ΠΡΟΣΚΕΚΛΗΜΕΝΕΣ ΞΕΝΟΓΛΩΣΣΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ ΕΛΛΗΝΩΝ ΕΡΕΥΝΗΤΩΝ

Primary and secondary antimicrobial resistance of *Helicobacter pylori (Hp)* clinical isolates from greek adult patients

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Helicobacter 2013;18(Suppl. 1)

The increasing prevalence of antimicrobial resistant *Helicobacter pylori* strains is a major cause of treatment failure. We evaluated primary and secondary resistance of *Hp* strains from Greek patients to amoxicillin-(AMO), metronidazole-(MET), tetracycline-(TET), clarithromycin-(CLA) and levofloxacin-(LEV). Furthermore, we determined *Hp* gene mutagions associated with CLA and LEV resistance. The study enrolled 266 *Hp* isolates from adult patients (age 52.3±14.3), 160 of which had undergone at least one failed course of treatment and 106 that have not received any previous eradication therapy or PPIs. *Hp* strain susceptibility was assessed by the E-test method, according to the 3rd European Multicentre Study Group adopting the MIC breakpoints for AMO and LEV (>0.5 mg/L), CLA and TET (>1 mg/L) and MET (>8 mg/L). Presence of genetic mutations were determined by Real-Time PCR (Light Cycler, Roche) in CLA-resistant and by sequencing analysis of *Hp gyrase A* gene in LEV-resistant strains.

No resistance to AMO or TET was detected. Primary resistance levels to MET and CLA were determined at 37.7% (40/106) and 23.6% (25/106), respectively. High levels of secondary resistance to MET (50.6%, 81/160) and CLA (72.3%, 114/160) were also observed. Primary (11.3%) and secondary (15.6%) LEV resistance was observed in isolates that were also resistant to MET and/or CLA. The predominant mutations correlated with CLA-resistance were A2143G and A2142G in *23S rRNA* gene and the Asn87Lys mutation in *gyrA* gene for LEV-resistance strains.

Antimicrobial susceptibility testing should be considered, prior to the selection of the proper antibiotic scheme, in order to achieve greater eradication rates in Greece.

Sequential treatment against *H. pylori* infection in a Greek population-a multivariant analysis

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Helicobacter 2013;18(Suppl. 1)

Introduction: In Greece high clarithromycin and metronidazole resistance plus unavailability of the alternative first-line treatments make sequential treatment (ST), the realistic first-line choice for *H. pylori* eradication.

Aim: Presentation of preliminary results of a prospective study using as first-line the ST in a Greek population and search for possible factors affecting successful *H. pylori* eradication.

Method: Sixty-two outpatients (average age:51.84 years), diagnosed as H.P-positive, were interviewed using a preformed questionnaire, recording sex, age, smoking, alcohol consumption, NSAID and long-term PPI intake, symptoms, anemia, upper GI personal history and family history (FH) of gastric Ca. All received 10-day ST: PPIs x 2 with: Amoxicillin 1gX2, the first 5 days and Clarithromycin 500mgX2 plus Tinidazole 500mgX2, the next 5 days. Six to eight weeks after ending of treatment, all patients received UBT and compliance control.

Results were analyzed by ITT (intension-to-treat) and PP (per-protocol) analysis and were compared with x^2 (Yates correction) and logistic regression analysis.

Results: Sixty-two treated patients. Four lost to eradication control. Fifty-three eradicated and five not eradicated H.P.

PP: 91.37%-ITT: 85.48%.

Eradicate	/	Age 45>/45<	Smoking -/+/ex	Alcohol -/+	NSAID -/+	Dyspepsia -/+	GOR -/+
+	25/28 2/3	34/19 5/0	23/17/13 3/0/2	49/4 3/2	48/5 5/0	18/35 1/4	20/33 2/3
Eradicate	Anemia -/+		Gastric ulcer -/+	Duodenal ulcer -/+		PPIs -/+	FH Ca -/+
+ -	37/16 3/2		50/3 3/2	42/11 5/0		47/6 3/2	50/3 5/0

Statistical analysis revealed a reverse correlation between patient's age and the probability to eradicate H.P. (p=0.028).

