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Ahmed Outlioua, Wafaa Badre, Christophe Desterke, Zerif Echarki, Nadira El Hammani, Monsef Rabhi, Myriam Riyad, Mehdi Karkouri, Damien Arnoult, Abdelouahed Khalil, et al.

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Abstract: Introduction: *Helicobacter pylori* induces acute gastritis that can progress to serious diseases such as gastric cancer. *H. pylori* interacts with host cells within the gastric mucosa, resulting in activation of multiple innate immune signalling pathways, leading to pro-inflammatory cytokines production and immune cells recruitment. Various studies have shown that there are ethnic- and population-related differences in the expression of these cytokines. Although the *H. pylori* infection is a major public health problem in Morocco, to our knowledge, no study has been carried out in gastric cytokine expression from *H. pylori*-infected Moroccan patients. Thus we aimed to i) determine the IL-1 β , IL-8 and IL-17A gene expression in gastric biopsies from Moroccan patients infected with *H. pylori*, and ii) to determine the cytokine signature of each pathological stages associated with this infection. Material and methods: 71 patients with epigastralgic pain were included in this study. The *H. pylori* detection on gastric biopsies was performed by histopathological and PCR analysis. The IL-1 β , IL-8 and IL-17A mRNA expression in the antrum and fundus biopsies was performed by RT-qPCR. Results: The histopathological and PCR analyses revealed that 87.32% of the patients were infected with *H. pylori*. IL-1 β mRNA expression was significantly lower in the antral mucosa of *H. pylori*-infected patients ($p = 0.0038$) than in the uninfected while there was no significant difference in the expression of IL-8 and IL-17A mRNA. The expression of the three cytokines was higher in the fundic mucosa of *H. pylori*-infected patients than in the uninfected patients, but only IL-8 and IL-17A expression reached statistical significance ($p = 0.042$ and $p = 0.0179$ respectively). Furthermore, the multivariate predictive analysis highlighted a cytokine signature that may predict metaplasia during the infection progression that involves a specific down-regulation of IL17A and an up-regulation of IL1 β in antral and fundic metaplasia respectively.

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Casablanca, July 29th, 2019

To the editor,
Cytokine journal

Dear editor,

On behalf of my colleagues, please find enclosed the manuscript entitled " **Gastric IL-1 β , IL-8, and IL-17A expression in Moroccan patients infected with *Helicobacter pylori* may be a predictive signature of severe pathological stages**" by Ahmed OUTLIOUA and colleagues, that we would like to submit for publication in Cytokine as a Research Article.

Helicobacter pylori induces acute gastritis that can progress to serious diseases such as gastric cancer. It interacts with host cells, resulting in activation of multiple innate immune signalling pathways, leading to pro-inflammatory cytokines production and immune cells recruitment.

Although the *H. pylori* infection is a major public health problem in Morocco, to our knowledge, no study on gastric cytokine expression from *H. pylori*-infected patients has been carried out in Morocco. Our survey included 71 Moroccan patients presenting with epigastralgic pain. The histopathological and PCR analyses revealed that 87.32% of these patients were infected with *H. pylori*. We analyzed IL-1 β , IL-8 and IL-17A gene expression in antrum and fundus biopsies from infected patients. Our results showed that IL-1 β mRNA expression was significantly lower in the antral mucosa of *H. pylori*-infected patients. The IL-1 β , IL-8 and IL-17A expression was higher in the fundic mucosa of *H. pylori*-infected patients. Furthermore, the multivariate predictive model highlighted a cytokine signature that may predict metaplasia during the infection progression that involves a specific down-regulation of IL-17A and an up-regulation of IL-1 β in antral and fundic metaplasia respectively. Taken together, our results may be helpful to improve clinical management of *H. pylori* infection.

Finally, neither this paper nor any similar paper, in whole or in part, has been or will be submitted to, or published in any other scientific journal while it is under revision.

Sincerely

Khadija Akarid, PhD

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Gastric IL-1 β , IL-8, and IL-17A expression in Moroccan patients infected with *Helicobacter pylori* may be a predictive signature of severe pathological stages.

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1 **Gastric IL-1 β , IL-8, and IL-17A expression in Moroccan patients infected with *Helicobacter***
2 ***pylori* may be a predictive signature of severe pathological stages.**

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28 **ABSTRACT**

29 **Introduction:** *Helicobacter pylori* induces acute gastritis that can progress to serious diseases
30 such as gastric cancer. *H. pylori* interacts with host cells within the gastric mucosa, resulting in
31 activation of multiple innate immune signalling pathways, leading to pro-inflammatory cytokines
32 production and immune cells recruitment. Various studies have shown that there are ethnic- and
33 population-related differences in the expression of these cytokines. Although the *H. pylori*
34 infection is a major public health problem in Morocco, to our knowledge, no study has been
35 carried out in gastric cytokine expression from *H. pylori*-infected Moroccan patients. Thus we
36 aimed to i) determine the IL-1 β , IL-8 and IL-17A gene expression in gastric biopsies from
37 Moroccan patients infected with *H. pylori*, and ii) to determine the cytokine signature of each
38 pathological stages associated with this infection.

39 **Material and methods:** 71 patients with epigastralgic pain were included in this study. The *H.*
40 *pylori* detection on gastric biopsies was performed by histopathological and PCR analysis. The
41 IL-1 β , IL-8 and IL-17A mRNA expression in the antrum and fundus biopsies was performed by
42 RT-qPCR.

43 **Results:** The histopathological and PCR analyses revealed that 87.32% of the patients were
44 infected with *H. pylori*. IL-1 β mRNA expression was significantly lower in the antral mucosa of
45 *H. pylori*-infected patients ($p = 0.0038$) than in the uninfected while there was no significant
46 difference in the expression of IL-8 and IL-17A mRNA. The expression of the three cytokines
47 was higher in the fundic mucosa of *H. pylori*-infected patients than in the uninfected patients, but
48 only IL-8 and IL-17A expression reached statistical significance ($p = 0.042$ and $p = 0.0179$
49 respectively). Furthermore, the multivariate predictive analysis highlighted a cytokine signature
50 that may predict metaplasia during the infection progression that involves a specific down-
51 regulation of IL17A and an up-regulation of IL1 β in antral and fundic metaplasia respectively.

