



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

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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Ahmed Outlioua, Wafaa Badre, Christophe Desterke, Zerif Echarki, Nadira El Hammani, et al.. 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. Cytokines, 2020. hal-03008966

HAL Id: hal-03008966

<https://hal.science/hal-03008966>

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Manuscript Number:

Title: 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.

Article Type: Full length article

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

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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

Coordinator of Molecular Genetics and Immunophysiopathology Research Team
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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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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.

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

1. INTRODUCTION

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

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

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

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

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

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

2. PATIENTS & METHODS

2.1. Ethics statement

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

2.2. Patients

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

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

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

2.3. Histopathological analyses

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

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

2.4. DNA extraction and *H. pylori* genotyping

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

2.5. Extraction of total RNA and semiquantitative RT-PCR

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

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'

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

2.6. Statistical analysis

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

3. RESULTS

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

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

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

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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%)

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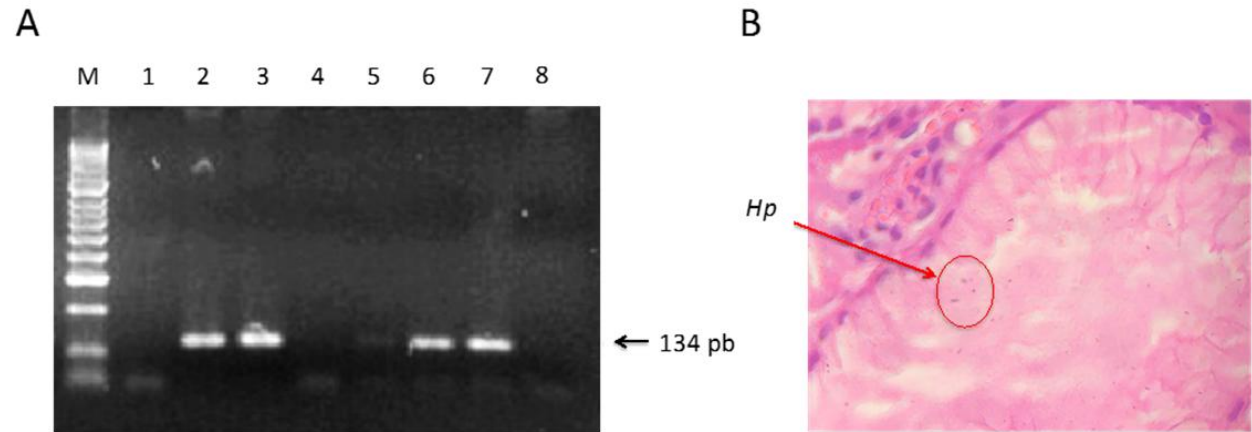
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Sixty-two patients (87.32%) were diagnosed as being *H. pylori*-infected (Fig. 1). The nine remaining patients diagnosed as *H. pylori* uninfected by the two methods were used as a control group. There was no difference between the two groups with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



