# ΠΡΟΣΚΕΚΛΗΜΕΝΕΣ ΞΕΝΟΓΛΩΣΣΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ ΕΛΛΗΝΩΝ ΕΡΕΥΝΗΤΩΝ

**A MODIFIED QUIKCHANGE II SITE-DIRECTED MUTAGENESIS METHOD FOR HE 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* status 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 p cagA sequence downstearn 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 repretitive gene sequences is desirable.

**EVALUATION OF APLICABILITY 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/*2 type patients. Five patients were homozygous extensive metaolizers (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) + amoxicillin 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.

**TEN DAYS SEQUENTIAL TREATMENT FOR *HELICOBACTER PYLORI* ERADICATION IN CLINICAL PRACTICE IN GREECE**

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**Introduction:** Eradication rates of *Helicobacter pylori* infection with standard triple therapy are extremely low. Sequential treatment seems very effective, but the majority of studies were performed in Italy.
**Aims and Methods:** To access, through a pilot study, the cure rate, acceptability and safety of the sequential treatment, as first line therapy for *Helicobacter pylori*. In Greece the reported clarithromycin resistance approximates 20% or even higher, while that to metronidazole has been reported at 49%.
Prospective single center study of two years duration. All patients included in the study were diagnosed with peptic ulcer disease or non ulcer dyspepsia by endoscopy. *Helicobacter pylori* infection was documented by at least two positive tests among rapid urease test, gastric histology, 13 C Urea Breath test. Patients were randomly assigned to one of the following therapies: A. 10 days conventional triple therapy (CT) including a proton pump inhibitor 20 mg plus Clarithromycin 500 mg and Amoxicillin 1000 mg, all twice daily. B. 10 days sequential treatment (ST) consisting of a proton pump inhibitor 20 mg and Amoxicillin 1000 mg for the first five days following by proton pump inhibitor 20 mg. Clarithromycin 500 mg and Metronidazole 500 mg for the remaining five days, all twice daily. *Helicobacter pylori* eradication was checked using a 13 C Urea Breath test 8 weeks after treatment. Intention to treat (ITT) and per protocol (PP) eradication rates were determinate.
**Results:** 135 patients per arm of therapy 60% men, mean age 51.9$\pm$9.2 years in CT and 50.8$\pm$8.8 years inST (p NS). Eradication was achieved in 95/122 who returned for the follow up in the CT thus ITT was 70.4% (95% CI 58-74%) and PP was 78% (95% CI 55-84%). In ST eradication was achieved in 110/121 who returned for the follow up thus ITT was 81.4% (95% CI 78-94%) and PP was 91% (95%CI 83-97%). Difference in ITT 11% (95% CI 3-25%) and in PP 13% (95%CI 5-25%). Adverse events were 14.8% in the CT. 15.7% in the ST (p NS). Drugs compliance was 95% and 96% in the two treatment groups.
**Conclusion:** The sequential treatment appears to be an effective and well tolerated treatment option as first line treatment in Greece, although its performance in our area in inferior than the reported from Italy and Spain.

**A COMPARATIVE EPIDEMIOLOGIC STUDY OF PEPTIC ULCER DISEASE IN GREECE. A TALE OF TWO CITIES**

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**Introduction:** The incidence of peptic ulcer disease seems to be declining in Western countries. Possible explanations could be the eradication of *Helicobacter pylori* (H pylori) and the rise in living standards.
**Aims and Methods:** The aim of this study was to compare epidemiological data on peptic ulcer disease in two Prefectures of Greece with similar prevalence of *H. pylori* infection and different living standards in 2005 and 2006. The prefecture of Ioannina a rather poor part of Greece with living conditions below average and the prefecture of Chios with the third best living conditions in the country. Data on endoscopic findings of peptic ulcer in the two general Hospitals of Chios and Ioannina were compared.
**Results:** The prevalence of *H. pylori* infection in Chios was 11.6% and in Ioannina 9.9% (p=NS). During 2005, in Ioannina 119 peptic ulcers were documented on 756 Upper GI endoscopies (UGIE) (15.7%), whereas in Chios 40 peptic ulcers were documented after 392 UGIE (10.2%), p=NS). During 2006, in Ioannina 103 peptic ulcers were documented on 815 Upper GI endoscopies (UGIE) (12.6%), whereas in Chios 45 peptic ulcers were documented after 390 UGIE (10.1%) (p=NS). When we looked at bleeding ulcers and ulcers with a high risk for rebleeding in 2005, 47% of ulcers in Ioannina fell into that category whereas in Chios only 27% fell into that category ($p<0.05$). For 2006 percentages were 46% for Ioannina and 21% for Chios ($p<0.03$). We were able to demonstrate previous proton pump inhibitor use in 25.1% of patients undergoing UGIE in Ioannina and in 80.8% in Chios.
**Conclusion:** Differences in living standards within Greece do not change the epidemiology of peptic ulcer disease. Prior use of a PPI seems to prevent the genesis of high risk ulcers.