52

53 **Keywords:** *Helicobacter pylori*, fundus, antrum, cytokine signature, Morocco.

54 1. INTRODUCTION

55 *Helicobacter pylori* is a spiral-shaped, gram-negative bacillus that infects the stomach of half of
56 the world's population and almost two-thirds of people in developing countries. The prevalence
57 of *H. pylori* infections varies according to country, age, ethnic origin, socio-economic conditions,
58 promiscuity, and hygiene. Approximately 80% of the populations of these countries is infected by
59 age 20 [1].

60 Epidemiological studies have shown that *H. pylori* infections are a major public health problem
61 in Morocco, affecting approximately 70% of the population. Epigastric pain is common, and
62 ulcerative disease affects about 5% of the Moroccan population, including young children and
63 adults [2, 3].

64 Since 1994, *H. pylori* has been classified by the World Health Organization (WHO) as the first
65 bacterial species that cause cancer in humans. Persistent *H. pylori* infections induce chronic
66 inflammation of the gastric mucosa that, in susceptible individuals, may progress to gastric
67 cancer [4]. The clinical outcome of the infection depends on complex interactions among the
68 infecting bacterial strain, the host immune response, and the environment. The initial
69 inflammatory response to *H. pylori* infection results in the secretion of a wide panel of cytokines
70 by epithelial and immune cells, including IL-1 β , TNF- α , IL-6, IL-8, and IL-17. These cytokines
71 have pleiotropic effects such as regulation of cell death, proliferation, differentiation, and
72 migration [5-7]. The increase in inflammatory cytokine levels seems to play a critical role in the
73 initiation and progression of gastric cancer. IL-1 β , a pro-inflammatory cytokine, combined with
74 *H. pylori* infection appears to be a key factor in the development of gastric cancer.
75 Polymorphisms of the IL-1 β gene and elevated levels of IL-1 β are associated with a higher risk
76 for developing hypochlorhydria, gastric atrophy, and gastric adenocarcinoma [8]. IL-17 and IL-8
77 also play important roles in the inflammatory response caused by *H. pylori* colonization. An
78 increase in IL-17 and IL8 mRNA expression has been observed in cancer tissues [9-11].

79 The progression to carcinogenesis appears thus to depend on the nature of the host's
80 inflammatory response. Various studies have clearly shown that there are ethnic- and population-
81 related differences in the expression of these cytokines [12]. Although the immune response is
82 considered to be one of the pathological determinants of *H. pylori* pathogenesis, to our
83 knowledge, no studies on the immune response in *H. pylori* infected Moroccan patients have yet
84 been carried out. Investigating the expression levels of IL-1 β , IL-8, and IL-17 in gastric biopsies

85 from *H. pylori*-infected patients could be a good prognostic tool for understanding gastritis
86 progression to more severe pathological stages and would allow a better management of infected
87 patients.

88 The goals of this work were (i) to determine the expression levels of IL-1 β , IL-8, and IL-17A in
89 gastric biopsies from *H. pylori*-infected Moroccan patients, and (ii) to determine the cytokine
90 signature of each pathological stage associated with *H. pylori* infections in order to assign an
91 expression profile to each stage of infection.

92

93 **2. PATIENTS & METHODS**

94 **2.1. Ethics statement**

95 This research was conducted according to the principles set out in the Declaration of Helsinki and
96 in local ethical guidelines. The study was approved by the Ethics Committee for Biomedical
97 Research, Ibn Rochd Hospital, Casablanca, Morocco. Written informed consent was obtained
98 from all patients.

99

100 **2.2. Patients**

101 Seventy-one patients referred for an upper gastrointestinal endoscopy to diagnose the cause of
102 abdominal pain were recruited by the Gastroenterology Department of Ibn Rochd Hospital,
103 Casablanca, Morocco.

104 Patients who had previous gastroduodenal surgery, who had been treated to eradicate an *H. pylori*
105 infection, or who had taken proton pump inhibitors or anti-inflammatory drugs during the 3
106 months preceding the endoscopy were excluded from the present study. Medical history was
107 obtained from each patient included in our study.

108 Six biopsies were taken from each patient, three from the antrum and three from the fundus. The
109 biopsies were separately processed to extract DNA, assess mRNA levels, and perform
110 histopathological analyses. The tissue sampling procedures as well as the method of sample
111 handling were identical for the biopsies from the antrum and the fundus.

112

113 **2.3. Histopathological analyses**

114 The gastric mucosal tissue samples were fixed in 10% formalin for 24 hours, and embedded in
115 paraffin. Sequential 3-5 μ m sections were cut and stained with hematoxylin-eosin and modified

116 Giemsa for routine histopathological analyses and were assessed according to the updated
117 Sydney system [13].

118

119 **2.4. DNA extraction and *H. pylori* genotyping**

120 The presence of *H. pylori* was assessed by histology and PCR. Genomic DNA was extracted
121 from the biopsies using the standard phenol-chloroform protocol. The presence of *H. pylori* was
122 confirmed using the 23S rRNA gene and the following primers: forward 3'-GGA GCT GTC
123 TCA ACC AGA TTC-5' and reverse 3'-CGC ATG ATA TTC CCA TTA GCA G-5'. For the
124 PCR, 200 ng of total DNA was mixed with 1.5 mM MgCl₂, 10 mM dNTP, 10 μM of each primer,
125 and 1 U of Taq DNA polymerase (Invitrogen, Canada) in a final volume of 25 μL. The mixture
126 was subjected to 40 amplification cycles of 3 min at 94°C, 45 s at 94°C, 30 s at 55°C, and 90 s at
127 72°C followed by a 10-min final extension at 72°C (Thermocycler Biometra). The amplicons
128 were separated by agarose gel electrophoresis and were visualized with ethidium bromide.

