and point mutations in genes responsible for clarithromycin fluorquinolone resistance in gastric biopsies. The sensitivity of the commercial assay was 100% compared to the sensitivity of conventional culture methods which was found to be 84%. Specificity and positive predictive value of both systems was 100%. However, the negative predictive value for culture was determined to be 66% which is highly unsatisfactory. The HelicoDR assay identified *H. pylori* in nine specimens that had previously been reported as culture negative.
In-house assays based on end-point PCR and sequence analysis were designed to detect mutations associated with resistance to tetracycline and amoxicillin. Twenty-nine specimens were tested using the tetracycline assay and the *pbp1* assay including the nine specimens that were culture negative and HelicoDR positive. Twenty specimens were tested using the *fts1* and *pbp2* assays. The tetracycline assay assay detected 27/29, while the *pbp1* assay detected 19/29. *fts1* detected 11/20 and *pbp2* detected 17/20.
A molecular method to complement culture in the case of culture failure would ensure the best possible service to the patient.

Abstract no.: P1.02

INHIBITION OF THE ADHESION OF *HELICOBACTER PYLORI* TO HUMAN CELL LINE OR PORCINE MUCIN

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Objectives: Different naturally occurring compounds inhibit the binding of *Helicobacter pylori* to the human gastric mucosa or human cell lines. These same compounds did not inhibit the in vitro growth of the bacteria and pre-incubation of bacteria with compounds did not have any effect on the bacterial binding to gastric tissue. This suggests that the anti-adhesive properties might be due to blocking of the *H. pylori* receptors that interacts with host tissue.
The aims of this study was to test the anti-adhesive effects of different compounds on *H. pylori* by looking at the agglutination between the compounds and *H. pylori* and gastric mucus respectively and to test the blocking- and the displacement effect of the extracts on both human cell lines and gastric porcine.
Methods: Agglutination of 57 fungal-, 69 tea- or 106 fish extracts with either two *H. pylori* strains or one porcine gastric mucin was tested. Blocking effect of extracts both on mucin and cells (AGS and HT-29) is tested by blocking assay and the ability of extracts to displace cell-/or mucinbound bacteria is tested by displacement assay.
Results: See table. Blocking- and displacement assay is ongoing.
Conclusion: Some fungal-, tea- and fish extracts might have anti-adhesive effects on *H. pylori* due to agglutination with bacteria or mucin. Furthermore, some of the extracts did indeed inhibit the in vitro growth of *H. pylori*.

Agglutination results

Extract from	*H. pylori*	Mucin
Fungi	4	1
Tea	24	-
Fish	1	4

Abstract no.: P1.03

NEW DNA EXTRACTION METHOD BASED IN A CARD FOR *HELICOBACTER PYLORI* STORED IN GLYCEROL

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The aim of this study was to evaluate a new DNA extraction method for *H. pylori* strains stored with glycerol and to determine the presence of the allelic variants of the gene *vacA* and the *cagA* gene.
Methods: Fifty-three strains stored more than 1.5 years (17 in 2008, three in 2009 and 33 in 2010) in Trypticase soy broth with 15% glycerol were studied. Suspensions of bacteria stored at -80°C were defrosted, 15 µL placed on a BlackLight Card and allowed to dry. PCR reactions for cagA gene and vacA allele were performed adding one disc from the BlackLight card containing the extracted DNA.
Strains were defrosted by plating 200 mcl of glycerol broth in blood agar and incubated in microaerobic atmosphere up to 5–7 days.
Results: PCR was positive for vacA allele s1 or s2 in 51 strains (96.2%). 12 were s1 (23.5%) and 39 were s2 (76.5%). Nine out of 33 strains tested (27.3%) were cagA positive. In two strains the PCR for vacA was negative; one was stored at the end of 2008 and the other at the beginning of 2009. All the strains stored in 2010 were positive for the vacA PCR. Culture was negative for all strains defrosted.
Conclusions: DNA extraction by using a card is a very easy method, non demanding technically and it is useful for doing PCR from *H. pylori* strains stored at -80°C in cryopreservative solution. PCR of vacA gene is useful to check the DNA status.

Abstract no.: P1.04

IN VITRO ACTIVITY OF GRAPE EXTRACTS AGAINST *HELICOBACTER PYLORI* CLINICAL ISOLATES

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Objective: The aim of this study was to determine in vitro activity of different phenolic compounds against *H. pylori* clinical isolates by a disc diffusion method.
Methods Twenty-five *H. pylori* clinical isolates were obtained from gastric biopsies from patients suffering of gastric symptomatology. Biopsies were processed following standard methodology. The in vitro activity of two different grape-derived extracts (grape extract and grape seed extract), rich in phenolic compounds, were studied by a disc diffusion method. Blank disc were impregnated with 10 mcl of each compound and laid in agar Columbia plus 7% sheep blood inoculated with a suspension of a two McFarland *H. pylori*. Plates were incubated 4 days at 37°C in a 10% CO2 atmosphere.
Results: Grape seed extract produced inhibition in all the strains tested and grape extract produced inhibitions in seven out of 25 strains. Fifty-two percentage and 80% of the strains were susceptible to clarithromycin and metronidazol, respectively. Grape extract was more active against CLA-R (four out of 12, 33.3%) than CLA-S strains (three out of 13, 23.1%). The strains with >19 mm inhibition zone using grape seed extract, presented also some zone inhibition using grape extract.
Conclusion: The grape seed extract tested in this study showed in vitro activity against all *H. pylori* clinical isolates, however grape extract showed in vitro activity against 28% of the *H. pylori* isolates. Number of strains inhibited for each compounds.

	Nordm; strains/total (%)	Zone inhibition: 7–14 mm	Zone inhibition: 15–20 mm
Grape extract	7/25 (28%)	5	2
Grape seed extract	25/25 (100%)	10	15

Abstract no.: P1.05

PROLONGED PRIMARY INCUBATION IN THE ISOLATION OF *HELICOBACTER PYLORI* FROM A PATIENT WITH FAILED ERADICATION THERAPY: A CASE REPORT

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Helicobacter pylori, a gastric pathogen, is strongly associated with peptic ulcer disease, and is an important risk factor for the development of gastric malignancies. Culture of the bacterium from gastric biopsy is the ''gold standard'' in confirmation of the diagnosis of *H. pylori* infection, and is essential for determination the drug resistance of *H. pylori*. However, primary isolation of *H. pylori* from gastric biopsies is rather demanding, and is affected widely by number of factors such as biopsy preparation, transport and culture media, and the method adopted. The duration of incubation for isolation of *H. pylori* has been recommended to be 5–7 days.

However, in the present case, we found that a prolonged incubation period of up to 14 days allowed successful isolation of *H. pylori* from a patient with an *H. pylori* positive duodenal ulcer who received triple therapy that failed to eradicate the bacterium. The biopsies were placed directly into transport medium and processed for culture within 2 hours. On day 14, some suspected *H. pylori*-like colony appeared on one of the plates. The isolate was confirmed to be *H. pylori* based on its typical colony morphology, negative Gram's stain, and positive urease, catalase, and oxidase tests. The isolate requiring 14 days recovery, later exhibited normal growth characteristics of *H. pylori* strains, indicating its unusually long incubation requirement was a temporary predicament.

Our report demonstrates that longer incubation time is needed for some strains, especially those enduring hostile environment or a period of antibiotic force.

Abstract no.: P1.06

HELICOBACTER PYLORI INFECTION AND COLON DISBIOSIS: IS THERE A LINK?

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Objectives: Disorders of colon microbiota in patients infected with *Helicobacter pylori* are widely investigated. We decided to evaluate content of *Bifidobacteria, Lactobacilli, Enterococci* and *Candida albicans* in stool of *H. pylori* infected patients and answer on the question: is there a link between *H. pylori* and colon microorganisms?

Methods: One hundred and three persons infected with *H. pylori* were observed. For all patients gastroduodenoscopy with biopsies from a stomach body and antrum were performed for verification of *H. pylori* infection (rapid urease test, polymerase chain reaction and histological method). Amount of *H. pylori* was estimate in stomach body and antrum by evaluation quantity of microbes (microscopic analysis): <20 microbes - mild (1 grade), 20–50 – moderate (2 grade), >50 – high (3 grade). Bacteriological analysis of stool was performed to evaluate content of microorganisms in colon and degree of colon disbiosis (1–4 grades). Statistical analysis was performed in Statistica 6.0 for Windows XP.

Results: We build two models of multiple linear regression: (1) $Y_{HPb} = 1.539 – 0.280X_1 + 0.279X_2$, where Y_{HPb} - amount of *H. pylori* in stomach body (1–3 grades), X_1 – content of *Bifidobacteria* in colon, lgcfu/g, X_2 – content of *Candida albicans* in colon, lgcfu/g ($p < .05$). (2) $Y_{HPa} = 0.927 + 0.253X_1 + 0.161X_2$, where Y_{HPa} – amount of *H. pylori* in stomach antrum (1–3 grade), X_1 – content of *Candida albicans* in colon, lgcfu/g, X_2 – degree of colon disbiosis (1–4 grades) ($p < .05$).

Conclusion: The link between *H. pylori* and colon microbiota is possible. It is need to perform next studies to confirm it.

Abstract no.: P1.07

POSITIVE SELECTION IN THE EVOLUTION OF *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEINS

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Homologous recombination in *Helicobacter pylori* has been extensively described to occur via Outer Membrane Proteins (OMPs), regulating protein expression and generating allelic diversity, while the importance of single nucleotide polymorphisms (SNP) remains little studied.

We used an OMP-encoding gene, *homC*, as a model to evaluate the weight of positive selection in the evolution of *H. pylori*, by using ≍200 sequences obtained from strains collected worldwide. N-site and branch-site phylogenetic analysis by maximum likelihood models were used to identify specific codons that may be important in *homC* evolution, and to evaluate the impact of selective pressure on the geographic segregation of strains, respectively.

The N-site overall analysis showed that 14 of the 742 (1.9%) *homC* codons are likely under positive selection (likelihood-ratio test (LRT), $p < 10^{-61}$). Four of these codons are located in the most variable allelic gene middle region, probably reflecting recombination-derived hitchhiking events. On the other hand, eight codons are located in the more conserved 5'and 3' gene regions, although the significance of this distribution remains to be clarified.

Branch-site analysis revealed 36 codons (4.9%) under positive selection (LRT, $p < 10^{-41}$), showing a non-random distribution, and 89% of these particular codons ($p < 10^{-3}$) support the phylogenetic segregation of European strains from both African and East Asian strains. The lack of visible recombination within this segment suggests an important biological role of point mutations in the evolution of *H. pylori* OMPs.

In conclusion, *homC* SNP analysis suggests that, besides recombination, positive selection contributes as well to the evolution of *H. pylori* OMPs.

Abstract no.: P1.08

ASSEMBLY COMPARISONS: RE-SEQUENCING OF THE *H. PYLORI* J99 AND 26695 STRAINS USING ION TORRENT AND ILLUMINA MISEQ NEXT GENERATION SEQUENCING TECHNOLOGIES

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Molecular epidemiology by whole genome sequencing is a rapidly growing and evolving area. Benchtop sequencing machines that produce millions of reads of short DNA sequences are becoming standard tools for laboratory analysis. We aimed to understand the accuracy of de novo genome assemblies derived from both the Ion Torrent and the MiSeq sequencing machines. We analysed the accuracy of each coding sequence (CDS) by re-sequencing and assembling the two completely sequenced and finished strains, J99 and 26695. We found that despite high quality data, the genome assemblies displayed limited accuracy and varying results. J99 was assembled more accurately than 26 695 by data derived by both machines. The number of coding sequences for that were 100% accurate in the J99 assemblies were 1028 (69%) for Ion Torrent and 1207 (81%) for MiSeq out of the annoatated total of 1491. In 26695, the number of correct genes were substantially fewer with 693 (44%) for Ion Torrent and 965 (62%) for MiSeq out of 1566 annotated genes.

Abstract no.: P1.09

SINGLE NUCLEOTIDE POLYMORPHISMS IN PRO- AND ANTI-INFLAMMATORY CYTOKINES AND THE RISK OF GASTRIC CANCER IN IRAN

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Interleukins depending on their tumor promoting or suppressing functions are known to affect cancer risk. IL-2 and IL-4 are respectively known as pro and anti-inflammatory cytokines which are affected by *H. pylori* infection and involved in predisposition to gastric cancer. We have, herein, investigated the risk of gastric cancer associated with of IL-2 -384G/T and IL-4 -590C/T SNPs and its interaction with *H. pylori* infection.

Gastric cancer patients (N = 254) and healthy controls (N = 251) were evaluated for *H. pylori*-specific serum IgG antibodies by ELISA as well as IL-2 -384G/T and

IL-4 -590C/T SNPs by PCR-RFLP. Age and gender adjusted odds ratio and the corresponding 95% confidence intervals was estimated by unconditional logistic regression model.
Subjects carrying the low producer IL-2 T allele had a significantly reduced risk of gastric cardia cancer (OR = 0.5 95% CI = 0.3–0.9). Additionally, male T carriers demonstrated reduced risk of gastric cancer of the cardia subsite (OR = 0.237, 95% CI = 0.067–0.843) and of the intestinal subtype (OR = 0.296, 95% CI = 0.088–0.997). In the *H. pylori*-seropositive group, these subjects had a drastically reduced risk of gastric cardia cancer (OR = 0.087, 95% CI = 0.008–0.943).
As for IL-4, carrying the high producer T allele increased the risk of gastric cancer (OR = 1.7, 95% CI = 1.0–3.0), mainly directed toward the non-cardia subsite (OR = 2.4, 95% CI = 1.2–4.6). This risk which was further amplified in the *H. pylori*-seropositive group (OR = 2.5, 95% CI = 1.3–5.1) of the non cardia subsite (OR = 3.7, 95% CI = 1.7–8.4) and intestinal subtype (OR = 3.4, 95% CI = 1.2–9.6).
Our results indicate contradicting roles for IL-2 -384G/T and IL-4 -590C/T SNPs in predisposition to gastric cancer.

Abstract no.: P1.10

HELICOBACTER PYLORI VIRULENCE FACTORS IN FIRST DEGREE RELATIVES OF GASTRIC CANCER PATIENTS

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Introduction: The presence of *Helicobacter pylori* (HP) virulence factors and family history play important roles in the pathogenesis of gastric cancer (GC) during a complex multi-step process. The aim of this study was to investigate the genomic HP profile in first degree relatives of GC patients (FDR), at high risk of GC and with different gastric precancerous lesions, in comparison with that in subjects of the general population (GP).
Material and Methods: Twenty-seven subjects with histological evidence of different levels of precancerous lesions, were submitted to gastroscopic examination with collection of gastric biopsies and HP culture: 14 were FDR and 13 were subjects from GP. Ten to 20 HP single colonies per biopsy, representing the possible HP genetic heterogeneity in the gastric niche, were analyzed for the presence of several cagPAI genes (CagA, CagE, VirB11) by PCR. Hom gene and VacA polymorphisms were also evaluated.
Results: A total of 311 HP colonies were analysed. Concerning cagPAI genes, HP isolated from FDR showed lower genetic heterogeneity (three vs. six genotypes) and a more frequent intact cagPAI than GP (53.6 vs. 37.5, $X^2_2 = 69.9$, $p < .0001$). The most frequent profile in HP from FDR was virB11 (+)/cagE(+)/cagA(+)/vacAs1i1m1 (46.3% tested colonies) while s2i2m2 genotype was less represented (27.2% vs. 52.3%). HomB had a high frequency (78.2%) and was present in 71.4% of colonies with at least one CagIsland gene deletion.
Conclusions: Strains isolated from FDR present a highly virulent genetic profile which can well represent the risk of these subjects to develop a severe disease.

Abstract no.: P1.11

GENETIC ALTERATION AND INTERACTION BETWEEN ISOLATED *HELICOBACTER PYLORI* STRAINS FROM GASTRIC ANTRUM AND BODY

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Background/Aims: We investigated the interaction between the *H. pylori* strains isolated from gastric antrum and body of same patients in vitro and in vivo.
Method: Among 48 pairs of cultured *H. pylori* isolates, only six were proved different each other in antrum and body by PCR-RAPD fingerprinting. In vitro, each pair of isolated strains was mixed together and subsequently subcultured five times every 3 days. In vivo, 5-week-old C_{57}BL/6 mice were infected with each pair of *H. pylori* strains for 12 weeks. The harvested stomach was divided into three parts. The histological examination, CLO test, and culture on the selective media were performed. All cultured strains were amplified for *UreC* with PCR, then analyzed by PCR-RAPD fingerprinting.
Results: In vitro, all of the strains showed positive for *UreC*. The DNA patterns of the mixture from antrum and body of five patients are same as those of the strains isolated from antrum of five corresponding patients. In the rest one patient, it shows the same as that of the strain isolated from body. In vivo, the strains from mice inoculated with the mixture showed the same DNA patterns as those from the strains inoculated with the strains of antrum or body, separately.
Conclusion: There was no genetic alteration in mixture of *H. pylori* isolates from antrum and body. This suggests that there is no interaction between *H. pylori* strains of antrum and body in the same patient.

Abstract no.: P1.12

ANALYSIS OF GENE MUTATIONS ASSOCIATED WITH ANTIBIOTIC RESISTANCE IN *HELICOBACTER PYLORI* STRAINS ISOLATED FROM 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. We aimed to identify the resistance of antibiotics and evaluate the effect of point mutations on treatment outcomes in Korean patients.
Methods: From August 2009 to December 2010, 82 HP strains were isolated gastric mucosal biopsy specimens. Specimens were cultivated and the resistance to five antibiotics was assessed using agar gel dilution method. DNA sequencing was carried out to detect the resistance-related gene mutations.
Results: In five clarithromycin resistant strains, a mutation was observed at A2143G point in 23S rRNA. Among them, eradication was failed in two patients with clarithromycin resistant and amoxicillin sensitive strains. In four amoxicillin resistant strains (MIC 0.5–1.0 μg/mL), mutations were not observed at S414 and N562 in *PBP1*, but in a strain (MIC 2.0 μg/mL) substitution was detected at N562Y. In 8 strains (28.5%) of 28 metronidazole resistant strains, the mutation was observed such as nucleotide deletion, insertion or stop codon in *rdxA*. There were not found the tetracycline resistant strains. 16s rRNA genes were sequenced but mutations were not found. Out of 19 levofloxacin resistant strains, 11 (57.8%) showed amino acid substitution at N87K (eight strains), N87I, A88V and D91N in *gyrA*. Conclusion:
We studied point mutations affecting the eradication of HP from Korean strains. There were observed clarithromycin mutations at A2143 in 23S rRNA gene and levofloxacin mutations at N87, A88 and D91 in *gyrA* will be determined the efficacy of HP therapy.

Abstract no.: P1.14

HELICOBACTER PYLORI CAGA-POSITIVE STRAINS ACTIVATE MATRIX METALLOPROTEINASE-10 IN GASTRIC CELLS *VIA* ERK AND JNK PATHWAYS

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Helicobacter pylori infection leads to up-regulation of the expression and activity of several matrix-metalloproteinases (MMPs), both in gastric cell lines and in the gastric mucosa. The aim of this study was to analyze the mechanisms leading to up-regulation of MMP-10 in gastric epithelial cells induced by *H. pylori*.
Infection of AGS cells with *H. pylori* led to an increase in MMP-10 mRNA, protein secretion and activity. *H. pylori* strains that were mutant for *cagA* or for *cagE* (lacking the ability to translocate CagA) failed to increase MMP-10 expression. These results were confirmed with a panel of *H. pylori* clinical isolates with known *cagA* status.
Specific inhibitors of MEK1/2 and JNK significantly decreased or abolished *H. pylori*-induced MMP-10 expression, whereas an inhibitor of p38 enhanced MMP-10 expression.
Treatment of AGS cells with EGF led to an increase in MMP-10 expression, and inhibition of EGFR with siRNAs or with chemical inhibitors abrogated *H. pylori*-induced MMP-10 expression, suggesting that EGFR is involved in MMP-10 up-regulation induced by the infection.
In conclusion, MMP-10 expression is stimulated by *H. pylori* strains containing CagA via the ERK and JNK pathways, and involving the EGFR.
Acknowledgements: ERA-PTG/0002/2006.

Abstract no.: P1.15

INHIBITION OF LYMPHOCYTE PROLIFERATION MEDIATED BY *HELICOBACTER SUIS* Γ-GLUTAMYL TRANSPEPTIDASE

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Helicobacter (H.) suis has been shown to cause gastric disease, both in pigs and humans. In the present study, we investigated the effect of *H. suis* γ-glutamyl transpeptidase (GGT) on the proliferation of lymphocytes. Incubation of Jurkat T cells with the enzyme resulted in a reduction of nearly 60% of cellular proliferation. Both cell death and cell cycle arrest were shown to be involved in this process. The inhibitory effect on purified mouse splenocytes was even more pronounced. Prior to and during *H. suis* GGT treatment, CD4+ and CD8+ T cells were stimulated with anti- CD3/CD28 mAb, and CD19+ B cells were stimulated with anti-IgM mAb and IL-2. Incubation of stimulated cells with 1 μg/mL *H. suis* GGT reduced the proliferation with about 80% for CD4+ and CD8+ T cells and with more than 95% for B cells. Supplementation of treated Jurkat cells with known *H. suis* GGT substrates was able to modulate the observed effects. Glutamine was able to restore the normal proliferation of the cells whereas supplementation with reduced glutathione (GSH) aggravated the inhibition of lymphocyte proliferation induced by *H. suis* GGT. In conclusion, this is the first report of a *H. suis* virulence factor involved in immune evasion. We showed that modulation of lymphocyte proliferation inhibition by *H. suis* GGT depends on the interaction of the enzyme with two important substrates, glutamine and glutathione. Inhibition of lymphocyte proliferation mediated by *H. suis* may be of importance for the chronic persistence of the bacterium in its preferred niche.

Abstract no.: P1.16

A MODIFIED QUIKCHANGE II SITE-DIRECTED MUTAGENESIS METHOD FOR THE GENERATION OF REPETITIVE GENE SEQUENCES: THE PARADIGM OF THE EPIYA-C CODING MOTIFS IN *CAGA*

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There is considerable variability amongst cagA-positive clinical isolates with regards to the number and combination of EPIYA motifs at the carboxyl-terminal end of the protein. In clinical isolates of Western origin, CagA protein harbors EPIYA-A, EPIYA-B and variable numbers of EPIYA-C motifs and combinations vary in infected symptomatic patients. The aim of this study was, to design a simple method in order to produce isogenic *H. pylori* strains expressing CagA protein harboring variable numbers of EPIYA-C motifs based on the parental P12 reference strain. To accomplish that, we ligated in line three copies of the EPIYA-C coding region, followed by the 140 bp cagA sequence downstream of EPIYA-C coding region and used this construction as a megaprimer in a QuikChange II Site-Directed mutagenesis procedure. As a template we utilized the P12 full length *cagA* gene sequence followed by the *C. jejuni* kanamycin cassette and a sequence of P12 genome spanning 1200 bp downstream of the *cagA* gene. Clones in DH5a cells were screened by EPIYA PCR assay (Panayotopoulou, 2007). In a single reaction we were able to generate all combinations of EPIYA-C motifs (AB, ABC, ABCCC). P12 isogenic strains were generated through *H. pylori* natural transformation and homologous gene recombination. All P12 isogenic strains exhibited the same growth properties and ability to adhere to AGS cells. Functionality of type IV secretion system was assessed by effective expression, translocation, phosphorylation of CagA protein and IL-8 secretion. Our modified method can be applied to similar cases when addition of repetitive gene sequences is desirable.

Abstract no.: P1.17

HELICOBACTER PYLORI PROTEIN JHP0940 ACTS AS A DUAL SPECIFIC KINASE AND INDUCES APOPTOSIS THROUGH INFLAMMASOME DEPENDENT PATHWAY

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It has been shown that JHP0940 protein of *H. pylori* induces cytokines relevant in chronic gastric inflammation and also acts as auto-phosphorylating ser/thr kinase. Extending these findings, we found that HP0940 also acts as an auto-phosphorylating tyrosine kinase; this points to its ''oncogene like'' role relevant in triggering the development of gastric cancer.
Our in vitro studies using tyrosine kinase assay have shown that JHP0940 also acts as auto-phosphorylating tyrosine kinase and induces proinflammatory cytokines in cultured RAW264.7 cells (mouse macrophage cell line). Upon treatment with JHP0940, these cells secreted IL1-beta, TNF-alpha and IL-6, in a dose and time dependent manner, as detected by ELISA and transcript profiling by q-RT-PCR. Further, this protein was found to decrease the viability of RAW264.7 cells up to 55% in 24 hours. The decreased viability was found to be due to apoptosis which was confirmed by TUNEL assay and, Fas expression analysis by flow-cytometry. When the severity of inflammation was determined, involvement of caspase-1 and IL-1beta was confirmed. This points to a possible action through inflammasome.
The proinflammatory and the enzyme regulatory responses triggered by JHP0940 lead to the assumption of its possible role in the form of a survival strategy; inducing inflammation to feed on exudates and triggering apoptosis to escape innate defence.

Abstract no.: P1.18

HELICOBACTER PYLORI INFECTION INDUCES MUTATIONS IN D-LOOP REGION OF MITOCHONDRIAL DNA

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Introduction: Genetic instability is a cancer hallmark. Mitochondrial DNA (mtDNA) is a genetic material particularly susceptible to ROS generated by the respiratory chain, being D-loop region a hot spot for mutations.
Aims: To determine if the D-loop region mutations are related to the *H. pylori* virulence and clinical outcomes.
Methods: AGS cells were coinfected (72 hours, 10^8CFU/mL) with *H. pylori* strains: [cagA(-), HP1], [urease(-), HP3], or [cagA(+), HP4]. Moreover, we obtained 8 *H. pylori* (+) gastric biopsies: four gastritis, two ulcers and two intestinal metaplasias. After mtDNA isolation by differential centrifugation and phenol-chloroform extraction, D-loop region was amplified using two pairs of primers. D-loop sequences of infected AGS, were compared with those corresponding to AGS control, and D-loop in biopsies with those of GeneBank database.
Results: We detected seven, nine and 18 mutations in AGS incubated with HP3, HP1 and HP4, respectively. In HP3 and HP1-infected cells, 55.6% of mutations were localized in hypervariables regions, but only 11.1% in HP4-infected cells. These mutations were localized in same positions in all samples, and corresponded to transitions and C insertions. In biopsies, the mean number mutations was: 5.8 (gastritis), 9.5 (ulcers) and 20 (metaplasias).
Discussion: *H. pylori* plays a role in the appearance of mtDNA mutations from initial steps of infection (gastritis) until more serious clinical outcomes (ulcers, metaplasia). The increase in mtDNA mutations is related to bacterial virulence factors (CagA) and the higher numbers are associated with premalignant lesions. This could be a new field of research to explain the influence of bacterial infections in the development of cancer.

Abstract no.: P1.19

CD277: A NEW CO-INHIBITORY MOLECULE ACTIVATED BY *HELICOBACTER PYLORI*

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Introduction: *H. pylori* promotes a vigorous immune response that is unable to get spontaneous eradication of the bacteria, but leads to a persistent inflammatory process. This aberrant immune response, is triggered by the interactions between pathogen and host cells. Co-inhibitory molecules play a crucial role in the abrogation of T cell responses in the context of chronic infections, and signals mediated by B7 family members (PD-L1), appear to be crucial. Butyrophilins (CD277) share significant sequence homology with B7 members, and little is known about their functions.
Aims: To assess if *H. pylori* modifies CD277 expression in gastric epithelial cells (AGS), and its relationship with density, bacterial genotype and clinical outcomes.
Methods: AGS were coinfected (24 hours, 10^8–2×10^8CFU/mL) with different *H. pylori* strains: [cagA(-), HP1], [urease(-), HP3], or [cagA(+), HP4]. We evaluated HLA-DR, marker of HLA-II on non-professional ''antigen presenting cells (APC)'', and CD277 expression. Moreover, we analyzed CD277 in 10

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H. pylori-infected gastric biopsies (five gastritis, five ulcers). Tests were performed in a FACSCalibur.

Results: In *H. pylori*-infected AGS, CD277 (control = 16.4 ± 6.6, [HP2 × 10^8] = 35.3 ± 15.1*), and HLA-DR (control = 12.5 ± 4.9, [HP2 × 10^8] = 20.2 ± 7.5*) expression, were increased independently of colonization density and genotype. In biopsies we detected a 2.2-fold increase CD277 values in ulcers compared to gastritis specimens.

Conclusions: *H. pylori* allows gastric epithelial cells to behave as APC, and increases CD277 expression. Due to the inhibitory properties of butyrophilins the host cells could collaborate to chronicity and severity of infection inducting anergy in T cells. CD277 emerges as a new target for interventions to overcome immune evasion and boost immunity in infected patients.

Abstract no.: P1.20

OXIDATIVE STRESS CAUSED BY *H. PYLORI* DECIDES THE MITOCHONDRIAL NETWORK FRAGMENTATION BY PROTEINS TRANSLOCATION (BAX AND DRP-1) TO FISSION SITES

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Objectives: Apoptosis has been associated with *H. pylori* infection. The crucial step in the apoptotic intrinsic pathway is outer mitochondrial membrane permeabilization, being the mitochondrial pores opening (OMP) responsible for it, although are not clearly identified neither the initialization signals nor the molecules involved.

Aims: To determine oxidative stress role and mechanism that drives the OMP in *H. pylori* infection. Involvement of Bcl-2 and fission proteins family.

Methods: AGS cells were *H. pylori*-infected (10^8 CFU/mL, 24 hours), and incubated with or without VitE or V5''Bax-translocation inhibitor'' (10^{-4} mol/L). It was studied:

- OMP (Calcein-AM with $CoCl_2$) by Confocal Microscopy (CM)
- Mitochondrial network phenotype (NAO) by CM
- Bax and Drp1 oligomerization by cross-linked and Western blot assays
- Bax and Drp1 colocalization by CM

Results: Calcein fluorescence in presence of $CoCl_2$, was reduced in mitochondria of coinfected AGS compared to control, showing that OMP has happened. *H. pylori* switched mitochondrial morphology from ''tubular'' (control) to ''punctuate and swollen'' phenotype (co-infected cells). Mitochondrial Bax in AGS-infected was as monomer, dimmer, trimmer, and heteromultimer with Drp1 (Bax-Drp1 and Bax-Bax-Drp1). Bax and Drp1 colocalized in mitochondria forming clusters at fission prospective sites. Vit E and V5 pretreatment avoided these alterations.

Discussion: Oxidative stress observed in *H. pylori*-infected gastric epithelial cells, is able to initiate an alterations cascade that leads cells to autoelimination, being OMP a crucial step. In the OMP are involved Bax and Drp-1 that are translocated to mitochondria to close proximity. Antioxidants and/or Bax translocation inhibitors treatment could prevent the OMP, the apoptosis development, and consequently, reduce the bacterial toxic effect on gastric epithelium.

Abstract no.: P1.21

COMBINED PRESENCE OF THE *HELICOBACTER PYLORI* JHP0562 AND TNPA GENES PREDICTS THE PRESENCE OF DUODENAL ULCER

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Helicobacter pylori infection is now accepted as the main cause of superficial gastritis and is associated with other gastroduodenal disorders. Infection usually occurs during childhood and when left untreated it will become chronic and last for life. During the first years of the infection the presence of *H. pylori* probably only results in asymptomatic chronic gastritis, but prolonged infection can lead to a variety of digestive illnesses, including peptic ulcer disease and gastric cancer. Recently, several putative virulence factors have been identified but discrepant data exist on their association with disease. The aim of this study was to evaluate a putative relationship between the presence of the jhp0562, cagA, sabB, tnpA and tnpB genes and disease. Patients were collected in the Imam Khomeini hospital between the May 2007 and April 2011. *H. pylori* could be isolated from 360/376 patients. The presence of jhp0562, cagA, sabB, tnpA and tnpB genes in these strains was examined by using a specific Real-Time PCR reaction on the purified DNA from these strains. Of 376 included patients (196 males, average age 42.1 years, range: 17–73) 108 were diagnosed with Duodenal Ulcer, 92 with Gastric Ulcer, 65 with Gastric Cancer, and 95 with Gastritis. A significant association (95% CI = 4.24–9.01; OR = 19.5) was observed between the presence of duodenal ulcers and the combined jhp0562+, tnpA+ genotype. The combined jhp0562+ tnpA+ genotype shows a strong correlation with the presence of duodenal ulcers in *H. pylori* infected patients and might serve to predict the induction of duodenal ulcers before they are clinically manifest.

Abstract no.: P1.22

DEVELOPMENT OF A NOVEL *HELICOBACTER PYLORI* BABA2 GENE-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ASSAY

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Background: *Helicobacter pylori* (*H. pylori*) babA2 is the ABO blood group antigen binding adhesin, which has a closely related paralogue babB with unknown function. Some studies showed that babA2 gene-positive *H. pylori* strains are associated with severe clinical outcome in Western populations. The ability to detect babA2, however, depends on the used PCR method. It has been shown recently that available babA2 primers may generate both false-negative and false-positive results due to sequence variation among *H. pylori* strains and cross-reactivity with babB gene.

Objective: To develop and evaluate a novel babA2 PCR in comparison to two widely used PCRs targeting 850-bp (PNAS USA 1999;96:12778–83) and 271-bp (Gut 2003;52:927–32) fragments of babA2.

Material and Methods: BabA2 primers were designed according to the multiple alignment of 94 babA2 and 24 babB sequences available in GenBank. A total of 217 *H. pylori* DNA isolates were consequently tested with the novel assay.

Results: Three forward and one reverse primer were selected to amplify 146-bp fragment of babA2 gene. Using novel PCR, babA2 was detected in 114/217 (52.5%) *H. pylori* isolates. Using 850-bp and 271-bp PCRs, babA2 was found in 74/217 (34.1%) and 174/217 (80.2%) cases, respectively. Sequencing of 146-bp and 850-bp PCR amplicons confirmed the presence of babA2, while it was not possible to distinguish reliably among babA2 and babB sequences in 271-bp amplicons.

Conclusion: Novel assay significantly improves the detection of babA2 gene over existing assays. Further validation of this assay is needed on a geographically more diverse collection of *H. pylori* strains.

Abstract no.: P1.23

THE ULCEROGENIC PROFILE OF *HELICOBACTER PYLORI* PAEDIATRIC STRAINS ASSOCIATED WITH PEPTIC ULCER DISEASE

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Helicobacter pylori infection is the major cause of paediatric peptic ulcer disease (PUD). In children with no other aetiology for the disease, this rare event occurs shortly after infection, presuming a still poorly understood higher susceptibility of the patient and highlighting the virulence of the implicated strain. Recently, we showed that the enhanced virulence of a group of paediatric ulcerogenic-strains result from a synergy between their ability to better adapt to the hostility of their niche and the expression of *cagA, vacAs1, oipA* ''on'' status, *homB* and *jhp562*[1]. Accordingly, these ulcerogenic strains share a particular proteome profile, providing them with better antioxidant defences, a metabolism favouring the biosynthesis of aromatic amino acids and higher motility[1]. Corroborating these findings, our preliminary data on electronic microscopic analyses demonstrated the presence of more abundant flagella in PUD-associated paediatric strains, in contrast to the control strain, a paediatric strain associated with non-ulcer dyspepsia (NUD). Compared with paediatric NUD-associated isolates, ulcerogenic

H. pylori strains present a greater ability to induce a marked decrease in the gastric cells' viability and to cause them severe cytoskeleton damage and mucins' production/secretion impairment[1]. To uncover the underlying molecular mechanisms, we are now characterizing the modifications induced by these strains in the proteome of human gastric cells, during in vitro infection, by two-dimensional gel electrophoresis followed by mass-spectrometry.

Work supported by Research Grant 2011 – Sociedade Portuguesa de Gastrenterologia.

Reference

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Abstract no.: P1.24

RETHINKING VACA: A TRUE MULTIFUCTIONAL TOXIN OR RATHER A NOVEL TYPE OF MONOFUNCTIONAL A-B TOXINS?