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

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

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

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

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

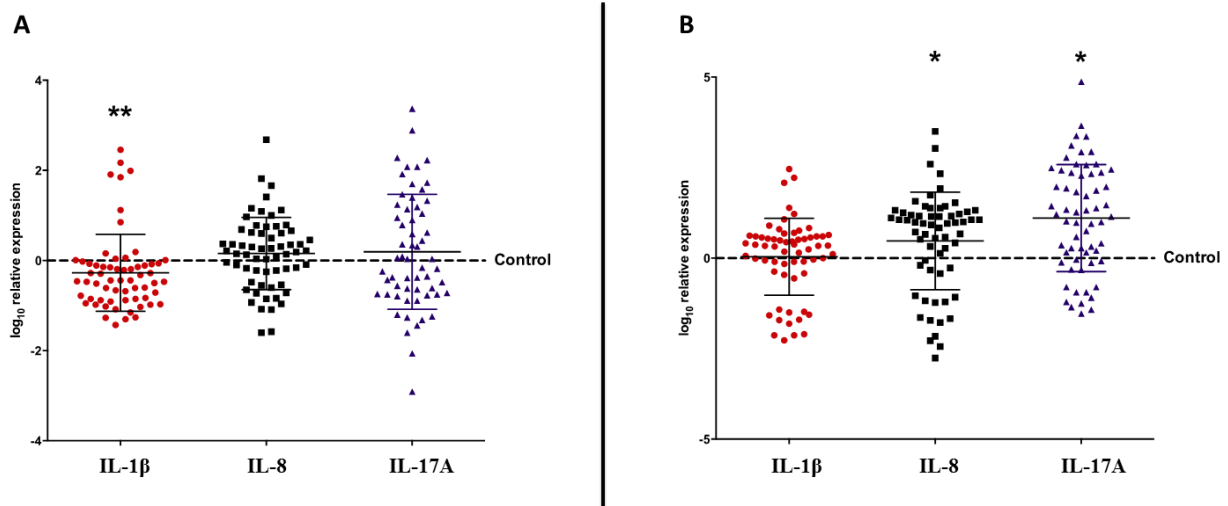


Fig. 2. IL-1 β , IL-8, and IL-17A cytokines mRNA expression in the antrum and fundus

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

3.3. Cytokines mRNA expression levels according to pathological stages

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

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

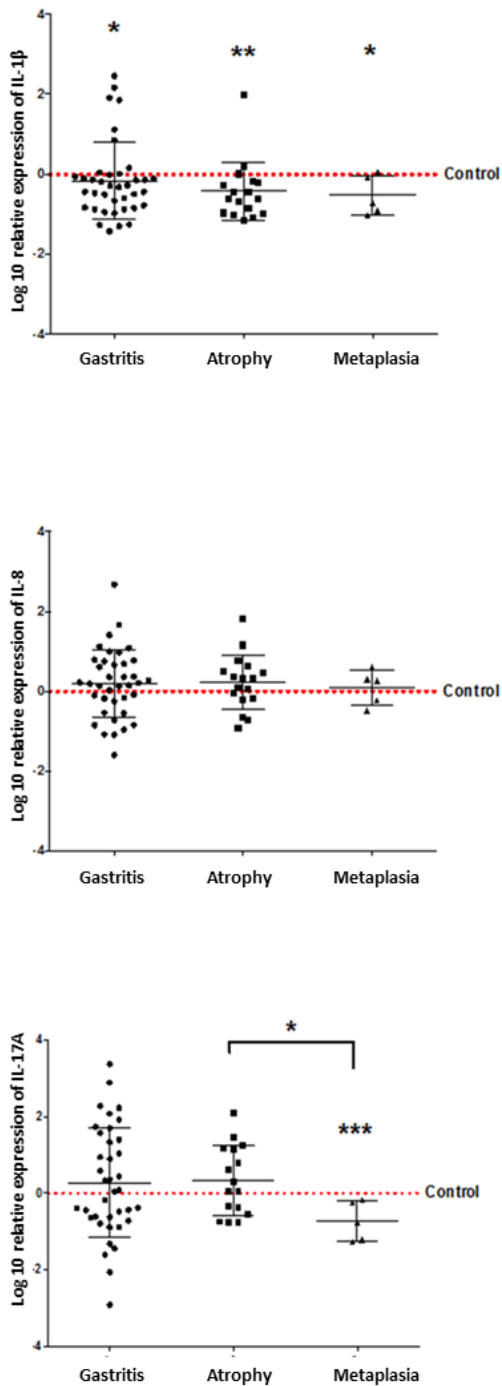
Table 3: Pathological stages of the precancerous cascade

