Pattern of gastritis as manipulated by current state of *Helicobacter pylori* infection

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**Abstract** - Helicobacter pylori (H. pylori) infection prevails from 60-80% in patients with gastric ulcer and 90-100% in those having duodenal ulcer. Patients with such type of chronic infection are at increased risk to develop peptic ulcers or gastric adenocarcinomas. The present work aims mainly to identify the pattern of chronic gastritis and potential effect of H. pylori infection using certain biomarkers, histological and immunochemical tests.

Fifty eight individuals, clinically diagnosed as having chronic gastritis, were participated in the present study. They were categorized into 2 groups, the first one (31%) demonstrated positive reaction to IgM antibodies of Helicobacter pylori (H. pylori) (>40u/ml) and the second group (69%) demonstrated negative reaction. Blood and antral biopsy samples were collected, directed to determination of serum gastrin, pepsinogen I (PgI), pepsinogen II (PgII), prostaglandin E$_2$ (PGE$_2$) and interleukin96 (IL96). Immunohistochemistry technique was also done in antral biopsy to demonstrate the expression of inducible nitric oxide synthase (iNOS), nitrotyrosine, DNA fragmentation, myeloperoxidase and histopathological examination.

Serum gastrin, PgI, PgII, PGE$_2$, IL-6 demonstrated significant increase in gastritis patients as compared to normal group. PgI, PgII showed significant increase joined with slight increase of IL-6 in IgM positive group as compared to negative one. Immunostaining testes in antral biopsy showed strong positive reactions for the above mentioned markers as compared to IgM negative group (mild positive reaction).

I. INTRODUCTION

Gastritis represents an inflammatory state of the stomach lining in response to injury which may be either acute or chronic and has many underlying causes [1]. Chronic type can be sub-classified as non-atrophic and special types (chemical, radiation, lymphocytic, non infectious, eosinophilic and others) [2]. Helicobacter pylori (H. pylori) may cause an acute or chronic gastritis and is associated with peptic ulcers, but the relationship to erosions is uncertain. H. pylori infection is ubiquitous; its prevalence ranges from 60-80% in patients with gastric ulcer and 90-100% in those having duodenal ulcer [3]. Most patients with peptic ulcers are infected with this organism mainly in the antrum, but the entire stomach may be involved [4]. The capacity of H. pylori to colonize the human stomach can be attributed to the production of specific bacterial products [5], urease here represents an essential virulence factor, potent antigen leading to immunoglobulin production (IgG and IgM).

Attachments of H. pylori to gastric epithelial cells results in activation of numerous signaling pathways and permits efficient delivery of toxin or other effectors molecules into the cells [6], where adherence is mainly through adhesions and receptors [7]. Bacterial adherence to host cell receptors triggers certain cellular changes (signal transduction cascades), leading to infiltration of inflammatory cells (neutrophils and monocytes), indeed persistence of the microorganism [7].

Another important virulence factor in H. pylori is the cytotoxin-associated protein (Cag A) identified as immune dominant antigen, located on the bacterial surface [8]. CagA protein is frequently co-expressed with vacuolat cytotoxin VacA, expressed as cytotoxin associated protein [9]. People with cytotoxin positive infection have endoscopically proven inflammation that is more pronounced than
Nitric oxide (NO) produced by these cells by forming pores in the mitochondrial membranes. Prostaglandin E_2 (PGE_2) in the stomach play an important role in maintenance of gastric mucosal integrity via several mechanisms including regulation of gastric mucosal blood flow, kinetics of epithelial cells, synthesis of mucus and inhibition of gastric acid secretion [17], referring to its protective potential to gastric mucosa.

H. pylori infection is associated with specific local and systemic immune responses. Early after 18 days of H. pylori infection, IgM response is detectable, whilst IgG and IgA response occur later after 60 days of infection, at which time IgM titers decline [18]. IgG and IgA serology is widely used as an accurate test for the diagnosis of H. pylori infections, but these two immunoglobulins remain detectable even after eradication of H. pylori and do not demonstrate the status of infection (acute, chronic, or previously treated infections) [19]. The use of IgM test would allow for direct screening of specimens and serve as a diagnostic tool for establishing active or recent infection. We used IgM serology in patients with H. pylori infection to study the differences in some gastric, inflammatory and oxidative biomarkers between those having positive IgM (active or recent infection) and those with negative IgM (chronic infection).