Conclusion: ST used on a Greek population as first-line eradication of H.P. meets international requirements and overcomes resistance and availability problems. Of factors studied, only younger age of treated patients seems to increase probability of eradication.

Sequential versus classical triple treatment study in a greek population

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Helicobacter 2013;18(Suppl. 1)

Introduction: Maastricht consensus IV data raises serious questions for *H. pylori* eradication in Greek population regarding the effectiveness of triple treatment as well as first-line treatments with metronidazole.

Aim: Presentation of the preliminary results of a first-line treatment prospective study of Sequential Treatment (ST) for eradication of *H. pylori* versus Classical Triple Treatment (CTT).

Methods: One hundred and fifty-two *H. pylori* positive out-patients were randomized to receive a 10 day regiment either with:

1) CTT: PPIs X 2, Clarithromycin 500 mg X 2, Amoxicillin 1 g X 2, 2) 90 patients (average age: 54.41 years, M:40, F:50) or ST: PPIs X 2 plus:

Amoxicillin 1 g X 2, for the first 5 days and Clarithromycin 500 mg X 2 and Tinidazole 500 mg X 2 for the next 5 days.

Sixty-two patients (average age: 51.84 years, M:29, F:33)

All patients underewent eradication test with UBT at least 6 weeks after completion of treatment. Results were analyzed by ITT (intension-to-treat) και PP (per-protocol) analysis and were compared with x^2 (Yates correction) and logistic regression analysis.

Results: There is a statistical significance among the eradication percentages of ST and CTT (P<0.001). ST has a 6.6 greater possibility to eradicate *H. pylori* compared to CTT.

	Total patients	Lost to control	Eradicated	Eradicated % PP	Eradicated % ITT	Not eradicated	Not eradicated % PP	Not eradicated % ITT
CTT	90	4	53	61,62%	58,88%	33	38,37%	36,66%
ST	62	4	53	91,37%	85,48%	5	8,62%	8,06%

Conclusion: ST should be the eradication regimen of choice for Greek *H. pylori* positive patients regarding the international criteria for *H. pylori* eradication.

A randomized study comparing 10 days concomitant and sequential treatments for the eradication of *Helicobacter pylori*, in a high clarithromycin resistance area

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Helicobacter 2013;18(Suppl. 1)

Aims: Our study compares the effectiveness and safety of quadruple non-bismuth "comcomitant" and "sequential" regimens for *H. pylori* eradication in a high clarithromycin resistance area.

Patients and methods: This is a prospective randomized clinical trial in three participating centers from Greece. Up to now we have included 90 *H. pylori* positive patients, without previous eradication attempt, with functional dyspepsia or peptic ulcer disease. All patients had a positive CLO-test and/or histology and/or culture. They were randomized to receive either sequential (esomeprazole 40mg and amoxicillin 1g bid for 5 days, followed by 5 days of esomeprazole 40 mg, clarithromycin 500 mg and metronidazole 500 mg), or concomitant treatment (all drugs taken concomitantly for 10 days). Eradication was confirmed by 13C-urea breath test or histology 4-6 weeks after treatment. Adverse events and adherence to treatment were evaluated.

Results: Forty-five patients (22F/23M, aged 18-81, mean 55 years, 25.5% smokers, 21.4% with ulcer disease) allocated to concomitant and 45 (20F/25M, aged 24-94, mean 50.8 years, 28.5% smokers, 23.2% with ulcer disease) to sequential treatment. Eradication rates were, respectively, 89% versus 82% by intention to treat (p=ns) and 93% versus 84% (p=ns) per protocol. Adherence to treatment was overall 98% (95%CI 95.9-99.6) and comparable among treatments. Treatments related side effects were reported in 45% of patients, without differences among treatment arms. Only one patient under sequential experienced severe abdominal distension.