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

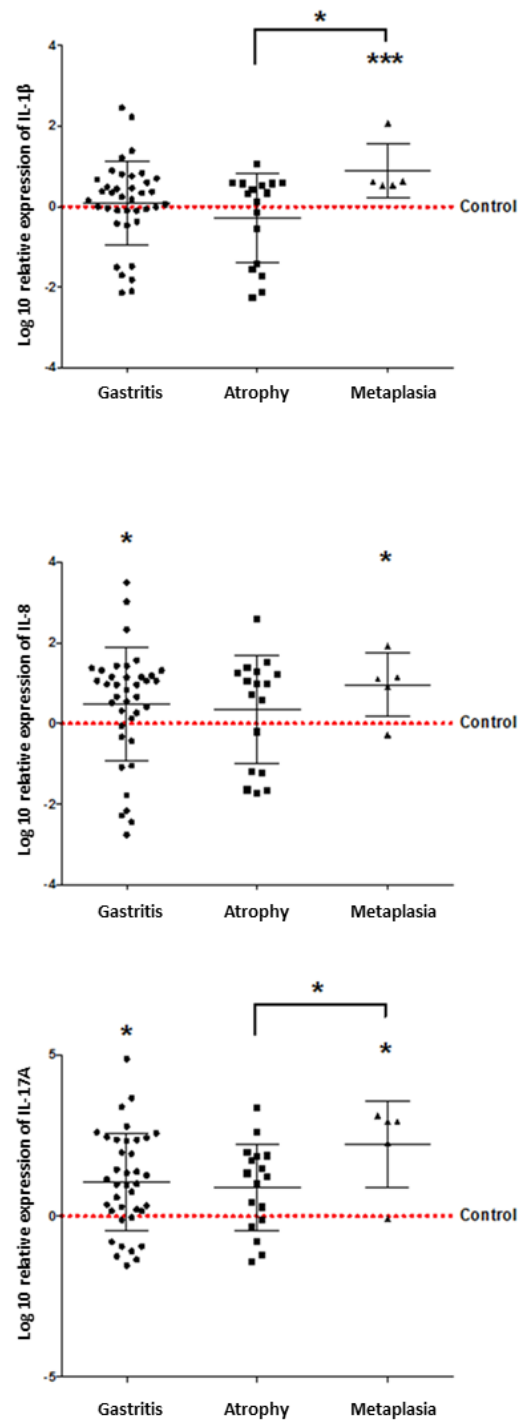
The relative expression of IL-1 β was significantly down-regulated in the antrum of *H. pylori*-infected patients with gastritis, atrophy, or metaplasia compared to the *H. pylori*-uninfected patients (Fig. 3A). However, the expression of IL-1 β was significantly higher in the fundus of the patients with metaplasia compared to the *H. pylori*-uninfected patients and the other pathological stages (Fig. 3B). There was no significant difference between the IL-8 expression in the antrum of patients with gastritis, atrophy, or metaplasia and *H. pylori*-uninfected patients (Fig. 3A). IL-8 expression in the fundus was significantly higher in patients with gastritis and metaplasia. It was also slightly higher in patients with atrophy compared to *H. pylori*-uninfected patients, but the 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).

