Biochemical effect of Helicobacter Pylori infection on immune stress biomarkers

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A B S T R A C T

The aim of this study was based on the fact that the H. pylori infection has been associated with the disturbance of the immune system. So, it was to evaluate the serum concentrations of Anti-nuclear Antibodies (ANA), Anti-Double Strand DNA (anti-ds DNA), Interlukin-2 (IL-2), Cortisol, and Immunoglobulin IgA, IgM, IgG and IgE in H. Pylori-infected patients and the healthy control group. In order to achieve this aim 80 Helicobacter pylori infected patients, and 20 clinically healthy subjects used as control they are classified according to ages which ranged between less than 20 to over 65 years are applied in the experiment. The result of the present study showed that there are great associations between serum ANA, Anti-ds DNA, IL-2, IgM, IgA, IgG and IgE with Helicobacter pylori infection. These immune stress biomarkers regarded as predictors or risk factors for Gastritis, Gastric cancer and autoimmune disorders.

Key words: ANA, Anti-dsDNA, Cortisol, H. pylori, IL-2, IgM, IgG, IgA and IgE

1. INTRODUCTION

Helicobacter pylori (HP) microaerophilic, spiral-shaped gram-negative bacteria, are organisms that can survive in the highly acidic stomach environment, and are known to cause chronic gastric inflammation and cancer (Mahachai et al., 2016). The infection is very common among Asians, with a prevalence rate of up to 50–80% in some countries (Xie and Lu, 2015). Interestingly, eradication of HP in some patients with associated autoimmune diseases leads to long-term remission of the autoimmune disease (Kuwana, 2014). Although HP infection has shown a protective effect on the development of lupus in a case control study, especially among African-American patients, the relationship of lupus-HP is still intriguing. HP infection has been associated with other autoimmune diseases such as immune thrombocytopenic purpura and membranous nephropathy (Hasni et al., 2011). The presence of H pylori antibodies signify this chronic infection and their prevalence increases with age in all populations, mainly due to the birth of cohort phenomenon (Kosunen et al., 2005). The roles of cytokines and chemokines in the carcinogenicity of H. pylori have been investigated. H. Pylori infection is a multi-factorial pathology and each of the host and...
bacterium dependent factors have their influences on the immune system. The inflammatory response in infection by H. Pylori determines the production of gastric cytokines, the alteration of acid homeostasis, which directly impacts the colonization pattern of the bacterium and the extension of the gastritis (Romero-Andria et al., 2015).

H. pylori infection may also induce abnormalities indirectly by affecting the brain-gut axis, similar to other microorganisms present in the alimentary tract. The brain-gut axis integrates the central, peripheral, enteric and autonomic nervous systems, as well as the endocrine and immunological systems, with gastrointestinal functions and environmental stimuli, including gastric and intestinal microbiota. The bidirectional relationship between H. pylori infection and the brain-gut axis influences both the contagion process and the host’s neuroendocrine-immunological reaction to it, resulting in alterations in cognitive functions, food intake and appetite, immunological response, and modification of symptom sensitivity thresholds (Budzyński and Klopoćka., 2014).

2. MATERIALS AND METHODS

2.1. Patients:
The study taken out on 100 individuals from both sex 20 of them healthy person and 80 patients infected with H pylori, with age range between 20 to over 65 years, they came to treatment and follow up in Gamal Abdel Naser General Hospital in medicine and endemic medicine departments for sustained from continuous (pyloric burning) heart burning and upon analysis them stool samples for the presence of H pylori antigen they are positive. Who classified according to age into four equal groups as follow:

- **Group I** (Control group) consist of 20 clinically healthy individuals with ages ranged from 20 to over 65 years.
- **Group II**: Consisted from 20-infected patients with age less than 20 years.
- **Group III**: Consisted from 20-infected patients with age ranged from 20-35 years.
- **Group VI**: Consisted from 20-infected patients with age ranged from 35 to 50 years.
- **Group V**: Consisted from 20-diseased patients with age ranged from 50 to 65 years.
- **Group VI**: Consisted from 20-diseased patients with age up to 65 years.