The present study aims mainly to determine i) the pattern of chronic gastritis, whether either H. pylori infection or not has any potential effect (current state of infection) using certain biomarkers, e.g. gastrin, pepsinogen I (PgI), pepsinogen II (PgII), PGE_2 and interleukin-6 (IL-6). ii) mucosal immunostaining test for nitrotyrosine, iNOS, myeloperoxidase and DNA fragmentation in antral biopsies isolated from individuals having positive IgM and those with negative IgM, followed by histopathological examination of the tissues.

II. MATERIALS and METHODS

A. Subjects

Age and BMI-matched 58 patients (49 male and 9 females) were recruited from those attending gastroenterology department, Ain Shams university hospital, Cairo, Egypt, for esophagogastroduodenoscopy and diagnosed as having H. pylori infection. All these patients are newly diagnosed and none of them had previously undergone anti-H. pylori treatment or had received antibiotics within the previous 2 months. Further 20 healthy volunteers (17 male and 3 females) participated in the current study. Patients received consents before going through the endoscopy procedures. Both patients and healthy volunteers received consent for the study which was approved by local ethical committee.

B. Sampling and biopsy

Before going through endoscopy, blood was collected from all patients and kept at 4ºC. Blood was centrifuged at 3300 xg for 15 minutes to separate serum, divided into aliquots and stored at -20ºC to be used later for immunoassays. During endoscopy, gastric tissue, antral biopsy, was obtained by means of routine biopsy forceps. The biopsy collected from each patient was kept in 10% formalin to be processed later for histological and immunohistochemical staining.

C. Immunoassay serum measurements

The level of IgM antibody was measured in the serum of all patients using AccuBind ELISA Kits (Monobind Inc, Lake Forest, CA, USA) and hence these patients were then categorized into IgM positive [IgM(+)] and IgM negative [IgM(-)] groups. Gastrin-17, PgI and PgII were measured in serum using sandwich enzyme immunoassay (ELISA) kits provided from Biohit Pic, Helsinki, Finland following manufacturer instructions. IL-6 was measured using a sandwich ELISA kit supplied from DRG international, Inc, Mountainside, USA. Serum PGE_2 were determined by enzyme immunoassay kits supplied from R&D Systems.
Inc., Minneapolis, USA following the instructions of manufacturer.

D. Histopathological and immunostaining of antral biopsy

Standard histological technique was employed using Haematoxylin and eosin staining. Immunohistochemical staining iNOS was determined using polyclonal antibody kit, highly purified from rabbit antiserum by peptide affinity chromatography, supplied from Zymed Lab, Inc, San Francisco, CA, USA. Nitrotyrosine, a stable marker of peroxynitrite formation, was done immunohistochemically to evaluate nitrosative stress involved in H. pylori gastritis using monoclonal mouse anti-nitrotyrosine kit supplied from Zymed Lab Inc, CA, USA. Immunostaining for DNA fragmentation, a marker for apoptosis, was determined using polyclonal antibody kit (DFF45), supplied from Lab Vision Corp, Fermont, CA, USA.

E. Statistical analysis

Statistical analyses of data were done by the Statistical Package for Social Sciences software (SPSS, Illinois, USA). Results were expressed as mean ± SD. Student t test was used to study any statistical differences between gastritis groups and healthy volunteers taking P < 0.05 as statistically significant.

III. RESULTS and DISCUSSION

H. pylori infection represents an etiological agent, acting as inducer for active chronic gastritis, reaching 70-95% [20]. Although it is highly associated with chronic gastritis, studies revealed that not all peoples exposed become infected and children may be able to spontaneously clear an acute infection [21]. Present work has been focused on well diagnosed chronic gastritis cases. The 58 patients diagnosed as having H. pylori infection were predominantly male (84%). This was in consistent with other studies, including a Meta analysis, showing the male predominance of H. pylori infection in adults [12]. Due to different factors, 31% (15 male, 3 females) of those H. pylori infected individuals (43 ± 7 years old) demonstrated positive results for IgM antibodies in their sera, while the remaining 69% (45 ± 6 years old) revealed IgM negative reaction of whom only 6 were female (Table 1). Detection of anti-H. Pylori IgM is highly specific, western blot analysis revealed a variable IgM response to H. pylori antigens among patients with the most reactive antigenic fractions being in the range of 55-100 kDa [22].