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Helicobacter pylori is the paradigm of a bacterium which favors a carcinogenic process. Into the last decade, careful analyses of two of its most important virulence factors, the vacuolating toxin VacA and the cytotoxin-associated gene A product CagA, have led to important breakthroughs for the study of bacterial-host relationships. From the cell biology point of view, VacA is a fascinating protein toxin which, although classified as a pore-forming toxin, apparently exerts pleiotropic effects on mammalian cells and tissues. It has thus been proposed that VacA may be considered a paradigm for toxin multifunctionality. However, an increasing body of evidence now suggests that VacA may rather be the prototype of a new class of monofunctional A-B toxins in which the A subunit exhibits pore-forming instead of enzymatic activity. Thus a peculiar mechanism of action for VacA, which allows it to intoxicate the human stomach, may be depicted. By combining the action of a cell-binding domain, a specific intracellular trafficking pathway and a novel mitochondrion-targeting sequence, the VacA pore-forming domain is selectively delivered to the inner mitochondrial membrane to efficiently kill target epithelial cells. VacA action on the human host could be exploited and controlled by *H. pylori* through a functional relationship with another virulence factor, CagA, to achieve the best interaction between the bacterium and the hostile gastric environment that represents its ecological niche.

Abstract no.: P1.25

PREVALENCE OF BACTERIAL VIRULENCE FACTORS IN *H. PYLORI* STRAINS ISOLATED IN PATIENTS WITH GASTROINTESTINAL DISEASES IN A PROSPECTIVELY ENROLLED COHORT IN MAGDEBURG (EAST GERMANY) 2011 AND 2012

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Background: The development of gastric cancer is dependent from host-related, environmental and bacterial virulence factors. The aim was to study the presence of *H. pylori* CagA and VacA variants in patients with different types of gastritis.

Methods: From all included patients gastric biopsies were obtained and the *H. pylori* status was determined. VacA and CagA variants were identified by PCR from *H. pylori* DNA. Anti-*H. pylori* and anti-CagA IgG was quantified by ELISA.

Results: As shown in table 1, 186 patients with different diseases were included and 1/3 were infected with *H. pylori*. Overall, 86.7% of all *H. pylori* strains contained the *cagA* gene. Interestingly, a remarkable number (n = 30, 63.8%) of those was not associated with an anti-CagA IgG response in the corresponding patients. Pilot investigation concerning CagA variants (number of EPIYA motifs) in 31 patients revealed predominant presence of ABC (65%), followed by ABCCC (16%), ABCC (13%) and AB (6%). In five patients, colonization with multiple strains having at least three CagA variants was detected. Variants of the vacA gene s1m1 and s2m2 were identified in 40% and 35% of the strains, respectively. Due to small numbers of cases at this moment, statistical analysis was not performed.

Conclusions: *H. pylori* strains isolated from patients in Magdeburg (East Germany) demonstrate a high degree of variability in regard to isoforms of CagA and VacA gene.

Table 1 Clinical data of study group

Diagnosis	Number of patients (Number of patients with isolated strains)	CagA Gene	VacAs 1m1	VacAs 2m2
Normal control	61 (2)	2 (100%)	0	1
Antrum predominant gastritis	15 (13)	9 (69.2%)	3	6
Corpus predominant gastritis	7 (6)	5 (83.3%)	1	4
Pangastritis	14 (10)	8 (80%)	5	2
Atrophic gastrits /intestinal metaplasia	63 (20)	20 (100%)	9	5
Peptic ulcer disease	15 (5)	4 (80%)	2	3
Gastric cancer	11 (4)	4 (100%)	4	0
Total number	186 (60)	52 (86.7%)	24	21

Abstract no.: P1.26

PREVALENCE AND CLINICAL RELEVANCE OF *CAGA*, *VACA*, *ICEA* AND *BABA2* GENES IN SLOVENIAN PEDIATRIC POPULATION INFECTED WITH *HELICOBACTER PYLORI*

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Aims: (1) To determine the prevalence and genetic diversity of *H. pylori cagA, vacA, iceA* and *babA2* genes in Slovenian pediatric population, and (2) to analyze the relationship between infections with different strains and the severity of antral inflammation.

Methods: DNA was extracted from 190 *Helicobacter pylori* (*H. pylori*) positive gastric biopsies. *H. pylori cagA, vacA and iceA* status was determined, as described previously (*J Pediatr Gastroenterol Nutr* 2009;49:289–96). *The presence of babA2* gene was determined by using two different primer sets targeting 850-bp (*Proc Natl Acad Sci USA* 1999;96:12778–83) and 271-bp (*Gut* 2003;52:927–32) fragments of *babA2*. Single gene was compared with density, activity and chronicity of *H. pylori* infection according to the Updated Sydney histological Classification.

Results: Multiple *H. pylori* genotypes were found in 20/190 (10.5%) children which were excluded from further analysis The *cagA* gene, and *s1* and *m1* alleles of the *vacA* gene were found in 66.4%, 73.9% and 40.6% of *H. pylori* isolates, respectively. *IceA1* positive strains were identified in 68.8%. Using 850-bp and 271-bp PCR assays, *babA2* gene was found in 52/170 (30.6%) and 137/170 (80.6%) cases, respectively. The severity of antral inflammation was associated with *cagA, vacAs1, vacAm1* and *babA2* (850–bp) positivity.

Conclusion: The results of this study showed that in contrast to *iceA1, cagA, vacAs1* and *vacAm1* are important virulence determinants of *H. pylori* in Slovenian children. The importance of *babA2* gene for clinical outcome is not clear yet as *babA2* status significantly depends on the used primer set.

Abstract no.: P1.27

CLINICAL VALUE OF HOPQI GENOTYPE OF *HELICOBACTER PYLORI*; ASSOCIATION WITH RESISTANCE PHENOMENA

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Eradication of *Helicobacter pylori* infection is accepted as the first-line treatment among patients with digestive diseases. To date, no previous studies have been carried out to investigate the status of *H. pylori* hopQ genotypes and the pattern of antibiotic resistance. The aim of our study was to investigate the association between hopQ types I and II genotypes of *H. pylori* in patients with resistant and

susceptible strains. One hundred-fifty-two consecutive *H. pylori* positive patients were enrolled. During upper gastroscopy, two antral biopsy specimens were taken and shipped to the diagnostic laboratory for routine bacterial culture according to the standard method. Antimicrobial susceptibility tests were performed with agar dilution method. Subsequently, DNA extraction and PCR assay for detection of hopQ genotypes were carried out. Of 155 participants, 152 were *H. pylori* positive (84: gastritis; GERD: 23 and 45 with gastric cancer, mean age: 43 with an age range of 19–65 years). The prevalence of resistance rate to clarithromycin, metronidazole, amoxicillin and tetracycline were 29.6%, 72.3%, 25.6% and 16.4%, respectively. hopQII was found in 7.2% (11/152) strains and hopQI was present in 85.5% (130/152) strains. The presence of hopQI was significantly associated with gastritis, GERD and gastric cancer patients (p = .004, .0024 and .0035, respectively). Our data strongly support the hypothesis that *H. pylori* hopQ I positive strains are more prone to be resistant to antibiotics. Detection of hopQ I genotype can be applied as a rapid and cost efficient method for diagnosis of resistant strains of *H. pylori*.

Abstract no.: P1.28

PREVALENCE OF *VACA* ALLELES IN *HELICOBACTER PYLORI* STRAINS ISOLATED IN TURKEY

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Aim: To determine the prevalence of the *vacA* signal and middle regions in Turkish isolates and to correlate with distribution of CagA positivity and clarithromycin susceptibility.

Methods: Two-hundred-thirty-four patients with dyspepsia (65 M, 169 F; mean age 43.8 ± 14.0 years) were studied. Antrum and corpus biopsy specimens were obtained for RUT, histopathology and culture. E-test was used to assess clarithromycin susceptibility in the isolated *H. pylori* strains and *cagA* status and *vacA* typing of *H. pylori* strains was established by PCR. DNA was extracted from all isolated *H. pylori* strains of antrum and corpus by QIAamp®DNA mini kit (Qiagen). One-hundred-sixty-four (70.1%) patients were *H. pylori* positive. One-hundred-fourteen (69.5%) of 164 patients were culture positive. A total of 102 *H. pylori* strains from antrum and 96 *H. pylori* strains from corpus were isolated from dyspeptic patients gastric biopsies.

Results: The combined assessment of isolated *H. pylori* strains from antrum and/or corpus biopsy specimens for each patient yield the following results: *vacA* m1s1 was observed in 29 (25.4%) patients (20 [69%] CagA+); *vacA* m1s2 in 1 (0.9%) patient; *vacA* m2s2 in 40 (35.1%) patients (4 [10%] CagA+); m2s1 alleles in 35 (30.7%) patients (25 [71.4%] CagA+). Nine patients have mixed strains: As expected, *vacA* m1s1 and m2s1 were associated with CagA positivity and *vacA* m2s2 was associated with CagA negativity. According to E-test results, 82 patients (71.9%) were clarithromycin-susceptible, 32 (28.1%) patients were clarithromycin-resistant. No relationship between vacA types and clarithromycin susceptibility was shown. No correlation was found between histopathological features and the types of vacA.

Conclusion: Our results confirmed that m1s1, m2s1 and m2s2 genotypes were common in patients with dyspepsia. *vacA* s1 genotype was associated with positivity of the *cagA* gene.

P2 – Epidemiology, Pediatrics and Diagnosis

Abstract no.: P2.01

COCCOID FORMS OF *HELICOBACTER PYLORI*: AN ADAPTATION TO OXIDATIVE STRESS

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Helicobacter pylori colonizes human stomach to maintain long-term persistent infection. This microaerophilic microorganism possess urease and detoxification enzymes including superoxide dismutase (SOD) and neutrophil- activating protein (NapA) which protect *H. pylori* DNA to oxidative stress caused by reactive oxygen species and aerobic conditions. It has been suggested that the morphological transformation to coccoid form may occur as an adaptation to aerobic conditions. The aim of this study was to evaluate the effect of oxygen on morphological changes, culturability, nucleic acid integrity, ureA, sodB and napA gene expression of *H. pylori* strains. *H. pylori* NCTC11638 and HP796 strains were exposed to atmospheric oxygen. Morphological changes from spiral to coccoid forms were observed by optical microscopy. The culturability status was determined by colony counting using Mueller Hinton agar supplemented with 7% horse blood. Genomic DNA and RNA were extracted at 0, 24, 48, 72 and 96 hours. The integrity of genomic DNA was examined by electrophoresis in a 1.5% agarose gel. The ureA, napA and sod gene expression were determined by RT-PCR. The rate of coccoids forms increased with time (95% at 96 hours), conserving their culturability status. These forms showed a highly specific DNA fragmentation pattern. The urea, sodA and napA gene expression decreased progressively over time and disappear at 96 hours.

These results demonstrate that coccoid *H. pylori* cells could adapt to aerobic environments in a ''silent'' state regulating the virulence-gene expression that contribute to species preservation, retain viability and thereby facilitate transmission to new hosts.

Abstract no.: P2.02

THE PREVALENCE OF PEPTIC ULCER DISEASE AND *HELICOBACTER PYLORI* INFECTION IN THE URBAN POPULATION OF SIBERIA OVER 45 YEARS OLD

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Aim: To study prevalence of peptic ulcer disease and *Helicobacter pylori* in the urban population of Siberia over 45 years old.

Methods: For the study we selected 801 persons (387 males, 414 females) aged over 45 years old living in the Krasnoyarsk city by the method of random sampling. To all patients were performed clinical and endoscopic examination and determination content antibodies to *Helicobacter pylori* in blood serum by immunoassay method using test kits ''GastroPanel'' (producer ''Biohit'', Finland).

Results: The prevalence of gastric ulcer in the surveyed population was 3.0% (males – 4.1%, females – 1.9%), duodenal ulcer – 5.5% (males – 7.2%, females – 3.9%). Among patients with peptic ulcer 91.2% people were smokers, among persons without peptic ulcer 38.6% people were smokers (OR = 15.28, CI 6.72–34.74, $p < .001$). The prevalence of *H. pylori* infection among surveyed persons was 90.0% (males – 89.7%, females – 90.3%). *H. pylori* was determined in 97.1% people with peptic ulcer and in 89.4% persons without peptic ulcer (OR = 3.55, CI 1.0–12.81, $p = .04$).

Conclusion: The prevalence of *Helicobacter pylori* is high in the urban population of Siberia. *H. pylori* infection was occurs more frequently in patients with peptic ulcer disease than in patients without peptic ulcer disease. Tobacco smoking is a risk factor for peptic ulcer disease.

Abstract no.: P2.03

PREVALENCE OF *H. PYLORI* INFECTION AND ATROPHIC GASTRITIS IN LATVIA

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Background: *H. pylori* infection and atrophic gastritis are related to an increased risk of gastric cancer. There is a decrease in global *H. pylori* prevalence. We have analyzed the prevalence in Latvia of *H. pylori* infection and atrophy as determined by pepsinogen testing.

Material and Methods: This sub-analysis was done on a randomly selected cross-sectional adult general population sample to access cardiovascular risk factors. Plasma samples were screened for *H. pylori* IgG (Mikrogen Diagnostik, Germany; cut-off value 24 U/mL) and pepsinogens (Pg) I and II (Eiken Chemical Co., Japan; cut-off PgI/PgII ≤3 and PgI ≤70 ng/mL for atrophy of any grade, and PgI/PgII ≤2 and PgI ≤30 ng/mL for advanced atrophy).

Results: Altogether, 3564 serum samples were available for the study (2346 women, 1218 men; median age 54). 79.21% of the tested individuals were *H. pylori* positive, with no difference between the genders. The prevalence increased with age ($p < .001$). Atrophy of any grade was identified in 1444 individuals (40.52%), and advanced atrophy positivity – in 475 individuals (13.33%). Linear association with the age was present in both response types ($p < .001$). The prevalence of atrophy of any grade was higher in women (41.73%) than men (38.18%; $p = .04$); this difference was lost for advanced atrophy (women 13.98%, men 12.07%; $p = .1$).

Conclusions: The prevalence of *H. pylori* infection or atrophy remains high in Latvia. For pepsinogen-based atrophy detection in Europe to stratify objectively the gastric cancer risk, this is critically important to set the right cut-off value.

Abstract no.: P2.04

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE IN LITHUANIA

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Introduction: An incidence of inflammatory bowel disease (IBD) is increasing worldwide, 25 percent of IBD is diagnosed in childhood. Data concerning prevalence rate of *Helicobacter pylori* (*H. pylori*) infection in inflammatory bowel disease (IBD) patients is conflicting, especially in children.

Aims and Methods: The aim of our study was to assess the seroprevalence of *H. pylori* infection in children with IBD and age and sex-matched normal controls. IgG antibodies to *H. pylori* were detected in blood samples. Only new IBD patients without use of antibiotics (metronidazole, amoxicillin, clarythromycin) during last 6 months were included into study. The prevalence of *H. pylori* in controls was also compared with data of seroprevalence of infection in 1998 (13 years ago).

Results: Thirty five children (21 with UC and 14 with CD; male/female ratio – 17/16), and 100 controls were studied. The children mean age were 15.14 ± 2.5 in IBD group and 15.10 ± 2.52 years in controls. The seroprevalence of *H. pylori* was 8.6% in patients with IBD group; (14.3% in patients with CD and 4.8% with UC), and 10.0% in controls ($p < .05$). In comparison to data obtain in 1998 year (167 healthy children) we revealed significant decrease of infection during last 13 years (10.0% and 41.0%, $p < .05$) in Lithuania.

Conclusions: We have not revealed differences in prevalence of *H. pylori* infection in IBD patients in comparison to controls. The prevalence of *H. pylori* infection in children is low and significantly decreased during the last 13 years in Lithuania.

Abstract no.: P2.05

HELICOBACTER PYLORI RECURRENCE AFTER ERADICATION THERAPY IN KOREA

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Background: Recurrence of *Helicobacter pylori* (*H. pylori*) infection is the result of either recrudescence or reinfection. Annual recurrence rates per patient-year of follow-up have been reported to vary from country to country. The aim of this study was to analyze recurrence rates of *H. pylori* after first-line and second-line eradication therapy in Korea.
Methods: From 2007 to 2010, 2691 patients with *H. pylori* infection received the first-line therapy and 573 patients who failed to the first-line therapy received the second-line therapy. *H. pylori* infection and the success of eradication were assessed by endoscopic biopsy and rapid urease test, or 13C-urea breath test. Patients were recommended to perform the follow-up urea breath test or upper digestive endoscopy with biopsy, 6 months after eradication and then annually.
Results: The eradication rate of the first-line therapy was 79.9% (1283/1605) and that of the second-line therapy was 90.4% (394/436) by per protocol analysis. Recrudescence rate was 4.2% (26/619) and 2.1% (5/237), and annual reinfection rate was 5.6% and 3.7% after the first-line and the second-line therapy, respectively. There were no significant differences in recrudescence and reinfection rate between the first-line and the second-line therapy.
Conclusions: The *H. pylori* recrudescence and reinfection rate in Korean adults is higher than in Western countries but lower than anticipated. Follow-up and reevaluation of patients should be considered when there are symptoms after eradication.

Abstract no.: P2.06

THE POLYMORPHISM OF INTERLEUKIN 8 -251 T/A, BUT NOT MANNOSE BINDING LECTIN 2 CODON 54 G/A, INFLUENCES THE SUSCEPTIBILITY OF GASTRIC CANCER WITH *HELICOBACTER PYLORI* INFECTION IN KOREAN POPULATION

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Background & Aims: Mannose-binding lectin (MBL) is a key component in innate systemic immunity. Increased mucosal IL-8 production due to *H. pylori* infection is a major factor in the immunopathogenesis of peptic ulcer disease (PUD) and gastric cancer (GC). Recent studies reported that the polymorphism of *MBL2* codon 54 and *IL-8* -251 are associated with *H. pylori*-infected atrophic gastritis (AG) and GC risk.
Material and Methods: Two hundred and nine patients with functional dyspepsia (FD), 70 AG, 128 PUD, and 203 GC were included in this study. The polymorphism of interleukin 8 (*IL-8*) -251 T/A and *MBL2* codon 54 G/A were examined by PCR-based restriction fragment length polymorphism analysis. The concentrations of serum MBL protein and gastric mucosal IL-8 were measured by ELISA.
Results: In *H. pylori*-positive gastric disease group, gastric mucosal concentration of IL-8 was not significantly different according to the IL-8 -251 genotypes (T/T, T/A, or A/A). IL-8 -251 T/A polymorphism was associated with the significantly higher risk of GC compared with FD or AG. There were no significant differences in MBL2 codon 54 G/A polymorphism among the FD, PUD, AG and GC groups. Serum concentration of MBL protein was not different among the gastric disease groups. However, Serum concentration of MBL was significantly different according to *MBL2* codon 54 G/G (1731 ± 335.5 ng/mL), G/A (166.6 ± 45.9 ng/mL), and A/A genotype (4.8 ± 5.8 ng/mL).
Conclusion: The polymorphism of *IL-8* -251 T/A, but not *MBL2* codon 54 G/A, influences the susceptibility of GC in Korean population.

Abstract no.: P2.07

HIGH RATE OF *HELICOBACTER PYLORI* REINFECTION IN LITHUANIAN PEPTIC ULCER PATIENTS

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Introduction: There are little data about the reinfection of HP from Eastern and Central Europe, which are medium to high HP prevalence areas.
Aim: To evaluate the frequency of HP reinfection in peptic ulcer patients during 9 years after HP eradication.
Patients and Methods: We invited 117 peptic ulcer patients in whom eradication of HP was confirmed 1 year after the eradication treatment. HP was tested by a rapid urease test and histology if endoscopy was performed. If endoscopy was refused the HP was tested by C14-urea breath test and serology. HP-positivity was established, if at least one of the tests was positive.
Results: Fifty-seven patients were available for the study procedures. The follow-up duration was 8.9 ± 1.0 years (6–12). Mean age – 52.3 ± 13.0 years. Endoscopy performed in 43 (75.4%) patients. HP has been established in 15 patients. In 2 HP-negative patients HP had been established during the follow-up period and eradicated. Therefore, we consider that reinfection occurred in 17 patients. If per protocol analysis is performed - reinfection is established in 17 of 57 (29.8% [95% CI: 19.2–42.2]) patients, the annual rate is 3.4%. If most optimistic analysis is applied (considering that all non-responders are HP-negative), reinfection could be in 14.5% (17/117) patients, the annual rate is 1.6%.
Conclusions: HP reinfection rate is high in Lithuania, suggesting that the prevalence of HP remains high. This probably reflects the differences in the socioeconomic status between Western and Eastern European countries.

Abstract no.: P2.08

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN IN SASAYAMA-CITY

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Objectives: Last year we reported that prevalence of *H. pylori* infection was low in Japanese children. To confirm this is not by chance or not by any biases, we measured the prevalence in the same area, again.
Subjects and Methods: One thousand nine hundred nine children from 0 to 11 years old in 16 schools (seven elementary schools, six kindergartens, and three nursery schools) in Sasayama-city were invited to this study in November, 2011. In their stool samples *H. pylori* antigen was detected using TestMate *Helicobacter pylori* Antigen EIA (Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan). According to the manufacturer's instruction, the cutoff value was decided at 0.1.
Results: Participation rate was 44% (835/1.909). Stool antigen positive% was 1.8% (15/835) in total: 0.0% (0/6) in children aged <1 year, 0.0% (0/26) in 1, 2.9% (1/34) in 2, 2.3% (1/44) in 3, 0.0% (0/104) in 4, 0.9% (1/115) in 5, 3.3% (4/120) in 6, 1.4% (1/71) in 7, 1.0% (1/100) in 8, 1.5% (1/69) in 9, 4.9% (4/82) in 10 and 1.6% (1/64) in 11 years.
Conclusion: Prevalence of *H. pylori* stool antigen was 1.8% in Japanese children. The results were similar with ones in 2010. In Japan, prevalence of *H. pylori* infection in children seems much lower than that in adults.

Abstract no.: P2.09

EVALUATION OF *HELICOBACTER PYLORI* STOOL ANTIGEN TEST RESULTS REQUIRED FROM DIFFERENT OUTPATIENT CLINICS AT UNIVERSITY HOSPITAL

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Objective: As a non-invasive test *H. pylori* stool antigen test has been accepted to determine *H. pylori* infection and for the treatment follow-up by Maastrich Consensus report 2005 in clinical laboratory settings.
Aim: To determine the prevalence of *H. pylori* infection among patients and to reveal influence of age, sex and to search for association with different clinical status.
Methods: Three-thousand-three-hundred and eightyseven patients (2155 females and 1232 males, mean age, 48.7 ± 16.6 years) who admitted to different outpatient clinics at Dokuz Eylül University Hospital were included in this study between January 2010 and April 2012. Stool specimens were collected and studied to determine the presence of *H. pylori* antigen in the feces by *H. pylori* stool antigen test (Pylori-Strip CORIS BioConcept).
Results: The prevalence of *H. pylori* infection was 18.1% (n = 612 of 3387) and the frequency among males and females was similar (17.5% and 18.4%, respectively). No significant statistical difference was found in *H. pylori* prevalence between two genders ($p = .54$). The prevalence of *H. pylori* antigen in stool specimens in various age groups revealed that in children (n = 8 of 612), aged 2–18 years (1.3%) and reaches maximum levels in adults aged 19–83 years (98.7%) mostly with dyspepsia. The mean age of overall *H. pylori* infection positive patients was 47.7 ± 14.7 and 48.9 ± 17.0 in *H. pylori* infection negative patients. No

statistical difference was found between age and the positivity of *H. pylori* infection (*t*-test, $p = .077$).
Conclusion: It was very interesting to find out the low rate of *H. pylori* positivity. This leads us to question ourselves how, when and which test to use in the diagnosis and/or treatment follow-up of *H. pylori* infection among our patients in our University Hospital.

Abstract no.: P2.10

MOLECULAR CHARACTERIZATION OF *HELICOBACTER PYLORI* INFECTIONS IN NIGERIA

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Background: *Helicobacter pylori* is the causative agent of gastritis, ulcer and is a risk factor in the development of gastric cancer (Blaser et al. 1995). In Nigeria, seroprevalence of *H. pylori* could be as high as 85% and there is no standard method of *H. pylori* diagnosis. The study is aimed at looking at more efficient methods for proper *H. pylori* diagnosis as well as checking the virulence potential of the Nigerian *H. pylori* isolates.
Methods: A total of 40 biopsies obtained from 20 patients positive for UBT were screened for culture, CLO test, PCR using glmM, Hpy and cagA genes. The virulence potential of the isolates were assessed using PCR for the vacA s1, s2, m1 and m2 regions. In order to find out if the cagA and cagY genes were expressed, western blotting was carried out on the isolates.
Results: A total of 34 *H. pylori* isolates were obtained from 20 patients. CLO was positive in 31 (91.2%), culture (100%), glmM (97.1%), Hpy (100%), cag A (97.1%). All the isolates (100%) were vacA s1, m1. Western blotting showed that cagA gene was expressed in 85.3% of the isolates while the cagY gene was expressed in 67.7% of the isolates.
Discussion: This study shows that the UBT, Hpy gene could be reliable methods for correct and accurate *H. pylori* diagnosis in Nigeria in cases where the culture technique is impossible due to constant power outages. All isolates carry virulence genes and majority were expressed irrespective of their clinical diagnosis.

Abstract no.: P2.11

PREVALENCE OF *HELICOBACTER PYLORI* AMONG MEDICAL STUDENTS IN LITHUANIA DECREASED DURING LAST 17 YEARS

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Introduction: The prevalence of *Helicobacter pylori* infection is decreasing in Western well economically developed countries, but remains comparably high in developing regions. There are very limited data on the changes of the prevalence of *Helicobacter pylori* in Eastern and Central European region.
Aims: To establish the prevalence of *Helicobacter pylori* among medical students and to compare with the data obtained 17 years ago.
Methods: The students of faculty of medicine of Lithuanian university of health sciences were investigated serologically from finger blood for the presence of antibodies against *Helicobacter pylori* in the years 1995 and 2012. In 1995 the ''Helisal'' test used in 120 students (mean age – 21.3 ± 1.0 years. In the year 2012 the SureScreen HPSC *Helicobacter pylori* test was used in 187 students (mean age - 22.4 ± 0.7 years).
Results: In 1995 *Helicobacter pylori* infection was established in 62 (52%) students, in 2012 *Helicobacter pylori* found in 57 (30.4%) students, $p < .05$. No difference was found between genders, having or not the peptic ulcer in anamnesis, presence or not of peptic ulcer and (or) gastric cancer among first degree relatives.
Conclusion: The prevalence of *Helicobacter pylori* among Lithuanian medical students decreased significantly during last 17 years.

Abstract no.: P2.12

HELICOBACTER PYLORI INFECTION IN SPAIN: IS ITS PREVALENCE REALLY DECREASING?

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Background: Knowing the *H. pylori* prevalence in the general population is important in order to estimate the population's risk to develop diseases related with this infection. It has been suggested that *H. pylori's* prevalence is decreasing over time in developed countries.
Aim: To review the studies evaluating *H. pylori* prevalence in the Spanish general population.
Methods: MEDLINE and congress abstract searches to identify studies evaluating *H. pylori* infection prevalence in the Spanish general population. A sub-analysis was performed considering only young adults (20–30 years). Data was meta-analyzed (inverse generic variance method).
Results: Nineteen studies were included (7668 individuals). Two studies included only children while eight studies included both children and adults. Reports were published between 1989 and 2007. The mean infection prevalence combining all studies was 48% (95% CI = 40–57%). Data were highly heterogeneous ($I^2 = 98\%$), reporting prevalences ranging from 16% to 78%. No time-dependant descending prevalence was demonstrated. In fact, the two most recent studies (from 2006 to 2007) reported prevalences of 69% and 60% respectively. Data from studies including only adults (nine studies) showed highly heterogeneous results ($I^2 = 96\%$), with a mean prevalence of 53% (95%CI = 43–63%). The prevalence in young adults (649 individuals) was lower but still considerably high (40%, 95% CI = 33–47%; $I^2 = 75\%$).
Conclusion: Prevalence of *H. pylori* infection in Spain is considerably high, approximately 50%. Contrary to what has been reported in other countries, the frequency of infection does not show a descending tendency over time. Even nowadays, the prevalence in young adults is still high (40%). Therefore, we can assume that the diseases related to this infection such as peptic ulcer and gastric cancer will continue to be very prevalent over the next few decades.

Abstract no.: P2.13

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION MEASURED WITH URINARY ANTIBODY IN JAPANESE FEMALE UNIVERSITY STUDENTS, 2010–2011

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Background: *Helicobacter pylori* infection decreased in the general population of Japan by improvement of general hygiene conditions. Mother-to-child transmission of *H. pylori* is one of important epidemiologic problems in Japan. Although there were many studies on the prevalence of *H. pylori* infection among populations aged 40 years or older in Japan, there were only limited sources tracking its latest prevalence among younger adults. The aims of the present study were to investigate the prevalence of *H. pylori* infection and identify the risk factors for *H. pylori* infection among healthy young women with potential to become mothers in the near future.
Methods: Subjects are 207 Japanese female students aged 20–24 years in a certain university. *H. pylori* infection status was determined by a urine-based ELISA. Data on their childhood and present living environments were collected using a questionnaire.
Results: The prevalence of *H. pylori* infection detected by the urinary test was 6.3% (95% CI: 2.9–9.6%). The odds ratios (95% CI) were 1.78 (0.56–5.62) for second or more in birth order, 2.50 (0.50–12.5) for drinking natural or well water in childhood. The number of siblings and sanitary conditions were not significant associated with *H. pylori* infection.
Conclusions: Our results suggest that the prevalence of *H. pylori* infection among Japanese women aged 20–24 years may be lower than 10%. Because the number of *H. pylori* positive subjects was small (13/207) in the present study, we would need to identify the risk factors for *H. pylori* infection among Japanese younger adults by further research.

Abstract no.: P2.14

DETECTION OF THE *H. PYLORI* -SPECIFIC ANTIGENS IN *CANDIDA ALBICANS* BY WESTERN-BLOTTING METHOD

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Introduction: In our previous studies, the intracellular existence of *H. pylori* inside the vacuole of *candida yeast* was studied through the microscopic observation and the identification of *H. pylori*-specific genes; *16S rRNA, ure AB, cagA* and *vacA* by PCR. In this study, occurrence of *H. pylori* specific -antigens in *candida* yeasts was investigated using Western-blotting method.
Methods: Ten PCR- confirmed *H. pylori*- positive *Candida* yeasts were lysed to release the proteins. Native proteins were separated by SDS-gel electrophoresis and transferred to a Polyvinylidene fluoride membrane. Chicken anti-*H. pylori* immunoglobulin Y (primary antibody) and goat-anti chicken IgY (secondary antibody) were used to detect proteins. Proteins extracted from a PCR-confirmed *H. pylori* were used as the positive control.
Result: After Electrophoresis and washing steps, protein bands were visualized using 3, 3′, 5, 5′-tetramethylbenzidine (TMB) detecting reagent. The size of protein bands obtained from two out of 10 *Candida* yeasts was similar to those of control.
Discussion: Detection of *H. pylori* -specific genes in *Candida* yeast reveals the intracellular occurrence of the bacterium but raises the question whether the organism is alive inside the yeast. Synthesis of proteins could be the indicator of viability of a cell. Accordingly, detection of *H. pylori* -specific proteins in yeast could indicate the existence of a live and functional bacterium inside the yeast.

Abstract no.: P2.15

DETECTION OF *HELICOBACTER PYLORI 16S RRNA* GENE IN THE YEAST ISOLATES FROM PATIENTS WITH DENTURE

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Introduction: There are several proposals suggesting that inflammations of the oral cavity and gastric mucosa have common bacterial or fungal etiology. *H. pylori* is responsible for several gastric diseases such as gastritis, peptic ulcer and gastric cancer, but its route of transmission is still not clear. The oral cavity has been proposed as a reservoir for gastric *H. pylori* because the bacterial genes were detected in both dental plaque and saliva by PCR. A significantly high density of oral yeasts has been observed in humans with dentures. This study proposes that yeast which colonizes the denture could serve as the possible reservoir of *H. pylori*.
Methods: Twenty four oral yeasts were isolated from 17 humans with denture. Samples were cultured on Yeast extract Glucose Chloramphenicol Agar to eliminate any bacterial contamination. Colony count was performed for all samples. Total DNAs were extracted from yeasts and PCR was performed to amplify *H. pylori- 16S rRNA* gene.
Results: A heavy colonization of yeasts was observed in dentures. *H. pylori-16S r RNA* gene was amplified from 13/24 yeasts. The size of the PCR products (519 bp) of all yeasts was similar to that of control.
Discussion: Detection of *H. pylori*-specific gene in yeast isolates from denture wearers indicates the intracellular existence of *H. pylori* in yeast, protecting the bacterium against stressful conditions of the oral cavity. Since all the denture wearers showed heavy colonization of yeasts in the oral cavity, they might be more susceptible to *H. pylori* infection.

Abstract no.: P2.16

AMPLIFICATION OF *16S RRNA* GENE OF *HELICOBACTER PYLORI* FROM VAGINAL YEASTS OF UBT POSITIVE WOMEN

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Introduction: *Helicobacter pylori* infection is associated with peptic ulcer and gastric carcinoma, although the route of bacterial transmission is still unrecognized. Previous studies indicated that intracellular symbiosis of *H. pylori* with oral and gastric yeasts might play an important role in the bacterial survival and transmission. In this study occurrence of *H. pylori* in the stomach of female patients was assessed by UBT. Furthermore, the vaginal yeasts of the same patients were examined for detection of *H. pylori-16S rRNA* gene.
Methods: UBT was performed in fifteen women and results were recorded. Fifteen vaginal yeasts were isolated. DNAs were extracted from yeasts and Polymerase chain reaction (PCR) was performed for detection of *16S rRNA* gene. PCR products were analyzed by electrophoresis.
Results: Six of fifteen women were UBT positive, two questionable and seven negative. The *H. pylori-16S rRNA* gene was amplified from five of six UBT positive patients (83.3%), one from two questionable (50%) and one from seven negative women (14.2%). The size of PCR products was 519 bp and homologous to that of control *H. Pylori*.
Discussion: In this study there was a significant relationship between result of UBT and amplification of *H. pylori*-specific gene in the vaginal yeasts. It is accepted that mothers' vaginal yeasts could be transmitted to oral cavity of newborn through normal delivery. Accordingly, it is proposed that the vaginal yeast might serve as a potent reservoir of *H. pylori* and play an important role in the bacterial transmission to newborn.

Abstract no.: P2.17

SEROPREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN A SOUTH ALGERIAN REGION

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Background: Previously studies of seroprevalence of *Helicobacter pylori* (Hp) infection in north algerian regions have shown a high positivity level in adult population, but no study has been done in the south algerian population.
Aim: to evaluate the seroprevalence of Hp infection in blood donors in south algerian region.
Materiel and Methods: Two hundred and ten sera of blood donors in Ghardaia, a south algerian city, are collected during 2009. The donors were from 18 to 63 years old (mean age 29 years); 70 were women and 140 men, Sera were tested by ELISA (platelia *H. pylori* IgG. Biorad).
Results: Over all 79% of sera were H.p positive; 75.5% and 80.7% respectively of women and men were positive: (there is no statsticaly significant difference.) The positivity according to different age was as follows:
Conclusion: The seroprevalence of Hp Infection is so high in south than in north algerian population.