Conclusions: Concomitant treatment has a non-statistically significant advantage (9%) over sequential therapy and was the only one overcoming 90% per protocol in a high clarithromycin resistance area. Both regimens were well tolerated and safe for the patients.

Phosphorilation of bacterial effector CAGA may be required for the induction of molecules involved in extracellular matrix remodeling in *Helicobacter pylori* experimental in vitro infection of gastric epithelial cells

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Helicobacter 2013;18(Suppl. 1)

Following its translocation inside gastric epithelial cells, CagA is hierarchically tyrosine phosphorylated by Src and Abl kinases, on repetitive EPIYA sequences, inducing the appearance of a scattering phenotype resembling the epithelial to mesenchymal transition. In western clinical isolates the type of EPIYA motifs varies depending on the surrounding sequence namely, EPIYA-A:EPIYAKVNK, EPIYA-B:EPIYAQVAKK and EPIYA-C:EPIYATIDDLG. The number of EPIYA-C repeats has been positively correlated to scattering. We investigated the potential involvement of CagA protein in the activation of matrix metalloproteinase-9 (MMP-9) and its activator MMP-3 in *H. pylori*-infected gastric epithelial cells (AGS). We utilized isogenic P12 *H. pylori* mutants, expressing CagA protein with variable numbers of functional EPIYA-C and phosphorylation-deficient EPIFA-C motifs, as well as the corresponding P12 cagA- and cage- knock out strains. AGS cells were infected in vitro and MMP-specific transcriptional activation was measured by quantitative reverse transciptase Real Time PCR, at several time points. MMP expression in total cell lysates and cell culture supernatants was also determined by western blot analysis at 24 hours post-infection. Nearly 100-fold increase in MMP-3 and 80-fold increase in MMP-9 was observed in the presence of CagA protein and proportional to the number of EPIYA-C terminal motifs. On the contrary, infection with CagA phosphorylation-deficient or the cagA-and cage-knock out mutants, induced only background levels of MMP transcription. CagA-dependent increase in gelatinase and caseinolytic activity was detected in infected supernatants, utilizing zymography. Phosphorylation of the CagA effector may be required for induction and secretion of MMP-3 and MMP-9 in experimental *H. pylori* infection.

Prevalence of CYP2C19 polymorphisms in a subgroup of *Helicobacter pylori* positive (HP+) Greek patients

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Helicobacter 2013;18(Suppl. 1)

Background and Aim: Proton pump inhibitors (PPI's) related differences in HP eradication are partly due to CYP2C19 polymorphisms. Their prevalence, correlation with antibiotic resistance molecular tests and role in eradication treatment regimes has not been studied in HP+ Greek patients.
Patients and Methods: One hundred twenty-three patients undergone upper GI endoscopy for various GI symptoms and 59 were tested (+) for HP infection. Molecular genetic test is available to identify HP (GenoType Helico DR Test-HAIN). A multiplex PCR and DNA strip hybridization were performed for restistance to clarithromycin (significant

mutation of 23S gene -positions 2146 and 2147) and fluoroquinolones (gyr A gene-codons 87 and 91). 59 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 27/59 patients (45.7%). Only 2 patients (3.38%) were poor metabolizers (PM, *2/*2). There were no *3/*1 or *3/*2 type patients. 11 patients were homozygous extensive metabolizers (HomEM, wild type, *1/*1) and 3 patients were poor metabolizers (PM, *2/*2) from the clarithromycin resistant HP+ patients group (13/59, 22%). The three HP+ patient who were resistant to fluoroquilolones were HetEM (*2/*1). Eradication with 14 days regime (PPI+clarithromycin+amoxycilin) was near 96%.

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.