Cytokine profile in patients with concurrent Schistosoma mansoni infection with Helicobacter pylori associated chronic gastritis

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Abstract: The response of the host to parasitic infections represents a complex interaction between non specific inflammatory mechanisms and specific immunologically adaptive events. The type of effector mechanisms involved depends on the type of organism. Schistosoma mansoni infection is characterized by a strong T-helper type 2 (Th2) cell-associated immune response. However, bacterial infection is associated with induction of Th1 immune response. Few data are available about the immune response of cases infected with combined Helicobacter pylori (H. pylori) and schistosomiasis. Thus, the investigation of the cytokine pattern in patients coinfected with both H. pylori and schistosomiasis was our rationale. This study included four patient groups: Group I included 24 patients infected with chronic schistosomiasis alone, Group II included 24 patients infected with H. pylori alone, Group III included 24 healthy control individuals with matched age and sex and Group IV patients with chronic H. pylori and schistosomiasis. Serum levels of IFN-gamma, interleukin (IL)-4 were measured in all groups by enzyme-linked immunosorbent assay. The results showed that the patients infected with H. pylori had significantly higher serum levels of IFN-gamma compared with the controls and the patients with schistosomiasis and coinfection (P < 0.001). On the other hand, serum levels of IL-4 were significantly higher in patients with schistosomiasis and coinfection compared with the control group and with the H. pylori patients. Schistosomiasis appeared to induce a Th2 cytokine profile, with increase in serum levels of IL-4, even in the presence of H. pylori coinfection. In conclusion, schistosomiasis may down regulate the stimulatory effect of H. pylori on Th1 cytokines.

Key words: cytokines, Schistosomiasis and H. Pylori.

1. Introduction

Most Helminthic infections are chronic, where the worms are long living and may survive within their host for many years. To survive such extended periods of time, these organisms have developed sophisticated survival substances that induce ant-inflammatory and/or regulatory immune responses (Maizels et al., 2004). This ability of helminthic parasites to modulate immune response and immune responsiveness has generated a great deal of interest (Helmy, 2009). The beneficial effects of helminthic infections and/or products have been demonstrated using experimental models in conditions such as inflammatory bowel disease, diabetes and allergy (Zaccone et al., 2006).

Schistosomiasis is a helminthic infection caused by the blood fluke of the genus Schistosoma. Despite intensive control efforts, disease caused by these worms remains a major public health concern in Egypt and so many other developing countries (ElSaied et al., 2009). Although, schistosomiasis is endemic in Egypt where Helicobacter pylori (H. pylori) is a widespread problem and coinfections are frequent, limited data exists on the effect of schistosomiasis on the severity of H. pylori infection (Elshal et al., 2004).

Helicobacter pylori (H. pylori) causes gastritis, peptic ulceration and is an important risk factor for gastric adenocarcinoma (the second highest cause of cancer deaths worldwide). The disease process is thought to have a multifactorial etiology (Hussein, 2010). The presence of H. pylori invariably induces gastric inflammation with the release of chemokines and cytokines, which in turn recruit and activate lymphocytes. It has been reported that H. pylori causes a predominant Th1 type response which enables it to eliminate the organism and might even benefit the bacteria by providing nutrients and growth factors. A prolonged Th1 response damages the mucosa and may lead to gastroduodenal disease (Torres et al., 2003).

An imbalance between Th1/Th2 immune response caused by helminth infection has been found to play a role in immune activation and/or dysregulation of the host immune response to concurrent bacterial infection. The increased pathology observed with concurrent S. mansoni and H. pylori infection demonstrates that the severity of H. pylori infection is exacerbated by the concurrent infection with S. mansoni and that schistosomiasis may be a risk factor for aggravate H. pylori infection.
pathogenicity (Brady et al., 1999; Mansfield et al., 2003).

2. Subjects and methods

The present study included 96 individuals of different age groups ranging from 18-65 years old. They were selected from those attending National Liver Institute Hospital, and Shebin El-Kom Educational Hospital (Hepatology Department).

Patients and controls were subjected to:

**Full clinical examination**

With special attention to gastrointestinal complaints as well as abdominal examination and assessment of the severity of the liver disease and its complications such as cirrhosis was carried out. History of parasitic infection within the previous three months was excluded.

**Stool examination**

Formol ether concentration technique to exclude protozoal infection was performed (Garcia, and Bruckner, 1998).

In addition, Kato Katz technique for diagnosis of helminthic infections (Katz et al., 1970).

**Diagnosis of Schistosomiasis:**

Confirmation of *S. mansoni* infection was carried out through detection of *S. mansoni* ova in stool as determined by quantitative examination of repeated stool samples or rectal snap and a serological test (indirect haemagglutination assay; Femouz Laboratories, Asnières, France).

**Diagnosis of *H. pylori* in serum:** *H. pylori* infected patients were screened for the presence of anti- *H. pylori* antibodies. SERION ELISA classic IgG kit was used; an enzyme immunoassay that utilizes enzyme-linked immunosorbent assay (ELISA; Sorin Biomedica, Saluggia, Italy).