Age (Years)	17–20 (n = 20)	21–30 (n = 110)	31–40 (n = 51)	41–50 (n = 16)	51–63 (n = 13)
% Hp (+)	75	76.36	88.23	93.75	76.93

Abstract no.: P2.18

DETECTION OF *H. PYLORI*-SPECIFIC GENES IN ORAL AND GASTRIC YEASTS FROM *H. PYLORI*-POSITIVE PATIENTS

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Introduction: Despite the widespread occurrence of *H. pylori* within human populations, this bacterium has not been isolated from any source except the human stomach. The exact mechanism of bacterial transmission from person to person is not clear yet. Several studies show the co-existence of *H. pylori* and *Candida albicans* in peptic ulcer and chronic gastritis. In this study, we examined oral and gastric yeasts from 3 *H. pylori* positive- patients for the existence of *H. pylori*-specific *16S rRNA* and urease (*ureAB*) genes.
Methods: Gastric biopsies and oral samples were collected from three patients. The samples were cultured on selective media to isolate *H. pylori* and yeasts. Three *H. pylori* strains were isolated from gastric biopsies. Three oral and three gastric yeasts were identified on CHROM Agar as *Candida* spp. All the six yeasts were subcultured for more than 10 times to remove exogenous bacterial contamination. Total DNAs were extracted from bacteria and yeasts and PCR was performed

to detect *H. pylori-16S rRNA* and *ureAB* genes in all six yeasts and three *H. pylori* isolates.
Results: Attempts to culture *H. pylori* from oral samples were negative. The size of PCR products of *H. pylori-16S rRNA and ureAB* gene from all oral and gastric yeasts and three *H. pylori* was 519 bp and 406 bp, respectively and homologous to the previously identified gastric *H. pylori*.
Discussion: Oral and gastric yeasts could have a common source. Accordingly, oral yeast could carry *H. pylori* to stomach. Oral yeast could serve as a reservoir of *H. pylori* which facilitates transmission of *H. pylori* from person to person.

Abstract no.: P2.19

IMPLICATION OF MOTHERS' VAGINAL YEAST IN TRANSMISSION OF *H. PYLORI* TO THEIR NEWBORNS

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Introduction: Some studies showed the important role of infected mothers in transmission of *H. pylori* to their newborns. Previous studies revealed the intracellular occurrence of *H. pylori* inside the yeast which could facilitate the spread of bacterium within human populations. Yeasts, mainly *Candida* species are carried in the oral cavity, stomach, anus and vagina. In this study vaginal yeasts of mothers and oral yeasts of their newborns were examined for the presence of *H. pylori 16S rRNA* gene using PCR.
Methods: Five yeasts were isolated from the vagina of pregnant mothers and five from the oral cavity of their newborns. Yeasts were cultured in yeast extract glucose medium with chloramphenicole to eliminate exogenous bacterial contamination. Microscopic observations revealed the occurrence of bacterium like bodies inside the yeast vacuoles. PCR was performed for detection of *H. pylori 16S rRNA* gene in DNAs extracted from yeasts.
Results: The size of PCR products was 519 bp and homologous to that from control *H. pylori*. Two out of five mothers and their newborns carried *H. pylori* in their vaginal and oral *Candida* yeasts, respectively.
Discussion: Mother's vagina yeast and the oral cavity of her newborn could both harbour *H. pylori* and thus might have a common origin. It has been indicated that newborns became colonized with yeasts within hours after birth and mother's vagina could be the source. Accordingly, *Candida* yeast could play an important role in vertical transmission of *H. pylori*.

Abstract no.: P2.20

YEASTS OF DENTAL PLAQUE COULD SERVE AS RESERVOIRS OF *HELICOBACTER PYLORI* IN THE ORAL CAVITY

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Introduction: Current studies indicate that *Helicobacter pylori* occurs in dental plaque. However, culture of *H. pylori* from dental plaque has not been successful due to the complexity of the oral microflora and fastidious nature of *H. pylori*. Accordingly, there is a little chance for the bacterial survival in dental plaque. In this study we examined yeast isolates from dental plaque as the possible reservoirs of *H. pylori* that could protect the intracellular bacterium in a hostile and competitive environment of the oral cavity.
Methods: Supragingival dental plaque samples were collected from 10 healthy subjects. The samples were cultured on selective media to isolate *H. pylori* and yeasts. Two *Candida* yeasts were isolated from 10 samples. The two yeasts were examined for the occurrence of *H. pylori-16S rRNA* gene. Total DNAs were extracted and PCR was performed using appropriate primers.
Results: Attempts to culture *H. pylori* from samples were not successful. However *H. pylori-16S rRNA* gene was amplified from one yeast. The size of PCR product was similar to that of control *H. pylori*.
Discussion: Recent studies have shown the invasive nature of *H. pylori* and its ability to penetrate into different eukaryotic cells. It is proposed that yeast in dental plaque could provide a safe niche for *H. pylori* with ample supply of nutrients for growth. Yeasts could protect the bacterium from harsh conditions of dental plaque. Furthermore detection of *H. pylori DNA* in dental plaque could be related to occurrence of live but non-culturable *H. pylori* inside the oral yeasts.

Abstract no.: P2.21

HELICOBACTER PYLORI IN ORAL CAVITY IN PATIENTS WITH CHRONIC GASTRITIS

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Objectives: *Helicobacter pylori* migration from stomach to oral cavity is possible. Also this microorganism is one of the strongest urease producing microbes. The aim: to detect of urease-producing microorganisms *Helicobacter pylori*) in oral cavity and stomach in patients with chronic gastritis.
Methods: Sixteen patients with chronic gastritis were observed. Detection of urease-producing microorganisms was performed by breath ''HELIK-test'' (Association of Medicine and Analytic, St-Petersburg, Russia) in four steps: (1) Measurement of concentration of ammonia in exhaled air on a empty stomach (basal level 1); (2) Measurement of concentration of ammonia after the patient rinsed a mouth with solution of 0.5 g of urea (loading level 1 – definition of urease-producing microorganisms in an oral cavity); (3) Measurement of concentration of ammonia after the patient rinsed a mouth a large amount of water (basal level 2); (4) Measurement of concentration of ammonia after the patient drank a portion of urea (0.5 g) in 20 mL of the distilled water inside (loading level 2 – definition of urease-producing microorganisms in a stomach). Results: increase of urease activity of microorganisms in an oral cavity was revealed in nine patients from 16 (56%). Breath HELIK-test for detection of *H. pylori* in stomach was positive at the same patients. Conclusion: it is impossible to exclude existence of *H. pylori* infection not only in a stomach, but also in an oral cavity. Hence further researches are needed for specification of occurrence of an infection in a mouth.

Abstract no.: P2.22

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

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Background: *Helicobacter pylori* (Hp) infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis and gastric cancer. Its acquisition is related with poor socioeconomic conditions while the relationship of nutrition and Hp is still a question.
Aim: To analyzed if socioeconomic factors and dietary contribute to Hp re-infection in pediatric age.
Patients and Methods: One hundred and fifty patients (92 males; age range 5–16 years) with Hp infection treated and eradicated in the past. Fifty patients with Hp re-infection and 95 patients not re-infected. We interviewed the children with questionnaire about socioeconomics factors, hygiene, living conditions and their dietary habits.
Results: A lower frequency of fermented dairy food, fruits and vegetable consumption was registred among children with Hp re-infection as compared to not been re-infected. Among persons with Hp re-infection were noted low socio-economic markers such as croweded living conditions, a large number of siblings and unclean water.
Conclusions: Might decrease the risk of Hp re-infection the use of probiotic, vitamin C, antioxidants contained in fruit and vegetables. Risk factors for Hp re-infection are low socioeconomics factors, hygiene and living conditions.

Abstract no.: P2.23

ANTIMICROBIAL RESISTANCE IN *HELICOBACTER PYLORI* SPANISH CLINICAL ISOLATES OBTAINED FROM PAEDIATRIC PATIENTS

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The aim of this study was to determine the resistance to several antimicrobial agents in *H. pylori* clinical isolates obtained from paediatric patients.
Methods: Two hundred and eighty-seven clinical isolates of *H. pylori* were studied for metronidazole, clarithromycin, amoxicillin, tetracycline, rifampicin and levofloxacin. Sixty-one strains were obtained from Hospital 12 de Octubre, 57 from Hospital de Fuenlabrada, 152 from Hospital Niño Jesus and 17 from other centers. The in vitro activity of the antimicrobial agents was determined by E-test using an inoculum of three McFarland. Plates were incubated at 37°C during 2–5 days in a CO_2 increased atmosphere. MIC was determined as the point where ellipse cross the E-test strip. EUCAST recommendations were used to consider a strain as resistant.

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Results: Metronidazoleresistance was found in 33.45%, clarithromycin resistance in 49.83%, levofloxacin resistance in 2.11%, rifampicin resistance in 26.52%, amoxicillin resistance in 10.49% and tetracycline resistance in 0.35%. The resistance according to the center is shown in the following tables.
Conclusions: Resistance to claritromycin was very high in the *H. pylori* strains obtained from children included in his study. Tetracycline and levofloxacin resistance was unfrequent. Differences in resistance rate were observed amongt the stains obtained from the different center.

Center	AMX-R	CLA-R	MET-R
HOSPITAL 12 DE OCTUBRE	16.39%	50.82%	54.10%
HOSPITAL FUENLABRADA	1.75%	47.37%	21.05%
HOSPITAL NIÑO JESUS	12.58%	51.32%	31.58%

Center	RIF-R	LEV-R	TET-R
HOSPITAL 12 DE OCTUBRE	31.58%	1.64%	0.00%
HOSPITAL FUENLABRADA	19.30%	5.26%	0.00%
HOSPITAL NIÑO JESUS	27.33%	1.33%	0.66%

Abstract no.: P2.24

HELICOBACTER PYLORI CARDITIS IN DYSPEPTIC CHILDREN

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Aims: To assess the association between *Helicobacter pylori* (*H. pylori*) infection and inflammation of antrum, corpus and cardia, and to correlate the level of carditis in *H. pylori* infected and non-infected group of children.
Methods: The gastric antrum, corpus and cardia biopsies were histologically evaluated according to the Updated Sydney Classification in 40 children undergoing upper endoscopy for chronic dyspeptic symptoms (mean age 13.2 years [range 1–18 years]). Half of them were infected with *H. pylori* and the infection was detected by rapid urease test and histology.
Results: Carditis was present in 32/40 (80%) of the patients. Inflammation of cardia was detected in 18/20 (90%) of *H. pylori* positive children and in 14/20 (70%) of non-infected patients. The degree of activity ($p < .01$) and chronic inflammation ($p < .01$) of cardia strongly correlated with *H. pylori* positive status. *H. pylori* was present in the antrum (20/40; 50%), corpus (17/40; 42.5%) and cardia (12/40; 30%) biopsy specimens. Higher bacterial load ($p < .01$) and degree of activity ($p = .05$), but not chronical inflammation ($p = .42$), were detected in antral biopsies compared to cardia specimens of children infected with *H. pylori*.
Conclusion: Our study demonstrated that carditis is strongly associated with *H. pylori* infection in children. The activity of inflammation and bacterial infiltration was significantly higher in the antral mucosa than in cardia of infected pediatric patients.

Abstract no.: P2.25

EFFICACY OF THE DIAGNOSTIC TESTS IN PEDIATRIC *HELICOBACTER PYLORI* INFECTION

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Because *Helicobacter pylori* infection is acquired mostly in childhood, accurate diagnosis of the pediatric infection remains a very important problem.
To investigate the accuracy of invasive and noninvasive diagnostic tests.
We conducted a prospective study of 145 children with active *H. pylori* infection, who were diagnosed by invasive [urease test, histopathology, culture, polymerase chain reaction (PCR)] and non-invasive tests (serum antibodies, fecal antigen), during 2009–2011.
Sensitivity, specificity and predictive value were analyzed, using the GraphPad Prism program.
Of 145 children with *H. pylori* infection, the urease test was positive in 115 children (sensitivity 85.19%; specificity 93.94%), histology in 129 cases (sensitivity 89.58%; specificity 99.36%) and culture in 108 cases (sensitivity 74.48%; specificity 100%). The *H pylori* virulence genotype was identified by PCR in 140 children (sensitivity 96.55%; specificity 100%), significantly higher compared with the other invasive tests. The *cag* A gene was positive in 96 cases, compared with *vac* A, which was identified in all 140 cases. *H. pylori* fecal antigen was identified in 132 children with a significantly higher sensitivity (92.96%), specificity (98.10%) compared with the most widely used biopsy-based tests, respectively lower in comparison with PCR. The serum antibodies were positive in 78 cases (IgG), respectively 80 cases (IgA), with a lower sensitivity and specificity than invasive tests and fecal antigen.
Our data suggest that among invasive test PCR, had a significantly higher sensitivity and specificity ($p < 0.0001$) compared with noninvasive ones. Among noninvasive tests *H. pylori* fecal antigen has shown high sensitivity and specificity ($p < 0.0001$).

Abstract no.: P2.26

LOWER LEVELS OF PANCREATIC ELASTASE1 IN *HELICOBACTER PYLORI* POSITIVE CHILDREN

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Background and Aims: Controversial data exist about the association of *Helicobacter pylori* (*H. pylori*) infection and symptoms. The aim of the study was to compare symptoms in *H. pylori* positive and negative children.
Methods: The patient sample included 194 children (aged 2–10 years) in kindergartens, primary care centres and ambulatory allergologist consultation. The parents were asked to bring a stool sample of a child and to fill out a questionnaire (socioeconomic factors, demographic data, previous symptoms and treatment with antibiotics, diagnosis of allergy). Presence of active *H. pylori* infection was detected by rapid stool antigen test (Coris BioConcept, Belgium). The activity of human pancreatic faecal elastase1 concentration in stool was detected by Pancreatic elastase1monoclonal ELISA test (ScheBo®, Germany). Moderate to mild exocrine pancreatic insufficiency was considered if the concentration of elastase1 was <400 μg/g stool. Statistical analysis: χ^2 test, *log* regression.
Results: The total prevalence of *H. pylori* infection was 11% (22/195). *H. pylori* positivity was 8% (9/103) in children with allergic disease and 14% (13/92) in children without allergy ($p > 0.05$). The mean level of concentration of elastase1 was significantly lower in *H. pylori* positive individuals: 471 (95% CI 460–483) vs. 418 (5% CI 350–487) microg/g stool ($p = 0.008$). *H. pylori* infection was negatively associated with abdominal pain and loose stools during the previous year and antibacterial treatment during the 1st year of life. In logistic regression analysis *H. pylori* was significantly associated with mild pancreatic insufficiency.
Conclusions: Association of *H. pylori* positivity with lower levels of pancreatic elastase1points to a possible pancreatic exocrine insufficiency, although the direct relationship remains unclear.

Abstract no.: P2.27

SOCIODEMOGRAPHIC EVALUATION OF CHILDREN WITH *H. PYLORI* GASTRITIS

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Helicobacter pylori (*H. pylori*) is a cause of gastritis among children. This microorganism is more frequent among individuals living in developing countries. We aimed to look for some sociodemographic features of children with *H. pylori* gastritis. Parents of children who underwent an upper gastrointestinal system endoscopy fullfilled a questionnaire before the procedure. The correlation between these demographic data and modified Sydney gastritis scores was searched. A total of 114 forms were fullfilled. Mean age of child, mother and father was 7–17 (12.2 ± 2.7), 27–48 (36.2 ± 4.8) and 28–58 (39.7 ± 5.3) respectively. Number of children and total number of persons living in house was 1–7 (2.7 ± 0.9) and 3–11 (5.1 ± 1.6). Smoking was present in 41.2% houses, animal (sheep and cow) was present in 65.8% houses. Source of drinking water was tap water in 81.6% houses and bottle water in the rest. A history of intestinal parasites was present in 63.2% children. 40.4% children had their own rooms and 51.78% children were sleeping with either mother or brother/sister. Central heating was present in 24.6% houses and stoves were used in the rest. 70.1% families lived in family houses and the rest in apartments. There was statistically negative correlation between *H. plori* density and mothers' school degree, mothers' working condition, source of drinking water and way of house heating.

In conclusion this study favors the knowledge that *H. pylori* is more common in low socioeconomic parts of the population. Educating the mothers may help lowering the acquisition of the disease.

Abstract no.: P2.28

DECREASED GHRELIN AND OBESTATIN EXPRESSION IN THE HUMAN GASTRIC MUCOSA WITH *H. PYLORI*: ANY ROLE IN DECREASED APPETITE IN CHILDREN WITH *H. PYLORI*

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Background and Aims: *Helicobacter pylori* is a gram-negative bacterium that colonizes the stomach and causes chronic gastritis. Ghrelin and obestatin are mainly produced by endocrine cells in the gastric mucosa. Therefore, the aim of the present study was to examine how gastric tissues of express ghrelin and obestatin with and without *H. pylori* infection.
Material and Methods: Twenty three tissues were obtained from endoscopically removed material after study was approved by the local ethics committee. Immunohistochemical staining was performed using the Avidin Biotin Complex (ABC) Method. The intensity was scored from 0 (no staining) to 3 (most intense staining) based on the strongest staining of the core.
Results: While 16 of the 23 children (70%) tested were positive for *H. pylori* according to pathological evaluation, seven children (30%) had negative *H. pylori* status. Both peptides showed a similar immunostaining pattern in the gastric mucosa. It was also observed markedly *lower* tissue *ghrelin* and obestatin levels in gastric tissue of patients with *H. pylori* bacterium (Figure).
Conclusion: The current results indicate that lower expression of ghrelin and obestatin might be due to histologically altered gastric mucosa in patients with *H. pylori* bacterium and suggest that decreased ghrelin and obestatin expression might cause in decreased appetite in children with *H. pylori* bacterium.

Abstract no.: P2.29

MICROBIAL DYSBIOSIS IN PEDIATRIC PATIENTS WITH CROHN'S DISEASE

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Introduction: Microbial dysbiosis has been suggested to play a role in the pathogenesis of Crohn's disease (CD), however the results of studies investigating gut microbial communities in CD patients vs. controls have been inconsistent, which likely relates to the presence of confounding factors. In this study we compared the microbial flora of children newly diagnosed with CD with that in matched controls.
Methods: Fecal samples from 22 symptomatic children undergoing diagnostic endoscopy at Sydney Children's Hospital and eighteen healthy controls were collected. No child had undergone antibiotic or anti-inflammatory therapy in the 4-week period prior to collection. Differences in microbial composition were analyzed using Tag-encoded FLX amplicon pyrosequencing using Gray28F (5'TTTGATCNTGGCTCAG) and Gray519r (5' GTNTTACNGCGGCKGCTG) primers. PCDAI scores of patients were determined.
Results: Analysis of microbial composition revealed that Firmicutes (57.9 ± 6.7%) were significantly lower in CD patients than controls (80.0 ± 2.4%) ($p = .0035$) which was largely due to changes in the class Clostridia. In contrast, Bacteroidetes and Proteobacteria percentages were higher and significantly higher in CD patients (22.5 ± 6.0% and 11.0 ± 4.2%, respectively) than in controls (11.1 ± 2.4% ($p = .086$) and 1.6 ± 0.5% ($p = .040$), respectively). Detection frequencies of Bacteroidetes and Firmicutes correlated with patient's PCDAI scores. Further analysis identified differences in the microbial compositions of patients with mild disease and moderate to severe disease. No Helicobacteraceae were detected.
Conclusion: A combination of different bacterial species or a dynamic interplay between individual species appears important for disease, which is consistent with the dysbiosis hypothesis of CD.

Abstract no.: P2.30

IS THERE ANY RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* INFECTION AND CELIAC DISEASE WHICH CAUSES IRON DEFICIENCY ANEMIA IN CHILDHOOD?

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Objective: *H. pylori* infection occurs mainly in childhood. Celiac disease (CD) is a constant gluten intolerance that may affect the morphology and function of the entire gastrointestinal tract. Both diseases may cause iron deficiency anemia.
Aim: To evaluate the association between CD and *H. pylori* infection in children with growth retardation and determine the relationship with anemia.
Methods: Eighty-nine children (mean age: 8.2 ± 4.8 years) who were admitted to our pediatric outpatient clinic were studied between March and May 2012 by the special study module group of the third-year medical school students. Serum specimens were obtained and all serology tests were performed to determine the presence of CD. Anti-Gliadin (deamidated) (AGA) IgA, anti-Endomysium (EMA) IgA antibodies by IFA (EUROIMMUN) and anti-tissue-transglutaminase (tTG) IgA antibody and anti-gliadin IgG antibodies by ELISA (EUROIMMUN) tests were performed. Anti-*H. pylori* IgA antibodies were also detected in their sera by ELISA (EUROIMMUN) and confirmed by Western Blot (EUROIMMUN) tests.
Results: At least one celiac antibody was found positive in 15 (16%) of 89 children and subsequently 23 (25%) children was found anti-*H. pylori* IgA antibody positive. There was no any relationship between celiac antibody positivity and anti *H. pylori* IgA antibody positivity ($p > .05$). Anemia was found in seven children with positivity in celiac antibody and *H. pylori* positivity was determined in three of these seven children. Four children without anemia were *H. pylori* positive. This may be related with anemia associated with limited number of children.
Conclusion: In our study no relationship was found between *H. pylori* antibody positivity and the precence of CD in children with growth retardation. This research should be studied in a larger group of children.

Abstract no.: P2.31

^{13}C-UREA BREATH TEST VALUES: A LARGE DATABASE STUDY FOCUSING AT GENDER DIFFERENCES

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Introduction: Although 13C-urea breath test (UBT) constitutes one of the most accurate non-invasive test for diagnosis of *H. pylori* infection, persist uncertain if the UBT values are similar in males and females.
Aim: To determine the delta over baseline (DOB) UBT values in males and females in a large cohort of patients in a university hospital.
Methods: Retrospective (2003–2011), cross-sectional study conducted at Federal University of Minas Gerais, Belo Horizonte, Brazil. Previously validated UBT was performed with an infrared analyzer (IRIS, Wagner Analysen-Technik, Germany), using 75 mg 13C-urea in 200 mL of orange juice after overnight fasting. Breath samples were collected at 0 and 30 minutes. All tests with a delta over baseline (DOB) >4‰ at 30 minutes were considered positive. Database analyses were performed using Minitab-16, Excel-2007, and Mann–Whitney test.
Results: After excluding multiple testing for an individual, 12 988 UBTs (7659 in females [59%] and 5239 [41%] in males] were performed. The majority of the patients were adults (mean age: 46 years, range 1–107, SD 16.8). UBT was positive in 3911 (30%) and negative in 9077 (70%). The median DOB positive UBT values in females (23.4 ‰) were significantly higher than in males (18.4 ‰) ($p < .000$). Among the positive UBT tests, there were no significant gender differences between age (females: 46 years, males: 44 years) and body mass index (BMI) range (females: 24.8 kg/m^2, males: 24.3 kg/m^2).
Conclusions: *H. pylori* positive females have an unexplained significantly higher UBT values than infected males and these findings might have clinical consequences.

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Abstract no.: P2.32

CORRELATION OF SERUM PEPSINOGEN WITH HISTOLOGICAL ATROPHY FOLLOWING SUCCESSFUL *H. PYLORI* ERADICATION

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Background: Levels of pepsinogen (PG) have been reported to correlate with the degree of gastric atrophy in *Helicobacter pylori* (*H. pylori*)-infected gastric mucosa.
Aims: We investigated the relationship between PG levels and histological atrophy before and after *H. pylori* eradication.
Methods: Eradication therapy was conducted on 180 *H. pylori*-positive patients with upper gastrointestinal conditions. Endoscopy was done prior to and at 2, 12 and 24 months after successful eradication therapy. Histological findings were scored using the updated Sydney System in the antrum and the corpus of the stomach. PG was measured.
Results: PG levels dropped significantly at 2 months after the eradication. By 12 months and 24 months post-eradication PG levels had risen again. Histological improvement was seen a little in atrophy at all sites at 12 and 24 months after the eradication. A significant correlation (coefficient: -0.19) between atrophy and PG levels were seen from prior to eradication. The correlation coefficient was greater at 2 months post-eradication (-0.31), decreased again at 12 months (-0.22), and was no longer significant at 24 months (-0.03).
Conclusion: Our results suggest that PG levels correlate with the degree of gastric mucosal atrophy prior to and soon after successful eradication therapy, but that the degree of correlation subsequently declines over time.

Abstract no.: P2.33

COMBINATION OF PHENOTYPIC AND GENOTYPIC METHODS FOR THE ANTIMICROBIAL RESISTANCE DETECTION OF *HELICOBACTER PYLORI* - AN ALGORITHMIC APPROACH

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Background: Antimicrobial resistance is the predominant cause for low success rates of *Helicobacter pylori* eradication therapy. In the era of empiric rather than specific selection of appropriate antimicrobial agent, susceptibility testing is usually performed only after multiple treatment failures. The importance of the resistance detection in this clinical setting becomes very high, as it usually is the last hope for patient. Culture is prone to fail because of the fastidious nature of *H. pylori*. With the advent of molecular methods for the resistance detection, we can complement the two methodologies and so improve laboratory yield from biopsy specimens.
Methods: Fourteen months experience with two-stage sequential diagnostic algorithm is presented. All culture negative specimens were further tested with at least one molecular method. Culture using three different agar media, in-house PCR and GenoType HelicoDR test was performed. Positivity rates for both culture-only and algorithmic approach are shown.
Results: Six hundred and seventy-four *H. pylori* cultures were performed, 507 (75%) of which were positive. Among 167 (25%) negative culture specimens, 50 (30%) were positive with at least one molecular test. The net positivity rates for culture-only and algorithmic approach are 75% and 83%, respectively.
Conclusion: Culture of *H. pylori* is negative in approximatelly one quarter of patient that previously tested positive by at least one *H. pylori* diagnostic test: UBT, rapid urease, histology or stool antigen. Negative culture precludes phenotypic susceptibility testing. Molecular resistance detection performed on all culture negative biopsies - an algorithmic approach – increases diagnostic yield from gastric biopsies and as for that seems reasonable.

Abstract no.: P2.34

HELICOBACTER PYLORI INFECTION IN PRIMARY CARE: CLINICAL USEFULNESS OF GASTROPANEL IN ASSESSING GASTRIC MUCOSAL DAMAGE

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Introduction: Upper gastrointestinal endoscopy represents the main diagnostic test in patients with dyspeptic symptoms, despite its invasiveness. Several tests have been proposed for evaluation of dyspepsia as non-invasive screening for gastritis. GastroPanel (Biohit, Helsinki, Finland) is a serological kit for a non-invasive determination of stomach-specific biomarkers, namely pepsinogen I, pepsinogen II, gastrin-17 and IgG anti-Hp antibodies and recently the IV Maastricht Consensus proposed pepsinogens as the best non-invasive test for screening of gastric malignant lesions.
Aims and Methods: To evaluate the performance of GastroPanel as a non-invasive test, useful to reduce in a population of dyspeptic patients the burden of upper gastrointestinal endoscopy.
Results: Among these 1000 pts, 368 (36.8%, 262 females, mean age 54 ± 16 years) demonstrated both a normal gastric function and morphology, 418 suspected for gastroesophageal reflux disease (GERD) (41.8%, 256 females, mean age 51 ± 15 years), 187 showed a picture of Hp-related non atrophic gastritis (18.7%, 120 females, mean age 57 ± 14 years) and 27 have a chronic atrophic gastritis (CAG) (2.7%, 18 females, mean age 61 ± 15 years).
Conclusion: In this preliminary experience only in 2.7% of cases EGDS is strictly recommended, namely in the group of the CAG subjects. In the remaining 97.3% of cases indication for Upper gastrointestinal endoscopy may be delayed and revaluated after Hp eradication or GERD therapy, taking in to account additional factors: age, cancer familiarity or failure of pharmacological therapy. Further data to estimate the negative predictive value of GastroPanel for atrophic gastritis in GERD patients are required.

Abstract no.: P2.35

EFFECT OF PROTON PUMP INHIBITOR THERAPY ON DETECTION OF *HELICOBACTER PYLORI* IN BLEEDING PEPTIC ULCER PATIENTS

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Background/Aims: Although proton pump inhibitor (PPI) therapy causes false negative results on diagnostic test for *Helicobacter pylori* (HP), it is not clear how rapidly PPI therapy affect on it. Aim of this study was to investigate effect of short-term, high dose PPI therapy on diagnosis of HP infection in bleeding peptic ulcer patients.
Methods: We retrospectively analyzed patients of bleeding peptic ulcer by endoscopy. HP infection was confirmed by rapid urease test, histology or urea breath test. We divided patients into two groups (PPI group, control group) at the time of diagnostic test for HP. PPI group was divided into subgroups by cumulative dose of PPI. (160MG-group; <160 mg, 160MG+ group; >160 mg).
Results: Total of 98 patients enrolled. Fifty-five patients (56.1%) showed HP infection at initial tests. Positive rate of HP infection in PPI group was 52.9% (46 of 87), significantly lower than that of control group, 81.8% (nine of 11) ($p < .01$). Positive rates of HP infection were 76.1% (51 of 67) in 160MG-group and 12.9% (four of 31) in 160MG+group ($p < .01$). Positive rates of HP infection by cumulative dose of <80, 80–160, 160–240, 240–320 mg, more than 320 mg of PPI were 84.4%, 59.1%, 18.8%, 12.5%, 0%, respectively. As cumulative dose of PPI increases, the positive rates of HP infection decreased ($p < .01$).
Conclusions: Our results suggest PPI therapy rapidly suppresses HP colonization within 3 days, dose dependently. Therefore, in patients with bleeding peptic ulcer treated with PPI, diagnostic test for HP should be performed as soon as possible.

Abstract no.: P2.36

PEPSINOGEN II AS A MARKER FOR SUCCESSFUL *H. PYLORI* ERADICATION

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Background: Pepsinogen (Pg) levels in plasma are increased by the presence of inflammation in the gastric mucosa, including as the result of *H. pylori* infection. The decrease in PgII level has been suggested a reliable marker for confirming the success of eradication. The aim of our study was to evaluate the appropriateness of this hypothesis.
Methods: Altogether 42 patients (25 women/17 men), mean age 45 (range 23–74) were enrolled. PgI and PgII were measured in plasma before the eradication and at least 4 weeks after completing the treatment with an ELISA test (Biohit,

Plc., Finland). The success of eradication was assayed during the control blood sample withdrawal with a UBT.
Results: The eradication was successful in 31 patients (74%), unsuccessful – in 11 patients (26%). PgII level decreased significantly either the group with successful (p = .029) or unsuccessful (p = .042) eradication. In the group with successful eradication the mean baseline Pg II level was 13.4 μg/L, the mean follow-up level – 8.3 μg/L, therefore the mean level decrease was 5.0 μg/L (95% CI 0.9–9.1); correspondingly the values in the group with unsuccessful eradication were 13.6, 7.8, and 5.8 (95% CI 0.8–10.8). Significant increase in PgI/PgII was observed in cases with successful eradication (p = .0018), but not in the group with eradication failure (p = .12).
Conclusions: The decrease in PgII levels cannot be used as a reliable marker for success in *H. pylori* eradication. The improvement in PgI/PgII ratio is reflecting differences in the pepsinogen proportion following the eradication but not diminishing of atrophy.

Abstract no.: P2.37

THE EFFECTIVENESS OF NON-INVASIVE TESTS IN DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN

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On the basis of the Children's City Hospital 5 St. Petersburg, Russia we examined 153 children from 10 to 17 years with HP-associated gastroduodenal diseases. Before eradication therapy and 6 weeks after the treatment esophagogastroduodenoscopy, *Helicobacter pylori* AMA RAPID UREASE TEST, non-invasive HELIC Ammonia Breath Test, HELIC-Device Ammonia Breath Test (Association of Medicine and Analytic, St. Petersburg), Urea Breath Test («Heli-View», Medi-Chem Ltd., South Korea), ACON *H. pylori* RAPID TEST STRIP (USA), PCR in feces and in antrum biopsies were performed.
This study confirmed the absence of a universal ''gold standard'' method.
Sensitivity and specificity of methods are presented in Table.
Conclusion: Non-invasive HELIC Ammonia Breath Test, HELIC-Device Ammonia Breath Test have high sensitivity and specificity, low cost, do not require expensive equipment and highly specialized personnel, can be used in children from 3 years of age, have no contraindications, and no complications.

Methods	Sensitivity (%)	Specificity (%)	$\chi 2 >$	p
Histology	99.5	100	282.01	<.001
AMA RAPID UREASE TEST	87.3	93.6	163.53	<.001
HELIC Ammonia Breath Test	91.5	91.1	180.94	<.001
HELIC-Device Ammonia Breath Test	93.0	89.7	158.07	<.001
Urea Breath Test	67.7	89.7	24.14	<.001
PCR in feces	53.1	95.6	31.53	<.001
PCR in biopsies	77.7	89.1	24.14	<.001

Abstract no.: P2.38

CAREFUL CLOSE-UP OBSERVATION OF GASTRIC MUCOSAL PATTERN BY NON-MAGNIFYING STANDARD ENDOSCOPY CAN PREDICT *HELICOBACTER PYLORI* INFECTION STATUS

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Objectives: Various endoscopic findings of *Helicobacter pylori* (*H. pylori*)-infected stomach such as antral nodularity, thickened gastric folds, and visible submucosal vessel, have been suggested on standard endoscopy, but, they are not reliable because of low sensitivity and specificity. Magnifying endoscopy revealed precisely the abnormal mucosal patterns in *H. pylori*-infected stomach. However, it takes more examination time and needs more training and experience. In this study, we aimed to find the abnormal mucosal patterns in *H. pylori*-infected stomach using non-magnifying standard endoscopy alone.
Methods: A total of 564 participants who underwent upper gastrointestinal endoscopy for a routine health check were consecutively enrolled from July 2011 to April 2012. We performed a careful close-up examination of the corpus mucosa at the greater curvature maintaining a distance ≤10 mm between endoscope tip and mucosal surface. We used three categories such as, normal regular arrangement of collecting venules (RAC), abnormal mosaic pattern (type A) and diffuse homogenous reddish surface (type B) to predict *H. pylori*-infected stomach.
Results: The frequencies of *H. pylori* infection in normal RAC pattern and types A, B patterns were 8.5%, 86.8%, and 97.9%, respectively. The sensitivity, specificity, positive and negative predictive values of all abnormal patterns for predicting *H. pylori*-infected stomach were 93.3%, 89.7%, 91.8%, and 91.5%, respectively. The overall accuracy was 91.7%. Distinct atrophic changes in the stomach body increased the diagnostic accuracy (p = .038).
Conclusion: Careful close-up observation of gastric mucosal pattern with standard endoscopy can predict *H. pylori* infection status.

Abstract no.: P2.39

MODIFIED 13C-UREA BREATH TEST IN THE DIAGNOSIS OF INFECTION *H. PYLORI* IN CRIMEAN POPULATION PATIENTS WITH GASTRITIS

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Background: The accuracy of the 13C-urea breath test (UBT) results can affect the contamination degree of *H. pylori* and atrophy gastric mucosa.
Aim: To evaluate effectiveness of low doses of 13C-urea with citric acid in comparison with a standard method of UBT in patients with chronic non-atrophic and atrophic gastritis.
Methods: Were examined 160 *H. pylori*-positive patients: 105 – with non-atrophic gastritis, 36 – with antrum, 12 body, seven multiply atrophy. *H. pylori* infection was confirmed by: rapid urease test, UBT and histologically. Patients were divided into four equal groups. In the control group used 75 mg. 13C-urea with orange juice. In the three groups as solvent for reagent used 2.0 g citric acid (1st gr.), 1.0g ascorbic acid (2nd gr.) or distilled water (3nd gr.).
Results: In evaluating the delta over baseline (DOB) at 30 minutes did not reveal differences in ability of the protocols of 13C-urea breath test to detected *H. pylori* (p = .86).
DOB at 15 minutes were higher in the 1st group – 12.15 ± 4.22‰ (control gr. – 4.22 ± 2.63‰; 2nd gr. – 6.15 ± 2.63‰; 3rd gr. – 4.51 ± 2.13‰; p < .05) with minimum value – 7.87 ‰. The DOBmin on 15 minutes in the other groups were below cut-off value for UBT and were lower than in the first group (p < .001).
Conclusions: The modified 13C-UBT with 50 mg. 13C-urea and citric acid allows obtaining diagnostically significant results already a 15 minutes of study, reduce the dose and the cost of the used reagent, increase the diagnostic accuracy of techniques in atrophic gastritis.