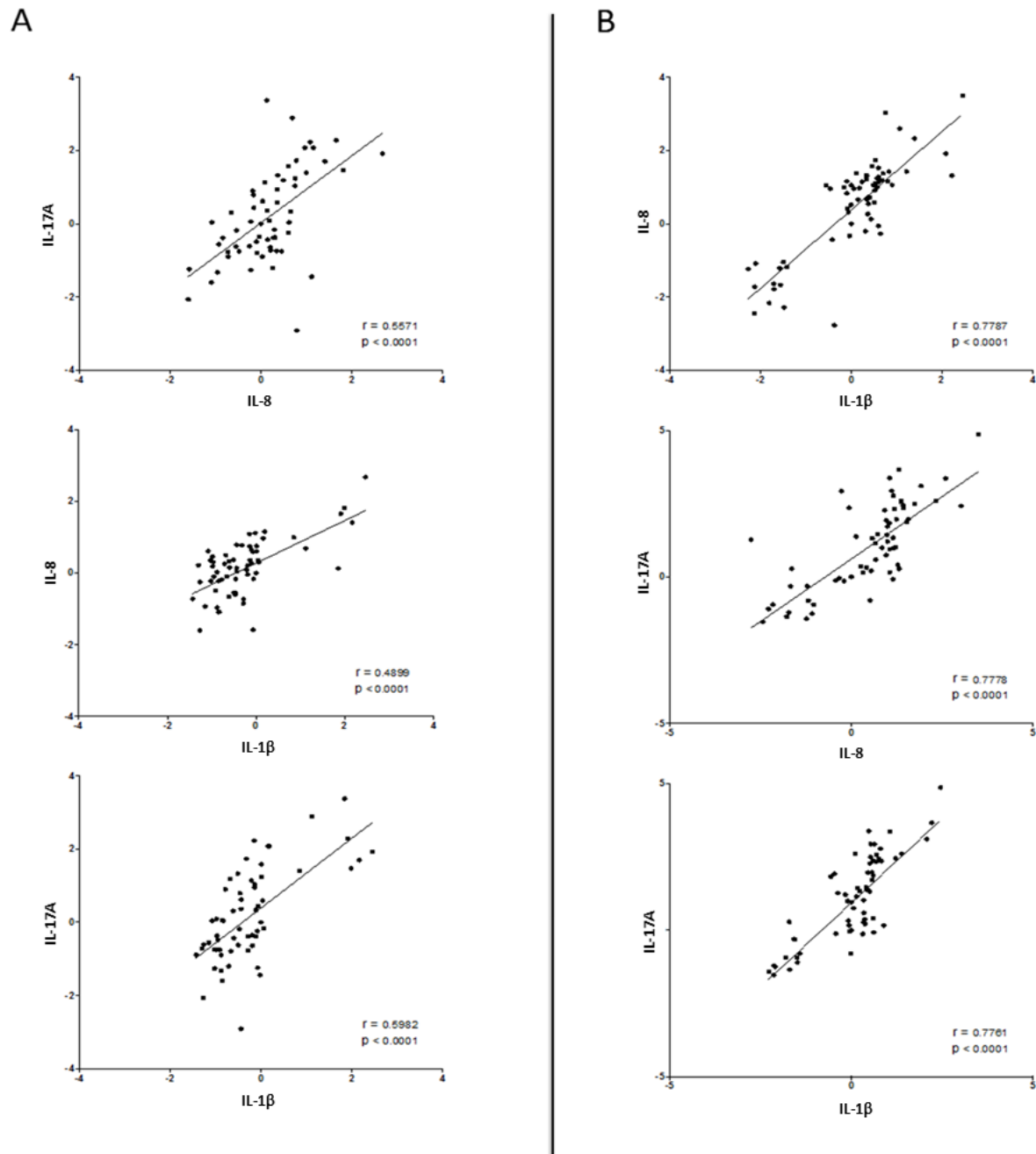


Fig. 4. Cytokines genes expression levels in antral (A) and fundic (B) regions

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

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).