129

130 **2.5. Extraction of total RNA and semiquantitative RT-PCR**

131 Gastric mucosal IL-1β, IL-8, and IL-17 mRNA levels were analyzed (table 1). Gastric specimens
132 were placed in RNAlater (QIAGEN, Canada) and were stored at -80°C until use. Total mRNA
133 was extracted from fresh biopsies using TRIZOL reagent according to the manufacturer's
134 protocol. One μg of total RNA was reversed transcribed using reverse transcriptase. The cDNA
135 was amplified for 40 cycles (annealing temperature 60°C) using standard conditions.

136

137 **Table 1.** Primer sequences for the RT-PCR analysis

Primers	Forward	Reverse
IL-1β	3'-TCC CCA GCC CTT TTG TTG A-5'	3'-TTA GAA CCA AAT GTG GCC GTG-5'
IL-17A	3'- CTC ATT GGT GTC ACT GCT ACT G-5'	3'- CCT GGA TTT CGT GGG ATT GTG-5'
IL-8	3'- CCA GGA AGA AAC CAC CGG A-5'	3'- GAA ATC AGG AAG GCT GCC AAG-5'
β-actin	3'-GAA CGG TGA AGG TGA CA-5'	3'-TAG AGA GAA GTG GGG TGG-5'

138

139 The conditions for the reactions were as follows: an initial 3-min denaturation step at 95°C,
140 followed by 40-s cycles at 95°C, 60°C, and 72°C. The RT-PCR was performed using an
141 Mx3005Pw QPCR system (Agilent Technologies, Canada) and a Brilliant II SYBR Green QPCR
142 Master Mix. IL1-β, IL-8, IL-17A, and β-actin mRNA levels were quantified using Agilent
143 Mx3000P QPCR System. Results were calculated using the $2^{-\Delta\Delta Ct}$ relative quantification method
144 normalized to β-actin.

145

146 **2.6. Statistical analysis**

147 The results were analyzed using the Mann-Whitney U test for nonparametric data. A $p < 0.05$ was
148 considered statistically significant. All analyses were performed using GraphPad Prism software
149 and version 3.4.3 of the R environment. An unsupervised principal component analysis was built
150 in version 1.40 of the FactoMineR R-package for the quantification of IL-1β, IL-8, and IL-17A
151 [14]. Boxplots were performed using the ggplot2 graphical definition [15], and statistical
152 significance between groups was assessed using the Kruskal-Wallis ranking test.

153

154 **3. RESULTS**

155 **3.1. Prevalence of *H. pylori* infection and patients' distribution according to age, sex, 156 and lifestyle parameters**

157 Patients' distribution according to *H. pylori* infection and age, sex, lifestyle parameters is
158 presented in Table 2.

159 Patients were considered *H. pylori*-infected when the PCR and/or histological analyses were
160 positive.

161

162

163

164

165

166

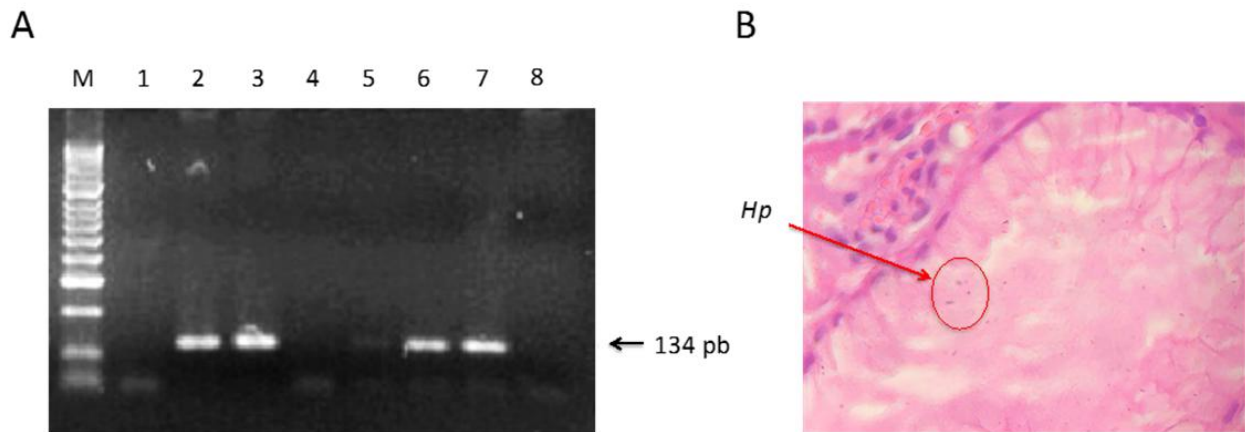
Table 2: Characteristics of the patients included in the study

	<i>H. pylori</i> -positive	<i>H. pylori</i> -negative
Mean age	46.18 ± 17.30	42.5 ± 21.95
Sex		
▪ Women	29 (40.84%)	3 (4.22%)
▪ Men	33 (46.47%)	6 (8.45%)
Lifestyle		
▪ Smokers	14 (19.71%)	2 (2.81%)
▪ Urban	56 (78.87%)	8 (11.26%)
▪ Rural	6 (8.45%)	1 (1.40%)
Total (n = 71)	62 (87.32%)	9 (12.67%)

168

169 Sixty-two patients (87.32%) were diagnosed as being *H. pylori*-infected (Fig. 1). The nine
 170 remaining patients diagnosed as *H. pylori* uninfected by the two methods were used as a control
 171 group. There was no difference between the two groups with respect to age, sex, or lifestyle.