Abstract no.: P2.40

COMPARISON OF SUCCESS RATES OF NONINVASIVE AND INVASIVE METHODS IN DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

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Background: Because *Helicobacter pylori* (HP) diagnosis is important in management of many gastroenterological entities, sensitivity and specificity data of both invasive and noninvasive tests for HP are important.
Methods: We report preliminary data on 48 outpatients tested for HP. The presence of HP was confirmed by a positive culture or histology or at least two positive tests from the five remaining invasive or noninvasive tests performed. Invasive tests performed on gastric mucosa samples comprised of Rapid Urease Test (RUT) and histology staining in all patients and a further culture in all RUT, histology or noninvasive testing positive patients. Noninvasive tests performed were: 13C Urea Breath Test (UBT), anti HP IgG and IgA using commercial ELISA, HP antigen stools tests and a saliva-based HP test.
Results: Twenty out of 48 patients tested were positive for HP. Sensitivity and specificity results were: RUT 90.5% and 100%; UBT 100% and 100%; HP stool antigen test 57.9% and 78.9%, the Saliva-based test 56.3% and 66.7%, for anti - HP IgG and IgA testing sensitivity was 94.7% and 63% respectively and specificity 90% and 85%. Concordance between culture (in 20 out of 48 patients) and histology results was 100%.
Conclusion: There is high concordance between histological and culture based diagnosis of HP. RUT is very specific, but of lower sensitivity. Between noninvasive tests available UBT performs as well as the reference invasive tests. ELISA testing and antigen detection in stool and saliva are less accurate and should probably not be routinely used as diagnostic tools.

Abstract no.: P2.41

UREASE ACTIVITY OF MOUTH CAVITY

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There are bacteria-urease producers in mouth cavity, *H. pylori* is among them. Reacting with urea solution during urea breath test (both CO_2 and NH_3 measurements) these bacteria can influence to the result of the test and deform it.
Aim: To evaluate the total urease activity of mouth cavity during UBT and to find the way to reduce it's influence.
Method: We have tested 62 patients by Helic-device, which allows determining the level of ammonia continuously. After the measurement of the ''basal'' level of ammonia in mouth cavity patient rinses their mouth by the urea solutions (concentration 0.2–10 mg/mL), not swallow it. Then we have measured the level of ammonia again – ''load'' level.
Results: Load level after rinsing was higher than basal level, and increase depend on urea concentration. For urea concentration 10 mg/mL increase was in 92% (57 patients), ammonia level was mostly higher 20 ppm. We have fixed dynamic characteristics of these increases.
To cope this problem we have investigated few possible ''stop-solutions'' (stomatidin, two tooth balms), which are able to influent the bacteria-urease producers and suppress the urea hydrolysis as desinfectants.
These stop-solutions were not effective enough, most productive was water rinsing out by special procedure.
Conclusions: • Urease producers from mouth cavity are able to generate big amounts of ammonia.
• Proper procedure of urease breath test should take this side urease activity into consideration.

Abstract no.: P2.42

EVALUATION OF EFFECTIVENESS OF DIFFERENT METHODS OF *HELICOBACTER PYLORI* DETECTION IN PATIENTS WITH CHRONIC HCV-INFECTION

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Aim: To determine frequency of *H. pylori* infection in patients with chronic HCV-infection followed by comparative evaluation of effectiveness of different methods of identifying of helicobacteriosis.
Material and Methods: Twenty-five patients (men – 64%) with chronic HCV-infection at the age of 19–64 years were involved in the study. Patients aged 21–40 years were dominated. In 48% of patients liver cirrhosis was determined (stage A-B – 30%, B – 40%, B-C – 30%). All patients were performed esophagogastroduodenoscopy with biopsy of gastric mucosa (antrum) and duodenum. Diagnosis of *H. pylori* was carried out by rapid urease test («Helpyl», AMA, Saint-Petersbourg), urea breath test («Helic-test», AMA, Saint-Petersbourg). A commercial kit («Helicopol») for DNA isolation from biopsy specimens was applied. The presence of *H. pylori* was confirmed by PCR using specific primers for ureC according to the recommendations of the manufacturer «Lytech» (Moscow, Russia).
Results: At endoscopy all patients were detected gastrobulbit (32% – erosive nature), in 36% of cases – chronic reflux esophagitis, in 38% – duodeno-gastric reflux. Varicose veins of esophagus I, II, III degrees were found in 40% of patients. PCR was positive in all samples. The positive detection of *H. pylori* by urease test in gastric and duodenal biopsy specimens was observed in 100% of cases. Sensitivity of ''Helic-test'' by using Helic-tubes was 72%.
Thus, a range of concomitant disorders of esophagus, stomach and duodenum associated with *H. pylori* are revealed in patients with chronic HCV-infection. The highest sensitivity was observed in PCR detection and urease ''Helpyl'' test.

Abstract no.: P2.43

IS FISH ALTERNATIVE TO CULTURE TO DETECT *H. PYLORI* AND CLARITHROMYCIN SUSCEPTIBILITY IN A PEDIATRIC PATIENT?

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Aim: To evaluate the treatment failure's reason and choose the treatment protocol.
Methods: Ten years old boy was referred to our outpatient clinic with resistant to *H. pylori* standard eradication therapy. RUT, histopathology and culture were applied to biopsy specimens. Antrum and corpus biopsy specimens were cultured on Columbia blood agar containing 7% defibrinated horse blood and supplemented with *H. pylori* Selective Supplement (DENT), and incubated at 37°C under microaerobic condition by using GasPak Campy Container System for 3–10 days. At the time of culturing a piece of the biopsies was prepared to examine spiral morphology by methylene-blue and Gram stains. The paraffin embedded antrum and corpus biopsy specimens were examined by fluorescence in situ hybridization (FISH) (BactFISH *H. pylori* Combi Kit) method to detect *H. pylori* and to determine clarithromycin susceptibility.
Results: *H. pylori* infection was positive in both antrum and corpus by histopathology. RUT and *H. pylori* stool antigen tests were negative for *H. pylori*. Unfortunately there was no growth on the culture media after 10 days and *H. pylori* culture was also negative. However, surprisingly *H. pylori* was detected and clarithromycin susceptibility was determined in patient's both antrum and corpus biospies by FISH method. So the reason of treatment failure was not clarithromycin resistance. This may result from metronidazole resistant strains.
Conclusion: FISH significantly increases the sensitivity to detect *H. pylori* in the clinical microbiology laboratory when compared with traditional culture. It is fast, reliable, rapid method and also suitable for determination of susceptibility of *H. pylori* to clarithromycin, especially when a quick decision is necessary for treating patient with treatment failure.

Abstract no.: P2.44

RELATION OF *HELICOBACTER PYLORI* WITH DYSPEPTIC SYMPTOMS AMONG MEDICAL STUDENTS IN LITHUANIA

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Introduction: The relation of *Helicobacter pylori* induced chronic gastritis with dyspepsia remains unclear.
Aims: To compare the frequency and intensity of different dyspeptic symptom in *Helicobacter pylori*-positive and *Helicobacter pylori*-negative persons.
Methods: The students of Lithuanian university of health sciences were investigated serologically for the presence of antibodies against *Helicobacter pylori* with the SureScreen ''HPSC – *Helicobacter pylori* test''. The students anonymously filled-in the gastrointestinal symptoms rating scale questionnaires. The intensity of different dyspeptic symptoms (epigastric pain or discomfort, heartburn, regurgitation, hunger-like-pain, nausea, borborygmus, epigastric fullness, belching, abdominal distention, constipation, diarrhea, urgency to defecate) was assessed during last week rating symptoms in 7 grade Likert scale.
Results: We investigated 187 students (mean age -22.4 ± 0.7 years). *Helicobacter pylori* found in 57 (30.4%) students. The overall prevalence of symptoms was not different between *Helicobacter pylori*-positive and *Helicobacter pylori* -negative students. Among students in whom pain or discomfort in epigastric region was present, the intensity of symptom was 2.3 ± 1.1 in *Helicobacter pylori*-positive students and 1.7 ± 0.9 in *Helicobacter pylori*-negatives, $p < .05$. Among the students with borborygmus (abdominal rumbling), the intensity of symptom was 1.9 ± 1.0 in *Helicobacter pylori*-positive students and 2.4 ± 1.3 in *Helicobacter pylori*-negatives, $p < .05$. Intensities of other symptoms were not significantly different.
Conclusion: In general we did not find any correlations between the status of *Helicobacter pylori* and the prevalence and intensity of different dyspeptic symptoms. Though, the intensity of epigastric pain and discomfort was significantly higher in *Helicobacter pylori*-positive students; the intensity of abdominal rumbling was significantly higher in *Helicobacter pylori*-negative students.

Abstract no.: P2.45

THE EFFECT OF *HELICOBACTER PYLORI* ERADICATION ON UPPER GASTROINTESTINAL SYMPTOMS AND CIRCULATING GHRELIN AND LEPTIN CONCENTRATIONS IN GASTRIC ULCER

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Aims: Ghrelin and leptin are gastric peptide hormones related to food intake and energy expenditure. We aimed to evaluate whether *H. pylori* eradication in gastric ulcer increase plasma ghrelin and leptin levels and to investigate whether upper gastrointestinal symptoms correlate with plasma ghrelin and leptin levels.
Methods: Total of 31 patients with gastric ulcer (19 *H. pylori* [+] and 12 *H. pylori* [-]) and 14 healthy controls were enrolled. Plasma ghrelin and leptin levels were measured via radioimmunoassay after overnight fast before and after treatment of gastric ulcer or *H. pylori*. Patients with *H. pylori* received esomeprazole, clarithromycin, and amoxicillin for 7 days and esomeprazole following 3 weeks, while

those with *H. pylori* (-) only esomeprazole for 4 weeks. The gastrointestinal symptoms questionnaires included nausea, vomiting, epigastric pain, heartburn, acid reflux, early satiety, and bloating. The treatment effects were determined by urease breath tests 4 weeks after the treatment completion.
Results: There were no differences in the mean plasma levels of ghrelin and leptin between the gastric ulcer patients and control. After ulcer treatment, the changes of the mean plasma levels of ghrelin (4.3 ± 1.5 vs. 4.5 ± 1.6 ng/mL, $p = .101$) and leptin (5.47 ± 2.07 vs. 6.07 ± 2.40 ng/mL, $p = .450$) were not significant. However, after successful eradication of *H. pylori*, the mean plasma ghrelin levels were significantly increased (4.0 ± 1.3 vs. 4.4 ± 1.5 ng/mL, $p = .039$). Only early satiety seemed to be correlated with the mean plasma ghrelin levels ($r = -0.434$, $p = .063$).
Conclusions: Status of *H. pylori* in gastric ulcer may affect gastric secretion of ghrelin.

Abstract no.: P2.46

ASSESSMENT OF POLYMORPHISMS AND EXPRESSION OF ABCB1 GENE IN THE GROUP OF PATIENTS INFECTED WITH *HELICOBACTER PYLORI*

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H. pylori is gram negative bacterium with ability to produce urease which spread urea. *Helicobacter pylori* infection is one of the most common in the world. Colonization of stomach mucosa by *H. pylori* is one of the reasons of gastric mucosa inflammatory. Ineffective eradication therapy leads to gastritis, which is gastric cancer risk factor.
Eradication schemes are based on PPI and two antibiotics. Lack of effective eradication is connected with widely understood multidrug resistance, which one of the reasons are transporters encoded by genes from ABC family.
ABCB1 gene encodes P-glycoprotein, typical ATP-dependent membrane pump for which substrates are among other antibiotics used in *H. pylori* eradication.
For ABCB1 gene over 50 SNPs were identified, including those at positions: C1236T, G2677T/A, C3435T. They can influence on gene expression and thus change the level and/or functions encodes by this gene P-glycoprotein.
The aim of the study is genotyping at positions: 1236 2677 3435 and evaluation of ABCB1 gene expression in biopsy sample of stomach mucosa from patients with *Helicobacter pylori* infection and comparison the frequency of obtained genotypes distribution with control group.
Investigated group: biopsy samples of stomach mucosa collected during gastroscopy. Control group: blood taken from blood donors.
Methods: Urease test, PCR-RFLP, sequencing, qualitative and quantitative PCR Mutant TT homozygotes and T allele of 3435 polymorphism occurred more frequently ($p = .0090$; $p = .0014$ respectively) in the group of *H. pylori* infected patients and also in the subgroup of men. For SNP at position 1236 homozygous TT occurred more frequently ($p = .0298$) in the *H. pylori* infected women.

P3 – Inflammation and Host Response, Immunity

Abstract no.: P3.1

A20 UPREGULATION IS INDUCED BY *HELICOBACTER PYLORI* INDUCES IN GASTRIC EPITHELIAL CELLS

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H. pylori is capable to induce inflammatory responses from host cells through NF-kB. However, little has been known about the process by which gastric epithelial cells counteract *H. pylori* induced-NF-kB activation to avoid overreactions. A20 is well-known as a NF-kB suppressor and proapoptotic protein. In this study, we investigated the role of A20 in *H. pylori* -gastric epithelium interaction. We found that upon *H. pylori* infection, A20 was upregulated in a time- and MOI-dependent manner. A20 mRNA increased sharply within 3 hours and then decreased gradually to the baseline after 36 hours. Nevertheless, cagA mutant strain is still able to trigger A20 upregulation but this ability was reduced considerably, indicating that A20 induction was partly cagA-dependent. Because *H. pylori* injects CagA protein and its peptidoglycan into the host cell via type IV secretion system, we suspected that both of them involved in the induction of A20. To confirm this hypothesis we transfected cagA vector and *H. pylori* peptidoglycan into gastric epithelial cells. We found that the transfection of these two agents resulted in a significant increase of A20 expression. To investigate the role of A20 in the interaction between *H. pylori* and host cell, we overexpressed A20 in gastric cells and infected them with *H. pylori*. We saw that the activation of NF-kB in these cells was suppressed significantly. These findings indicate that *H. pylori* induces A20 expression in host cell to restrain *H. pylori* -activated NF-kB. This negative feed-back loop will establish the balance between host and infectant.

Abstract no.: P3.2

HELICOBACTER PYLORI INFECTION: CURCUMIN REDUCES GASTRIC INFLAMMATION BY MODULATING INTESTINAL MICROBIOTA?

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Aim: Evaluate the role of a nutraceutical (curcumin) in the outcome of *Helicobacter pylori* gastric inflammation through modulation of the intestinal microbiota.

Material and Methods: Twenty five C57BL/6 mice were used: Control group (CG), n = 5, Infected Group (IG), n = 10 and Curcumin Group (CurG), n = 10. IG and CurG were inoculated intragastrically with *H. pylori* SS1 cell suspension. Mice were then treated with either PBS (CG and IG) or Curcumin (CurG). Five mice from each group were sacrificed at week 6 and the remaining five from IG and CurG at week 18. At each time point, stomach samples were collected for immunohistochemistry and histology. Faeces samples to evaluate intestinal microbiota composition by FISH were also collected.

Results: Immunohistochemistry confirmed that all IG and CurcG mice were infected. Histological examination showed that 40% (2/5) of IG mice had intramucosal inflammation, at both 6 and 18 weeks, while no inflammation was observed in CurG mice, at the same time points. Microbiota analysis at week 6, comparing IG with CG, showed a decrease of *Lactobacillus casei/paracasei* (-46.6×), *L. plantarum* (-5.7×) and *Bifidobacterium* (-11.3×); comparing CurcG with IG, an increase of *L. casei/ paracasei* (7.4×), *L. plantarum* (12.7×) and *Bifidobacterium* (15.8×) was observed. At week 18, in the CurG mice, intestinal colonization with these three Genera was similar to the CG, except for *Fusobacterium*, which was 6.0 times higher than in IG mice.

Conclusions: Our experimental data suggest that modulation of the intestinal microbiota by curcumin may represent a promising strategy within the scope of *H. pylori* infection.

Abstract no.: P3.3

ASSOCIATION OF IL-8 -251T/A SINGLE NUCLEOTIDE POLYMORPHISM AND SERUM PROTEIN LEVELS WITH GASTRIC CANCER IN AN IRANIAN CASE-CONTROL STUDY

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IL-8 is a well-studied pro-inflammatory cytokine which is known to potentiate gastritis in response to *H. pylori* infection. Furthermore, its elevated circulatory levels are associated with poor prognosis of gastric cancer patients. IL-8 -251T/A single nucleotide polymorphism (SNP) results in higher production of this cytokine among its carriers. We have, therefore, studied the state of IL-8 -251T/A SNP and its serum protein levels with gastric cancer.

Our case-control study included 364 gastric cancer patients and 404 healthy controls. Serum *H. pylori*-specific IgG antibodies were measured by ELISA. IL-8 T251A SNP was detected by PCR-RFLP and serum protein levels were measured by magnetic-based ELISA. Age /gender adjusted odds ratio and the corresponding 95% confidence intervals was estimated by unconditional logistic regression model.

IL-8 T251A carriers over the age of 50 showed an increased risk of gastric cancer (OR = 1.5, 95% CI = 1.0–2.3), directed toward the non-cardia subsite (OR = 1.6, 95% CI = 1.0–2.7) and intestinal subtype (OR = 1.6, 95% CI = 1.0–2.7). Moreover, the risk of intestinal gastric cancer was inflated in the female carriers (OR = 2.7, 95% CI = 1.0–7.4). Serum IL-8 levels were significantly higher in patients with gastric tumors of both subsites as compared to the controls ($p < .001$). Furthermore, IL-8 T251A carriers possessed higher levels of serum IL-8 as compared to subjects with the wild genotype ($p < .05$).

In conclusion, our results indicate that IL-8 as a highly pro-inflammatory cytokine may be considered as a candidate risk marker for noncardia/intestinal type gastric cancer at both gene and protein level, each of which may reflect the status of the other.

Abstract no.: P3.4

EXPRESSION OF TH17 CYTOKINES AND ANTIMICROBIAL PEPTIDES IN THE GASTRIC INFECTIONS INDUCED BY *HELICOBACTER PYLORI*

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Helicobacter pylori (*H. pylori*) infection causes a gastric mucosal inflammation which can lead to severe pathologies. Pathological determinism depends on host genetic factors, bacterial virulence factors and environmental factors. The gastric inflammatory response is associated with an increase of cytokines and antimicrobial peptides production. The major *H. pylori* virulence factor is the *cag* pathogenicity island encoding the effector protein CagA and a type IV secretion system. From a large collection of gastric biopsies, the detection of *H. pylori* was determined by culture and PCR. Each sample positive for *H. pylori* has been classified according to clinical and histological results. Forty-one gastritis, 13 precancerous or cancerous diseases, 12 ulcers (seven duodenal and five gastric) and a control group including 70 healthy mucosa (absence of detection of *H. pylori*) were studied. The expression of genes encoding inflammatory mediators such as IL-8, IL-17A, IL-22 and antimicrobial peptides such as S100A9 and BD2 was analyzed by quantitative RT-PCR. Among all strains of *H. pylori* isolated, the presence of the gene encoding the CagA protein was investigated. High expression of IL-8, IL-17A, IL-22, S100A9 and BD2 was detected in biopsies infected with *H. pylori* and more strongly in ulcers and precancerous or cancerous diseases. Moreover, in gastritis, the increased expression of these mediators appears to be CagA dependent. This study may allow to correlate cytokine profiles with specific gastric pathologies and to better understand the role of Cag A in the gastric mucosa inflammatory status of patients.

Abstract no.: P3.5

DIFFERENTIAL EXPRESSION OF HUMAN BETA DEFENSIN- 2 AND -3 IN GASTRIC MUCOSA OF *HELICOBACTER PYLORI*-INFECTED INDIVIDUALS

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Background: Antimicrobial peptides are keyplayers of initial innate immune responses to human pathogens. Two major representatives, the human beta defensin 2 and 3 (hBD2, hBD3) are both known to be induced by *Helicobacter pylori*. Previously, it was demonstrated in vitro that *H. pylori* actively abrogates hBD3 expression during prolonged infections. Here we comprehensively assessed hBD2 and hBD3 expression ex vivo in the gastric mucosa of healthy individuals.
Materials and Methods: Twenty volunteers (*H. pylori* positive and *H. pylori* negative: n = 10) were enrolled. *H. pylori* positive subjects underwent eradication therapy and repeated the protocol. Expression of both defensins were assessed by quantitative RT-PCR and ELISA, and correlated with histopathological degree of gastritis.
Results: hBD2 and hBD3 were found to be ubiquitously expressed in all three groups. In general, hBD2 levels were elevated in relation to *H. pylori* infection (up to 40-fold). This upregulation correlated with degree of gastritis in corpus and antrum. In contrast, hBD3 mRNA amounts were significantly decreased, while corresponding protein levels remained unchanged. Eradication therapy led to normalization of mucosal hBD2 expression, while hBD3 expression demonstrated high interindividual variations among individuals.
Conclusions: Both defensins are ubiquitously but differentially expressed in gastric mucosa in relation to *H. pylori* infection. Ex vivo data support previous in vitro findings that *H. pylori* infection is associated with reduced hBD3 expression in chronic active gastritis.

Abstract no.: P3.6

BLOOD AND LYMPHATIC MICROVESSELS DENSITY IN GASTRIC MUCOSA OF DYSPEPTIC PATIENTS

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Introduction: Published data on the role of *H. pylori* in angiogenesis are contradictory, while its role in lymphangiogenesis has not been investigated.
Aims: To investigate the density of lymphatic and blood vessels, together with *H. pylori* infection, in biopsies of gastric body and antrum of dyspeptic patients.
Methods: Biopsies from patients subjected to gastroscopy according to clinical criteria were studied by immunohistochemistry. Exclusion criteria were: previous eradication therapy, ulcer disease, regular non-steroidal anti-inflammatory use, and proton pump inhibitor or antibiotic treatment 15 and 30 days prior to recruitment respectively. Microvessel density was determined with CD34 and D2.40 monoclonal antibodies (DAKO, Glostrup, Denmark), which are markers of blood and lymphatic vasculature respectively. Quantification was performed by direct counting of microvessels in four fields at 40× magnification. *H. pylori* infection was assessed by ^{13}C-urea breath test and/or histopathological diagnosis.
Results: Twenty-three biopsies (13 antral) from 13 patients were studied. Their median age was 62 years, 46% males, 73% gastritis and/or atrophy and 24% *H. pylori* positive. Higher blood microvessels densitiy was associated to *H. pylori* infection ($p = .03$) (Table 1). Microvessels count was slightly elevated, without statistical signification, among biopsies with histological diagnosis of gastritis. Further associations with inflammatory activity or location were not found. Lymphangiogenesis was not related with any of the studied variables.
Conclusions: (1) *H. pylori* infection was associated with increased gastric angiogenesis (2) Lymphangiogenesis was not related with the studied clinicopathological features.

Abstract no.: P3.7

COX-2 INHIBITION WITH NUTRACEUTICALS: A NEW THERAPEUTIC APPROACH AGAINST *HELICOBACTER PYLORI* INFECTION?

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Accumulated evidence in humans and animals shows that *H. pylori* up-regulate the expression of cyclooxygenase (COX)-2 both at mRNA and protein levels which might be the one of the mechanisms leading to several gastric diseases.
Aim: To study the expression of COX-2 on mice gastric mucosa during long-term treatment with two nutraceuticals: curcumin and synbiotic 2000® on *H. pylori* experimental chronic infection.
Materials and Methods: We infected 45 C57BL/6 mice with SS1 – *H. pylori* strain. After infection confirmation by ^{13}C-urea breath test mice where then treated with either PBS, curcumin (10 mg/mouse) or Synbiotic 2000® (50 mg/mouse), three times per week. Five mice from each treatment group were euthanized at week 6, 18 and 27. Gastric samples were removed for COX-2 immunohistochemistry analysis.
Results: All the 45 mice were Hp positive by ^{13}C-urea breath test and immunohistochemistry. In the PBS group the production of COX-2 was significantly up-regulated at week 6 (area of positive immunostaining 393–544 $\times 10^3$ pixels), 18 (area of positive immunostaining 242–614 $\times 10^3$ pixels) and 27 week (area of positive immunostaining 129–175 $\times 10^3$ pixels). The treatment with either curcumin or synbiotic significantly decreased the expression of COX-2 at all time points.
Conclusions: These results suggest the therapeutic usefulness of both nutraceuticals on COX-2 inhibition during chronic experimental mice *H. pylori* infection. The supplementation of diet in humans with curcumin or Synbiotic 2000® may be a novel therapeutic approach against gastric inflammation induced by Hp infection.

Abstract no.: P3.8

PRODUCTION OF ANTI-*H. PYLORI* IMMUNOGLOBULIN Y (IGY) IN THE CHICKEN EGG YOLK

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Introduction: Immunizing chickens with certain antigens and collecting the related antibodies have been proposed as a useful method for production of edible immunoglobulins (Igs). Oral IgY could have applications in the control of gastric infections such as those caused by *Escherichia coli* and *Salmonella enterica*. In this study immunization of chicken and production of anti-*H. pylori* IgY in egg yolk was studied
Methods: A suspension of one heat-killed and PCR-confirmed *H. pylori* isolate mixed with freund's adjuvant was intramuscularly injected to two 5 months-old chickens, once a week for three consecutive weeks. Ten days after the last immunization, eggs were collected and total antibody was purified from egg yolk. The presence of anti-*H. pylori* IgY was assessed using dot blotting method on Polyvinylidene fluoride membrane. PCR-confirmed *E. coli* and *Salmonella entrica* were used to eliminate the possibility of cross-reaction.
Results: Specific binding of extracted antibodies to *H. pylori*-specific antigens was observed as colored spots on the membrane, indicating the presence of anti-*H. pylori* ploycolonal antibodies in the chicken eggs. Colorimetric reaction of antibodies with *E. coli* and *S. entrica*-specific antigens were not observed.
Discussion: Immunization of chickens for the production of antibodies has several advantages such as no need for blood sampling from animals and production of a large amount of antibodies in egg yolk. Furthermore, egg yolk containing anti-*H. pylori* antibody administered orally could provide a novel and effective approach to prevent *H. pylori* infection.

P4 – Clinical Trials and Drug Resistance

Abstract no.: P4.01

BISMUTH-CONTAINING QUADRUPLE THERAPY VS. STANDARD TRIPLE THERAPY FOR EMPIRIC PRIMARY TREATMENT OF *HELICOBACTER PYLORI* INFECTION: SYSTEMATIC REVIEW AND META-ANALYSIS OF EFFICACY, TOLERABILITY AND ROLE OF ANTIBIOTIC RESISTANCE

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Object: Systematic review and meta-analysis to compare the efficacy and tolerability of quadruple therapy (proton pump inhibitor (PPI), bismuth, tetracycline, and metronidazole) and triple therapy (PPI, clarithromycin, and amoxicillin) as first-line treatment for *Helicobacter pylori* (*H. pylori*) eradication. The effect of resistance to clarithromycin and metronidazole on the efficacy of treatments was also analyzed.
Methods: A search of MEDLINE, EMBASE, and Cochrane Library of randomized controlled trials comparing both regimens was carried out. Statistical analysis was conducted with RevMan 5.1 software. Funnel plots and subgroup analyses were carried out.
Results: Twelve papers were included in the analysis. Different duration of treatment regimens account for the high heterogeneity among the studies ($I^2 = 78\%$). In the subgroup analysis similar eradication rates were yielded when the duration of treatment was the same for both regimens. The 10-day quadruple therapy was superior to the 7-day triple therapy (Eradication rate of 82.5% and 57.7% respectively, (RD) = 0.25, 95% CI: 0.18–0.32, $p < .00001$). There were no differences in side effect rates yielded by quadruple vs. triple therapy (RD = 0.92, 95% CI: 0.76–1.12). Four out of 12 studies assessed also the effect of resistance to antibiotics on eradication rates. Clarithromycin resistance significantly affected the efficacy of triple therapy (RD = 0.75, 95% CI: 0.63–0.87) whereas metronidazole resistance did not affect the efficacy of quadruple therapy (RD = 0.09, 95% CI: -0.06 to 0.25).
Conclusions: The 10-day quadruple therapy is more effective than the 7-day triple therapy, overcomes clarithromycin resistance and its efficacy is not affected by metronidazole resistance in vitro.

Abstract no.: P4.02

RELIABLE EFFICACY OF 14-DAY HIGH DOSE PPI TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION INDEPENDENT EFFECT OF CYP2C19 GENOTYPE AND HIGH PREVALENCE OF METRONIDAZOLE RESISTANCE IN THAILAND

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Aim: Eradication rate of standard triple therapy has decreased worldwide. Newer regimen is required to achieve better outcome. This randomized control study was designed to determine eradication rate of 7-day and 14-day high dose PPI triple therapy for *H. pylori* eradication in association with CYP2C19 genotype and antibiotic resistance in Thailand.
Methods: Between December 2010-December 2011, patients underwent EGD at Thammasat Hospital for dyspepsia were recruited. Three biopsy samples from antrum were obtained for RUT, CYP2C19 genotype, GenoType® HelicoDR, culture and E-test. Patients were randomized to receive 7-day or 14-day high dose PPI triple therapy which consisted of lansoprazole 60 mg BID, amoxicillin 1 g BID, and clarithromycin 500 mg BID. UBT was performed 4 weeks after therapy to assess eradication.
Results: Total of 110 patients were enrolled in this study (55 received 7-day regimen and 55 received 14-day regimen). Antibiotic sensitivity tests demonstrated 40% of metronidazole resistant without clarithromycin resistant strains in both groups. Prevalence of CYP2C19 genotype was 65.4% (7-day arm) vs. 57.6% (14-day arm) with RM, 30.8% (7-day arm) vs. 33.3% (14-day arm) with IM and 3.8% (7-day arm) vs. 9% (14-day arm) with PM without any difference between 2 groups. Eradication rate of 14-day regimen was higher than 7-day regimen (100% vs. 92.7%; p-value = .05). Minor side effects were reported including bitter taste (10%) and nausea (4%).
Conclusions: Fourteen-day high dose PPI triple therapy provide high eradication rate regardless of CYP2C19 genotype and metronidazole resistance and can used as first line *H. pylori* eradication in Thailand.

Abstract no.: P4.03

FOURTH-LINE RESCUE THERAPY WITH RIFABUTIN IN PATIENTS WITH THREE *H. PYLORI* ERADICATION FAILURES

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Background: In some cases, *Helicobater pylori* infection persists even after three eradication treatments.
Aim: To evaluate the efficacy of an empirical forth-line rescue regimen with rifabutin in patients with three eradication failures, extending the experience of an ongoing multicenter study.
Methods: *Design*: Multicenter, prospective study. *Patients*: Patients in whom the following three eradication treatments had consecutively failed: 1st treatment: PPI + clarithromycin + amoxicillin; 2nd treatment: quadruple therapy (PPI + bismuth + tetracycline + metronidazole); 3rd treatment: PPI + amoxicillin + levofloxacin. *Intervention*: In patients failing these three regimens, a 4th regimen with rifabutin (150 mg b.i.d.), amoxicillin (1 g b.i.d.) and a PPI (standard dose b.i.d.) was prescribed for 10 days. *Outcome*: Eradication was confirmed using the ^{13}C-urea breath test 4–8 weeks after therapy. *Compliance and tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: One-hundred patients (mean age 50 years, 39% males, 31% peptic ulcer/69% functional dyspepsia) were included. *Compliance*: eight patients did not take correctly the medication (in six cases due to adverse effects). Per-protocol and intention-to-treat eradication rates were 52% (95% CI = 41–63%) and 50% (40–60%). Adverse effects were reported in 30 (30%) patients: nausea/vomiting (13 patients), asthenia/anorexia (8), abdominal pain (7), diarrhoea (5), fever (4), metallic taste (4), myalgia (4), hypertransaminasemia (2), leucopoenia (<1500 neutrophils) (2), thrombopoenia (<150 000 platelets) (2), headache (1), and aphthous stomatitis (1). 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.: P4.04

TEN-DAY SEQUENTIAL THERAPY IS A PROMISING THERAPEUTIC APPROACH FOR *HELICOBACTER PYLORI* INFECTION IN NAÏVE PATIENTS: A RANDOMIZED MULTICENTER TRIAL

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Introduction: The eradication rates of standard triple therapy for *Helicobacter pylori* infection are disappointingly declining worldwide. It has been reported in Europe that a 10-day sequential therapy had good eradication success. This trial aimed at comparing the efficacy and tolerability of sequential regimen as first-line treatment of *H. pylori* infection with standard triple regimen.
Methods: A total of 348 naïve *H. pylori*-infected patients from six hospitals in Korea were randomly assigned to one of two groups. The standard triple therapy group consisted of 20 mg of rabeprazole, 1 g of amoxicillin, and 500 mg of clarithromycin, twice daily for 7 days. The sequential therapy group consisted of a 5-day dual therapy (20mg of rabeprazole, and 1 g of amoxicillin, twice daily) followed by a 5-day triple therapy (20 mg of rabeprazole, 500 mg of clarithromycin, and 500 mg of metronidazole, twice daily).
Results: The intention-to-treat (ITT) and per-protocol (PP) eradication rates were 62.2% (95% CI 54.8–69.6%) and 76.0% (95% CI 68.5–83.5%) in the standard group, and 77.8% (95% CI 71.4–84.2%) and 87.9% (95% CI 82.3–93.5%) in the sequential group, respectively. The eradication rate was significantly higher in the sequential group compared with the standard group in both

the ITT and PP populations ($p = .002$ and $p = .013$, respectively), whereas the incidence of adverse events was similar between two groups.
Conclusion: Ten-day sequential therapy is more effective, equally tolerated for eradication of *H. pylori* infection compared to standard triple therapy. As a result, sequential therapy may have a role as a first-line treatment for *H. pylori* infection.

Abstract no.: P4.05

SECOND-LINE RESCUE THERAPY WITH LEVOFLOXACIN AFTER FAILURE OF TREATMENT TO ERADICATE *HELICOBACTER PYLORI* INFECTION: TIME TRENDS IN A SPANISH MULTICENTER STUDY OF 1000 PATIENTS

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Background: Second-line bismuth-containing quadruple therapy is complex and frequently induces adverse effects. A triple rescue regimen containing levofloxacin is a potential alternative; however, resistance to quinolones is rapidly increasing.
Aim: To evaluate the efficacy and tolerability of a second-line triple regimen containing levofloxacin in patients whose *Helicobacter pylori* eradication treatment failed and to assess whether the efficacy of the regimen decreases with time.
Methods: *Design*: Prospective multicenter study. *Patients*: Patients in whom treatment with a regimen comprising a PPI, clarithromycin, and amoxicillin had failed. *Intervention*: Levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.), and omeprazole (20 mg b.i.d.) for 10 days. *Outcome*: Eradication was confirmed using the ^{13}C-urea-breath test 4–8 weeks after therapy. *Compliance/tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: The study sample comprised 1000 consecutive patients (mean age: 49 ± 15 years, 42% men, 33% peptic ulcer) of whom 97% took all medications correctly. Per-protocol and intention-to-treat eradication rates were 75.1% (95% CI, 72–78%) and 73.8% (95% CI, 71–77%). Efficacy (intention-to-treat) was 76% in the year 2006, 68% in 2007, 70% in 2008, 76% in 2009, 74% in 2010, and 81% in 2011. In the multivariate analysis, none of the studied variables (including diagnosis and year of treatment) were associated with success of eradication. Adverse effects were reported in 20% of patients, most commonly nausea (7.9%), metallic taste (3.9%), myalgia (3.1%), and abdominal pain (2.9%).
Conclusion: Ten-day levofloxacin-containing therapy is an encouraging second-line strategy, providing a safe and simple alternative to quadruple therapy in patients whose previous standard triple therapy has failed. The efficacy of this regimen remains stable with time.