2.2. Collection of sample:
Stool and venous blood samples of all infected and healthy persons were collected. The Stool samples were analyzed for H.pylori Antigen using ELISA stool antigen test (Golden Bio Technologies Corp H. pylori Antigen ELISA Kit, Upland CA 91786 , U.S.A) and The collected blood samples were transferred to 5 ml vacutainer tubes, stand about 30 minutes in room temperature, then centrifuged (at 3000 r.p.m for 5 minutes) and all available sera were stored at -20 °C, diluted for biochemical analysis.

2.3. Biochemical parameters:
Serum Anti-dsDNA; ANA (Homburger et al.,1998); IL2 (Sautto et al.,20016); Cortisol (Rosalki, 1998); IgM, IgA and IgG (Dati et al., 1996) and IgE (Homburger, 1991).

2.4. Statistical analysis:
The Statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (Version 22). Data were treated as complete randomization design according to (Steel et al., 1997). Multiple comparisons were carried out applying LSD.

3. RESULTS
The presented data in table (1) revealed that the mean values of serum Anti-dsDNA double stranded and serum ANA concentration in the infected human groups showed a significant increase at different ages in comparison with the recorded values of control healthy group. The highest significant increase was noticed in patients at age more than 65 years old, while the lowest significant increase was seen in patients less than 20 years old.

The data presented in table (2) revealed that the mean values of serum cortisol and serum (IL-2) concentration in the infected human...
groups showed a significant increase at different ages in comparison with the recorded values of control healthy group. The highest significant increase in serum cortisol was noticed in patients at age less than 20 years old and the highest significant increase in serum (IL-2) was noticed in patients at age more than 65 years old, while the lowest significant increase in serum cortisol was seen in patients more than 65 years old and the lowest significant increase in serum (IL-2) was seen in patients less than 20 years old.

Table (1) Effect of Helicobacter pylori infection on serum Anti-DNA double stranded and ANA pattern.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anti-DNA (ds) (U/ml)</th>
<th>ANA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I</td>
<td>20.18 ± 1.89^d</td>
<td>1.14 ± 0.15^d</td>
</tr>
<tr>
<td>G II</td>
<td>31.03 ± 1.53^e</td>
<td>2.56 ± 0.22^e</td>
</tr>
<tr>
<td>G III</td>
<td>38.15 ± 0.90^b</td>
<td>2.70 ± 0.26^b</td>
</tr>
<tr>
<td>G IV</td>
<td>37.69 ± 0.88^b</td>
<td>3.48 ± 0.23^b</td>
</tr>
<tr>
<td>G V</td>
<td>37.12 ± 1.16^b</td>
<td>3.86 ± 0.21^b</td>
</tr>
<tr>
<td>G VI</td>
<td>6.14 ± 0.42^a</td>
<td>4.71 ± 0.16^a</td>
</tr>
</tbody>
</table>

Table (2) Effect of Helicobacter pylori infection on serum Cortisol and (IL2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anti-DNA (ds) (U/ml)</th>
<th>ANA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I</td>
<td>20.18 ± 1.89^d</td>
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</tr>
</tbody>
</table>

The data presented in table (3) revealed that the mean values of serum Ig-G, IgA, IgM and IgE concentration in the infected human groups showed a significant increase at different ages in comparison with the recorded values of control healthy group. The highest significant increase in serum IgG, IgA, IgM and IgE was noticed in patients at age less than 20 years old, while the lowest significant increase in serum IgG, IgM and IgE was seen in patients more than 65 years old. And the lowest significant increase in serum Ig-A was seen in patients at 20-35 years old.