172



173

174 **Fig. 1. Diagnosis of *H. pylori* infection by PCR and histopathology analyses**

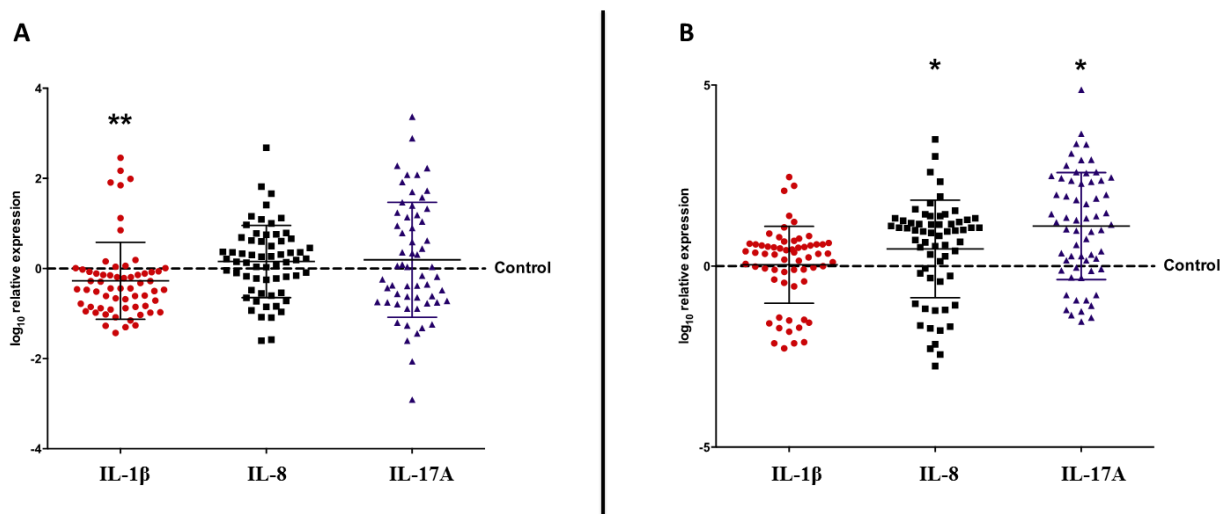
175 (A) Agarose gel showing the *H. pylori* 23S rRNA gene amplicons (134-pb). The amplicons were
176 visualized with ethidium bromide. Line M: 50-bp DNA ladder; Lines 1, 4, and 8: *H. pylori*-
177 negative samples; Lines 2, 3, 5, 6, and 7: *H. pylori*-positive samples. (B) The biopsy specimens
178 were fixed in 10% buffered formalin, embedded in paraffin, cut in sequential sections, and
179 stained with hematoxylin-eosin and modified Giemsa. The arrow points to *H. pylori* bacilli.
180

181 **3.2. IL-1 β , IL-8, and IL-17A cytokines mRNA expression in the antrum and fundus**

182 Paired biopsies from the antrum and fundus of *H. pylori*-infected (n = 62) and *H. pylori*-
183 uninfected (n = 9) patients were analyzed for the expression of IL-1 β , IL-8, and IL-17A genes
184 (Figs. 2A and 2B).

185 IL-1 β mRNA expression was significantly lower in the antrum of *H. pylori*-infected patients than
186 in the *H. pylori*-uninfected patients (p = 0.003). There was no significant difference in the
187 expression levels of IL-8 and IL-17A in the antrum of *H. pylori*-infected and *H. pylori*-uninfected
188 patients (Fig. 2A). In the fundus, IL-8 and IL-17A mRNA expression were significantly higher in
189 *H. pylori*-infected than in *H. pylori*-uninfected patients (p = 0.04 and p = 0.02 respectively). IL-
190 1 β mRNA expression in the fundus of *H. pylori*-infected patients was slightly higher than in *H.*
191 *pylori*-uninfected patients. However, this difference did not reach significant levels (Fig. 2B).

192



193

194 **Fig. 2. IL-1 β , IL-8, and IL-17A cytokines mRNA expression in the antrum and fundus**

195 The relative IL-1 β , IL-8, and IL-17A genes expression was analyzed by qRT-PCR in paired
196 biopsies from the antral (A) and fundic (B) regions of the *H. pylori* - infected patients. IL-1 β , IL-
197 8, and IL-17A mRNA expression levels relative to the β -actin gene are indicated on the y axis.
198 The 62 infected patients were normalized to the 9 uninfected patients (control) as indicated by the
199 dashed lines. The results are expressed with standard deviations. The statistical analyses were
200 performed using the Mann-Whitney test for non-parametric data. * p<0.05, ** p<0.01.

201

202 **3.3. Cytokines mRNA expression levels according to pathological stages**

203 In order to understand the involvement of these cytokines in the outcome of *H. pylori* infections,
204 the patients were stratified into pathological stages based on their histopathology scores and
205 endoscopic findings (Table 3).

206 According to the classification of the updated Sydney system and the Correa cascade each patient
207 was assigned to one of the three pathological stages [13, 16]: gastritis (n = 38), atrophy (n = 17),
208 or metaplasia (n = 5).

209

210 **Table 3: Pathological stages of the precancerous cascade**

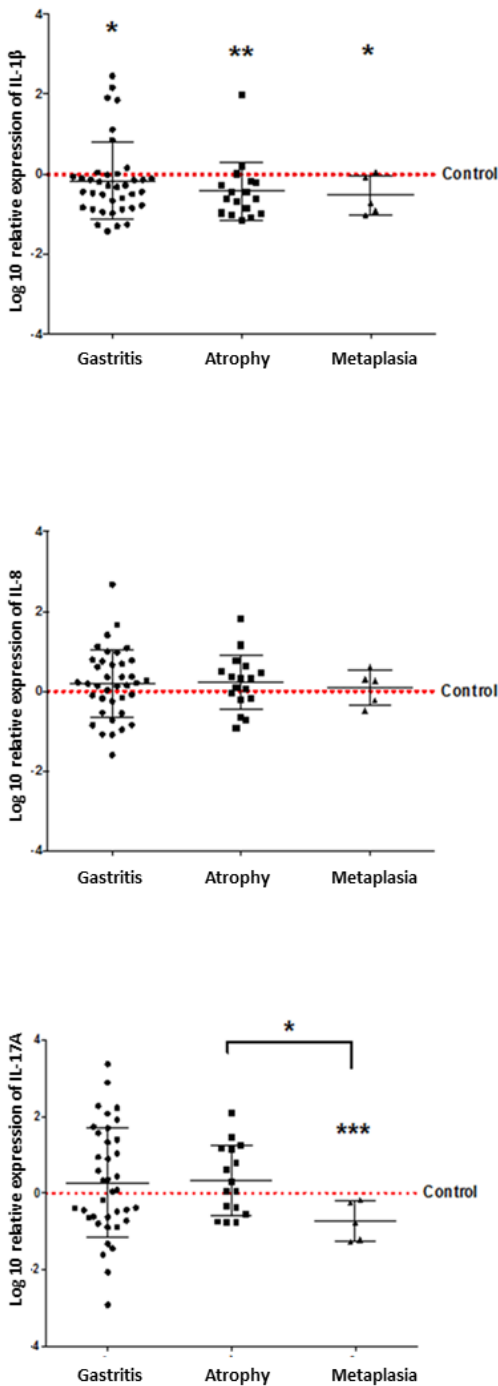
Pathological stages	Gastritis	Atrophy	Metaplasia
<i>H. pylori</i> -infected patients	38 (61.29%)	17 (27.41%)	5 (8.06%)