Table (1) demonstrated significant gastremia in both patient groups (IgM+ and IgM- groups, *P*<0.001), which is mostly attributed to intragastric increase of H. pylori inducing corpus atrophy and G cells damage in the antrum part. It may be also depends on alkalinization in G cells environment caused by H. pylori urease and in agreement with previous reports [23-25].

 Serum pepsinogen (I&II) are higher in patients with H. pylori infection than in normal controls (*P*<0.001). The IgM+ group of patients demonstrated significantly higher levels of serum pepsinogen (I&II) than the IgM- group (*P*<0.05) as illustrated in Table 1. This may be attributed to a polypeptide secreted by H. pylori during earlier infection which stimulates chief cells directly and promotes pepsinogen synthesis and secretion, specifically PgII. This is mainly through intracellular mechanisms of increasing Ca++, cyclic adenosine monophosphate (cAMP) and phosphoinositide. Certain reported studies concluded similar findings, referred to higher PgI and lower ratio of PgI/PgII in IgM(+) group than in IgM(-) group [26,27]. Recent study has been expressed pepsinogen as a biomarker of gastric mucosal status including atrophic change and inflammation i.e as the fundic mucosa reduces, serum pepsinogen level gradually decreases. This can detect extensive atrophic gastritis regardless of their H. pylori status [28].

Table 1: Gastrin-17 (pmol/l), pepsinogen I (Pgl; µg/l) pepsinogen II (PgII; µg/l), prostaglandin E2 (PGE2; pg/ml) and interleukin-6 (IL-6; pg/ml) measured in serum from patients having H. pylori either with IgM(+) or IgM(-) and healthy volunteers.

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<th>Normal controls</th>
<th>IgM (+)</th>
<th>IgM (-)</th>
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<tr>
<td>N</td>
<td>20</td>
<td>18</td>
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<td>(M/F)</td>
<td>(17/3)</td>
<td>(15/3)</td>
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<td>Age</td>
<td>47</td>
<td>43</td>
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Chronic gastritis can be turned to atrophy, intestinal metaplasia and dysplasia which are precancerous [29,30]. Accordingly histological and physiological improvements through treatment of patients having positive atrophic gastritis are promising in prevention of gastric cancer [31]. Those authors have considered serum pepsinogen level as a non endoscopic blood test in the diagnosis of atrophic gastritis, H. pylori eradication and a screening tool for high risk subjects having atrophic gastritis rather than a test for cancer itself.

Vaaninen et al (2003) [32] concluded that diagnosis of atrophic gastritis using test panel of serum gastrin-17, PgI and H. pylori antibodies were in good agreement with the endoscopic and biopsy findings, considering such panel a non endoscopic diagnostic and screening tool.

Serum IL-6 level showed also significant increase in both patient groups as compared to control, mostly marked in IgM(+) group only although non significant (Table 1). H. pylori infection can induce IL-6 in monocyte/macrophages and in chronically inflamed tissues, leading to the development of gastritis. However, activated macrophages represent the main sources [33]. Oxidative stress can also influence the expression of multiple genes in monocytes and other cells including signal molecules such as protein kinase C (PKC), nuclear factor kappa (NF-kB) [34] and overexpression of these genes stimulates the secretion of pro-inflammatory cytokines [35]. Serum PGE2 showed also significant increase as shown in Table 1. This may be attributed to increased cyclooxygenase-2 (COX-2) expression in subsequent to leukocytes infiltration [36].

Haematoxyline and eosin staining for IgM(-) category showed irregular lumen (LU), damaged epithelium(E), irregular shape of fundic gland (FG) and multiple inflammatory cells (Fig.1a and Fig.1b).