Abstract no.: P4.06

ANTIBACTERIAL ACTIVITY AND MECHANISM OF ACTION OF POLYETHYLENEIMINE FUNCTIONALIZED ZINC OXIDE NANO-PARTICLES ON METRONIDAZOLE-RESISTANT *HELICOBACTER PYLORI*

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Helicobacter pylori, a gram-negative bacterium inhabits the stomach of about 50% of the world population and its correlation with disease states like peptic ulcer and gastric cancer is well established. A major concern is the emergence of *H. pylori* variants that are resistant to antibiotics being used in current therapies. In this context we have attempted to develop nanoparticles that may function as an efficient inhibitor of *H. pylori*. Zinc oxide (ZnO) nano particles capped with polyethyleneimine (PEI) have been engineered which when compared to conventional ZnO has been demonstrated to be more water soluble, relatively acid stable, more potent in generating reactive oxygen species (ROS) and more specifically attracted towards gram negative bacteria by virtue of its ability to intercalate into the outer membrane lipopolysaccharide (LPS). Exposure to the ZnO-PEI nanoparticles resulted in significant loss of viability of *H. pylori* including a metronidazole-resistant strain. Imaging studies suggest that ZnO-PEI nano particles are internalized into *H. pylori* cells without any visible agglomeration. Subsequently, huge amount of intracellular ROS is generated and very significant membrane damage has been observed. Associated with this is the classical spiral to coccoid morphological transition and large scale degradation of 16S and 23S rRNA. Estimation of cellular ATP levels suggests that ZnO-PEI treated *H. pylori* are metabolically compromised. Toxicity studies have been conducted and the results obtained suggest that ZnO-PEI may be considered as a relatively safe, potential therapeutic agent which functions synergistically when used in combination with antibiotics for *H. pylori* treatment.

Abstract no.: P4.07

WHEN STANDARD TRIPLE THERAPY WITH PPI + AMOXICILLIN + CLARITHROMYCIN FAILS IN THE ERADICATION OF *HELICOBACTER PYLORI*, WHAT'S NEXT?

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Background: Standard triple therapy with PPI + amoxicillin + clarithromycin is the treatment of choice for *Helicobacter pylori* infection but it fails in approximately 20% of patients.
Aim: To conduct a meta-analysis of studies assessing the efficacy of rescue treatments after failure of standard triple therapy with PPI + amoxicillin + clarithromycin in *H. pylori* eradication.
Methods: *Selection of studies*: Studies reporting efficacy were used for generic inverse variance, and randomized clinical trials for meta-analyses. Inclusion criteria: studies treating *H. pylori*-positive patients after PPI + amoxicillin + clarithromycin failure. Studies were excluded if diagnostic/confirmation of eradication were made only by serology, PCR or polyclonal stool antigen, or if second-line treatment was selected depending on antibiotic sensitivity. *Search strategy*: Bibliographical searches were performed in PubMed up to May 2012. *Data synthesis*: Intention-to-treat eradication rate.
Results: Although 34 RCT met inclusion criteria, there was not enough information to perform a meta-analysis comparing treatments because of the huge number of different comparisons found (n = 26). The efficacy of second-line treatment with PPI + levofloxacin + amoxicillin was analyzed by inverse variance: 13 studies were included (1925 patients); the eradication effect was 74% (CI 95% = 0.68–0.80; $p < .001$; $I^2 = 89\%$), and this effect was higher when treatment was given 10 days instead of 7 (82% vs. 65%). For inverse-variance of PPI + bismuth + tetracycline + metronidazole (24 studies, 1937 patients) the eradication rate was 77% (95%CI = 0.72–0.81; $p < .001$; $I^2 = 87\%$). For PPI + amoxicillin + metronidazole (18 studies, 1357 patients), eradication rate was 90% (95% CI = 0.87–0.92; $p < .001$; $I^2 = 58\%$). Finally, ranitidine-bismuth-citrate + tetracycline + niroimidazole achieved a 76% eradication rate (six studies, 358 patients, 95% CI = 0.64–0.88; $p < .001$; $I^2 = 86\%$).
Conclusion: After failure of first-line eradication treatment with PPI + amoxicillin + clarithromycin, a combination of PPI + amoxicillin+metronidazole or PPI + levofloxacin + amoxicillin for 10 days are good candidates for second-line treatment.

Abstract no.: P4.08

A RANDOMIZED CONTROLLED STUDY FOR THE THIRD-LINE RESCUER THERAPY AFTER FAILURE OF TWO *H. PYLORI* ERADICATION IN JAPAN

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Background: In Japan, the first and second-line treatment for *H. pylori* are covered by the national health insurance system. However, the third-line treatment is not established for 2–3% of patients with persistent *H. pylori* after two eradication therapy. A randomized controlled study was conducted to establish the standard third-line treatment.
Method: A total of 204 patients were enrolled after failure of the first and second-line eradication using one week triple therapy with PPI + AMPC + CAM and PPI + AMPC + MNZ. They were randomly assigned to three treatment groups as follows; LA: lansoprazole 30 mg qid + AMPC 500 mg qid for 2 weeks, LAL: lansoprazole 30 mg bid + AMPC 750 mg bid + levofloxacin 300 mg bid for 1 week, and LAS: lansoprazole 30 mg bid + AMPC 750 mg bid + sitafloxacin 100 mg bid for 1 week. Eradication of *H. pylori* was assessed by 13C-urea breath test performed.

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Results: Eradication rates by intention-to treat and per-protocol analyses in LA were 54.3% (95% CI: 42.6–66.0) and 56.7% (44.9–68.5). Those in LAL were 43.1% (31.1–55.1) and 43.7% (31.6–55.8). Those in LAS were 70.6% (59.9–81.3) and 71.6% (60.9–82.3), which were significantly higher than those with LAL ($p < .001$) and LA ($p < .05$). Resistant rates of CAM, MNZ, and AMPC were 86.4%, 71.3%, and 1.8%, respectively.
Conclusion: The standard third-line regimen of *H. pylori* eradication was PPI based triple therapy with AMPC and STFX in Japan.

Abstract no.: P4.09

EFFICACY OF LEVOFLOXACIN-BASED TRIPLE THERAPY AS A SECOND-LINE THERAPY AFTER TRIPLE THERAPY FAILURE

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Aim: Eradication rate of legacy triple therapy in the Asia-Pacific has declined in recent years due to increasing rate of clarithromycin and metronidazole resistance. Newer regimens are required to improve eradication rate. This study was designed to determine the efficacy of levofloxacin-based triple therapy as a second-line eradication therapy after failed triple therapy.
Methods: Total of 58 patients with the mean age of 54.8 years who previously failed to respond to 7–10 days of standard triple therapy as indicated by positive ^{13}C-UBT or positive CLO-test were enrolled. Antral biopsy samples were obtained for culture and sensitivity using standard E-test for amoxicillin and levofloxacin resistance. Patients were received 10-day treatment of levofloxacin (500 mg) once a day plus lanzoprazole (30 mg) bid and amoxicillin 1000 mg bid. ^{13}C-UBT was performed 4 weeks after therapy to assess eradication.
Results: Eradication rate of levofloxacin given once daily plus lanzoprazole and amoxicillin resulted in 82% eradication rate. In vitro levofloxacin resistance was not associated with eradication failure using this regimen as second-line therapy. Age and sex did not predict eradication failure. About 10% of patients reported side effects but none of the patients dropped out.
Conclusions: Levofloxacin-based triple therapy using levofloxacin 500 mg once daily is effective in *H. pylori* eradication after failed triple therapy with eradication rate of 82%. The regimen is simple and tolerable. Accumulative eradication rate with triple therapy followed by levofloxacin-based regimen is about 94%. In-vitro resistance, age, sex were not associated with eradication failure with this second-line therapy

Abstract no.: P4.10

THIRD-LINE RESCUE THERAPY WITH LEVOFLOXACIN AFTER FAILURE OF TWO TREATMENTS TO ERADICATE *HELICOBACTER PYLORI* INFECTION

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Background: *Helicobacter pylori* eradication therapy with a proton pump inhibitor, clarithromycin, and amoxicillin fails in a considerable number of cases. A rescue therapy still fails in more than 20% of patients.
Aim: To evaluate the efficacy and tolerability of a third-line triple regimen containing levofloxacin in patients with two consecutive *H. pylori* eradication failures.
Methods: *Design*: Prospective multicenter study. *Patients*: In whom a first treatment with omeprazole-clarithromycin-amoxicillin and a second with omeprazole-bismuth-tetracycline-metronidazole had failed. *Intervention*: A third eradication regimen with levofloxacin (500 mg b.i.d.), amoxicillin (1 g b.i.d.) and omeprazole (20 mg b.i.d.) was prescribed for 10 days. *Outcome*: Eradication was confirmed using the ^{13}C-urea breath test 4–8 weeks after therapy. *Compliance and tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: Two-hundred patients (mean age 51 ± 13 years, 45% males, 101 with peptic ulcer disease and 99 with uninvestigated dyspepsia) were initially included, and 12 were lost to follow-up. All but seven patients complied with the protocol. Per-protocol and intention-to-treat eradication rates were 73% (95% CI, 66–80%) and 68% (61–75%). Adverse effects were reported in 19% of the patients, the most common being myalgia (8%), nausea (6%), metallic taste (5%), abdominal pain (3%), and diarrhea (3%).
Conclusion: A 10-day levofloxacin-containing triple regimen is an encouraging third-line strategy and a safe and simple alternative after multiple previous *H. pylori* eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole and tetracycline.

Abstract no.: P4.11

THIRD-LINE RESCUE THERAPY WITH BISMUTH-CONTAINING QUADRUPLE REGIMEN AFTER FAILURE OF TWO TREATMENTS (WITH CLARITHROMYCIN AND LEVOFLOXACIN) TO ERADICATE *HELICOBACTER PYLORI* INFECTION

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Background: *Helicobacter pylori* eradication therapy with a proton pump inhibitor, clarithromycin, and amoxicillin fails in a considerable number of cases. A rescue therapy with PPI-amoxicillin-levofloxacin still fails in more than 20% of patients.
Aim: To evaluate the efficacy and tolerability of a bismuth-containing quadruple regimen in patients with two consecutive *H. pylori* eradication failures.
Methods: *Design*: Prospective multicenter study. *Patients*: In whom a first treatment with PPI-clarithromycin-amoxicillin and a second with PPI-amoxicillin-levofloxacin had failed. *Intervention*: A third eradication regimen with a PPI (standard dose b.i.d.), bismuth subcitrate (120 mg q.i.d. or 240 mg b.i.d.), tetracycline (from 250 mg t.i.d. to 500 mg q.i.d.) and metronidazole (from 250 mg t.i.d. to 500 mg q.i.d.) was prescribed for 7–14 days. *Outcome*: Eradication was confirmed using the ^{13}C-urea breath test 4–8 weeks after therapy. *Compliance and tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.
Results: Two-hundred patients (mean age 50 years, 55% females, 20% with peptic ulcer disease and 80% with uninvestigated or functional dyspepsia) were initially included, and two were lost to follow-up. Ninety-seven per cent of patients complied with the protocol. Per-protocol and intention-to-treat eradication rates were 66% (95% CI, 59–73%) and 65% (58–72%). Adverse effects were reported in 22% of the patients, the most common being nausea (11%), metallic taste (8%), asthenia (7%), vomiting (6%), abdominal pain (10%), and diarrhea (7%); all of them were mild.
Conclusion: A bismuth-containing quadruple regimen is an acceptable third-line strategy and a safe alternative after two previous *H. pylori* eradication failures with standard clarithromycin-containing and levofloxacin-containing triple therapies.

Abstract no.: P4.12

SECOND-LINE RESCUE TRIPLE THERAPY WITH LEVOFLOXACIN AFTER FAILURE OF QUADRUPLE NON-BISMUTH ''SEQUENTIAL'' OR ''CONCOMITANT'' TREATMENT

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Background: Quadruple non-bismuth containing ''sequential'' and ''concomitant'' regimens, including a PPI, amoxicillin, clarithromycin and a nitroimidazole, are increasingly used as first-line treatments for *Helicobacter pylori* infection. Eradication with rescue regimens may be challenging after failure of 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 quadruple non-bismuth containing treatment failure.
Methods: *Design*: Prospective multicenter study. *Patients*: In whom a quadruple non-bismuth containing regimen, administered either sequentially (PPI + amoxicillin for 5 days followed by PPI + clarithromycin + metronidazole

for 5 more days) or concomitantly (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 ^{13}C-urea breath test 4–8 weeks after therapy. *Compliance and tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: Up to now, 86 consecutive patients have been included (mean age 50 years, 41% males, 22% smokers, 14% peptic ulcer and 86% dyspepsia):30 after ''sequential'', and 56 after ''concomitant'' treatment failure. All patients took all medications correctly. Overall, per-protocol and intention-to-treat *H. pylori* eradication rates were both 78% (95%CI = 69–87%). Respective cure rates for ''sequential'' and ''concomitant'' failure regimens were 83% (25/30) and 75% (42/56). Adverse effects were reported in six (7%) patients: metallic taste and heartburn, vomiting, diarrhea, aphthous stomatitis, vaginal candidiasis, and asthenia; all of them were mild.

Conclusion: Ten-day levofloxacin-containing triple therapy constitutes an encouraging second-line strategy in patients with previous quadruple non-bismuth ''sequential'' or ''concomitant'' treatment failure.

Abstract no.: P4.13

PYLERA® + OMEPRAZOLE QUADRUPLE THERAPY FOR *HELICOBACTER PYLORI* DEMONSTRATES HIGH EFFICACY WITH MINIMAL RISK OF BISMUTH-ASSOCIATED NEUROTOXIC SYMPTOMS

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Helicobacter pylori (*H. pylori*) is associated with dyspepsia and peptic ulcer disease (PUD), and is the primary risk factor for gastric carcinoma. International guidelines support ''test-and-treat'' strategies irrespective of PUD status. Bismuth-containing therapies for PUD are effective but have been criticized for potential neurotoxicity. This study evaluated PYLERA® + omeprazole in *H. pylori* eradication efficacy via urea breath test (UBT) and potential bismuth toxicity associated with chronic plasma alert levels >50 μg/L (Hillemand *et al*. 1977). 32 UBT- positive subjects received PYLERA® + omeprazole for 10 days under controlled conditions, i.e. standard meals and pharmacokinetic sampling (provided by 28 subjects). 31 subjects (97%) demonstrated *H. pylori* eradication on confirmatory UBT 28 days post-treatment. Average Day 10 steady state plasma and blood bismuth concentrations were <50 μg/L in all subjects. Transient (<1 hour) bismuth concentrations >50 μg/L were sporadically seen in 12 and 8/28 subjects, for plasma and blood, respectively. PYLERA® was well tolerated. 17/32 subjects (53%) reported 38 mild treatment-emergent adverse events (TEAEs) which resolved by treatment end. The most common TEAEs were headache (22%), dizziness (9%) and gastrointestinal disorders. 4/12 subjects with transient plasma bismuth C_{max} >50 μg/L (33%) experienced seven episodes of headache, compared to 3/16 patients (19%) with plasma bismuth C_{max} values <50 μg/L. All headaches were mild, occurred or started during treatment, and in most patients resolved the same day.

10 day PYLERA® + omeprazole quadruple therapy is effective in eradicating *H. pylori* and was well tolerated. No adverse events were suggestive of a potential central neurotoxicity with bismuth.

Abstract no.: P4.14

PROPOLIS IMPROVING ERADICATION OF *HELICOBACTER PYLORI*

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Propolis possess antibacterial effect, therefore it might improve the efficacy of eradication of *Helicobacter pylori* (HP). The aim of the study was to determine the ability of propolis to improve HP eradication.

Methods: Seventy four patients with HP-associated chronic gastritis were randomized into three groups: 28 patients in the first group were treated with omeprazole 20 mg twice a day, amoxicillin 2000 mg and clarithromicyn 1000 mg for 14 days; 24 patients in the second group took the same triple therapy plus 30% aqueous extract of propolis in 100 mL twice a day during 10 days; 22 patients in the third group (who were not agree take antibiotics for any reason) was prescribed omeprazole 20 mg twice a day and 30% aqueous extract of propolis in 100 mL twice a day for 14 days. Upper gastrointestinal endoscopy was performed for all the patients twice. Morphology and urea test were used to detect presence of HP. Grade and stage of gastritis were evaluated by OLGA system.

Results: Eradication rate was 78.6% in the first, 87.5% in the second and 40.9% in the third group. Side effects of therapy founded in 53.6% patients in first and in 20.8% in second group (p = .02). There were no intergroup difference for grade and stage of gastritis before the treatment. One month after the therapy grade of gastritis reduced in all the group of patients.

Conclusion: Our data suggests that propolis increases the rate of HP eradication and may reduce frequency of side effects of therapy.

Abstract no.: P4.15

HELICOBACTER PYLORI THERAPY AND BE ACCEPTED OR REJECTED FOR LOCAL USE BASED ON RATIONAL GROUNDS INSTEAD OF TRIAL AND ERROR

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Background: Optimization means identification of the preferred components of *H. pylori* eradication therapy (i.e. drugs, doses, formulations, number and timing of administrations per day, relation to meals, and duration of therapy that reliably maintains cure rates of ≥90 or 95%. Once optimized, the success in different regions can be reliably predicted provided one knows success rate with susceptible and resistant strains with each of the antibiotic combinations contained within the regimen.

Methods: Using the data from large trials with data for susceptible and resistant organism for each group: all susceptible, and with each resistance we developed formulae allowing prediction of the outcome in any population for 7 and 14 days triple therapy, for 10 days sequential therapy.

Results: General formula: Outcome = % with no resistance × success with no resistance + % with resistance with each component × outcome with each single resistance + % with two resistances × % with two resistances, etc.

Example: At 10% clar resistance, the outcome of sequential would vary from approximately 89% in the US (20% met resistance) to 74% in Latin America (60% resistance). In regions with low met resistance: sequential will fall below 90% at approximately 15% clari resistance, 14 days triple at approximately 11% and 7 days triple at 3–5%.

Conclusion: Whether Hp therapy is effective in any area can be reliably predicted.

	No res (%)	Clari res (%)	Met res (%)	Dual res (%)
10 days sequential	95	70	75	10
14 days triple	97	50	n/a	0
7 days triple	92	20	n/a	0

Abstract no.: P4.16

HELICOBACTER PYLORI FIRST-LINE TREATMENT WITH CLARITHROMYCIN AND METRONIDAZOLE IN PATIENTS ALLERGIC TO PENICILLIN: IS IT AN ACCEPTABLE OPTION?

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Background: In patients allergic to penicillin, which constitutes a relatively common subgroup, a triple therapy including a proton pump inhibitor (PPI), clarithromycin, and a nitroimidazole represents one of the most frequently recommended regimens for the treatment of *Helicobacter pylori* infection. However, this regimen may be relatively ineffective in areas of high clarithromycin resistance, where a bismuth-containing quadruple therapy may be preferred.

Aim: To assess the efficacy and safety of *H. pylori* first-line treatment with a PPI, clarithromycin and metronidazole in patients allergic to penicillin, extending the experience of an ongoing multicenter study.

Methods: *Design*: Prospective multicenter study including consecutive patients allergic to penicillin. *Intervention*: PPI (standard dose), clarithromycin (500 mg b.i.d.), and metronidazole (500 mg b.i.d.) for 7 days. *Outcome*: Eradication was confirmed by ^{13}C-urea-breath test 4–8 weeks after therapy. *Compliance and tolerance*: Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by means of a questionnaire.

Results: One hundred and fourteen patients allergic to penicillin were included (mean age 52 years, 40% males, 44% peptic ulcer/56% functional dyspepsia).

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Compliance: seven patients (6%) did not take the medication correctly (due to adverse effects in two cases). Per-protocol and intention-to-treat eradication rates were 59% (95%CI=49–68%) and 56% (47–66%). Adverse effects were reported in 15 patients (13%): metallic taste (seven patients), nausea (8), vomiting (1), and abdominal pain (3).
Conclusion: Allergic to penicillin *H. pylori* infected patients may be treated with a first-line treatment combining a PPI, clarithromycin and metronidazole, but its efficacy is clearly disappointing. Bismuth-containing quadruple therapy (that is, PPI, bismuth, tetracycline and metronidazole) may be a better option in areas of high clarithromycin resistance, but this will need to be confirmed in future studies.

Abstract no.: P4.17

ERADICATION RATE OF *HELICOBACTER PYLORI* INFECTION ACCORDING TO THE STAGES OF PEPTIC ULCER DISEASE

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Aims: The eradication rate of *Helicobacter pylori* infection might be affected by the inflammatory status of gastric mucosa and virulence factors such as CagA and VacA. The inflammatory status of gastric mucosa is different according to the stages of peptic ulcer disease. The aim of this study was to evaluate the eradication rate of *H. pylori* infection according to the stages of peptic ulcer disease.
Methods: We retrospectively investigated 1013 patients who received *H. pylori* eradication therapy from January 2004 to December 2010. The number of patients with gastric ulcer, duodenal ulcer and combined ulcer were 320, 622 and 71, respectively. The patients were sorted into three groups such as active stage (n = 264), healing stage (n = 262) and scar stage (n = 487) and then the eradiation rates were compared.
Results: No significant difference in eradication rate was observed between gastric and duodenal ulcer. The eradication rates in active stage, healing stage and scar stage were 88.6%, 83.2% and 82.3%, respectively. There was a significant difference in eradication rate between active and scar stage ulcer (p = .023). No significant difference was observed among the stages of gastric and duodenal ulcer.
Conclusions: There was a significant difference in eradication rate between active stage and scar stage ulcer. Different eradication regimen or treatment duration might be necessary.

Abstract no.: P4.18

COMPARISON OF 7-DAY AND 14-DAY TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION AND CHANGE OF ERADICATION RATE

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Background and Aims: Although triple combination therapy containing a proton pump inhibitor (PPI) and two antibiotics is considered as a standard regimen for the first-line anti-*Helicobacter pylori* treatment, there are still debates on the ideal duration of treatment and the changes over times in the eradication rate. The aim of this study was to compare the efficacies of 7-day and 14-day triple therapy and the changes of eradication rate over time.
Materials and Methods: We reviewed 1547 patients who underwent the therapy with a proton pump inhibitor (PPI) combined with two antibiotics (omeprazole 20 mg or equivalent dose of other PPIs, amoxicillin 1000 mg, and clarithromycin 500 mg twice daily) for 7-day or 14-day to eradicate *Helicobacter pylori*.
Results: A total of 1547 patients were enrolled this study. The overall eradication rate was 90.9% (1406/1547). The two groups were comparable in terms of baseline characteristics. The eradication rates of the 7-day triple anti-*H. pylori* therapy group were not inferior to those of the 14-day group (91.2% vs 89.1%). But, the eradication rate of the 7-day triple anti-*H. pylori* therapy group were inferior to those of the 14-day group in 2011 (65.12% vs 86.8%). The eradication rate of 90.9% in 2001 was decreased to 75.3% in 2011.
Conclusions: The eradication rate first-line triple therapy for *H. pylori* in Korea has decreased, 14-day triple therapy are expected to superior to 7-day triple therapy. At this point, a large randomized prospective study to establish the ideal duration of treatment is needed.

Abstract no.: P4.19

META-ANALYSIS: 10 DAY-SEQUENTIAL THERAPY VERSUS CONCOMITANT NONBISMUTH-BASED QUADRUPLE THERAPY FOR ERADICATION OF *HELICOBACTER PYLORI*

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Backgroud: The eradication rate of PPI based triple therapy has decreased due to increased antibiotic resistance in Korea. In recent studies of the first-line therapy, the eradication rate of sequential therapy is decreasing and as another option for the successful *Helicobacter pylori* (HP) eradication, concomitant nonbismuth-based quadruple therapy is tried. The aim of this study was to compare the efficacy of sequential therapy with concomitant nonbismuth-based quadruple therapy.
Methods: Between October 2009 and March 2012, 209 patients with HP infections allocated 2 groups. Group A: Sequential therapy group: lansoprazole 30 mg b.i.d, Amoxicillin (AMX) 500 mg t.i.d, for the first 5 days, followed lansoprazole 30 mg b.i.d, 500 mg clarithromycin (CLA) b.i.d, metronidazole (MTZ) 500 mg t.i.d for 5 days. Group B: Concomitant therapy group: lansoprazole 30 mg b.i.d, AMX 500 mg t.i.d., CLA 500 mg b.i.d, MTZ 500 mg t.i.d. for 1 week. After 4 weeks, the eradication of HP was assessed by urea breathing test and the side effects were assessed by questionnaire.
Results: The eradication rate of concomitant therapy was higher than sequential therapy in ITT analysis 76.9% (80/104) versus 72.4% (76/105) (p = .57) and PP analysis 80.0% (80/100) versus 73.1% (76/104) (p = .24). The side effects including taste alteration, nausea, epigastric discomfort were not different in both groups 37.5% (39/104) versus 40.0% s(42/105) (p < .1), and the withdrawal due to side effects was not significantly different between two groups (4/104 vs 1/105).
Conclusion: Concomitant nonbismuth-based quadruple therapy for 7 days was more effective than sequential therapy to eradicate HP as first line therapy but insignificant statistically with mild and moderate side effects in Korea.

Abstract no.: P4.20

THE 2 WEEKS SEQUENTIAL THERAPY AND THE CONCOMITANT THERAPY FOR *HELICOBACTER PYLORI* ERADICATION WERE EFFECTIVE AS A FIRST LINE THERAPY IN KOREA

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Backgrounds and Aims: The eradication rate of proton-pump inhibitor (PPI)-based triple therapy for *Helicobacter pylori* has decreased due to increasing antibiotics resistance. Recently, the sequential therapy and the concomitant therapy is tried to overcome antibiotics resistance and have produced good outcomes in many countries. The aim of this study was to assess the efficacy of those therapies in Korea.
Materials and Methods: A total 116 patients with proven *H. pylori* infection were enrolled in this study. Fifty eight patients (mean age 56.79 ± 12.28 male 48.3%) received the sequential therapy (20 mg of rabeprazole and 1 g of amoxicillin, twice daily for the first 7 days, followed by 20 mg of rabeprazole, 500 mg of clarithromycin, and 500 mg of metronidazole, twice daily for the remaining 7 days) and the other 58 patients (mean age 54.24 ± 12.62, male 41.4%) took the concomitant therapy (20 mg of rabeprazole, 1 g of amoxicillin, 500 mg of clarithrymcyin, and 500 mg of metronidazole, twice a day for 14 days). Successfulness of eradication was evaluated by the ^{13}C-urea breath test at least 4 weeks later after end of treatment.
Result: The eradication rate of the sequential and the concomitant therapies was 77.6% (45/58) and 82.8% (48/58) by intention-to-treat analysis, 81.1% (43/53) and 82.1% (46/56) by per-protocol analysis, but there was no difference statistically (p = .642 and p = 1.000). The adverse rate was 47.3% (26/55) in sequential therapy group and 51.8% (29/56) in concomitant therapy group, also there was no difference (p = .706), and almost all patients were well tolerable to each therapies.
Conclusion: Those therapies were effective to eradicate *Helicobacter pylori* in Korea as a first line therapy but not enough. They had relatively higher adverse rate, but those all were minor problems, both therapies were well tolerated.

Abstract no.: P4.21

EFFECTIVENESS AND SAFETY OF MODIFIED BISMUTH-BASED QUADRUPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION

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Background: Antibiotic resistance is a major cause of the failure of *Helicobacter pylori* (H.p.) eradication treatment. Quadruple therapy is a standard second-line therapy for *Helicobacter pylori* infection. Bismuth-based quadruple therapy consisting of PPI, bismuth, tetracycline and metronidazole is reported to have an effective from 64.6% to 76% (per-protocol) in patients who failed first-line therapy. The aim of this study was to evaluate the effectivness and safety of modified bismuth-based quadruple therapy.
Patients and Methods: Fourty-four (17.3%) of 254 patients treated with modified bismuth-based quadruple therapy, in whom the first-line therapy regimens had failed to eradicate H.p. infection between January 2010 and December 2011. They (44 patients) received colloidal bismuth subcitrate (120 mg-four times a day), pantoprazole (40 mg-twice a day)), metronidazole (500 mg-three a day), moxifloxacin (400 mg-once a day) for 2 weeks. *H. pylori* status was rechecked with ^{13}C- UBT 6 weeks after the end of therapy.
Results: The eradication rates were 75% and 82.5% (33/44; 33/40) according to the intention-to-treat (ITT) and per-protocol (PP) analyses. Three patients were lost to follow-up and one complied poorly with medication. The compliance was 87%.
Conclusion: The modified bismuth-based quadruple therapy may be safe and effective for patients who fail to respond to first-line therapy regimens. This therapy scheme was well tolerated.

Abstract no.: P4.22

HIGH DOSE (240 MG) DEXLANSOPRAZOLE PLUS AMOXICILLIN FOR *HELICOBACTER PYLORI* INFECTION

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Background: *Helicobacter pylori* infections have become increasingly difficult to treat.
Aim: To examine whether amoxicillin and high dose dexlansoprazole would reliably achieve *H. pylori* infections eradication ≥90%.
Methods: An open-label prospective pilot study of *H. pylori* eradication in treatment-naïve subjects with active *H. pylori* infection (positive by two tests). Each received amoxicillin 1 g and dexlansoprazole 120 mg each twice a day at approximately 12 hour intervals for 14 days. Success was accessed by urea breath test 4–6 weeks after the end of therapy. An effective therapy was defined as a per-protocol treatment success of 90% or greater; treatment success of 80% or less was prespecified as an unacceptable result.
Results: After 13 subjects were entered (12 men, one woman; average age 54 years) the prespecified stopping rule of six treatment failures was achieved (i.e. the 95% confidence interval excluded achieving the required 90% success rate even if 50 patients were entered) and enrollment was stopped. Per-protocol and intention-to-treat treatment success were both 53.8%; (7/13); 95%CI = 25–80%. Compliance was 100%. Three patients (23%) reported side-effects, all of which were mild and none interrupted therapy.
Conclusion: Theoretically, and from experience in Japan, dual PPI plus amoxicillin should successfully eradicate *H. pylori* provided the intragastric pH can be maintained as near neutral. Clearly, dexlansoprazole, despite being administered at high dose and twice a day (total daily dose 240 mg), failed to achieve an intragastric milieu consistent with dual PPI plus amoxicillin therapy being an effective anti-*H. pylori* regimen.

Abstract no.: P4.23

EFFICACY OF FREQUENT PPI DOSING FOR *HELICOBACTER PYLORI* ERADICATION IN RELATION TO CYP2C19 GENOTYPE STATUS

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Objectives: In the eradication therapy for *H. pylori* infection, 24-hour intragastric pH and percent time of pH 4.0 are required to attain >6.0 and <10%, respectively, during the eradication, regardless of CYP2C19 genotype. Majority of patients infected with *H. pylori* can eradicate, irrespective of the bacterial susceptibility to clarithromycin. However, the optimum treatment is unclear at present, as most previous reports have loss of sample power. Here, we assessed acid inhibitory effects of PPIs by different dosing methods.
Methods: Using 183 trials of pH monitoring, we compared acid inhibition of PPIs (omeprazole at 20 mg; lansoprazole at 30 mg; rabeprazole at 10 mg) in related with CYP2C19 genotypes. We then evaluated efficacy of divided treatment with rabeprazole (40 mg od; 20 mg bid; and 10 mg qid).
Results: Median 24-hour pHs with omeprazole, lansoprazole, and rabeprazole were 4.4 (2.1–7.3), 4.8 (3.5–6.4), 5.4 (3.3–7.5), respectively. Median 24-hour pH differed among different CYP2C19 genotype status in not only omeprazole and lansoprazole but also rabeprazole and the variation of pH attained with omeprazole was widest. In the efficacy of divided treatment, median pHs for rabeprazole 40 mg od, 20 mg bid, and 10 mg qid were 4.8 (3.6–6.4), 5.7 (4.1–7.4), 6.6 (4.9–8.4), respectively. Although dosing dose a day was same, increases in dosing times effectively increased pH (qid: percent time of pH <4: 3.4% [3.5–9.8%]), even in CYP2C19 rapid metabolizers.
Discussion: Four-times daily dosing achieved potent acid inhibition for a 24-hour, regardless of CYP2C19 genotypes, suggesting that this treatment has potential usefulness for patients infected with *H. pylori*.

Abstract no.: P4.24

THE ROLE OF *HELICOBACTER PYLORI* ERADICATION IN THE TREATMENT OF CHRONIC URTICARIA IN COMPARISON WITH ITS CLASSIC TREATMENTS

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Objectives: Given the high spread of *Helicobacter pylori* in the world (specially Iran) and this fact that the majority of Urticarial patients also carry this bacteria, we studied on two treatment groups to determine the eradicating effect of *H. pylori* on it.
Materials and Methods: On referral patients to the Quaem Hospital in Mashhad (Iran), 120 *H. pylori* infected patients, with positive Urease Breath Test (UBT), were selected. For 60, classic treatment of Urticaria (H1 and H2 Blockers) and for the rest, Triple drug therapy (20 mg Robeprazole/Pariet® twice daily, 500 mg Clarithromycin/Klacid® twice daily, and 1 g Amoxicillin/Amoxil® twice daily during 14 days) for *H. pylori* eradication were prescribed. Eradication was confirmed by UBT (Heliprobe®/Kibion). In the course of treatment (within one, 3 and 6 months after therapy) the signs and symptoms resolution were separately analyzed in two groups.
Results: The majority of patients who had been exposed to Triple drug treatment, were significantly better cured than the group exposed to classic treatment (p-value < .001). In addition, other skin disorders (such as itching), manifested a better recovery, too (p-value < .001). As to the age factor, no significant difference was found (p-value = .863). However, the average age with regard to gender, was significantly higher in men (p-value = .006)
Conclusion: In the majority of the patients suffering from Chronic Urticaria and *H. pylori* infection, Triple drug eradicative treatment was much more effective than the classic H1 and H2 Blockers for resolution of the Urticaria signs and symptoms.

Abstract no.: P4.25

A MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY OF TRIPLE THERAPY USING RABEPRAZOLE, AMOXICILLIN, AND METRONIDAZOLE FOR *HELICOBACTER PYLORI* IN JAPAN

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Background and Aims: Triple therapy with rabeprazole, amoxicillin and metronidazole was approved by the Japanese Ministry of Health, Labour and Welfare in August 2007 as a second-line therapy for *H. pylori*-positive gastric and duodenal ulcers in Japan. The present multicenter prospective observational study aimed to investigate the safety and efficacy of this therapy in light of its widespread use in clinical practice since approval.
Methods: Patients with *H. pylori*-positive gastric or duodenal ulcer in whom first-line therapy was unsuccessful were administered rabeprazole 10 mg, amoxicillin 750 mg and metronidazole 250 mg twice daily for 7 days, based on the approved dose and regimen. Patient background factors, including complications, previous medical history and concomitant drugs, eradication results and adverse events were recorded by the investigator.
Results: Of the 143 registered participants, safety and efficacy analyses were conducted on 136 and 125 subjects, respectively. The incidence of adverse drug

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reactions was 2.21% and the *H. pylori* eradication rate was 92.80%. Subgroup analyses to investigate patient background factors affecting safety and efficacy revealed no factors that significantly affected the incidence of adverse drug reactions or *H. pylori* eradication rate.
Conclusions: Amid reports of decreased eradication rates with clarithromycin-based first-line therapy, the >90% *H. pylori* eradication rate achieved in the present study demonstrates the clinical efficacy of rabeprazole-based triple therapy in subjects in whom first-line therapy is unsuccessful.