Table (3) Effect of Helicobacter pylori infection on Serum IgM, IgA, IgG, and IgE.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ig-G (mg/dl)</th>
<th>Ig-A (mg/dl)</th>
<th>Ig-M (mg/dl)</th>
<th>Ig-E (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G I</td>
<td>821.25 ± 36.96^f</td>
<td>330.22 ± 16.91^d</td>
<td>117.26 ± 3.83^d</td>
<td>55.68 ± 3.85^d</td>
</tr>
<tr>
<td>G II</td>
<td>1813.23 ± 78.56^d</td>
<td>585.99 ± 24.45^a</td>
<td>159.25 ± 4.15^a</td>
<td>117.02 ± 3.57^c</td>
</tr>
<tr>
<td>G III</td>
<td>1209.16 ± 56.90^i</td>
<td>369.60 ± 18.17^e</td>
<td>137.61 ± 3.18^b</td>
<td>131.41 ± 4.31^b</td>
</tr>
<tr>
<td>G IV</td>
<td>1616.64 ± 55.97^c</td>
<td>456.24 ± 20.12^b</td>
<td>140.49 ± 3.65^b</td>
<td>139.20 ± 4.09^b</td>
</tr>
<tr>
<td>G V</td>
<td>1771.20 ± 50.39^b</td>
<td>431.75 ± 21.25^b</td>
<td>145.99 ± 3.24^b</td>
<td>130.00 ± 3.95^b</td>
</tr>
<tr>
<td>G VI</td>
<td>988.86 ± 41.70^o</td>
<td>406.60 ± 16.55^b</td>
<td>129.80 ± 2.91^cd</td>
<td>154.34 ± 4.20^a</td>
</tr>
</tbody>
</table>
The results showed higher serum levels of ANA and Anti–dsDNA in H. pylori-infected patients which represent the H. pylori-related immune disturbance in these patients. The mean value of serum ANA was significantly higher in comparison to the control group this result was came in accordance with the results of Jafarzadeh et al. (2013), who found higher serum levels of ANA in H. pylori-infected patients with peptic ulcer disease. H. pylori infection causes not only a variety of gastroduodenal diseases but is also involved in the pathogenesis of various autoimmune disorders such as rheumatoid arthritis (RA), idiopathic thrombocytopenic purpura, Sjogren's syndrome, autoimmune gastric atrophy, anti-phospholipid antibody syndrome, autoimmune thyroiditis and Henoch-Schoenlein purpura (Hasni et al., 2011). The possible mechanisms responsible for the elevation of ANA levels in H. pylori-infected patients still remain to be determined. Some H. pylori components may be responsible for this phenomenon. It has been demonstrated that both cell surface-bound urease and purified urease isolated from H. pylori activate murine B cells to produce various autoantibodies, such as immunoglobulin M (IgM)-type RF, anti-single-stranded DNA and anti-phosphatidylcholine antibodies (Yamanishi et al., 2006). Moreover, it has also been reported that the direct interaction of extracellular toll like receptors (TLR2) on B-1 type cells with H. pylori urease induces the production of various autoantibodies in a T-cell-independent manner. This may account for the association of various autoimmune diseases with H. pylori infection. Accordingly, it has been suggested that H. pylori components such as urease may be among the environmental inducers that initiate several autoimmune diseases by inducing the production of autoantibodies via the activation of B-1 cells (Jafarzadeh et al., 2013). The significant increase of serum Anti-dsDNA came in agreement with those recorded by Surawut et al. (2018) their study finding that Helicobacter pylori infection increased anti-dsDNA. HP infection has been shown to enhance auto-antibodies (Yamanishi et al., 2006; Hasni et al., 2011), thus this study specifically looked at anti-dsDNA levels, a specific auto-antibody of lupus, which also helps determine disease severity. Indeed, HP induced anti-dsDNA in wild-types; the localized HP gastritis may induce the systemic inflammatory responses and enhance lupus progression in some patients with lupus. IL-2 is a pro-inflammatory cytokine that is mainly synthesized by immunoregulatory T helper cells and which plays an important role in antitumor immunity. Helicobacter pylori (H. pylori) are gram-negative bacteria that colonize the gastric mucosa and induce the production of IL-2. This process increases the magnitude of inflammation and may influence the development of gastric pathologies (Melchiades et al., 2017). A cellular inflammatory infiltrate of the lamina propria, comprising polymorphonuclear leukocytes and mononuclear cells, is present in the gastric mucosa after Hp colonization, and an increased cytokine synthesis is thought to play a crucial role in the pathogenesis of this type of gastritis (Basso et al., 1996). We found that Hp-infected patients had significantly higher level of serum interlukin-2 (IL-2) than uninfected patients. Our findings for IL-2 are in agreement with those reported by Hussey and Jones (2011) showing that H. pylori infection enhanced levels of serum IL-2. While Sundrud et al. (2004) showing that CD4 T cells are critical for protection against H. pylori infection . Thus, it seems possible that immune evasion strategies of H. pylori may involve the inhibition or modulation of T cell immunity. Indeed, two reports have recently demonstrated that VacA inhibits activation of Jurkat T cells (a human T cell lymphomaleukemia cell line) as well as human peripheral blood lymphocytes, indicates that the secreted Helicobacter pylori
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Vacuolating toxin (VacA) inhibits the activation of T cells. VacA blocks IL-2 secretion in transformed T cell lines by suppressing the activation of nuclear factor of activated T cells (NFAT).