The immunostaining toward nitrotyrosine and myeloperoxidase showed negative reaction in the epithelial (E) lining FG and inflammatory cells filling LP (Fig.2a and Fig.2b). Immunostaining for iNOS revealed a moderate positive reaction in the cells of the FG (Fig.2c) as well as mild positive reaction for DFF in the epithelial cells lining FG and cells filling LP (Fig.2d).

### Table 1

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<th>(year)</th>
<th>(6)</th>
<th>(7)</th>
<th>(6)</th>
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<tr>
<td>Serum gastrin-17 (pmol/l)</td>
<td>31.28</td>
<td>(2.78)</td>
<td>(6.3)</td>
<td>(5.28)</td>
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<tr>
<td>Serum PgI (µg/l)</td>
<td>90.8</td>
<td>(25.6)</td>
<td>(41.9)</td>
<td>(30.1)</td>
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<td>Serum PgII (µg/l)</td>
<td>10.26</td>
<td>(1.47)</td>
<td>(8.7)</td>
<td>(4.6)</td>
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<td>PgI/PgII</td>
<td>8.78</td>
<td>(2)</td>
<td>(3.45)</td>
<td>(2.61)</td>
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<tr>
<td>Serum PGE2 (pg/ml)</td>
<td>300.3</td>
<td>(22.38)</td>
<td>(26.6)</td>
<td>(23.36)</td>
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<tr>
<td>Serum IL-6 (pg/ml)</td>
<td>81.8</td>
<td>(9.55)</td>
<td>(34.91)</td>
<td>(34.77)</td>
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Results were expressed as mean (SD). Significantly different from healthy volunteers group* P< 0.001.
Fig. 2: Immunostaining section of human fundic gland from gastritis patients, with anti-H. pylori IgM(+) showing for (a) nitrotyrosine negative reaction in the epithelial (E) lining fundic gland (FG) and inflammatory cells (arrows) filling lamina propria (LP), (b) myeloperoxidase showing strong positive reaction in the inflammatory cells (arrows) infiltrating lamina propria (LP) (c) iNOS showing moderate positive reaction in the cells of the fundic gland (arrows), (d) DNA fragmentation factor (DFF) showing mild positive reaction in the epithelial cell (arrows) lining fundic gland (FG) (x200).

Histological examination of IgM(+) category demonstrated irregular short fundic gland (FG), wide gastric pit (GP), multiple inflammatory cells and blood vessels filling lamina propria (LP) as shown in Fig. 3a and Fig. 3b. Strong positive reaction for nitrotyrosine in the epithelial (E) lining FG and inflammatory cells filling LP (Figure 4a) as well as for MPO in the surface columnar epithelial cells (E) and other cells lining fundic gland (Fig. 4b). Immunostaining for iNOS showed positive reactions in the inflammatory cells filling LP (Fig. 4c). Furthermore, a strong positive reaction for DNA fragmentation factor (DFF) in the epithelial lining FG and inflammatory cells filling LP was also observed (Fig. 4d).

Inflammatory cells such as polymorphonuclear cells and macrophages can express iNOS in mammals [37,38], mostly associated with nitrotyrosine production additionally can induce apoptosis (DNA fragmentation) as mentioned before [39]. Ding and colleagues demonstrated that H. pylori can induce programmed cell death in cultured gastric epithelial cells as do pro-inflammatory cytokines which released during infection [40]. Others concluded that hypergastrinaemia can render epithelial cells within corpus tissues much more susceptible to apoptosis either by radiation or H. pylori infection [25].
IV. Conclusion

Individuals demonstrating IgM positive reactions recorded significant increase in pepsinogen I, II joined with slight increase in IL-6 level as compared to IgM negative group. Their fundic gland showed strong positive reactions for nitrotyrosine, iNOS, DNA fragmentation and myeloperoxidase as compared to IgM negative group.

References:


[37] F. Iacopini, A. Consolazio, D. Bosco, A. Marcheggiano, A. Bella, R. Pica, et al,
Oxidative damage of the gastric mucosa in Helicobacter pylori positive chronic atrophic and nonatrophic gastritis, before and after eradication. *Helicobacter*, 8, 2003, 503-512.