Abstract no.: P4.26

EFFICACY OF RIFABUTIN BASED TRIPLE THERAPY INCLUDING HIGH DOSE PPI AND AMOXICILLIN FOR THIRD LINE RESCUE THERAPY OF *HELICOBACTER PYLORI*

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Background: Rifabutin has been founded to be effective in rescue therapy for *Helicobacter pylori* (HP) infection. There were various trials of changing doses and duration of rifabutin (RFT) based rescue therapy. The efficacy was known about 60–80%. We investigated the efficacy of rifabutin based rescue therapy after first and second line treatment failures of HP infection.
Materials and Method: Between December 2007 and March 2012, 51 patients were allocated to two groups after first and second line treatment failures of HP infection. Group A: PPI double dose therapy: 32 patients were received lansoprazole 30 mg bid, Amoxicillin (AMX) 1.0 g tid, RFT 150 mg bid for 7 days. Group B: PPI quadruple dose therapy: 19 patients were received lansoprazole 60 mg bid, (AMX) 1.0 g tid, rifabutin (RFT) 150 mg bid for 7 days. Urea breath test was performed at 4 weeks later after the end of treatment. We evaluated the side effects.
Results: The eradication rates of PPI quadruple dose therapy were higher than PPI double dose therapy in ITT analysis were 94.7% (18/19) versus 78.1% (25/32) and PP analysis 94.7% (18/19) versus 80.6% (25/31). The side effects were epigastric pain in group A and B (9.3% vs 5.3%) and epigastric discomfort (6.2% vs 5.3%) and there were no significant differences between two groups. The treatment was not completed in one case of group A due to unacceptable side effects such as abdominal pain and vomiting.
Conclusion: Rifabutin based triple therapy including PPI quadruple dose and high dose amoxicillin were effective and safe for third line rescue therapy of HP.

Abstract no.: P4.27

EFFICACY OF ERADICATION IN PATIENTS INFECTED WITH *CAGA*(+) AND *CAGA*(-) STRAINS OF *HELICOBACTER PYLORI*

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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. According to some studies *cagA* (+) status of *H. pylori* is associated with lower eradication rate. The aim: to investigate efficacy of eradication in patients infected with *cagA* (+) and *cagA* (-) strains of *H. pylori*.
Methods: Sixty persons infected with *H. pylori* were under supervision. For all the surveyed patients gastroduodenoscopy with a biopsy from a stomach antrum was performed for verification of *H. pylori* infection by means of rapid urease test and polymerase chain reaction (PCR) with detection of genes of *H. pylori* pathogenicity island: *ureC* and *cagA*. All the surveyed patients have been divided into two groups: Patients
from the 1st group (15 patients) were infected with *cagA*(-) strains. Patients from the 2nd group (45 patients) were infected with *cagA*(+). Patients from both groups received standard eradication therapy: omeprasol 20 mg twice a day during 21 days, amoxicillin 1000 mg twice a day during 7 days, claritromicin 500 mg twice a day during 7 days. Statistical estimation was performed in programs Excel and Statistica 6.0 for Windows XP.
Results: Eradication rate was the same in 1st and 2nd group – 60%.
Conclusion: According to our study cagA(+) or cagA(-) status of *H. pylori* does not influence on eradication rate. Efficacy of eradication was low in both groups. It can be a result of short usage of antibiotics.

Abstract no.: P4.28

COMPARISON OF THE EFFICACY BETWEEN LANSOPRAZOLE FOR 4 AND 8 WEEKS IN ESD-INDUCED GASTRIC ULCERS

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Background/Aims: We compared the effectiveness between 4 and 8 weeks treatment of lansoprazole in iatrogenic gastric ulcer after endoscopic submucosal dissection (ESD).
Methods: Eighty-four patients diagnosed as gastric adenoma or early gastric cancer were enrolled. After ESD, the patients were randomly assigned to treatment with lansoprazole for 28 days (4-week group) or for 56 days (8-week group). At 8 weeks after ESD, we performed endoscopy to evaluate the degree of ulcer healing. Ulcer stage and size reduction (reduction ratio) were compared between the two groups. Ulcer reduction ratio was determined by dividing the ulcer dimension at 8 weeks after ESD by the initial ulcer dimension. Moreover, we evaluated the factors affecting complete healing.
Results: From 84 patients, 69 patients were finally analyzed (34 patients in the 4-week group and 35 patients in the 8-week group). At 8 weeks, the ulcer were mostly in scar stage or small-sized healing stage and no significant differences were observed between the two groups in terms of ulcer stage (scar stage 68% in 4-week group vs 69% in 8-week group, $p = .93$) and ulcer reduction ratio (0.81 and plusmn; 1.57% in 4-week group vs 0.37 and plusmn; 0.78% in 8-week group, $p = .15$). Complete ulcer healing at 8 weeks was correlated with only the initial ulcer size (35.2 and plusmn; 8.6 mm in scar stage vs 41.9 and plusmn; 10.1 mm in non-scar stage, $p = .006$).
Conclusion: For ESD-induced gastric ulcer, treatment with lansoprazole for 4 weeks was as effective as treatment for 8 weeks.

Abstract no.: P4.29

RESISTANCE TO RIFAMPICIN/RIFABUTIN IS STILL LOW IN *HELICOBACTER PYLORI* CLINICAL ISOLATES IN GERMANY

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Objectives: Rifabutin-based triple-therapies are frequently used in refractory *H. pylori* infections or in patient allergic to penicillin. The aim of this study was to update the rate of rifampicin/rifabutin resistance in *Helicobacter pylori* clinical isolates in Germany and to identify rifampicin/rifabutin resistance-associated mutations in the rpoB gene.
Methods: Susceptibility to rifampicin in a total of 3861 clinical *H. pylori* strains isolated between 2008 and 2011 was tested by the E-test method. Information on prior eradication therapies were gathered and resistant isolates were molecular genetically analysed by Sanger sequencing of those regions in the rpoB genes that have been shown to frequently carry resistance-associated mutations.
Results: From 3861 clinical isolates examined, 85 (2.2%) showed phenotypic resistance to rifampicin (MIC > 4 mg/L). The vast majority of patients harbouring rifampicin-resistant isolates had been unsuccessfully treated at least on one occasion and showed additional resistances to clarithromycin, metronidazole or quinolones; high resistant rifampicin-resistant isolates (MIC ≥ 32 mg/L) showed cross-resistance to rifabutin. Out of 50 isolates tested, 24 revealed point mutations in the resistance-associated regions, primarily affecting codons 530 and 540. Most strains, however, did not show any mutations in the genetic regions examined.
Conclusions: Resistance to rifampicin/rifabutin in *H. pylori* clinical isolates in Germany is still rare; hence rifabutin is still a reasonable alternative and might be used without prior antimicrobial susceptibility testing. Mutations other than those affecting the hot spots in-between amino acid 525 and 545 of RpoB might confer resistance to rifampicin/rifabutin and need to be considered when genotypically testing for resistance.

Abstract no.: P4.30

HELICOBACTER PYLORI ERADICATION RATE FOR ''EXOTIC'' QUADRUPLE THERAPIES IN WESTERN AUSTRALIA

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Background: This study describes the antibiotic resistance profile of *H. pylori* and eradication rates for salvage therapies at the *H. pylori* Research Laboratory in Perth, Western Australia.

Aim: Reports the antibiotic resistance of *H. pylori*, reviews the necessity of pre-antibiotic sensitivity testing, and ascertains the effectiveness of alternative antibiotics.
Methods: A total of 347 consecutive patients who had failed at least two courses of standard triple therapy initially prescribed by their physicians and were then referred to this laboratory between 2007 and 2011 were included in this study. Antibiotics were prescribed based on the pre-treatment antibiotic sensitivity tests.
Results: In 96.8% of the patients' samples, *H. pylori* was successfully cultured. The proportion resistant to clarithromycin and metronidazole were 92% and 66% respectively, with 63% resistant to both. For the ''second line'' quadruple therapy, with proton pump inhibitor, amoxicillin, rifabutin and ciprofloxacin ''PARC'', *H. pylori* was successfully eradicated in 95.8%. For the ''third line'' quadruple therapy using customised combinations of bismuth, rifabutin, ciprofloxacin, tetracycline and furazolidone; the eradication rate was 86%.
Conclusions: Patients who present with antibiotic resistant *H. pylori* can be confidently treated with PARC therapy or other ''exotic'' salvage therapies. The latter regimens should be guided by culture and sensitivity especially when treatment options are limited by penicillin allergy. Pre-treatment antibiotic sensitivity tests contributed to the high eradication rate in this study. The ''exotic'' salvage therapies described herein produced an eradication rate of 86%.

Abstract no.: P4.31

IMPROVED ALLELE-SPECIFIC PCR ASSAYS FOR DETECTION OF CLARITHROMYCIN AND FLUOROQUINOLONE RESISTANT OF *HELICOBACTER PYLORI* IN GASTRIC BIOPSIES: IDENTIFICATION OF N87I MUTATION IN GYRA

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Objective: To use Allele-Specific PCR (ASP-PCR) to predict clarithromycin and fluoroquinolone resistance Hp from gastric biopsies from Colombia.
Methods: Antimicrobial susceptibility to clarithromycin and levofloxacin were tested by agar dilution. DNA was extracted from gastric tissue biopsies and regions of the 23S rRNA gene gyrA gene were amplified by PCR and DNA sequencing. ASP-PCR described previously to determine 23S rRNA and gyrA mutation of Hp were performance in 55 and 53 susceptible strains to clarithromycin and levofloxacin respectably and 42 and 53 resistant to clarithromycin and levofloxacin. New primers were designed for the N87I mutation in the GyrA protein. The sensitivity and specificity was evaluated by comparison to agar dilution (phenotypic test) and the sequence of 23S rRNA and gyrA genes from Hp isolates with each ASP-PCR.
Results: Using agar dilution as the ''gold standard'', the sensitivity and specificity of ASP-PCR of 23S rRNA gene using gastric biopsies was 100% and confirmed by sequence analyses. The published ASP-PCR missed 25/53 (47%) of levofloxacin resistant strains. The sensitivity of modified ASP-PCR for gyrA gene was 100% with a specificity of 92.7%.
Conclusions: The published ASP-PCR under recognized the frequency of levo resistance in Colombia. We developed a new primer for detection of N87I mutations in levofloxacin resistant *H. pylori* and standardized two previously described ASP-PCRs for detection of mutations that confer clarithromycin and levofloxacin resistance in *H. pylori* from gastric biopsy specimens.

Abstract no.: P4.32

SUCCESS OF TARGETED ANTIMICROBIAL THERAPY IN CASES OF *HELICOBACTER PYLORI* FAILING STANDARD TRIPLE THERAPY

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Background: *Helicobacter pylori* is the most common chronic bacterial infection in humans and associated with numerous gastrointestinal complications; worldwide antimicrobial resistance and treatment failures are increasing. Eradication rates with standard triple therapy are now unacceptably low.
Aim: To assess patterns of antimicrobial resistance and success of targeted treatment in *H. pylori* cases failing standard triple therapy.
Methods: One hundred and twenty-seven cases of *H. pylori* cultured between 2008 and 2010 were evaluated retrospectively.
Results: 94.5% had received a minimum of two courses of standard triple therapy prior to culture and sensitivity. 73.2% showed resistance to metronidazole, 72.3% to clarithromycin, 2.4% to amoxicillin, 56.7% to metronidazole and clarithromycin, 0.8% to amoxicillin and clarithromycin and 1.6% to all three; there were no cases of tetracycline resistance. At analysis treatment outcomes were known for 84 cases; 33.3% showing successful eradication. 53.6% of successful and 64.3% of unsuccessful cases had targeted treatment documented. Regarding failed targeted treatment 69.4% cases were sensitive to 2 antibiotics used; in 27.8% sensitivity was only shown to one antibiotic used; whilst in 22.2% sensitivity to one antibiotic was unknown. 5.6% showed resistance to one antibiotic and 2.3% resistance to both used.
Conclusions: In this cohort success of targeted treatment was low despite use of appropriate antimicrobials as per culture and sensitivity. Antibiotic resistances were in keeping with international observation, as such would 3rd line empirical therapy not be more cost and time effective than culture and sensitivity. No treatment regimen used showed clear benefit; more research is required into the reasons underlying treatment failure.

Abstract no.: P4.33

EVALUATION OF APPLICABILITY AND PREVALENCE OF CYP2C19 POLYMORPHISMS IN A SUBGROUP OF *HELICOBACTER PYLORI* POSITIVE (HP+) GREEK PATIENTS

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Background and Aim: PPI-related differences in HP eradication are partly due to CYP2C19 polymorphisms. Their prevalence, correlation with antibiotic resistant molecular tests and role in treatment has not been studied in HP+ Greek patients.
Patients and Methods: Fifty patients undergone upper GI endoscopy for various GI symptoms. Molecular genetic test is available to identify HP (GenoType Helico DR Test-HAIN). A multiplex PCR and DNA strip hybridization were performed for resistance to clarithromycin (significant mutation of 23S gene -positions 2146 and 2147) and fluoroquinolones (gyr A gene-codons 87 and 91). 25 HP+ patients genotyped for CYP2C19*2 and *3 alleles. The CYP2C19*2*3 allele was genotyped by Real-Time PCR method using the Light Mix Kit human CYP2C19*2 and CYP2C19 *3 (TIB MOLBIOL) in Light Cycler 480 (Roche Diagnostic).
Results: Heterozygous extensive metabolizers (HetEM, *2/*1) were 12/25 patients (48%). Only one patient (4%) was poor metabolizer (PM, *2/*2). There were no *3/*1 or *3/*2 type patients. Five patients were homozygous extensive metabolizers (HomEM, wild type, *1/*1) and one patient was poor metabolizer (PM, *2/*2) from the clarithromycin resistant HP+ patients group (6/25, 24%). The only one HP+ patient who was resistant to fluoroquilolones was HetEM (*2/*1). Eradication regimes with PPI + clarithromycin/metronidazole (in clarithromycin resistants) + amoxycilin was near 100%.
Conclusions: More epidemiological data in Greek population are needed to establish the real prevalence of the CYP2C19 polymorphisms which, combined with the antibiotic resistant molecular test could be useful for difficult to treat patients.

Abstract no.: P4.34

PRIMARY RESISTANCE OF *HELICOBACTER PYLORI* IN SLOVENIA

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Background: Empirical antimicrobial therapy – still the major strategy for *Helicobacter pylori* eradication – is based on local surveillance data of antimicrobial resistance rates. This report presents data of the Slovenian surveillance system for the year 2011.
Methods: *H. pylori* isolates from all patients tested for culture and sensitivity in the laboratory serving the majority of gastroenterology services in Slovenia from 2011 were included. Susceptibility was performed with E-tests, Iso Sensitest agar supplemented with 10% horse blood incubated in microaerophilic atmosphere for 48–72 hours. Culture negative biopsies were tested with GenoType Helico DR test for molecular detection of clarithromycin and fluoroquinolon resistance. 138 isolates from patients with no previous eradication therapy and 188 isolates from patients with one or more treatment failures were analysed for primary and secondary resistance rates, respectively.
Results: The primary resistance rates for amoxicillin, clarithromycin, metronidazole, levofloxacin and tetracycline was 0%, 16%, 29%, 10% and 0% respectively. The secondary resistance rates in the same order were 2%, 82%, 81%, 20% and 0%. There were 55% of primary and only 8% of secondary isolates

susceptible to all tested antimicrobial agents. Amoxicillin resistance was detected for the first time in Slovenia in 2011.
Conclusion: Slovenia is among countries with high prevalence of clarithromycin, metronidazole and levofloxacin primary resistance and worrisome resistance rates and profiles among secondary isolates. Systematic surveillance is of paramount importance in such a clinical setting. In the view of low success rates of primary treatment a question of antibiogram supported – tailored therapy is more relevant today.

Abstract no.: P4.35

PRIMARY RESISTANCE OF *HELICOBACTER PYLORI* TO MACROLIDES AND METRONIDAZOLE IN THE NORTHERN PART OF CROATIA

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Objectives: The aim of this study was to assess primary antibiotic resistance rates in *Helicobacter pylori* isolates over a period of 12 years (1995–2011).
Methods: A total of 962 *H. pylori* were isolated from gastric biopsies taken from patients visiting CHC Zagreb and CHC Merkur in Zagreb, Croatia. Isolates from 1995 to 2011 were included in the study. From each patient four gastric biopsies were taken for histology and two were sent to the Department for Clinical and Molecular Microbiology CHC Zagreb for culture and determination of antibiotic activity against *H. pylori* by means of agar dilution. Susceptibility to clarithromycin, azithromycin, metronidazol, tetracycline and amoxicillin was determined. Primary resistance was assessed in *H. pylori* strains isolated before the first eradication therapy.
Results: Resistance of *H. pylori* to amoxicillin and tetracycline was not detected. Clarithromycin and azithromycin showed a common resistance pattern in all tested isolates. Primary resistance to macrolides was detected in 98 (10.2%) strains and 274 (29.9%) strains were resistant to metronidazole out of 962 pretreatment isolates. From 1994 to 1999 resistance to macrolides was 7% (43/618); metronidazole 32% (183/572). From 2000 to 2006 for macrolides there were 14.6% resistant strains (44 /301)and metronidazole 23.6% (71/301 strain). From 2007 to 2011 there were 43 pretreatment isolates; 25.6% (11) resistant to macrolides and 46.5% (20) resistant to metronidazole.
Conclusions: The resistance rates to metronidazole and macrolides are high in *H. pylori* from northern Croatia. There is increase in resistance to macrolides and metronidazole among pretherapy isolates in observed period.

Abstract no.: P4.36

IN VITRO ANTIMICROBIAL ACTIVITY OF RIFABUTIN AGAINST METRONIDAZOLE-RESISTANT *HELICOBACTER PYLORI* ISOLATES

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Introduction: Antibiotic resistance of *H. pylori* has been a growing problem in the current therapeutic measurements. The incidence of resistance has been one of the most important factors involved in treatment failure. The aim of this study was to determine the susceptibility of metronidazole-resistant *H. pylori* strains to rifabutin.
Methods: Fifty-six *H. pylori* strains were isolated from dyspeptic patients. Twenty-eight isolates were metronidazole resistant and 28 were susceptible. Bacterial Suspensions with the turbidity of 2 MacFarland's unit were surface inoculated on the Brucella agar containing 5% blood. Serial dilutions of rifabutin in dimethyl sulfoxide (1, 0.5, 0.25, 0.125, 0.06, 0.03 μg/mL) were inoculated into the blank discs deposited on the surface of Brucella agar. The size of growth inhibition zones were recorded after 3 days microaerophilic incubation at 37°C. Strains with inhibition zone diameters >21 mm were classified as susceptible to rifabutin. The MIC of rifabutin was determined as 0.06 μg/mL.
Results: All the metronidazole-suseptible isolates were also susceptible to rifabutin. However, 3/28 (10.71%) of metronidazole-resistant isolates showed resistance to rifabutin (MIC 0.06 μg/mL). The overall resistance rate to rifabutin was 3/56 (5.35%).
Discussion: Metronidazole resistance of *H. pylori* is a major problem in bacterial eradication. Rifabutin has been considered as a candidate antibiotic for the third-line eradication regimen. In this study most (89.29%) of metronidazole-resistant strains were susceptible to rifabutin. Our results show the high efficacy of rifabutin against metronidazole-resistant isolates. Accordingly, rifabutin could be recommended for treatment of refractory *H. pylori* infections.

Abstract no.: P4.37

ASSESSMENT OF CLARITHROMYCIN RESISTANT *HELICOBACTER PYLORI* STRAINS IN TURKISH DYSPEPTIC PATIENTS

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Objective: Clarithromycin resistance in *H. pylori* is considered an important cause for treatment failure. Aim:
To evaluate the efficacy of fluorescence *in situ* hybridization (FISH) to detect *H. pylori* and determine clarithromycin resistance due to mutations in positions 2143 and 2144 of 23S rRNA gene compared with culture and antimicrobial susceptibility testing. We also identified *cag*A positivity and look for its relation with clarithromycin susceptibility. Methods:
Two hundred and thirty-four patients with dyspepsia (65 M, 169 F; 43.8 ± 14.0 years) were studied. Antrum and corpus biopsy specimens were obtained for RUT, histopathology and culture. E-test was used to assess clarithromycin susceptibility in the isolated *H. pylori* strains and *cag*A status of *H. pylori* strains was established by PCR. *H. pylori* presence and clarithromycin susceptibility were determined by FISH in paraffin embedded antrum and corpus biopsy specimens.
Results: One hundred and sixty-four (70.1%) patients were *H. pylori* positive. One-hundred-fourteen (69.5%) of 164 patients were culture positive, and 137 (83.5%) were positive by FISH. The sensitivity of FISH was significantly better but not the specificity for detection of *H. pylori* when compared with culture (83.5% and 91.4% vs 69.5% and 100.0%, respectively). Among *H. pylori*-positive patients, FISH detected clarithromycin resistance in 20.2% and E-test in 28.0% of samples considering both antrum and corpus. The concordance between E-test and FISH was 89.5%. Among *H. pylori*-culture positive patients; 53 (46.5%) were *cag*A positive and no correlation was found between *cagA* positivity and clarithromycin resistance.
Conclusion: FISH significantly increases the sensitivity to detect *H. pylori* in the clinical microbiology laboratory when compare with traditional culture techniques. FISH technique is a reliable and highly sensitive method, especially useful when a quick decision is necessary for treating dyspeptic patient.

Abstract no.: P4.38

ANTIMICROBIAL SUSCEPTIBILITY OF *HELICOBACTER PYLORI* STRAINS ISOLATED FROM GASTRIC BIOPSIES TO CLARITHROMYCIN AND LEVOFLOXACIN CURRENTLY USED IN OUR HOSPITAL

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Objective: Antimicrobial resistance is the major cause of treatment failure of *H. pylori* infection.
Aim: To determine the primary clarithromycin and levofloxacin resistance in Turkish patients with dyspepsia.
Methods: Eighty *H. pylori* positive patients with dyspepsia according to at least two positivity of RUT, histopathology and culture were included in this study. All tests were performed in antrum and corpus biopsy specimens. Sixty patients were culture positive for *H. pylori*. Antimicrobial susceptibility of 105 isolated *H. pylori* strains of 60 culture positive patients from antrum and/or corpus biopsy specimens to clarithromycin and levofloxacin was tested on MuellerHintonAgar supplemented with 5% sheep blood and incubated in microaerophilic conditions for 3 days. The MIC of clarithromycin and levofloxacin were determined with the E-test method, MIC values used for clarithromycin and levofloxacin ≥1 μg/mL and >1 μg/mL, respectively. Agar dilution and disc diffusion tests were also used to evaluate and to compare susceptibility patterns as an alternative to E-test.
Results: Resistance to Levofloxacin was detected in 17 patients (26.7%) and to clarithromycin in 15 patients (25.0%). Seven (11.7%) patients were resistant to both clarithromycin and levofloxacin. Interestingly, we observed that the overall resistance rates were decreased in only antrum (21.8% for clarithromycin, 20% for levofloxacin)or only corpus (14% for clarithromycin, 20% for levofloxacin).

Conclusion: Our isolated strains showed a clarithromycin resistance percentage higher than that reported by Maastricht III Consensus Report as threshold (15–20%) for not to use it first line treatment. Levofloxacin resistance rate was also high. So levofloxacin and clarithromycin should be reconsidered for treatment of *H. pylori*. The problem of antibiotic treatment failure in case of resistant *H. pylori* strains can be overcome by routine culture and antimicrobial susceptibility testing in our University Hospital.

Abstract no.: P4.39

CLARITHROMYCIN RESISTANCE IN *HELICOBACTER PYLORI* ISOLATES DEPENDING ON GENETIC CHARACTERISTICS AND PREVIOUS TREATMENT

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Objective: The aim of this study was to determine the distribution of clarithromycin resistance levels among genetically different *Helicobacter pylori* strains obtained from treated and non-treated patients.
Methods: *Helicobacter pylori* strains were obtained from biopsies of symptomatic patients between 2008 and 2009. Biopsies were cultured in Blood and Pylori Agar plates incubated at 37 C in a 5% CO_2 atmosphere. Resistance to clarithromycin was determined by E-test considering resistant if CMI was ≥1 mg/L for clarithromycin. *vacA* and *cagA* characterization was determined by electrophoresis with 1.2% agarose gel after conventional PCR with previously described primers. DNA extraction of each strain was performed by using the automatic system EasyMag (BioMérieux).
Results: 78 patients were included, 23.75% of which had been treated previously for *H. pylori*. Resistance rates for clarithromycin raised to 38.46%. 26.25% of strains were characterized as vacA s1 and/or cagA positive.
Conclusion: Resistance level to clarithromycin was high among the population studied (more than 38%) but its distribution depends on the strain genotype and the previous treatment received: lower resistance levels correspond to strains which carry more virulent genotypes and were obtained from patients who had not received any previous treatment.

		Clarithromycin		p by χ^2
		Susceptible	Resistant	
Previous treatment	Yes	6	12	**0.004**
	No	40	**16**	
Virulence factor	s1	17	**4**	**0.032**
	s2	31	26	
	cagA positive	18	**3**	**0.008**
	cagA negative	30	27	

Abstract no.: P4.40

FREQUENCY OF ANTIBIOTIC RESISTANCE IN ELDERLY, *HELICOBACTER PYLORI*-ERADICATED PATIENTS WITH OR WITHOUT CONCOMITANT ANEMIA

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Background: HP eradication rates with standard triple therapy are disappointing: studies from several countries confirm this poor performance. In Ukraine has never conducted researches to determine antibiotic resistance, antibiotic susceptibility testing is also not always applicable.
The aim of this study was to assess the eradication rate of sequential treatment regimen compared with conventional triple therapy for the eradication of HP infection in complex of iron replacement therapy (IRT) in elderly, HP-infected patients.
Methods: Two hundred and fifty-one elderly (M = 71.5 ± 9.4 years) dyspeptic patients were studied prospectively. All HP-infected (n = 107) patients were randomized to receive 10-day sequential therapy or standard 7-day triple therapy treatment. Patients with concomitant IDA (n = 44) was additionally received IRT. An isolated patient group with mild IDA was only eradication therapy because of hypersensitivity to oral iron medications.
Results: Prevalence of HP-infection among investigated patients was 70.9%. Hemoglobin levels was significantly lower in HP-positive patients (113.6 ± 9.06 g/L) than in HP-negative (149.5 ± 6.36 g/L). Anemia was diagnosed in 40.1%, IDA – in 92.8% of all anemic cases. Latent iron deficiency was presented in 43.9% non-anemic cases. Higher eradication rates were found with the sequential regimen compared to the standard regimen (72% vs 95% $p < .05$).
Conclusions: Combined eradication and IRT is associated with rapid devolution of IDA and led to prolonged remission of anemic syndrome. Sequential treatment is effective for eradicating HP in elderly patients and maybe can be used as a first-line therapy, but only in areas with a low CLA resistance rate, what needs further studies.

Abstract no.: P4.41

ISOLATION AND MOLECULAR CHARACTERIZATION OF THE FIRST STABLE AMOXICILLIN RESISTANT *HELICOBACTER PYLORI* IN SLOVENIA

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Background: Amoxicillin-based regimens are the predominant treatment options for eradication of *Helicobacter pylori*. Resistance to amoxicillin among *H. pylori* isolates is rare and the clinical impact is so far unknown. Here, we present a case of an infection with phenotypically and genotypically amoxicillin resistant *H. pylori,* successfully treated with amoxicillin-based triple therapy.
Case: A 61-year-old female with *H. pylori* positive chronic gastritis presented to us after two unsuccessful eradication attempts with (1) amoxicillin, metronidazole and pantoprazole for 7 days, and (2) amoxicillin, clarithromycin and pantoprazole for 7 days. Culture and susceptibility testing was performed after the second treatment failure. *H. pylori* resistant to amoxicillin, clarithromycin and metronidazole and susceptible to tetracycline and levofloxacin was isolated. A third eradication regimen with amoxicillin, levofloxacin and omeprazole was successful as proven by negative UBT.
Microbiology: From gastric biopsy specimen *H. pylori* with high MIC for amoxicillin (2 μg/mL and 3 μg/mL) was isolated as determined by two independent laboratories using the E-test method. Sequence analysis of pbp1A revealed a previously described amino acid substitution at position 414 from Serine to Arginine, which had been shown to increase the MIC of amoxicillin.
Conclusion: With the recently provided epidemiological cut-off value for amoxicillin by EUCAST (>0.12 μg/mL), we will identify more amoxicillin resistant strains. There are limited clinical data regarding treatment of such isolates with amoxicillin. With restricted treatment options, especially for therapy refractory infections, amoxicillin may remain effective.

Abstract no.: P4.42

RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* CAGA GENOTYPE AND RESISTANCE TO METRONIDAZOLE

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Introduction: Antibiotic resistance is a major cause of failure in treatment of *H. pylori* infection. It has been proposed that treatment failure is significantly higher in patients infected with cagA-strains. Metronidazole is the most widely used antibiotic for *H. pylori* treatment, however metronidazole resistance is growing. The aim of this study was to evaluate the correlation between cagA genotype and metronidazole resistance in *H. pylori* isolates.
Methods: In this study 127 isolates were used. Resistance to serial dilutions (32, 16, 8, 4 μg/mL) of metronidazole was assessed by disc diffusion method. Suspensions of *H. pylori* isolates with the turbidity of 2 MacFarland's unit were surface inoculated on Brucella agar. Ten micro litres of metronidazole dilutions were inoculated into blank discs deposited on the surface of Brucella agar. After 3–5 days incubation under microaerophilic conditions, the sizes of inhibition zones were recorded. Strains with inhibitory zone <20 mm, at MIC 8 μg/mL were considered as metronidazole-resistant. Genomic DNA was extracted from bacterial isolates and PCR was performed for the detection of cagA gene.
Results: Results of this study showed that 54/127 (42.51%) strains were cagA+, 81/127 (63.77%) resistance to metronidazole and, 38/81 (46.91%) metronidazole-resistant strains were cagA+. However, no significant correlation was found between metronidazole resistance and cagA status ($p > .05$).

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Discussion: It has been proposed that failure in the treatment of *H. pylori* infection is correlated with cagA- strains. However other reports indicated no correlation between cagA status and treatment failure. Our results also indicated cagA genotype is not correlated with treatment outcome.

Abstract no.: P4.43

METALLOANTIBIOTICS: SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF BISMUTH-FLUOROQUINOLONE COMPLEXES AGAINST *HELICOBACTER PYLORI*

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Novel organometallic compounds have been prepared by complexing the fluoroquinolones, norfloxacin, ofloxacin, ciprofloxacin, sparfloxacin, lomefloxacin, pefloxacin and gatifloxacin, with bismuth. The complexes were characterized by UV, IR, atomic absorption spectroscopy, elemental analysis, differential scanning calorimetry, thermogravimetric analysis and mass spectrometry. Their antibacterial potential against *Helicobacter pylori* and other microorganisms was investigated. These compounds were found to possess strong activity against *Helicobacter pylori* with a minimum inhibitory concentration of 0.5 mg/L. They also exhibited moderate activity against *Escherichia coli, Staphylococcus aureus, Bacillus pumilus* and *Staphylococcus epidermidis*. These bismuth-fluoroquinolone complexes have the potential to be developed as drugs against *H. pylori* related ailments.

Abstract no.: P4.44

COMPARISON OF THE ANTI-*HELICOBACTER PYLORI* EFFECT OF LANSOPRAZOLE WITH OMEPRAZOLE

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Introduction: Benzimidazoles such as lansoprazole and omeprazole are gastric parietal cell proton pump inhibitors that by increasing the pH of stomach, improve the stability and efficacy of antibiotics in gastric mucosa. Their anti-*H. pylori* effect has also been demonstrated by in vitro studies. In this study, the anti-*H. pylori* activity of lansoprazole was compared with omeprazole, using disc diffusion method.
Methods: Eighty-nine strains of *H. pylori* were used. Bacterial suspensions in normal saline (density: 2 MacFarland's unit) were surface inoculated on Brucella blood agar. Serial dilutions (64, 32, 16, 8 μg/mL) of lansoprazole and omeorazole were prepared in dimethyl sulfoxide and inoculated into blank discs on surface of blood agar. Plates were incubated in a microaerobic atmosphere at 37°C and examined after 72 hours. Isolates with inhibitory zone of >20 mm were considered susceptible. The MICs were determined as 8 μg/mL for lansoprazole and 32 μg/mL for omeprazole.
Results: The number of susceptible strains to lansoprazole was 80/89 (89.88%) and to omeprazole was 70/89 (78.65%). Lansoprazole at MIC 8 produced inhibition zone of 38 mm, reaching 52 mm when its concentration was increased to 64 μg/mL. The anti-*H. pylori* effect was equivalent to metronidazole at 8 μg/mL. Omeprazole at MIC 32 μg/mL produced inhibition zone of 36 mm reaching 43 mm when increased to 64 μg/mL.
Discussion: Our results showed higher anti-bacterial activity of lansoprazole compared with omeprazole. Accordingly, combination treatment regimens, including lansoprazole, are recommended to increase the antibacterial activity and reduce the risk of developing resistance.

Abstract no.: P4.45

HELICOBACTER PYLORI RESISTANCE TO COMMON ANTIBIOTICS IN IRAN

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Introduction: The incidence of *H. pylori* resistance to current antibiotics is a growing problem and resistance rates vary in different geographical regions. In this study we evaluated the rates of *H. pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone and determined whether the resistance to current antibiotics has changed with time.
Method: Susceptibilities of 127 clinical *H. pylori* isolates to serial dilutions of metronidazole (32, 16, 8, 4 μg/mL), amoxicillin (2, 1, 0.5, 0.25 μg/mL), clarithromycin, Furazolidone and tetracycline (4, 2, 1, 0.5 μg/mL) were determined by disc diffusion method. After 3–5 microaerophilic incubation, inhibition zones diameter were recorded. MICs were determined for metronidazole (8 μg/mL), clarithromycin (2 μg/mL), amoxicillin (1 μg/mL), tetracycline (0.5 μg/mL) and furazolidone (0.5 μg/mL).
Results: Susceptibility of *H. pylori* strains was determined, according to inhibition zone diameter cut-off (>13 mm for furazolidone and >20 mm for other antibiotics). Resistance rates to metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone were found in 63.77%, 13.38%, 7.87%, 49.06% and 7.87% of isolates, respectively.
Discussion: Compared with our previous studies, resistance rates to metronidazole and tetracycline increased considerably from 55.6% to 63.77% and 38.1% to 49.06%, respectively. The increase in resistance rate to metronidazole may be due to its overuse in the treatment of parasitic, genital or dental infections. The misuse of tetracycline in food animal productions may play an important role in the increase of resistance rate. Accordingly, it would be necessary to administer these antibiotics with more caution.

Abstract no.: P4.46

EXTREMELY SUSCEPTIBILITY OF *HELICOBACTER PYLORI* TO MOXIFLOXACINE AS ONE OF NEWLY INTRODUCED FLUOROQUINOLONES

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Helicobacter pylori is a pathogen that infects more than 50% of the world population, associated with gastric disorders. Indeed, increasing rate of antibiotic resistance in *H. pylori* had a dramatic influence of efficacy of therapeutic regimens. New generation of fluoroquinolones has introduced while the status of exact efficacy is not determined in countries like Iran. Remarkably, determining the latest trend of resistance among these antibiotics can be useful for clinicians in treatment of this rouge infection. The aim of this research was to investigate the antimicrobial sensivity of the ethnical strains of *H. pylori* in Northern Iran isolated from patients with digestive disorders. Antral biopsy specimens obtained from dyspeptic patients were investigated for culturing the *H. pylori* accordingly. Bacterial culture and susceptibility tests were done based on standard methods. In the current study, 10% of strains were re-selected randomly and retested to confirm our susceptibility tests. Of 190 patients, 185 (97%) were identified as positive for *H. pylori*. There was no statistically significant difference between the age and gender with antimicrobial susceptibility ($p > .05$). In this study, 185 single colonies of *H. pylori* strains (75 women [40%], 110 men [60%]; mean age 41.5; 20–80 years) were collected. Primary resistance of *H. pylori* isolates were clarithromycin (35/185:18%), metronidazole (125/185:67%), tetracycline (20/185:10%), amoxicillin (10/185:5%), levofloxacin (2/185: 1%) and moxifloxacine (0/185: 0%). In conclusion, our results show that we have a great option for proscribing the moxifloxacine and levofloxacin as novel agents to deal with resistant strains. Whilst we are confronting to resistant strains to antibiotics like metronidazole, modifying the therapeutic regimens can be a reliable intervention for successful therapy.