Our results showed a significant increase in serum cortisol at different ages in comparison with the recorded values of control healthy group. Some studies showed that an increased serum cortisol level promoted H. pylori colonization. Koşan et al. (2008) found that, compared with those of the healthy control group, the serum cortisol level was increased in H. pylori-positive patients. In contrast, Katagiri et al. (2006) reported that H. pylori-infected patients had significantly decreased cortisol levels than H. pylori-negative patients. They also demonstrated that cortisol prevents H. pylori colonization through strengthening the host defense mechanisms.

Furthermore, disturbances in the upper and lower digestive tract permeability, motility and secretion can occur, mainly as a form of irritable bowel syndrome. Many of these abnormalities disappear following H. Pylori eradication. H. pylori may have direct neurotoxic effects that lead to alteration of the brain-gut axis through the activation of neurogenic inflammatory processes, or by microelement deficiency secondary to functional and morphological changes in the digestive tract. In digestive tissue, H. pylori can alter signaling in the brain-gut axis by mast cells, thereby indirectly influencing the brain-gut axis. These immune-mediators may stimulate mast cells (MC) in the gastric mucosa, as well as the hypothalamus and brain stem (via neuroendocrine-immuno crosstalk), thereby activating the sympathetic ANS and pituitary-suprarenal axis, resulting in increased cortisol and adrenaline secretion (Budzyński and Kłopocka., 2014).

6. CONCLUSIONS

H. pylori infection associated with increased in immune stress marker as Anti-ds DNA and ANA and immunoglobulin IgM, IgG, IgA and IgE, which are associated with autoimmune diseases, also this infection associated with increased serum IL-2 in response to this increase also cortisol level will increase. H. pylori is probably responsible of immune system disturbance and autoimmune disorder.

7. REFERENCES


methods. Archives of pathology & laboratory medicine, 122(11), 993.


Sundrud, M. S., Torres, V. J., Unutmaz, D., & Cover, T. L. (2004). Inhibition of primary human T cell proliferation by Helicobacter pylori vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proceedings of the National Academy of Sciences, 101(20), 7727-7732.

Surawut, S., Panpetch, W., Makjaroen, J., Tangtanatakul, P., Thim-Uam, A., Wongphoon, J., ... & Leelahavanichkul, A. (2018). Helicobacter pylori infection increased anti-dsDNA and enhanced lupus severity in symptomatic FcyRIIb-
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deficient lupus mice. Frontiers in Microbiology, 9, 1488.