211

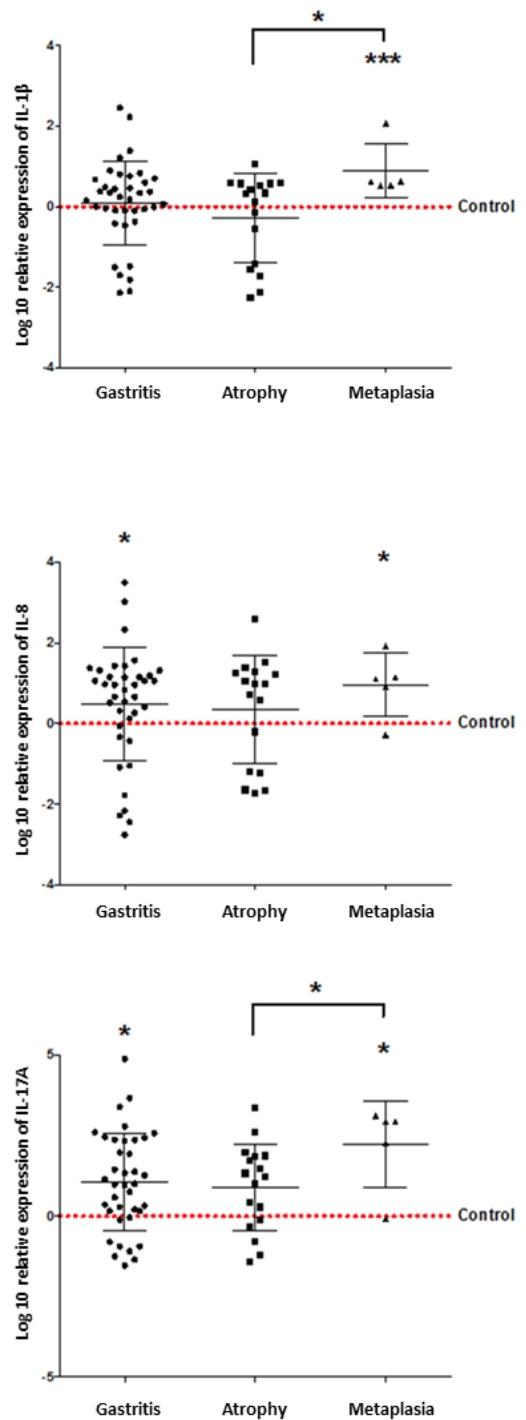
212 The relative expression of IL-1 β was significantly down-regulated in the antrum of *H. pylori*-
213 infected patients with gastritis, atrophy, or metaplasia compared to the *H. pylori*-uninfected
214 patients (Fig. 3A). However, the expression of IL-1 β was significantly higher in the fundus of the
215 patients with metaplasia compared to the *H. pylori*-uninfected patients and the other pathological
216 stages (Fig. 3B). There was no significant difference between the IL-8 expression in the antrum
217 of patients with gastritis, atrophy, or metaplasia and *H. pylori*-uninfected patients (Fig. 3A). IL-8
218 expression in the fundus was significantly higher in patients with gastritis and metaplasia. It was
219 also slightly higher in patients with atrophy compared to *H. pylori*-uninfected patients, but the
220 difference did not reach significance (Fig. 3B).

221 Similarly to IL-1 β , there was a significant decrease in IL-17A expression in the antrum of
222 patients with metaplasia compared to the *H. pylori*-uninfected patients and to patients with
223 gastritis or atrophy (Fig. 3A). However, the relative expression of IL-17A in the fundus was
224 significantly higher in patients with gastritis or metaplasia. IL-17A expression was also higher in
225 the fundic mucosa of patients with atrophy, but the difference did not reach significance (Fig.
226 3B). IL-17A expression was higher in patients with metaplasia than in patients with gastritis or
227 atrophy. To summarize, our results show that IL-1 β and IL-17A expression is down-regulated in
228 the antrum of patients with metaplasia and that the three cytokines studied (IL-1 β , IL-8, and IL-
229 17A) are up-regulated in the fundus of these patients.