Abstract no.: P4.47

PREVALANCE OF ANTIBIOTIC RESISTANCE OF *HELICOBACTER PYLORI* IN SAINT-PETERSBURG

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Introduction: Nowadays treatment of *Helicobacter pylori* (*H. pylori*) is complicated by the increasing rates of antibiotic resistance being reported worldwide. Using the molecular-genetic method the clinically significant 40% rate of resistance to clarithromycin was reported in Saint-Petersburg (Baryshnikova et al., 2011), which is much higher than the rate in other regions of Russia. Nevertheless there is still no data of primary antibiotic resistance to *H. pylori* in Saint-Petersburg by using the cultural method.

Aim: To determine the antibiotic susceptibility of *H. pylori* isolates from Saint-Petersburg residents by disk diffusion method.
Methods: The study group included 49 dyspeptic *H. pylori* -positive patients (median age of 40.1 ± 5.2) with endoscopical features of chronic gastritis. The main exclusion criteria was the prior use of clarithromycin, amoxicillin, levofloxacin, metronidazole, nifuratel or tinidazole. Susceptibility to antibiotics was determined by disk diffusion method by measuring the growth inhibition zone around the disk.
Results: Cultures for *H. pylori* yielded 26 isolates. Data showed that 69.2% were resistant to metronidazole, 42.3% to levofloxacin, whereas only two isolates (7.7%) were resistant to clarithromycin. No resistance was found to amoxicillin (table). Among the isolates 65.4% were sensitive to nifuratel, 46.2% to tinidazole.
Conclusion: We found a prevalence of primary clarithromycin resistance of 7.7% in patients of Saint-Petersburg, that allows using this antimicrobial in first-line therapy for *H. pylori* infection.

Antimicrobials	(S-susceptible, R-resistance) n (%)		
	S (%)	S/R (%)	R (%)
amoxicillin	21 (80.8)	5 (19.2)	0
clarithromycin	21 (80.8)	3 (11.5)	2 (7.7)
levofloxacin	4 (15.4)	11 (42.3)	11 (42.3)
nifuratel	17 (65.4)	6 (23.1)	3 (11.5)
metronidazole	1 (3.9)	7 (26.9)	18 (69.2)
tinidazole	12 (46.2)	8 (30.8)	6 (23.1)

Abstract no.: P4.48

A2147G IS MOST PREVALENT MUTATION AMONG CLARITHROMYCIN RESISTANCE *HELICOBACTER PYLORI* STRAINS IN MONGOLIAN PATIENTS

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Backround and Aims: The resistance of *H. pylori* to the recently available antibiotic treatment regimens has been a growing problem. Therefore aim of study was to determine the prevalence of antibiotic resistance among *H. pylori* strains isolated from Mongolians and to provide a new molecular method easily detect mutations predictive of clarithromycin resistance in *H. pylori*.
Method: One hundred fifty-seven samples of gastric biopsies were obtained during upper gastrointestinal endoscopy from the patients referred for the exploration of clinical gastritis. All urease positive samples were cultured according to standard microbiological procedures. All *H. pylori* strains were grown under microaerophilic conditions on selective Pylori agar. *H. pylori* antibiotic sensitivity was examined using E-test method. In addition, the mutations of the corresponding gene were studied by GenoType HelicoDR DNA strip testing.
Result: Total of 157 gastric biopsy specimens, 61.1% (96) were confirmed to have gastric *H. pylori* infection by ureasa test. We have successfully obtained 60.4% (58) pure *H. pylori* isolates. The overall *H. pylori* E-test antibiotic resistance rates were 33.3% for clarithromycin, 66.7% for metronidazole, 33,4% for amoxicillin, 40% for tetracycline, 26.7% for erythromycin and 13.3% for nitrofuranton. GenoType HelicoDR test result for detection of 14 clarithromycin resistance clinical strains. Overall, the most frequent mutation was A2147G (MUT3 profile), observed in five strains, followed by D91N (MUT1) in only one strain.
Conclusion: The prevalence of *H. pylori* infection increased among Mongolian population. In the present study, *H. pylori* metronidazol-resistant strains are more frequently found in Mongolians and clarithromycin-resistant strains frequently have mutations in the 23S rRNA gene.

Abstract no.: P4.49

PREVALENCE TO CLARITHROMYCIN RESISTANCE OF *HELICOBACTER PYLORI* STRAINS ISOLATED IN SICILY

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Helicobacter pylori, responsible for gastritis, peptic ulcers, gastric adenocarcinoma, infects half of the world's population but infection is more prevalent in developing countries and incidence is decreasing in Western countries. Because over 80% of people infected with *H. pylori* show no symptoms, the guidelines suggest eradication of the microorganism only in symptomatic patients or in the presence of severe gastric disease. Eradication therapy consist in a proton-pump-inhibitor combined with clarithromycin and amoxicillin or metronidazole. Clarithromycin is a key component of most treatments to eradicate *H. pylori,* and resistance to drug has become one of the major reasons for treatment failure. In fact, to reduce it, all consensus guidelines recommended clarithromycin as first choice treatment only in populations with <15–20% of resistance prevalence. The resistance is associated with point mutations in the peptidyltransferase region encoded in domain V of 23S rRNA gene. The most prevalent point mutations are the transitions A2142G and A2143G and the transversion A2142C. The prevalence of *H. pylori* resistance to clarithromycin varies among different countries, may exceed the threshold dictated by the guidelines, however they can not be detected in each case for the difficulties related to the isolation of microorganism and evaluation of drugs in vitro; for these reasons are required epidemiological data. The aim of study was to evaluate the resistance to clarithromycin in one hundred *H. pylori* strains isolated in Sicily, from patients with gastric pathology. In vitro the antibiotic susceptibility was determined by Kirby-Bauer test and confirmed by molecular method.

P5 – Gastric Cancer and Preneoplastic Lesions

Abstract no.: P5.02

CUTOFF VALUES SHOULD BE RECONSIDERED IN SEROLOGICAL RISK EVALUATION OF GASTRIC CANCER IN JAPAN

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Purpose: A combination of *H. pylori* antibody (Hp) and pepsinogen (PG) serum levels, which classified into four groups (A:Hp(-)PG(-), B:Hp(+)PG(-), C:Hp(+)PG(+), and D:Hp(-)PG(+)) has been used in risk evaluation of gastric cancer in Japan. We set different cutoff values (COVs) to define *H. pylori* infection and gastric atrophy, and proposed a method to determine optimal COVs.
Methods: The subjects were 275 gastric cancer patients (case) and 275 healthy control subjects (control) in our previous case-control study. We measured Hp and PG in their sera using J-HM-CAP and RIAbeads Pepsinogen I and II, and classified by three different COVs.
Results: When the COV for PG I was set at 70 ng/mL (constant), PG I/II ratio at 3.0, and Hp at 2.3, A, B, C, and D represented 32.4% 32.7%, 32.4%, and 2.5% in the controls, and 32.7%, 32.4%, 2.5%, and 2.9% in the cases, respectively. However, when the COV for PG I/II ratio at 4.0 and Hp at 1.3, A, B, C, and D represented 20.4%, 34.2%, 45.1%, and 0.4% in the controls, and 0.4%, 32.4%, 66.5%, and 0.7% in the cases, and when the COVs for PG I/II ratio at 4.0 and Hp at 1.0, 13.1%, 41.5%, 45.5%, and 0.0% in the controls, and 0.0%, 32.7%, 67.3%, and 0.0% in the cases.
Conclusion: Setting higher COV of pepsinogen and lower COV of *H. pylori* antibody gave a more appropriate classification in the serological risk evaluation of gastric cancer. We can decide optimal COVs by combining serological tests with cancer registry data.

Abstract no.: P5.03

HELICOBACTER PYLORI WITH HIGH-EXPRESSED THIOREDOXIN-1 IS RELATED TO GASTRIC CANCER

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Objective: To identify the pathogenic properties of *Helicobacter pylori* (Hp), and to investigate the protein identified from this map, which is perhaps related to gastric carcinogenesis.
Methods: Hp strains were isolated from endoscopic biopsy specimens of gastric mucosa of patients with gastric cancer, peptic ulcer, and gastritis. The protein maps of Hp were obtained by 2-DE and the different proteins were analyzed by mass spectrometry. Then thioredoxin (Trx) was analyzed. The expressions of Trx1 mRNA were analyzed by real-time PCR. Hp strains with highly and lowly expressed Trx1 were cocultured with gastric cancer cell BGC-823 and normal gastric epithelial cell GES-1. MTT, cell cycle and cell apoptosis were used to estimate the growth situation of cells. Western blot was used to measure the related proteins. The two Hp strains were also used to infect Mongolian gerbils for 91 weeks.
Results: Trx1 of Hp in mRNA level was higher-expressed in Hp from gastric cancer and peptic ulcer. After infected by Hp with high-expressed Trx1, the proliferation of BGC-823 was promoted and the cells coming into S phase were increased. However, the proliferation of GES-1 was decreased and the apoptosis rate was significantly higher. P21 of GES-1, CyclinD1 of BGC823 was up-regulated, CyclinD1 of GES-1 was down-regulated. High-expressed Trx1 Hp was highly pathogenic, resulting in seriously pathological changes in vivo.
Conclusion: Trx1 of Hp is related with the gastric carcinogenesis, and it could be thought as a biomarker of highly pathogenic Hp.

Abstract no.: P5.04

ROLE OF THE PROTEIN IQGAP1 DURING *HELICOBACTER PYLORI* INFECTION AND GASTRIC ADENOCARCINOMA

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IQGAP1, a member of the IQGAP family of scaffold proteins, plays a crucial role in the pathways in which Cdc42 or Rac1 modulates cadherin-based cell adhesion. Gastric adenocarcinomas are classified in two different types, the intestinal type and the diffuse type. Almost 30% of diffuse gastric cancers are associated with a mutation of the *cdh*1 coding for E-cadherin. However, the origin of the remaining 70% has not yet been elucidated. Our aim was to identify the role of IQGAP1 during *Helicobacter pylori* infection and development of gastric adenocarcinoma. Wild type or mutant IQGAP1 ± mice (n = 80) were infected with different *Helicobacter* sp. strains (*Helicobacter felis*, *H. pylori* SS1 and HPARE). Stomachs were recovered 6 months and 1 year post-infection. A histologic analysis was performed: inflammation, hyperplasia, atrophy, metaplasia, and dysplasia were evaluated for each mouse stomach. After 6 months of infection, IQGAP1 ± mice developed more mucous metaplasia than did the wild type mice. For the other lesions, no difference was shown at 6 months between wild type and IQGAP1 ± mice. However, all of the mice infected by *Helicobacter* sp. developed more inflammation, hyperplasia, atrophy and dysplasia than the non-infected mice. One year post-infection, differences between wild type mice and IQGAP1 ± mice were more significant. Finally, IQGAP1 ± mice infected with *H. pylori* HPARE developed significantly more hyperplasia, atrophy, mucous metaplasia, pseudo-intestinal metaplasia, and dysplasia than those infected with the other strains. These results suggest that IQGAP1 is a crucial protein in *H. pylori* induced gastric adenocarcinoma.

Abstract no.: P5.06

ERADICATION OF *HELICOBACTOR PYLORI* FOR THE PREVENTION OF DUODENAL ULCER REBLEEDING: 10 YEARS FOLLOW-UP STUDY

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Introduction and Background: *H. pylori* eradications can reduce peptic ulcer recurrence and complications. How long can *H. pylori* eradication prevent DU bleeding andrecurrence ? How about those failed in eradication?
Aim: To investigate the rebeeding rate and ulcer recurrence rate of patients with DU bleeding after eradication therapy after 10 years followup.
Material and Method We collected patients with DU bleeding from 2000 to 2002 restrospectively. We got those DU bleeding with positive HP infection. *H. pylori* eradication regimens including triple therapy, Denol-base quadruple therapy and sequential therapy were given. Du rebleeding and recurrence were checked with endoscopy in 2012.
Result: Nine hundred two patients with DU bleeding were found in 2000–2002. There were 870 patients with positive *H. pylori* infection. Triple therapy successfully eradicated 775 patients. Ninety-five failed patients received Denol-base quadruple therapy. Eighty-one patients eradicated and 14 patients failed in. Adjuvant sequential therapy were given for rescue with three successful eradication. Eleven patients with total failure in *H. pylori* eradicationand 859 patients with *H. pylori* eradication were resulted. In 2012, prospective endoscopy was done in 11 patients with total failure and no DU recurrence was found. No rebleeding had been found in 870 patients received *H. pylori* eradication since 2000–2002.
Conclusion: Duodenal ulcer rebleedingdoes not occur in patients with complicated duodenal ulcers bleeding after *H. pylori* eradication. The eradication rate of *H. pylori* in DU bleeding was as good as in DU. No futher recurrence of duodenal ulcer was found also in total failure of eradication of *H. pylori* 10 years later.

Abstract no.: P5.08

EXPLORATION OF THE ASSOCIATION BETWEEN *HELICOBACTER PYLORI* ANTIBODIES AND STOMACH CANCER USING MULTIPLEX SEROLOGY

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Aims: Infections with *Helicobacter pylori* (*H. pylori*) due to its various virulence genotypes or host differences may induce different immune responses. We hypothesized that certain antibody response patterns might be more closely linked to stomach cancer development than others. To test this hypothesis, we used multiplex serology in a Swedish population-based case-control study of stomach cancer.
Methods: Serum samples were obtained from 268 cases and 222 controls aged between 40 and 79 years. We quantitatively measured antibodies against 17 *H. pylori* proteins including CagA using a recently developed multiplex serology assay.
Results: Of the 17 antibodies, 15 showed significant associations with stomach cancer risk, with adjusted odds ratios (ORs) ranging from 1.6 to 4.5. The excess risks were confined to non-cardia stomach cancer, but showed no significant difference between intestinal and diffuse types. The relative risks elevated with increasing numbers of *H. pylori* antibody positivities. Using principal component analysis, we identified two significant factors: (1) A CagA-dominant factor (antibodies against CagA, VacA, Omp and GroEL loaded highly), and (2) a non-CagA factor (antibodies against NapA, UreA, and Catalase loaded highly). Scores from both factors showed a dose-dependent association with non-cardia stomach cancer risk (CagA-dominant factor, highest versus lowest quartiles, OR = 7.4 [95% CI = 3.1–17.7]; non-CagA factor, highest versus lowest quartiles, OR = 1.9 [95%CI = 1.1–3.4]).
Conclusion: Our multiplex serology added little to the discrimination already attained with existing serological markers. Further studies are warranted to search for new infection biomarkers that would be better suited for risk stratification among infected individuals.

Abstract no.: P5.09

MULTI-CENTER STUDY OF GASTRIC CANCERS DETECTED AFTER *HELICOBACTER PYLORI* ERADICATION

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Aim: It had been shown that *H. pylori* eradication reduced the risk of gastric cancer. However, gastric cancer is sometimes discovered after successful eradication of *H. pylori*. The aim of this study is to clarify the clinicopathlogical features of gastric cancers which detected after successful eradication.
Method: The multi-center, retrospective survey about the gastric cancer cases detected after *H. pylori* eradication was conducted in Japan. Successful eradication was dined as continually negative results of more than two diagnostic tests of *H. pylori*.
Results: A total 424 gastric cancer cases from 34 institutions (mean age = 64 years: 36–87, men/female = 338/86, primary/metachronous = 248/176) were registered this study. Seventy cases detected before 1 year after eradication, while 28 cases detected 10 years or more after eradication. The characteristics of the cases detected before 5 years (A) and the cases over 5 years (B) were compared. Advanced cancers and large lesions (>1 cm) were significantly more detected in the group B. When eradication had been done for peptic ulcer, the half of cancer cases detected over 5 years, while for gastric adenoma, MALT lymphoma and gastric cancers, most cases detected before 5 years. Morphological features such as depressed type and large lesions (<1 cm), and diffuse type in histology were significantly more detected in the primary cancers than in metachronous cancers.
Conclusion: Gastric cancer was detected even after 10 years or more, and the clinicopathlogical features were different by baseline diseases or degree of gastric mucosal atrophy. Our study revealed that follow-up endoscopic examination is necessary even after successful *H. pylori* eradication for a long time.

Abstract no.: P5.10

THE CELLULAR FIBRONECTIN IN GASTRIC CANCER PATIENTS WITH KNOWN *HELICOBACTER PYLORI* INFECTION STATUS

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Background: The *H. pylori* infection is a significant risk factor of developing GC. Cellular fibronectin is a glycoprotein involved in normal physiological processes such as cells adhesion, migration and differentiation. It is possibly also associated with the development and progression of the gastric cancer. This study was aimed to investigate this marker in the plasma of GC patients with known serologic status of *H. pylori* infection.
Methods: Serum samples from 84 gastric cancer patients, 82 patients with non-malignant gastrointestinal diseases (NMGID), and 34 blood donors were tested. cFN concentration were measured by a competitive EIA and was analyzed in *H. pylori* seropositive GC patients.
Results: Using receiver operating characteristic curve analysis, sensitivity level 80% and specificity levels higher than 60% was chosen for further analysis. cFN concentration ≥2.0773 mg/L was found to be relevant to the gastric cancer group compared with donors and NMGID patients ($p < .001$). Higher concentration of cFn was observed in pT3-T4b stages ($p < .036$), but pN stage was not associated with increased concentration of this serologic marker ($p = .915$). Mixed Lauren type of distal gastric cancer was relevant to cFN concentration >=2.0773 mg/L in gastric cancer patients with pT3-T4b stages ($p = .004$). There were no statistically significant differences of the cFn concentration between *H. pylori* positive and negative gastric cancer patients as determined by one-way ANOVA ($p = .173$).
Conclusions: cFn could be helpful in identifying patients with gastric cancer in pT3-pT4b stages as well as distally located mixed type of cancer. Increased concentration of cFn in GC is not associated to *H. pylori* infection.

Abstract no.: P5.11

BACTERIA OF THE KIND HELICOBACTER IN THE OMENTUM AND REGIONAL LYMPH NODES (RLN) IN PATIENTS WITH GASTRIC CANCER (GC)

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Objective: To establish the cause of rapid urease test positive reaction in the omentum and RLN at patients with GC (Senchukova et al., Helicobacter, 2010, 3, P. 62–63).
Method: The detection of *H. pylori* in the omentum and RLN was performed in 47 patients with GC by the HELPIL-test (HT, Russia) and immunohistochemistry (IHC) using antibodies against *H. pylori* (RB-9070; Thermo Fisher Scientific).
Results: In the omentum HT was positive at 42 (89.4%) patients with GC. On IHC *H. pylori* was identified in 29 samples (69.1%) of the omentum and 19 (45.2%)samples of RLN. In the omentum the small cocci (up to 0.2 microns in diameter) were located in the immediate vicinity of the RLN. An individual bacterial cells, their accumulations (up to 15–50 bacteria) and seldom the short direct sticks (up to 2 microns in diameter) were visualized. Sometimes cocci accumulations have been limited by thin-walled structures without endothelial cells. In a tissue of lymph nodes *H. pylori* were visualized seldom and have been presented both an individual cocci or small accumulations bacterial cells (up to 10 bacteria).
Conclusion: The positive test on the urease activity in the omentum and RLN and identification *H. pylori* by the IHC can testify about an ability of bacteria to translocation. Considering the presence of the cross reactions on IHC stains between bacteria of kind Helicobacter, the further researches on genotyping the described microorganism and to studying of a role translocation bacteria in gastric cancerogenesis are required.

Abstract no.: P5.12

THE SPECIFIC FEATURES OF *HELICOBACTER PYLORI* INFECTION IN GASTRIC MUCOSA (GM) IN PATIENTS WITH GASTRIC CANCER (GC)

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We study the features of *H. pylori* infection in GM in patients with GC. The detection of *H. pylori* in the GM was performed in 47 patients with GC by the immunoperoxidase stains using antibodies against *H. pylori*.

Results: In GM located near to the tumor prevailed coccoid forms of *H. pylori*. Only coccoid forms were identified in 28 (59.6%) specimens, coccoid and curved – in 15 (31.9%). Not found the signs of infection in 4 (8.5%). The diameter of cocci was 0.2–0.4 μm. Bacteria were both in the surface mucosa and within the epithelial cells (ECs). Their massive defeat was noted in five cases. In the deep gastric glands, within the ECs and lymphoid cells infiltrating the lamina propria (LP) we often observed multiple punctate inclusions, giving a positive reaction with antibodies to *H. pylori*. The reaction with antibodies to *H. pylori* in the ECs was from hardly appreciable to severe. The correlation between the expression of *H. pylori* in ECs and in LP ($r = 0.82$, $p = .004$). In the LP it was detected the separate ECs and their small accumulations also.

Conclusion: These data suggest that *H. pylori* may be the intracellular parasite, and perhaps capable to intracellular multiplication. A weak intensity of the specific reaction with antibodies to *H. pylori* described intracellular particles may indicate that at intracellular multiplication initially formed the primitive forms of bacteria, which lack expression of some antigens. Further research is needed to clarify the nature of these changes.

Abstract no.: P5.13

THE INCIDENCE OF GASTRIC CANCER BIOMARKERS IN RESIDENTS OF CIRCUMPOLAR COMMUNITY

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Gastric and esophageal cancers are both significantly prevalent in northern regions of Siberia. Chronic atrophic gastritis and *Helicobacter pylori* are risk factors for gastric cancer. To identify these issues in context of gastrointestinal disorders, random samples of circumpolar city of Yakutsk population of different ethnic origin (Caucasian and Mongoloid) aged 45–90 years were examined. A total of 153 randomly selected respondents participated, representing eighty-one newcomers (mostly of Slavic origin) and seventy-two persons of Yakut aboriginal nationality. Blood levels of pepsinogen I (PGI) and antibodies to *H. pylori* have been identified in examined persons using ''Gastropanel'' (Biohit, Finland). The criterion of atrophy was a level of PGI < 25 ng/mL). In the city of Yakutsk, the prevalence of atrophic gastritis in Caucasoid population was 12.3%, and in Mongoloid population – 15.3%. Low levels of PGI were significantly associated with the age of persons surveyed: atrophy was observed in 7.4% in persons aged under 60 years, in 10.5% aged 61–70 years and in 16.5% over the age of 70 years ($p = .03$). *Helicobacter pylori* infection was detected with similar high proportion in all surveyed (72.2–74.1%). The high prevalence of atrophic gastritis and *H. pylori* infection may partially explain the high incidence of gastric cancer in Russia, and, especially among the population of the republic of Yakutia (Sakha).

Abstract no.: P5.14

MULTIPLE SIMULTANEOUS GASTRIC LESIONS BEFORE ENDOSCOPIC RESECTION

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This study aimed to identify the clinical factors for development of multifocal lesions by endoscopic surveillance but not surgical resection. From April 2010 to April 2011, patients with EGC or adenoma who had underwent ESD were periodically performed follow up endoscopy. We checked baseline characteristics of patients, *H. pylori* status, the existence of gastric atrophy, intestinal metaplasia, pepsinogen I, II level and intra-gastric pH. A total 165 patients underwent ESD and among this patients, 34 patients were excluded and 131 patients were analyzed. Multiple simultaneous gastric lesions had be detected in 18 patients (13.74%), 37 lesions. Multivariate analyses showed that man ($p = .029$, Odds ratio 5.62, 95% CI 1.197–26.437) and old age ($p = .010$, Odds ratio 1.095, 95% CI 1.022–1.173) were independent risk factors of multifocal lesion. With multifocal lesions, there was no patients without atrophy, 14 patients of pangastritis and four patients of antral predorminant atrophic gastritis. The PG I level ≤70 was 12 patients (12/18, 66.7%) and PG I/II ratio ≤3 was 10 patients (10/18, 55.6%). PG I and PG I/II ratio is lower in patients with multifocal lesions than solitary lesions, but not significant. *H. pylori* status has not contributed in development of multifocal lesion. The prevalence of multifocal lesions was 13.74% in our study, main lesion was mainly EGC, additional lesion was mainly LGD. Main and additional lesion were mainly located at same territory and gross finding was similar. And old age and man were significantly associated with multifocal lesions.

Abstract no.: P5.15

DOWNREGULATION OF CDX2 EXPRESSION IN GASTRIC CELLS USING CHITOSAN/SIRNA NANOPARTICLES – A STRATEGY TO REVERT GASTRIC INTESTINAL METAPLASIA

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Introduction: Gastric intestinal metaplasia (IM) is a pre-malignant condition associated with increased risk of adenocarcinoma. *Helicobacter pylori* eradication is not able to revert or stop progression of IM in all patients. CDX2 is the key molecular mediator of intestinal differentiation, both in intestine and ectopic foci. CDX2 regulates its own expression, being hypothesised that this mechanism is crucial for the maintenance of the intestinal phenotype, making CDX2 an appealing therapeutic target.

Aims and Methods: To design and optimize a nanoparticle (NP)-based delivery system of siRNAs directed to CDX2 in gastric cells (AGS and IPA220), using chitosan, a polymer with mucoadhesive properties, modified with imidazol (CHimi). NPs with different CHimi to siRNA ratios (N/P) were prepared to optimize NP formation capacity, size and charge, determined, respectively, by competitive agarose gel electrophoresis, dynamic light scattering and electrophoresis measurements. NPs cytotoxity was evaluated using a resazurin-based assay. Lipofectamine™/siRNA complexes were used as controls. CDX2 downregulation was assessed at mRNA and protein levels.

Results: CHimi with a substitution degree of the primary amines of 10 and 16% was obtained. An N/P ratio of 50 rendered the preparation of NPs with a positive net charge (+33V) and an average size<500 nm, able to fully complex the total amount of siRNAs. A 50% decrease in the CDX2 protein was obtained with NPs, without compromising cell viability.

Conclusion: We report for the first time a NP-based delivery system directed to CDX2, that can be envisioned as a therapeutic strategy to revert gastric IM in vivo.

Abstract no.: P5.17

OLGA AND OLGIM STAGING SYSTEMS DO NOT CORRESPOND IN A PROSPECTIVE SETTING

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Background: OLGA (operative link on gastritis assessment using atrophy) and OLGIM (using intestinal metaplasia (IM) are histological staging systems for gastritis. Both are proposed to have prognostic value.

Aim: To compare OLGA and OLGIM in a prospective setting.

Methods: Gastric biopsies from antrum and corpus were obtained from 184 patients (age 19–94, median 56, f:m 119:65) with normal gastric mucosa (61), chronic gastritis (36), atrophy or IM (63 AG/IM), ulcer (15 UD) and first diagnosed gastric cancer (9 GC). Histopathological assessment, OLGA and OLGIM staging was performed by one expert pathologist. Gastrin 17, pepsinogen I (PI) and II (PII) were measured in serum of all patients.

Results: Seventy-five of 184 patients (41%) were classified as stage I-IV according to OLGA or OLGIM: 63 with AG/IM, 7/15 with UD and 5/9 with GC. OLGA and OLGIM stage were not consistent in 57/75 cases (76%). High OLGA stages more often had their correspondence with low OLGIM stages. Of all serum parameters, only the PI/PII ratio correlated with the OLGA stage ($r = -0.4$, $p < .0001$).

Conclusions: Patients with premalignant gastric conditions and GC were unequally distributed in OLGA and OLGIM stages. Gastric cancer at first diagnosis was not associated with high stages of OLGA or OLGIM.

Abstract no.: P5.18

HELICOBACTER PYLORI IS ASSOCIATED WITH UNINVESTIGATED DYSPEPSIA AND ATROPHIC GASTRITIS IN THE URBAN POPULATION OF SIBERIA OVER 45 YEARS OLD

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Aim: To study relationship of *Helicobacter pylori* with uninvestigated dyspepsia and atrophic gastritis in the urban population of Siberia over 45 years old.
Methods: For the study we selected 801 persons (387 males, 414 females) aged over 45 years old living in the Krasnoyarsk city by the method of random sampling. To all patients were performed clinical examination with the filling of standard questionnaire for the study of uninvestigated dyspepsia and determination content of pepsinogen-1, pepsinogen-2 and antibodies to *Helicobacter pylori* in blood serum by immunoassay method using test kits ''GastroPanel'' (producer ''Biohit'', Finland). Marker for severe atrophy of gastric body mucosa we considered the level of pepsinogen-1 <25 μg/L and the ratio pepsinogen-1/pepsinogen-2 <3.
Results: The prevalence of *H. pylori* infection among surveyed persons was 90.0% (males – 89.7%, females – 90.3%). The prevalence of uninvestigated dyspepsia in the surveyed population was 24.5% (males – 24.3%, females – 24.7%). The prevalence of severe gastric body atrophic gastritis in surveyed population was 10.9% (males – 11.9%, females – 9.9%). *H. pylori* was determined in 95.9% people with dyspepsia and in 88.1% persons without dyspepsia (OR=3.01, CI = 1.45–6.25) in 97.1% patients with gastritis and in 89.4% individuals without gastritis (OR=3.93, CI = 1.00–16.36).
Conclusion: The prevalence of uninvestigated dyspepsia, atrophic gastritis and *Helicobacter pylori* was high in the urban population of Siberia. *H. pylori* infection was associated with uninvestigated dyspepsia and atrophic gastritis in the surveyed population.

Abstract no.: P5.19

GASTRIC CANCER PREVENTIVE MECHANISMS OF KOREAN RED GINSENG THROUGH THE INHIBITION OF *HELICOBACTER PYLORI*-INDUCED HYDROGEN SULFIDE

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Helicobacter pylori-associated gastritis or gastric cancer is closely associated with increased levels of hydrogen sulfide (H_2S) and Korean red ginseng imposes a significant rescue from *H. pylori*-associated gastric diseases through suppressing H_2S. Since the incubation of endothelial cells with H_2S has been known to enhance their angiogenic activities, we hypothesized that ameliorating actions of H_2S-induced gastric inflammation or angiogenesis can explain the preventive effect of *H. pylori*-associated carcinogenesis. The changes of inflammatory mediators as well as angiogenic growth factors and angiogenic activities in the absence or presence or absence of Korean red ginseng extracts (KRGE) were checked in HUVEC stimulated with NaHS. KRGE efficiently decreased the expressions of cystathionine [[Unsupported Character – Symbol Font ]]-synthase (CBS) and cystathionine [[Unsupported Character – Symbol Font ]]-lyase (CSE) coincided with the significantly decreased expressions of either inflammatory mediators including COX-2, iNOS or several angiogenic factors including IL-8, HIF-1[[Unsupported Character – Symbol Font ]], VEGF, IL-6, and MMPs. NaHS significantly increased tube formation of endothelial cells, whereas KRGE pretreatment significantly attenuated. NaHS activated p38 and Akt related to increased angiogenic factors and increased proliferation of HUVEC, whereas KRGE effectively could abrogate these H_2S-activated angiogenesis. Antagonistic action of KRGE against H_2S-induced angiogenesis can explain the gastric cancer preventive mechanisms in *H. pylori* infection.

Abstract no.: P5.20

ANALYSIS OF GENETIC EXPRESSION ASSOCIATED WITH *HELICOBACTER PYLORI* INDUCED GASTRIC HYPERPLASTIC POLYPS

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Background/Aims: The eradication of *Helicobacter pylori* (*H. pylori*) can lead to the regression of gastric hyperplastic polyps. Although there is much attention on the relation between *H. pylori* and hyperplastic polyp, little information has been known about which molecular target is related with the formation of gastric polyps. We have investigated which genetic expression is related with the formation of *H. pylori*-induced gastric hyperplastic polyps.
Methods/Results: Six patients (three patients for control, three patients for hyperplastic polyp) were enrolled for this experiment. Real-time PCR was performed in the tissues of gastric polyps and corpus obtained during upper GI endoscopy. Genetic expressions of MMP-9 were increased in gastric polyps comparing with that of controls whereas inflammatory cytokines (IFN-r, IL-1b, IL-21, IL-22, IL-17A) and MMP-3 were unchanged.
Conclusion: These data suggest that MMP-9 might be related with the formation of *H. pylori*-induced gastric hyperplastic polyps.

Abstract no.: P5.21

EFFECTIVENESS OF *HELICOBACTER* ERADICATION IN THE TREATMENT OF GASTRIC MALTOMA

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Helicobacter pylori eradication induces remission in most patients with gastric mucosa-associated lymphoid tissue (MALT) lymphoma. However, there have been few reports about the effect of bacterial treatment on the gastric MALToma in Korea, a well-known *H. pylori* endemic area. From January 2000 to December 2010, consecutive patients with stage-I gastric MALToma were enrolled in single centre retrospectively. A total of 46 patients were enrolled and median age of the patients was 59 years (36–81 years). There were fewer male than female (M:F, 18:28) and male to female ratio was 1:1.6. The macroscopic tumour type was determined according to the classification of Watanabe, as follows : (1) ulceration (43.5%); (2) protruding (13.0%); (4) granular (8.7%); (4) infiltrative (28.3%); and (5) mixed (6.5%). Most of lesions were single (69.6%) and the neoplasm was located at the body in 68.8%, antrum in 25.0% and 6.2% in cardia or fundus. The median time of follow-up was 37.2 months (range 5–96 months). Among 45 of 46 patients who checked status of *H. pylori,* 31 (68.9%) was positive in *H. pylori* test. Eradication was done in 24 of 31 in *H. pylori*-positive patients. Among 22 of 24 assessable patients, 21 (95%) showed successful eradication. During the follow-up period, all of 21 showed CR (85.7%) or PR. Five of 14 *H. pylori*-negative MALToma had eradication. Among them, four patients showed CR and one showed no change. In conclusion, irrespective of the existence of bacteria, *H. pylori* eradication is effective in the treatment of gastric MALToma.

Abstract no.: P5.22

GASTRIC MORPHOLOGY IN PATIENTS WITH A POSITIVE PEPSINOGEN TEST: THE INITIAL RESULTS OF A POPULATION-BASED STUDY

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Background and Aims: Atrophy of the stomach mucosa is considered a premalignant condition. Pepsinogen tests are used for non-invasive detection of atrophy. The aim of this study was to evaluate the gastric morphology in patients with decreased pepsinogens.
Material and Methods: Pepsinogen I (PgI) and II (PgII) (Eiken, Japan) was measured in plasma samples from a population based study. Here we report the initial results from 54 individuals with decreased pepsinogens undergoing endoscopy. The cut-off level for atrophy of any grade (Group 1) was PgI/PgII ≤ 3 and PgI ≤ 70 ng/mL, but for advanced atrophy (Group 2) – PgI/PgII ≤ 2 and PgI ≤ 30 ng/mL. Spearmans rank correlation coefficient was used to measure the correlation between pepsinogens and morphology.
Results: Out of the total 54 cases 14 were Group 2 according to the pepsinogen results. One gastric adenocarcinoma was diagnosed in Group I (1.9%). In the Group I – 19 (35.2%) patients were found to have atrophy in the corpus (mild in 17, moderate in two cases) according to the histology results. In the Group 2 – 12 (85.7%) patients were diagnosed atrophy at histology (mild in 10, moderate – in 2 patients). The grade of corpus atrophy was found to correlate irreversibly to the value of PgI/PgII, $rs = -0.568$ ($p < .001$).
Conclusion: The majority of individuals with decreased pepsinogen levels had histological signs of atrophy. The grade of corpus atrophy irreversibly correlates to the value of PgI/PgII. Therefore, pepsinogens has a potential to detect premalignant and malignant gastric lesions.