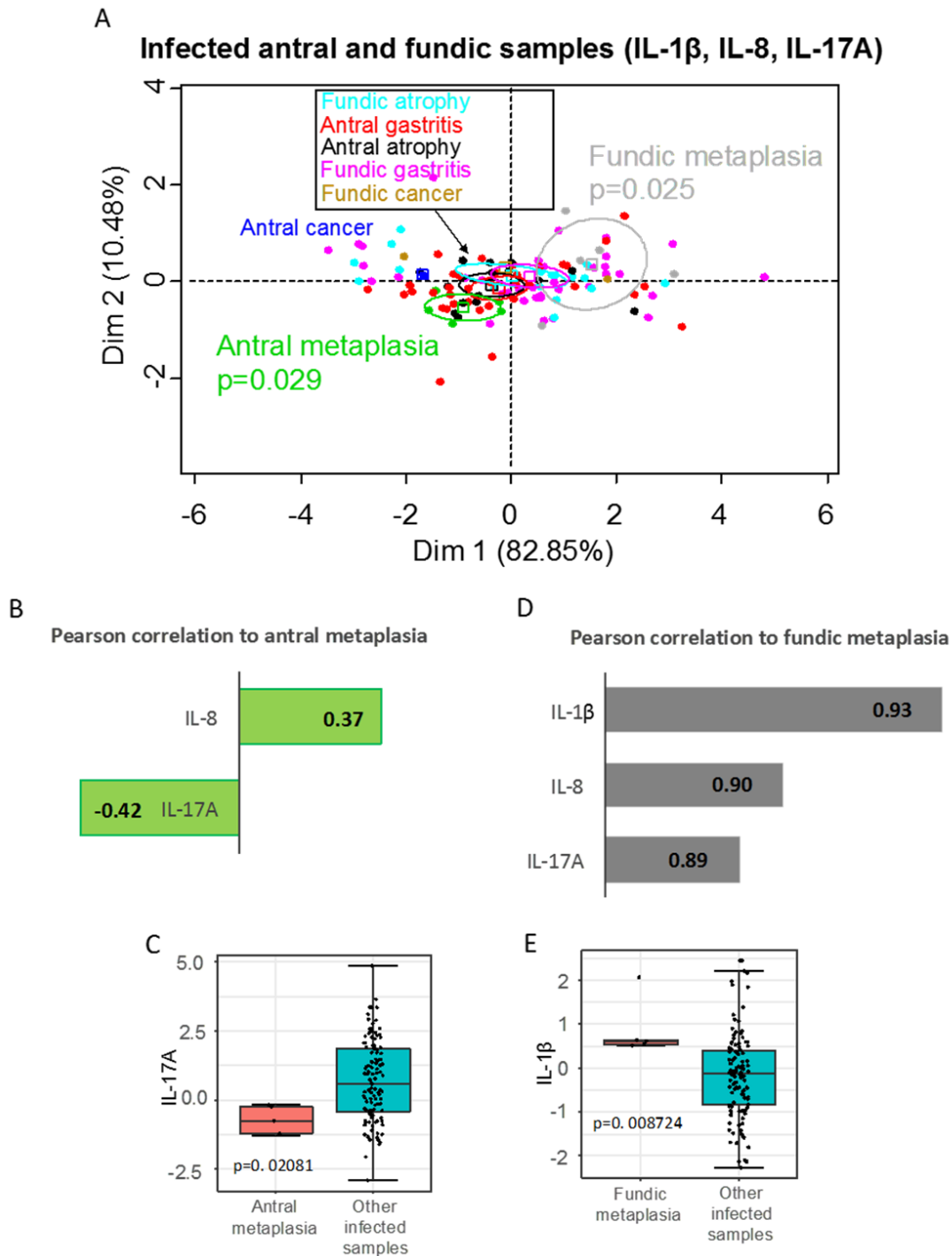


Fig. 5. Interleukin triad-predicted gastric metaplasia in *H. pylori*-infected antral and fundic gastric samples

(A) Unsupervised principal component analysis performed with the interleukin triad (IL-1 β , IL-8, IL-17A) on antral and fundic gastric biopsies from patients infected with Hp (the p values for

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

4. DISCUSSION

In the human stomach, *H. pylori* colonizes the mucosa overlying the gastric epithelial cells and induces chronic inflammation. Immune cells are recruited and communicate with epithelial cells via cytokines. Signalling through cytokine receptors expressed on immune and non-immune cells can promote inflammation and engage the host response against gastrointestinal bacteria. To our knowledge no study has been carried out in *H. pylori*-infected Moroccan patients. Thus we aimed to focus on three inflammatory cytokines (IL-1 β , IL-8, and IL-17A) that are known to play important roles in *H. pylori* infection and gastric precancerous cascade induced by this bacterium. In the present study, we investigated IL-1 β , IL-8, and IL-17A mRNA expression in the antral and fundic gastric mucosa of *H. pylori*-infected Moroccan patients using semiquantitative RT-PCR. The histopathological and PCR analyses revealed that 87.32% of the patients were infected with *H. pylori* independently of age, sex and lifestyle. This prevalence is close to other reported results in Moroccan population [17, 18]. Our results showed that IL-1 β , IL-8, and IL-17A mRNA expression levels were different in the antrum and the fundus of *H. pylori*-infected patients. Indeed the expression of IL-1 β mRNA was significantly lower in the antral mucosa of *H. pylori*-infected patients than uninfected patients, while there was no significant difference in the expression of IL-8 and IL-17 mRNA between the two groups. The expression of the three cytokines was higher in the fundic mucosa of *H. pylori*-infected patients, but only IL-8 and IL-17 expression reached statistical significance. Furthermore, there was a significant positive correlation of these cytokines expression in the antrum and fundus, indicating that they are closely related and associated with *H. pylori* infection pathogenesis. Patients were assigned to three pathological stages according to the updated Sydney system [13]. It has been suggested that the intestinal type of gastric cancer was the end result of progressive changes in the gastric mucosa, starting with chronic gastritis, followed by multifocal atrophic

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

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

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

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

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

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

5. CONCLUSION

IL-1 β , IL-8, and IL-17 cytokines are pleiotropic and involved in multiple signalling cascades that may enhance the precancerous process. To our knowledge, the present study is the first that investigates cytokine mRNA gene expression in paired biopsies from fundus and antrum in *H. pylori*-infected Moroccan patients. We 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. 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

371 **Acknowledgement**

372 This research was supported, in part, by a grant from Agence Universitaire de la Francophonie
373 (AUF), PIRAT program 2013.

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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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing 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.

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.