A



B



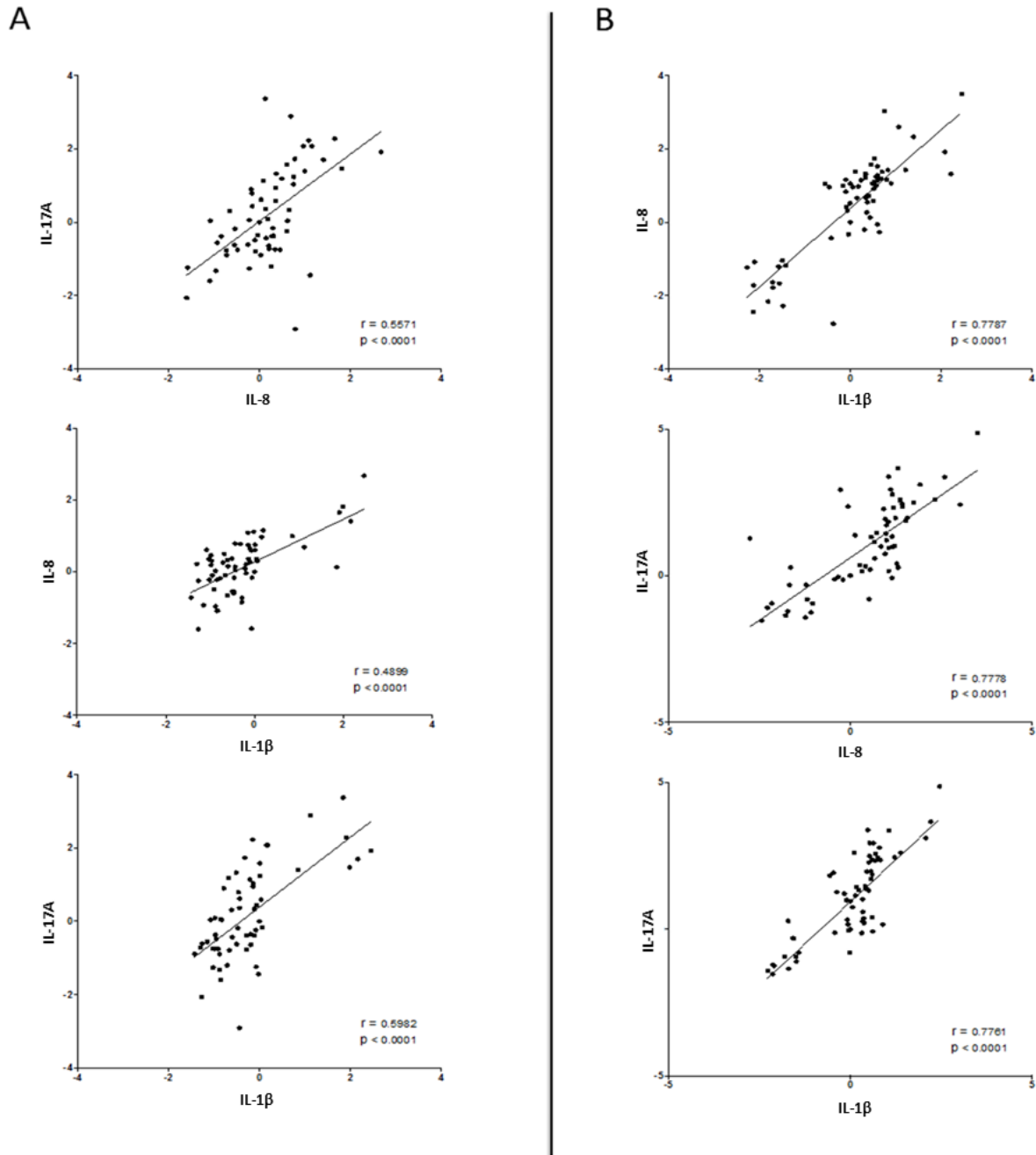
230

231 **Fig. 3. Cytokines mRNA expression levels according to pathological stages**

232 We stratified the patients into three pathological stages based on the classification of the updated
233 Sydney system and the Correa cascade [13, 16]. IL-1 β , IL-8, and IL-17 expression levels in the

234 antrum (A) and the fundus (B) according to pathological stages was normalized to the 9 controls
235 as indicated by the red dotted lines. Gastritis (n = 38), atrophy (n = 17), and metaplasia (n = 5).
236 * p<0.5, ** p<0.01, ***p<0.001.

237
238 Significant correlations were also found between IL-1 β , IL-17, and IL-8 expression in the antrum
239 (Fig. 4A) and fundus (Fig. 4B). The IL-1 β , IL-8, and IL-17A gene expression of *H. pylori* -
240 infected patients were significantly positively correlated in both the antrum (Fig. 4A) and the
241 fundus (Fig. 4B).



242

243 **Fig. 4. Cytokines genes expression levels in antral (A) and fundic (B) regions**

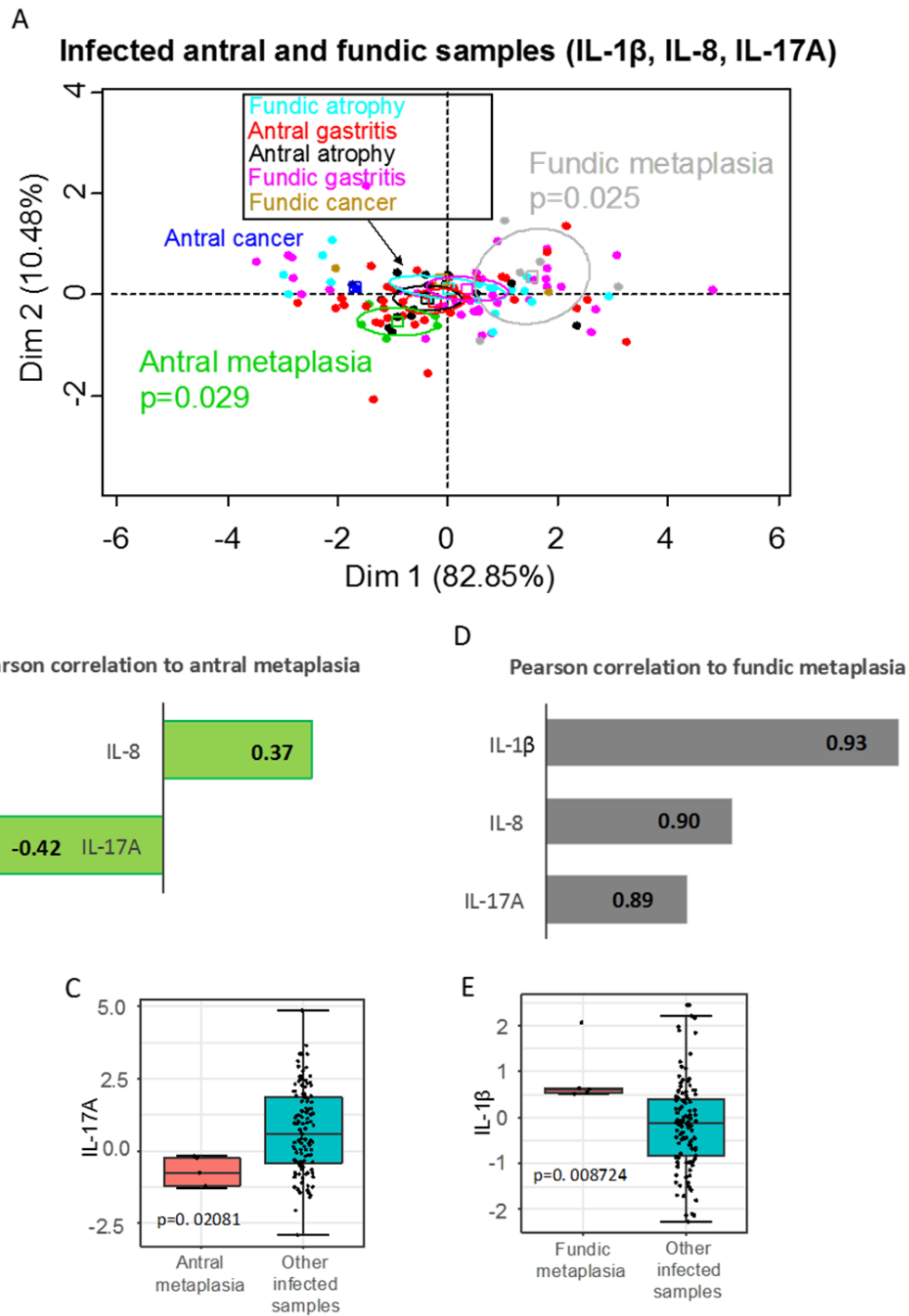
244 We correlated cytokines mRNA expression levels (IL-1 β , IL-8, IL-17A) of the *H. pylori*-
 245 infected patients in antral (A) and fundic regions (B): Correlation: IL-1 β vs IL-8 ; IL-1 β vs IL-
 246 17A ; IL-17A vs IL-8. *** p<0.001.