Abstract no.: P5.23

RELATIONSHIP BETWEEN OLGA AND GASTROPANEL IN PATIENTS WITH UPPER GASTROINTESTINAL DISORDERS

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Introduction: Chronic Atrophic Gastritis (CAG) is the most important independent risk condition for gastric cancer, histology being considered the gold standard for both diagnosis and follow up. The Operative Link for Gastritis Assessment (OLGA) is an innovative histological system proposed for staging atrophic gastritis. GastroPanel (Biohit, Helsinki, Finland) is a serological kit for a non-invasive diagnosis of CAG.
Aims and Methods: To evaluate correlation between GastroPanel and OLGA stage in a cohort of dyspeptic patients. Histological evaluation of gastric biopsies was performed in 125 patients (83 females, mean age 53 years). Atrophy was assessed according to the OLGA staging system, stages III and IV are considered at high risk for gastric cancer development. GastroPanel was performed in all patients.
Results: GastroPanel analysis showed that 63.2% of patients have no gastritis, 17.6% a Hp-related gastritis and 19.2% atrophic gastritis. Comparing GastroPanel results to the histological findings, 48 patients of the first group were confirmed normal at histology, 27 patients have an OLGA stage 0-II, four have an OLGA stage III, none stage IV. Among the patients with Hp-related gastritis only one has an OLGA stage III, none OLGA IV; 18 out of the 24 patients with evidence of CAG at GastroPanel have OLGA III and IV. The negative predictive value (NPV) of GastroPanel for CAG is 95.0%, being the positive predictive value (PPV) 75.0%.
Conclusion: GastroPanel shows an high NPV to predict absence of atrophic gastritis. The relationship between GastroPanel and OLGA system seems to confirm the clinical performances of the test.

Abstract no.: P5.24

THE EFFICIENCY OF SERUM PEPSINOGEN LEVELS AND *HELICOBACTER PYLORI* ANTIBODIES IN REFLECTING THE STATUS OF GASTRIC MUCOSA AMONG AN IRANIAN POPULATION

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The high incidence and mortality rate of gastric cancer (GC) in Iran and the poor survival rate due to its late detection are the major incentives in seeking non invasive approaches for early screening of high risk populations. Serum pepsinogen (PG) levels have long been established as potential indicators of the gastric mucosal integrity, abnormal levels of which are interpreted as defects in their corresponding producer cells. We have investigated the efficiency of these serum biomarkers plus serum *H. pylori* (Hp) status in detection of gastric inflammation, atrophy and gastric cancer among an Iranian population. Histopathological studies on gastric specimens were performed according to the updated Sydney system. Serum anti-Hp IgG and PG levels were measured by ELISA and their efficiency in predicting the risk associated with gastric tissue damage was investigated. Logistic regression analysis, adjusting for age and gender, revealed that Hp-positive subjects were at respectively 8.7 and 4.2-folds excess risk of developing gastritis and gastric cancer than Hp-negative subjects. The risk of gastritis was further (up to 9.8-folds) amplified in Hp-positive subjects with high serum PG II (>11.8 μg/L) levels. Moreover, the risk of gastric cancer was drastically (up to 17-folds) inflated in Hp-positive subjects with low serum PG I (<56 μg/L) levels.
Our data recommend evaluating serum Hp status in combination with serum PG I and II levels at the newly recommended cut-off values of 56 and 11.8 μg/L for better estimating the risk of gastric cancer and gastritis (inflammation and atrophy) respectively.

Abstract no.: P5.25

POLYMORPHISMS OF TNFA PROMOTER REGION IN PEPTICULCER PATIENTS

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Tumour necrosis factor alpha encoded by *TNFA* gen is known to be a key mediator in the inflammation process which is a preliminary condition for peptic ulceration. The constitutive immune response of the host determines the degree of the mucosal inflammation and could induce the development of a ulcer disease or even malignant tumour. Substitutions -308G>A, -857C>T, -863C>A, -1031T>C in the *TNFA* influence its transcription activity and thus are responsible for increase production of TNF-alpha. Accordingly, the polymorphisms could influence the risk of peptic ulceration and its connection with *H. pylori* infection. The aim of the study was to evaluate the relationship between frequency of allelic variants of -308G>A, -857C>T, -863C>A, -1031T>C and the presence of gastric ulcer on the ground of *H. pylori* infection. To estimate the *TNFA* haplotype frequencies and to evaluate their association with the occurrence of gastric ulcer. Gastric mucosa specimens taken from individuals with peptic ulcer disease. The presence of *H. pylori* infection was evaluated by rapid urease test. Subjects were genotyped by polymerase chain reaction with subsequent restriction enzyme digestion (PCR-RFLP). The results were analyzed statistically and frequencies of particular haplotypes were estimated. Investigated polymorphisms of *TNFA* gene showed the association with neither predisposition to development of peptic ulcer disease nor susceptibility to *H. pylori* infection in peptic ulcer patients. None of haplotypes composed of investigated polymorphisms was shown to be connected with increased predisposition to development of peptic ulcer disease or susceptibility to *H. pylori* infection among individuals with this disease.

P6 – Extra-Digestive Diseases and other Helicobacters, NSAIDs and Novelties

Abstract no.: P6.01

HELICOBACTER AND IDIOPATHIC PARKINSONISM: LEUKOCYTE-SUBSET COUNTS PROVIDE CLUES TO A SUBORDINATE PATHOGENIC PATHWAY

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Following *Helicobacter pylori* eradication, in idiopathic parkinsonism (IP), hypokinesia improves, flexor-rigidity increases (*Helicobacter* 2010;15:279–95). After antimicrobials for other indications, hypokinesia is unchanged, but rigidity increases (*Helicobacter* 2011;16 (Suppl 1):128). *Helicobacter* protects against lactulose-hydrogen-breath-test (LHBT) positivity for small-intestinal-bacterial-overgrowth (SIBO) (ibid). Despite relative lymphopenia, IP-probands have an increased proportion of circulating natural-killer-cells (Gut Pathogens 2009;1:20).

Methods: We explore relationship of IP-facets to peripheral immune/inflammatory-activation in light of presence/absence of *Helicobacter* (urea-breath- and/or stool-antigen-test: positivity confirmed by gastric-biopsy) or LHBT-positivity (4-hour, 25G lactulose).

Results: In 38 patients (untreated (17) or on stable long-t½, anti-parkinsonian medication), the higher the natural-killer-count, the shorter stride, slower gait and greater flexor-rigidity (by mean (95% CI) 49 (14, 85) mm, 54 (3, 104) mm/s, 89 (2, 177) Nm/10^3, respectively, per 100 cells/μL increment, after adjustment for patient-characteristics: $p = .007$, 0.04 and 0.04). T-helper-count was inversely-associated with flexor-rigidity before ($p = .01$) and after adjustment for natural-killer-count (-36 (-63, -10) Nm/10^3 per 100 cells/μL, $p = .007$). Neutrophil-count was inversely-associated with tremor (visual-analogue-scale, $p = .01$). Effect sizes were independent of anti-parkinsonian medication, not masked (except natural-killer-count/flexor-rigidity association) by including 13 patients receiving levodopa. Cellular associations held after allowing for potential confounding by hydrogen-breath-test- or *Helicobacter*-status. However, LHBT-positivity was associated with higher natural-killer- and T-helper-counts, lower neutrophils ($p = .005$, .02 and .008). Moreover, *Helicobacter*-positivity was associated with additional reduction in stride and speed (68 [24, 112] mm and 103 [38, 168] mm/s, each $p = .002$).

Conclusion: We propose a rigidity-associated subordinate pathway, flagged by higher natural-killer-count and lower T-helper, against which *Helicobacter* protects by keeping SIBO at bay.

Abstract no.: P6.02

GASTRIC CORPUS ATROPHY, BUT NOT *HELICOBACTER PYLORI* COLONIZATION IS ASSOCIATED WITH GASTROESOPHAGEAL REFLUX DISEASE

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Introduction: Prevalence of gastroesophageal reflux disease (GERD) is high and has a tendency to increase. Still controversial data exist about the association of *H. pylori* and GERD.

Aim: To study the association between GERD and gastric corpus atrophy (GCA), *Helicobacter pylori* colonization of gastric corpus and antrum mucosa (HCGC and HCGA, respectively) as well as the role of other risk factors: body mass index (BMI), smoking status (SS), gender.

Methods: Prospective patients due to current gastrointestinal symptoms underwent upper endoscopy with standard biopsy and further histological examination. Final sample contained 657 patients (mean age 47.5, range 18–84, Male 41.7%): 139 patients had erosive reflux esophagitis (ERD), 120 patients – non-ERD (NERD) and 398 patients were the control group. Statistical methods used *t*-test, χ^2 test, Mann–Whitney test and logistic regression analysis.

Results: HCGC and HCAC rate was significantly lower in ERD patients compared to the control group: 38.8% versus 50.5% ($p = .018$) and 38.8% versus 49% ($p = .039$), respectively. GCA (any grade) rate was significantly lower in ERD and NERD patients compared to the control group: 4.3% ($p = .001$) and 8.3% ($p = .039$), versus 15.8%, respectively. Mean BMI ($p = .001$) and rate of persons who ever smoked ($p = .002$) was significantly higher in the ERD group compared to control patients. In logistic regression analysis model GCA ($p = .016$), BMI ($p = .001$), gender ($p = .004$) and SS ($p = .023$) was significantly associated with the presence of ERD, while GERD was associated with GCA ($p = .016$), BMI ($p = .003$) and gender ($p = .024$). HCGC and HCAC were not independently associated with ERD or GERD.

Conclusions: Gastric corpus atrophy rather than *H. pylori* infection itself is negatively associated with GERD.

Abstract no.: P6.03

IMPACT OF *HELICOBACTER PYLORI* INFECTION ON ALZHEIMER'S DISEASE SEVERITY: A MOUSE MODEL STUDY

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Helicobacter pylori infection seems to play a critical role in extragastric diseases including Alzheimer's dementia (AD). We found that *H. pylori* infection was associated with a higher cognitive impairment in AD patients. Chronic *H. pylori* infection could worsen AD lesions *via* atherosclerosis and inflammation. Thus, our aim was to infect AD transgenic (Tg) mice and their wild type (WT) littermates by Helicobacter species in order to explore their cerebral and behavioural lesions. In a first study, C57Bl/6 mice were infected during 18 months with *H. pylori* SS1 strain (n = 6) or *Helicobacter felis* (n = 6) or left uninfected (n = 6). In a second study, two groups of Tg mice (APPswe+PS1dE9) and their WT littermates were infected with SS1 (n = 75) or *H. felis* (n = 75) or unfected (n = 75) and sacrificed after 3, 6 and 9 months. For the two studies, brain and stomach were processed to detect cerebral amyloid plaques (thioflavine S stain), astroglial and microglial cells (immunochemistry anti-GFAP and anti-iba one respectively) and gastric lesions (hematoxylin and eosin stain). For the second study only, spatial memory, social interaction and anxiety were tested before sacrifice. In the first study, no amyloïd plaque was observed in C57Bl/6 infected mice but astroglial activation tended to increase in the hippocampus of these mice compared to non-infected mice. Behavioural and histological experiments related to the Tg mice are currently in progress. Based on epidemiological arguments supporting an AD – *H. pylori* association, this murine model should provide elements on the impact of *H. pylori* infection on the brain.

Abstract no.: P6.04

MOLECULAR EPIDEMIOLOGIC ANALYSIS AND ANTIMICROBIAL RESISTANCE OF *HELICOBACTER CINAEDI* ISOLATED FROM SEVEN HOSPITALS IN JAPAN

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Helicobacter cinaedi colonizes the colons of human and animals and can cause colitis, cellulitis, and sepsis in humans, with infections in immunocompromised patients being increasingly recognized. However, methods for analyzing the molecular epidemiology of *H. cinaedi* are not yet established. A genotyping method was developed using multilocus sequence typing (MLST) and used to analyze 50 *H. cinaedi* isolates from Japanese hospitals in addition to 6 reference strains. Pulse-field gel electrophoresis (PFGE) results were also compared with the MLST results. Based on the genomic information from the CCUG18818 strain, 21 housekeeping genes were selected as candidates for MLST and were observed to have high homology (96.5–100%) between isolates. Following a comparison of the 21 housekeeping genes from eight *H. cinaedi* isolates, seven genes were chosen for MLST, revealing 14 sequence types (STs). The isolates from three hospitals had the same STs, but the isolates from the other four hospitals belonged to different STs. Isolates belonging to ST6 were analyzed by PFGE and showed similar

patterns, but the patterns were different between isolates. Isolates belonging to ST9, ST10, and ST11, which belonged to the same clonal complex, showed the same pattern between isolates. All isolates were found to contain mutations in GyrA and the 23S rRNA gene that confer ciprofloxacin and clarithromycin resistance, respectively, in *H. cinaedi*. These results raise concerns about the increase in *H. cinaedi* isolates resistant to clarithromycin and ciprofloxacin in Japan.

Abstract no.: P6.05

ENDOSCOPIC FINDINGS ACCORDING TO *HELICOBACTER PYLORI* IN PATIENTS WITH FUNCTIONAL DYSPEPSIA

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Background/Aims: This study verifies whether endoscopic findings differ according to *Helicobacter pylori* (*H. pylori*) infection in patients with functional dyspepsia.
Meterials and Methods: The study surveyed upper gastrointestinal symptoms of 382 patients with functional dyspepsia and conducted esophagogastroduodenoscopy. The endoscopic findings were classified according to the Sydney classification, as edema, erythema, friability, exudates, flat erosion, raised erosion, rugal hyperplasia, atrophy, visibility of vascular pattern, intramural bleeding spot, nodularity, respectively in antrum, body, and fundus.
Results: (1) The average age of 382 patients was 52.3 years. There were 176 males and 206 females, (2) Among 382 patients, 167 (43.7%) had epigastric pain syndrome, 215 (56.3%) had postprandial distress syndrome, (3) Among 167 epigastric pain syndrome, 85 (51.1%) patients were infected with *H. pylori;* whereas among 215 postprandial distress syndrome patients, 102 (47.6%) patients were infected with *H. pylori,* (4) Compared to *H. pylori* positive patients, those *H. pylori* negative patients had more raised erosions (44.1% vs 22.8%, $p < .05$), and intramural bleeding spots (11.8% vs 1.8%) in antrum, and more raised erosions (10.8% vs 5.3%, $p < .05$) and intramural bleeding spots (8.8% vs 0.9%, $p < .05$) in body.
Conclusions: Raised erosions and intramural bleeding spots in antrum and body were frequently observed in patients with *H. pylori* negative than positive.

Abstract no.: P6.06

THE PUTATIVE CONTINGENCY NATURE OF *HELICOBACTER BIZZOZERONII* NAD(P)H-NITROREDUCTASE HBZC1_00960

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The canine *H. bizzozeronii* (Hbz) is the only non-*H. pylori* gastric *Helicobacter* isolated from humans so far. Metronidazole (Mtz), in combination with tetracycline and lansoprazole, was used to treat a Hbz infection in a patient with chronic gastritis. However, the treatment failed to clean the stomach from Hbz, which was re-isolated after 5 months. SNPs analysis on isolates obtained after the treatment showed that, among the six possible nitroreductases present in the Hbz genome, only the oxygen-insensitive NAD(P)H-nitroreductase HBZC1_00960 (47% identity with *H. pylori RdxA* HP0954) was affected. We observed an insertion of a cytosine in a 3′ eight nucleotide stretch causing a frameshift of the C-terminal amino acid sequence of the protein. It has been shown that in Hpy the inactivation of *rdxA* confers resistance to Mtz with an increase of up to five times the MIC compared to the parental strains. In contrast, we reported only threefold increase of the MIC value for Mtz in mutant Hbz strains in which *rdxA* was deleted by insertion of a chloramphenicol resistance cassette. Furthermore, we have observed that Hbz is able to acquire spontaneously the same level of resistance with a frequency of about 10e-4 which is higher than value reported for Hpy. Spontaneous Hbz mutants resistant to Mtz showed instable poly cytosine stretch (C9-C10) in the 3′ terminal of *rdxA,* suggesting a possible contingency nature of this locus. The phase-variability of the Mtz resistance in Hbz is currently being studied.

Abstract no.: P6.07

THE SIGNIFICANCE OF POSITIVE GIEMSA STAIN CASES IN NEGATIVE UBT (UREA BREATH TEST) PATIENTS

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Background: The urea breath test is well known as a rapid diagnostic procedure used to identify infections by *H. pylori* which is based upon the ability of *H. pylori* to convert urea to ammonia and carbon dioxide. This study aimed to investigate the significance of positive Giemsa stain cases in human gastric biopsy specimens which were taken from negative UBT patients.
Methods: We selected 99 patients who took UBT and were taken endoscopic biopsy simultaneously. We compared Giemsa stain results and UBT results. Genetic analysis using Pyrosequencing technology was performed in positive Giemsa stain cases with negative UBT result.
Results: Fifty four out of 99 patients were negative for both UBT and Giemsa stain and thirty three were positive for both UBT and Giemsa stain. Two cases were positive for UBT, but negative for Giemsa stain. Ten cases were negative for UBT, but positive for Giemsa stain. All of 10 patients had no clinical symptoms and their gastric biopsy specimens were taken in the health care center. In the pyrosequencing analysis of 16S rRNA using nine human gastric biopsy specimens of these 10 cases, four *H. pylori* infected cases and five Campylobacter infected cases (three *C. hyointestinalis,* one *C. jejuni,* one *C. curvus*) were found.
Conclusion: *C. hyointestinalis* is the most common cause of non-*H. pylori* -related Giemsa positive infection in negative UBT patients.

Abstract no.: P6.08

PREVALENCE AND CHARACTERISTICS OF TYPE A GASTRITIS IN KOREA

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Background: *Helicobacter pylori* (*Hp*) has been shown to cause atrophic gastritis, but the progression process is still unknown. *Hp* infection is significantly associated with anti-gastric autoantibodies. The aim of study was to investigate whether autoimmune gastritis could affect the development of different spectrum of diseases in *Hp* infected persons.
Patients and Methods: In 212 outpatients referred to Youido St. Mary's Hospital, endoscopy was performed. *Hp* status was identified by biopsy specimens that were stained Warthin–Starry stain and/or urea breath test. Fasting serum pepsinogen I, II, gastrin and anti-parietal cell antibody (APCA) were checked.
Results: The fasting serum gastrin level of type A gastritis [n = 18, APCA(+), *Hp* (-)] was 96.9 ± 23.9 pg/mL, whereas type non-A/non-B gastritis [n = 96, APCA(-), *Hp* (-)] was 62.8 ± 5.8 pg/mL. Type A and B gastritis [n = 19, APCA(+), *Hp* (+)] was 144.1 ± 47.9 pg/mL [$p < .05$ vs APCA(-)], whereas type B gastritis [n = 79, APCA(-), *Hp* (+)] was 69.4 ± 6.6 pg/mL. Clinical spectrums of disease according to APCA and *Hp* were as following. The percentage of reflux esophagitis and duodenal ulcer were 33.3%, 2.1% in non-A/non-B gastritis group [APCA(-), *Hp* (-)], whereas 16.7%, 0% in type A gastritis group [APCA(+)/*Hp* (-)]. Type B gastritis group [APCA(-), *Hp* (+)] showed 34.2%, 6% and type A and B gastritis group [APCA(+), *Hp* (-)] showed 21.1%, 5.2%.
Conclusion: With this results, type A gastritis patients had a significant higher level of gastrin compared to non-A gastritis patients regardness of *Hp* infection. Autoimmune gastritis could affect the development of gastrin related disease in patients with *Hp* infection.

Abstract no.: P6.09

DETECTION OF *HELICOBACTER PYLORI* AND ENTEROHEPATIC HELICOBACTER IN BILE OF PATIENTS WITH CHRONIC HEPATITIS C

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Aim: Detection of Helicobacters in bile of patients with chronic hepatitis C and different pathologies of gastrointestinal tract.
Material and Methods: The material for study was bile samples of 33 patients, taken in duodenal intubation. The group of HCV patients consisted of seven patients aged from 24 to 49 years. The group of patients with gastrointestinal disorders included 26 people aged from 22 to 64 years. In 46% of cases chronic noncalculous cholecystitis was diagnosed. Detection of Helicobacter was performed by PCR. Specific primers were used to determine generic genes of Helicobacter, allowing to generate amplicons 16S rDNA. If result was positive, amplification with using specific primers for detection of *H. pylori, H. bilis, H. pullorum, H. rappini* was performed.
Results: DNA of bacteria of the genus Helicobacter was detected in 12 cases (36.4%): in the group of HCV patients – in 1 patient (14%, *H. rappini* was found), in patients with gastrointestinal disorders – 11 (42%). Positive for bacteria of the genus Helicobacter DNA samples were subsequently tested with species-specific primers. In seven cases, the samples were positive for 23S rRNA gene of *H. pylori* and in the same number – for gene ureB *H. rappini*. Mixed-infection with *H. pylori* + *H. rappini* was diagnosed in five patients (gastrointestinal disorders group). All samples for gene cdtB *H. pullorum* and cdtB *H. bilis* were negative.

Conclusions: These data indicate a high incidence of biliary tract colonization by bacteria of the genus Helicobacter, and in most cases with extragastric disorders, which may indicate their possible participation in the pathogenesis of these diseases.

Abstract no.: P6.10

RECENT ERADICATON RATE OF MOXIFLOXACIN-CONTAINING TRIPLE THERAPY AS SECOND-LINE TREATMENT FOR *HELICOBACTER PYLORI* IN KOREA

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Backgrounds and Aims: Eradication rate of 7-day moxifloxacin-containing triple therapy with a proton pump inhibitor, moxifloxacin, and amoxicillin for second-line treatment was reported up to 80%. The efficacy is expected to decrease continuously due to increasing antibiotics resistance. The aim of this study is to evaluate the efficacy and tolerability of the triple therapy regimen containing PPI, moxifloxacin, and amoxicillin as a 2nd-line eradication treatment in Korea recently.
Materials and Methods: A total 91 patients (mean 56.05 ± 11.7 male 49.5%) of *Helicobacter pylori* infection previously treated with conventional triple therapy and failed to treat were enrolled in this study between December 2009 and January 2012. All patients were treated with moxifloxacin-containing triple therapy as second-line therapy for 7 days. Successfulness of eradication was evaluated by the ^{13}C-urea breath test at least 4 weeks later after end of treatment. All patients were asked to fill in a validated questionnaire to report therapy-related side effects. Each symptom was graded from absent or present.
Result: The eradication rate of the moxifloxacin-containing triple therapy for 7-days was 56.0% (51/91) by intention-to-treat analysis, and 56.2% (50/89) by per-protocol analysis. The regimen was well tolerated and side effects were comparable.
Conclusion: The moxifloxacin containing-triple therapy failed to achieve the acceptable eradication rates, but had no significantly side effect in our study. This study shows that the rate might be decreased in Korea recently. This regimen may not seem to be a suitable choice as a second-line *H. pylori* eradication therapy in Korea.

Abstract no.: P6.11

RECENT ERADICATION RATE OF RESCUE QUADRUPLE THERAPY AS SECOND-LINE FOR *HELICOBACTER PYLORI* IN KOREA: A RANDOMIZED RETROSPECTIVE STUDY

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Backgrounds and Aims: Eradication rate of standard quadruple therapy using a proton-pump inhibitor, bismuth, metronidazole, and tetracycline was reported about 80% in Korea. The efficacy is expected to decrease continuously due to increasing antibiotics resistance. The aim of this study is to assess the eradication rate and evaluate the tolerability of standard quadruple therapies in recent years of Korea.
Materials and Methods: A total 136 (mean age 57.34 ± 13.1 male 58.1%) patients with proven *H. pylori* infection regardless of previous treatment regimen were enrolled in this study between July 2004 and February 2012. 114 (mean age 57.91 ± 12.8 male 59.6%) patients was treated for 7 days and 22 (mean age 54.36 ± 14.5 male 50.0%) patients was treated for 14 days. Successfulness of eradication was evaluated by the ^{13}C-urea breath test at least 4 weeks later after end of treatment.
Result: The eradication rate of the standard quadruple therapy for 7-day and 14-day was 74.6% (85/114) and 68.2% (15/22) by intention-to-treat analysis, 75.9% (85/112) and 71.4% (15/21) by per-protocol analysis. The difference between the two groups was not statistically significant ($p = .535$). Total eradication rate without regard for treatment duration was 73.5% (100/136) by intention-to-treat analysis and 75.2% (100/133) by per-protocol analysis.
Conclusion: Compared to previous report about eradication rate of standard quadruple therapy in Korea, this study shows that the rate might be decreased in recent years. Because the eradication rate could be more decreased, further study for alternative treatment regimen could be needed.

Abstract no.: P6.12

AN INNOVATIVE FORM OF PANTROPRAZOL IN THE TREATMENT OF *HELICOBACTER PYLORI* INFECTION ASSOCIATED WITH NSAID-GASTROPATHY

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Helicobacter pylori (H.p.) infection in patients on NSAIDs is a modifiable risk factor for complications. The lack of acid inhibition leads to the absence of epithelialization in erosions of the stomach and duodenum, as well as failure in Hp eradication. The purpose of our trial was to study the effect of acid suppression by innovative pantoprazole (Nolpaza) in the treatment of H.p associated NSAID-gastropathy. We examined 25 patients with erosive lesions in the mucous membrane of the antral stomach and duodenal ulcers in patients receiving non-steroidal anti-inflammatory drugs. In order to eradicate H.p., patients received a 7-day course of drug therapy: Nolpaza, 0.04 g × b.i.d., clarithromycin 0.5 g × b.i.d., amoxicillin, 1.0 g × b.i.d. To evaluate the effectiveness of prescribed treatment, we has performed the 24 hours gastroesophageal pH monitoring for 5 days from the initiation of treatment and control test of Hp eradication after 4 weeks of discontinuation of pantoprazole. Duration of acid suppresion action (intragastric pH >3.5 units) Pantoprazole was 23.2 ± 0.94 hours per day. In assessing the duration of time intragastric pH >5 units, duration was 21.4 ± 0.56 hours. We have not established the existence of ''the phenomenon of nocturnal acid breakthrough'' by 5 days treatment of pantoprazole. Hp eradication was achieved in 24 (96%) of patients with NSAID-gastropathy. Pantoprazole (Nolpaza) at a dose of 0.04 g × b.i.d.-meets the requirements of modern pharmacotherapy of acid suppresion and eradication of *H. pylori* in patients with *Helicobacter pylori* associated NSAID-gastropathy.

Abstract no.: P6.13

EFFICACY OF TRIPLE THERAPY INCLUDING A PPI PLUS AMOXICILLIN AND METRONIDAZOLE (PAM) FOR TREATMENT OF *H. PYLORI* INFECTION: PRELIMINARY REPORT OF A SYSTEMATIC REVIEW

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Introduction: Clarithromycin-including triple therapy does not achieve acceptable cure rates in areas where primary resistances to this drug are high. By contrast, the efficacy of triple therapy including a PPI plus amoxicillin and metronidazole (PAM) is largely independent of in vitro antibiotic resistances. Furthermore, as plasmatic half-lives of both amoxicillin and metronidazole are short, administering these drugs three times a day might increase cure rates. The present study aims to systematically review the efficacy of PAM, putting emphasis on the effect of drug dosing and treatment duration.
Methods: A systematic review was performed in Medline collecting articles published until May 2012 that included at least one branch of PAM therapy. Sub-analysis were performed according to the number of antibiotic and PPI daily doses and according to treatment duration. We calculated the efficacies and its 95% confidence intervals.
Results: One hundred and ten studies (144 treatment arms) with 10312 patients were included. Overall cure rates were 79% by intention to treat and 81% per protocol. Main sub-analysis results are shown in the table:
In addition, in the subgroup of 339 patients (six studies) who received a 14-day treatment with t.i.d. antibiotics and b.i.d. PPI, per protocol efficacy was 91%.
Conclusions: Fourteen-day three times a day PAM therapy may be a simple, cheap and efficacious alternative to currently recommended quadruple therapies for treatment of *H. pylori* infection.

Antibiotic schedule	Intention to treat			Per protocol		
	N	Efficacy (%)	IC 95%	N	Efficacy (%)	IC 95%
t.i.d	2405	81	80–83	2190	83	81–85
b.i.d.	5657	76	75–77	3938	80	79–81
Treatment duration						
14 days	3265	79	77–80	2333	83	81–85
10 days	862	71	68–74	708	76	73–80
7 days	5331	77	75–78	4816	80	79–81
PPI						
High dose	9337	79	78–80	7955	82	81–83
Low dose	975	69	66–72	744	74	71–77

Abstract no.: P6.14

LEPTIN IS ASSOCIATED WITH SYMPTOMS IN FUNCTIONAL DYSPEPSIA WITHOUT *HELICOBACTER PYLORI* INFECTION

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Background and Aims: Symptoms of functional dyspepsia (FD) are caused by certain pathophysiological mechanisms including impaired gastric motility. Leptin and ghrelin, known as the gastric hormones, have been shown to control gastrointestinal (GI) motility as well as feeding behavior. The present study aimed to investigate whether leptin or ghrelin is associated with GI symptoms in patients with FD.

Methods: Forty seven patients with FD according to the Rome II criteria, whose endoscopic biopsies revealed no *Helicobacter pylori* infection (18 males, 45.2 ± 1.8 years, 17 with dysmotility-like dyspepsia and 30 with ulcer-like dyspepsia) and 18 controls (8 males, 45.7 ± 3.4) were enrolled for one consecutive year. They all filled out a questionnaires composed of upper GI symptoms such as nausea, vomiting, abdominal discomfort, abdominal pain, epigastric sorness, and reflux. Serum levels and gastric mucosal mRNA expressions of leptin and ghrelin were measured and compared between each group by multivariate analyses.

Results: There were no differences between FD groups and control in the serum levels of leptin and ghrelin and in the gastric mucosal mRNA expressions of leptin and ghrelin. However, gastric mucosal mRNA expressions of leptin were significantly increased in dysmotility-like dyspepsia subgroup ($p = .029$). In the FD group, the patients with vomiting tended to express higher leptin mRNA at the fundus than those without ($p = .070$).

Conclusions: Our results suggest that gastric leptin expressions are associated with symptoms of FD, and particularly with vomiting. Leptin can be speculated to be a target to control symptoms in managing FD.

Abstract no.: P6.15

CLINICAL PICTURE OF DYSPEPSIA: ROLE OF HP INFECTION, LACTOSE INTOLLERANCE AND YELLOW STOMACH

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Background: Lactose intolerance, biliary reflux and Hp infection are common clinical conditions in patients with gastrointestinal disorders. Frequently symptoms reported in each condition are similar even if related to the presence of different pathogenetical factors. Therefore it would be useful to distinguish different pathophisiological pattern of symptoms in order to establish an appropriate treatment.

Aims: To evaluate the frequency of symptoms reported by patients with lactase deficiency and to assess whether the presence of Hp infection and/or biliary reflux modify clinical presentation.

Materials and Methods: We enrolled 114 dyspeptic patients undergoing upper endoscopy, referring history of lactose intolerance. These patients received a questionnaire to assess symptoms in relation with lactose intake. In addition to gastric biopsies, Quick Lactase Test (QLT, Biohit, Helsinki, Finland) is also performed to confirm the lactase deficiency.

Results: QLT was positive in 104 (91%, female 78, mean age 47 ± 14). 16% of patients with hypolactasia also had a Hp-related gastritis and 13% had biliary reflux in stomach. Only two patients experienced the three conditions at the same time. The abdominal distension is the most represented symptom (90%), followed by borborygms (62%), diarrhoea (56%), abdominal pain (52%) and nausea (35%). There were no differences in the distribution of clinical symptoms in relation to the severity of lactase deficiency or to presence of Hp infection and/or bilary reflux.

Conclusions: Our study show that in patients with persistent gastrointestinal disorders it is necessary to associate further investigations after the clinical evaluation, to identify the correct etiology for the sympthoms.

Abstract no.: P6.16

EVALUATION OF ANTIMICROBIAL AND UREASE INHIBITORY ACTIVITIES OF *ARISTOLOCHIA ARGENTINA* ON *HELICOBACTER PYLORI* STRAINS

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Helicobacter pylori survives and persists in human stomach due to the urease enzime, which provides the alkaline microenvironment. Antibiotic therapy can kill planktonic cells of *H. pylori,* however they are not effective in cells within a biofilm. The increase of antibiotic resistance demands the search for alternative strategies. *Aristolochia argentina*, is used in folk medicine for gastrointestinal disorders in Cuyo, Argentina. The aim of this study was to evaluate antimicrobial activity of *A. argentina* extract on planktonic and established *H. pylori* sessile cells and their urease inhibitory activity. The minimum inhibitory concentration (MIC) of *A. argentina* was assayed by agar dilution method using serial dilutions in range of 64 at 0.032 mg/mL. The *H. pylori* strains, NCTC11638 and five clinical isolates were assayed. The effect of 1.5 mg/mL *A. argentina* extract on *H. pylori* adhered to an abiotic surface was determined using plate counting; the morphologic changes were observed by optical microscopy and urease expression was determined by RT-PCR. A. argentina showed activity against *H. pylori* with MICs between 2 and 16 mg/mL. In established biofilms, the extract decreased biofilm biomass ($p \leq .05$), induced 100% *H. pylori* coccoid forms and inhibited the urease expression after 26 hours of treatment. *A. argentina* could be a new strategy for an effective therapy againts *H. pylori* infection due to its antimicrobial activities on *H. pylori* planktonic and sessile cells and the inhibition of urease expression at low concentrations.

Abstract no.: P6.17

MISSED SYNCHRONOUS GASTRIC NEOPLASM WITH ENDOSCOPIC SUBMUCOSAL DISSECTION FOR GASTRIC NEOPLASM: EXPERIENCE IN A SINLGE CENTER

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Purpose: The aim of this study was investigate the incidence and characteristics of missed SGN during follow-up endoscopy in patients who have undergone ESD.

Material and Methods: We investigated the clinicopathological features of 602 patients and gastric neoplasms treated by ESD from January 2005 through July 2009 at our institution. We defined any second neoplasm found within 1 year after ESD as a missed SGN.

Results: Out of 602 patients, 12 (2.0%) had missed SGNs. Among the 12 missed SGNs, 7 (58.3%) cases were carcinomas. All cases of missed synchronous gastric cancer (SGC) were exclusively discovered in the posterior wall of the stomach (seven of seven cases, 100%, $p = .016$). Missed SGNs were more frequently observed when the primary gastric neoplasm was adenoma (4.0% vs 1.0%; OR = 4.114; 95% CI = 1.224–13.831). Furthermore, the risk of missed SGC increased 12-fold in the primary gastric adenoma group compared to the primary gastric carcinoma group (2.9% vs 0.24%; OR = 12.308; 95% CI = 1.472–102.939).

Conclusions: Endoscopists need to make an effort to find SGN, especially when they perform ESD for an adenoma, which is a less serious lesion. The important blind spot in screening endoscopic examination before ESD is the PW of the upper third and middle third of the stomach.

Abstract no.: P6.18

CLINCAL IMPACT OF SECOND-LOOK ENDOSCOPY AFTER ENDOSCOPIC SUBMUCOSAL DISSECTION FOR GASTRIC NEOPLASMS

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Background/Aim: One major complication of endoscopic submucosal dissection (ESD) is delayed bleeding. Most hospitals routinely perform second-look endoscopy to reduce the chance of delayed bleeding without solid evidence supporting this practice. The aim of this study was to evaluate whether second-look endoscopy prevents delayed bleeding and to evaluate the clinicopathological features associated with delayed bleeding to identify lesions that may need second-look endoscopy.

Methods: We investigated 392 lesions in 388 patients who underwent ESD for early gastric cancer from January 2006 to July 2011. Delayed bleeding was defined as clinically evident bleeding from mucosal defects 24 hours after ESD. We reviewed data including characteristics of patients, lesions, and procedures. The incidence of delayed bleeding before and after second-look endoscopy performed within 3 days of ESD was also investigated to determine the utility of second-look endoscopy.

Results: Delayed bleeding was evident in 12 of 392 lesions (3.1%), all of which achieved endoscopic hemostasis. The only significant factor predicting delayed bleeding was a resected specimen over 40 mm in size (OR = 6.200, 95% CI = 1.912–20.108). Delayed bleeding occurred more frequently before second-look endoscopy than after (p = .022).

Conclusions: From our data on ESD for early gastric cancer it is too early to conclude that second-look endoscopy is not a valuable procedure; rather, our findings indicate that second-look endoscopy may be useful for preventing post-ESD bleeding, especially for resected specimens over 40 mm in size.