247

3.4. Interleukin triad-predicted gastric metaplasia in *H. pylori*-infected antral and fundic gastric samples

IL-1 β , IL-8, and IL-17A levels were quantified in antral and fundic gastric samples from *H. pylori*-infected patients at different stages of the pathology: atrophy, gastritis, and metaplasia. Seventy-one samples were included in a multivariate predictive model. An unsupervised principal component analysis of the levels of these three interleukins resulted in an effective representation of all the samples in the first principal map, with eigenvalues for the first two dimensions of 82.85% and 10.48%, respectively (Fig. 5A). The sample group discrimination in this analysis mainly showed a significant positive correlation of fundic metaplasia on the first principal axis (Fig. 5A, Pearson correlation, $p = 0.025$) and a significant negative correlation of antral metaplasia on the second principal axis (Fig. 5A, Pearson correlation, $p = 0.029$). These results suggest that the interleukin triad makes it possible to discriminate the metaplasia stage from the other stages of the disease (atrophy, gastritis), and to discriminate between fundic metaplasia and antral metaplasia, indicating that it is important to analyze biopsies from both regions in order to diagnose this disease.

To determine which interleukins would predict antral metaplasia, interleukin Pearson coefficients were analyzed during the principal component analysis. IL-17A was significantly negatively correlated with the second axis (Fig. 5B), thus predicting that it would be down-regulated in antral metaplasia compared to the other *H. pylori*-infected samples (Fig. 5C, $p = 0.02$). To determine which interleukins would predict fundic metaplasia, interleukin Pearson coefficients were analyzed during the principal component analysis. IL-1 β was significantly positively correlated with the first axis (Fig. 5D), predicting that it would be up-regulated in fundic metaplasia compared to the other *H. pylori*-infected samples ($p = 0.009$, Fig. 5E).



271
 272 **Fig. 5. Interleukin triad-predicted gastric metaplasia in *H. pylori*-infected antral and fundic**
 273 **gastric samples**
 274 (A) Unsupervised principal component analysis performed with the interleukin triad (IL-1 β , IL-8,
 275 IL-17A) on antral and fundic gastric biopsies from patients infected with Hp (the p values for

276 group discrimination are indicated); (B) Barplot of Pearson correlation coefficients for
277 interleukins that are correlated with antral metaplasia; (C) Boxplot of IL-17A levels in antral
278 metaplasia compared to other infected samples (the p values were calculated using the Kruskal-
279 Wallis test); (D) Barplot of Pearson correlation coefficients for interleukins correlated with
280 fundic metaplasia; (E) Boxplot of IL-1 β levels in fundic metaplasia compared to other infected
281 samples (the p values were calculated using the Kruskal-Wallis test).

282

283 **4. DISCUSSION**

284 In the human stomach, *H. pylori* colonizes the mucosa overlying the gastric epithelial cells and
285 induces chronic inflammation. Immune cells are recruited and communicate with epithelial cells
286 via cytokines. Signalling through cytokine receptors expressed on immune and non-immune cells
287 can promote inflammation and engage the host response against gastrointestinal bacteria. To our
288 knowledge no study has been carried out in *H. pylori*-infected Moroccan patients. Thus we aimed
289 to focus on three inflammatory cytokines (IL-1 β , IL-8, and IL-17A) that are known to play
290 important roles in *H. pylori* infection and gastric precancerous cascade induced by this bacterium.
291 In the present study, we investigated IL-1 β , IL-8, and IL-17A mRNA expression in the antral and
292 fundic gastric mucosa of *H. pylori*-infected Moroccan patients using semiquantitative RT-PCR.

293 The histopathological and PCR analyses revealed that 87.32% of the patients were infected with
294 *H. pylori* independently of age, sex and lifestyle. This prevalence is close to other reported results
295 in Moroccan population [17, 18]. Our results showed that IL-1 β , IL-8, and IL-17A mRNA
296 expression levels were different in the antrum and the fundus of *H. pylori*-infected patients.
297 Indeed the expression of IL-1 β mRNA was significantly lower in the antral mucosa of *H. pylori*-
298 infected patients than uninfected patients, while there was no significant difference in the
299 expression of IL-8 and IL-17 mRNA between the two groups. The expression of the three
300 cytokines was higher in the fundic mucosa of *H. pylori*-infected patients, but only IL-8 and IL-17
301 expression reached statistical significance. Furthermore, there was a significant positive
302 correlation of these cytokines expression in the antrum and fundus, indicating that they are
303 closely related and associated with *H. pylori* infection pathogenesis.

304 Patients were assigned to three pathological stages according to the updated Sydney system [13].
305 It has been suggested that the intestinal type of gastric cancer was the end result of progressive
306 changes in the gastric mucosa, starting with chronic gastritis, followed by multifocal atrophic

307 gastritis, and then intestinal metaplasia [19]. Our results showed that the relative expression of
308 IL-1 β and IL-17A in the antrum of *H. pylori*-infected patients is significantly down-regulated in
309 the metaplasia stage compared to uninfected patients and the other pathological stages. The IL-8
310 expression followed the same trend but the difference did not reach significance. The expression
311 of IL-1 β , IL-8, and IL-17A in the fundic mucosa of the *H. pylori*-infected patients with
312 metaplasia was up-regulated. Based on a multivariate predictive model, our results suggest that
313 this interleukin triad may be used to predict the metaplasia stage, mainly by a specific down-
314 regulation of IL-17A expression in the antrum and a specific up-regulation of IL-1 β in the
315 fundus. These cytokines could thus be used as markers of the progression of *H. pylori* infections
316 in Moroccan patients.

317 The down-regulation of the IL-1 β expression in the antrum of *H. pylori*-infected patients is
318 consistent with the results reported by Rossi et al. [7] but do not agree with those of Serelli et al.
319 [6]. Furthermore, the up regulation of IL-17A and IL-8 in the fundus was in agreement with
320 previous studies [9-11, 20]. The discrepancies in the different studies on cytokines expression
321 may be due to ethnic differences and/or to different sample sizes [12]. Furthermore, cytokines
322 expression in paired antral and fundic biopsies from *H. pylori*-infected individuals have not been
323 clearly reported.

324 IL-1 β , a pro-inflammatory cytokine, is a powerful inhibitor of gastric acid secretion, promoting
325 hypochlorhydria and favoring further colonization by *H. pylori* and then more severe gastritis
326 [21, 22]. IL-1 β may work in tandem with other inflammatory cytokines to recruit and activate
327 immune cells in the gastric mucosa, resulting in its inflammation. IL-1 β triggers the release of IL-
328 17A which is upregulated during *H. pylori* infections [23, 24]. The IL-17A involvement in *H.*
329 *pylori*-related gastritis is also supported by the fact that this cytokine is able to stimulate both
330 immune and non-immune cells to produce inflammatory mediators, including TNF α , IL-1, IL-6,
331 and matrix metalloproteinases (MMPs) that may also influence the outcome of *H. pylori*-
332 associated diseases such as gastritis [25]. It has been reported that *H. pylori* up-regulates IL-8
333 gene expression in gastric epithelial cells which could also be stimulated by IL-17A [20, 26].

334 Based on the stratification of our patients according to the updated Sydney classification, our
335 results clearly showed that the three inflammatory cytokines were upregulated in the fundic
336 mucosa of metaplasia patients. *H. pylori* infections initially causes antral gastritis, but
337 hypochlorhydria develops during persistent infections, allowing the bacteria to migrate

338 proximally, resulting in pangastritis and an increased risk for adenocarcinoma. In some cases,
339 gastritis is predominantly fundal, acid secretion decreases, and atrophy installs which is strongly
340 associated with increased IL-1 β [27]. It has been suggested that *H. pylori* is not normally able to
341 colonize the fundus unless acid secretion is reduced [28].

342 IL-1 β is upregulated in the breast, colon, lung, and gastric cancer as well as in melanomas. IL-1 β
343 participates in the carcinogenesis process by its ability to produce angiogenic and pro-metastatic
344 factors such as VEGF, IL8, IL6, TNF α , and TGF β [29]. Previous research using experimental
345 models has shown that local IL-1 β production influence tumor growth and metastasis by
346 potentiating cell proliferation or inducing inflammation and angiogenesis [30]. As IL-1 β also
347 promotes Th17 differentiation, it is possible that some of the pro-carcinogenic effects can be
348 mediated by these cells [23, 24]. Indeed, the recruitment and activation of phagocytes and other
349 pro-inflammatory cells by IL-17 signalling further boost oxygen and nitrogen radical levels and
350 oxidative stress [31, 32]. On the other hand, Kitadai *et al.* reported that IL-8 expression is directly
351 correlated with the vascularization of human gastric carcinomas, while its inhibition decreases
352 angiogenesis in stomach cancer [33]. Atrophic gastritis leads to a rise in pH and hypochlorhydria
353 that facilitates the colonization and proliferation of *H. pylori* [34]. IL-8, IL-1 β , and TNF α can
354 also promote epithelial cell proliferation, NF-KB activation, and cyclooxygenase-2 (COX-2)
355 expressions accelerating the progression of atrophic changes and induce intracellular signalling
356 transformation that may result in an accumulation of mitotic errors [4]. Some *H. pylori* antigens
357 are similar in structure to the gastric proton pump ATPase, which can lead to the autoimmune
358 destruction of the pump, further reducing acid secretion [35, 36]. The increase in gastric pH may
359 allow other pathogens to infect the stomach, further amplifying the inflammation.

360

361 **5. CONCLUSION**

362 IL-1 β , IL-8, and IL-17 cytokines are pleiotropic and involved in multiple signalling cascades that
363 may enhance the precancerous process. To our knowledge, the present study is the first that
364 investigates cytokine mRNA gene expression in paired biopsies from fundus and antrum in *H.*
365 *pylori*-infected Moroccan patients. We highlighted a cytokine signature that may predict
366 metaplasia during the infection progression that involves a specific down-regulation of IL-17A
367 and an up-regulation of IL-1 β in antral and fundic metaplasia respectively. Future research

368 should focus on identifying the expression of these cytokines in cancer stages and the molecular
369 pathways involved in the disease progression.

370

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

KA designed and coordinated the study, with the contribution of AK, DA, MyR, MoR and WB. AO carried out the experimental work with ZE. WB and NEH were in charge of patients' management and their sampling at the hospital. KA jointly with AK, DA, MyR, CD, AO and MK analyzed the whole data and drafted the manuscript. All authors read and approved the final manuscript.