**Assessment of cytokine profile.**

A blood sample was taken from each patient and control. Sera were separated and cryopreserved at 70 °C till tested by ELISA Kits (Pomi et al., 1997).

a- **Quantitative determination of serum IFN-γ**

The test was done according to the manufacturer’s instructions (Hygult biotechnology b.v). It is a solid phase sandwich ELISA. Samples and standards were incubated in microtitre wells coated with a mouse mAb against human IFN-γ. During incubation human IFN-γ was bound to solid phase wells. A preformed detector complex was added to the wells, where it was bound to the solid phase. Para nitrophenyl phosphate PNPP substrate was added and the optical density was measured in an ELISA plate reader.

b- **Quantitative determination of serum IL-4 by ELISA**

This test is a solid phase sandwich ELISA (Diaclone). Microtitre wells coated with mAb specific for IL-4 were used. Samples and standards of known IL-4 concentration were pipetted into the wells, and incubated. After washing, a biotinylated mAb specific for IL-4 was added and incubated. Then, the enzyme streptavidinperoxidase was added. After incubation and washing to remove all unbound enzyme, a substrate solution was added to induce a colored reaction product.

**Histopathology for gastric mucosa:**

Biopsy specimens were collected, frozen in Tissue Tek OCT compound (Miles, Inc., Elkhart, IN) and then stored at −80°C. Then, 5-μm sections were cut on a 2800 Frigocut cryostat (Reichert-Jung, Germany) and were stained with hematoxylin and eosin. Pathology was scored by using a modified histology scoring system based on published methods (Loher et al., 2003).

**Statistical analysis**

Data were coded and analyzed using the SPSS computer program. Quantitative data were presented as mean ±SD for patients and control. Qualitative data were compared using frequency and percentage. Student t test for comparison of means and Pearson correlation test for comparing the serum levels of both cytokines in the patient group were used.

3. Results

The study included analysis of 96 Egyptian patients. Table (1) summarizes the demographic and clinical findings of all patients. The study population included 65 (76.7%) men and 31 (32.3%) women. The male: female ratio was 2.1: 1. The age of the patients enrolled ranged from 18-65 years, mean age 41.31 ±11.06 years.

Some patients enrolled in the study had different degrees of liver cirrhosis (as diagnosed by their clinical, laboratory, and radiological findings).

Different methods for diagnosing schistosomiasis in all groups using stool examination, rectal snap, Kato thick smear and IHAT were assessed. Results revealed that all cases were positive by IHA test, followed by rectal snap (17 cases), then Kato thick smear (11 cases) and lastly stool analysis (8 cases) as shown in table (2).

The patients groups only prone to endoscopy were examined for histopathology. The prevalence of gut mucosal damage caused by *H. pylori* alone was significantly higher than that with concurrent *S. mansoni* infection. 58.3% of cases in group I had grade 2 pathological features with neutrophils in LP and glandular epithelial lining, while 20.8% of cases in group IV had neutrophils and glandular epithelial lining in LP (Table 3).

The mean value of IL-4 was 70.87±30.83 in group I, 15.5±8.37 in group II, 10.71±9.16 in group III and 58.29±13.33 in group IV. A significant increase in serum level of IL-4 was found in studied
schistosomiasis cases compared to controls and *H. pylori* infected patients (*p* <0.001). The co-infected patients have the highest levels of IL-4 than *H. pylori* infected patients and control group (Table 4).

In the current study serum level of INF γ in patients with schistosomiasis and *H. pylori* infection and its role in disease progression was assessed. The mean value of INF- γ was 1.54±1.54 in group I, 13.77±11.82 in group II, 2.15±1.85 in group III and 3.28±1.66 in group IV. INF- γ was significantly higher in group II in comparison with other studied groups (Table 5).

### Table 1: demographic and clinical data of all patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>NO %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demography</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.31 ±11.06 (18-65)</td>
</tr>
<tr>
<td>Males</td>
<td>65 (76.7%)</td>
</tr>
<tr>
<td>Females</td>
<td>31 (32.3%)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>8 (8.3%)</td>
</tr>
<tr>
<td>No Cirrhosis</td>
<td>88 (91.7%)</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td>39 (40.6%)</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>48 (50%)</td>
</tr>
</tbody>
</table>

### Table 2: Comparison between the studied groups as regards stool analysis, rectal snip, Kato thick smear and IHA results for diagnosis of schistosomiasis

<table>
<thead>
<tr>
<th>IHA</th>
<th>Kato thick smear</th>
<th>Rectal snip</th>
<th>Stool examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive %</td>
<td>Negative %</td>
<td>Positive %</td>
<td>Negative %</td>
</tr>
<tr>
<td>NO (100)</td>
<td>0 (0.0)</td>
<td>NO (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>24 (100)</td>
<td>0 (0.0)</td>
<td>7 (29.2)</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
</tr>
<tr>
<td>0 (0.0)</td>
<td>24 (100)</td>
<td>0 (0.0)</td>
<td>24 (100)</td>
</tr>
<tr>
<td>24 (100)</td>
<td>0 (0.0)</td>
<td>4 (16.7)</td>
<td>20 (83.3)</td>
</tr>
</tbody>
</table>

### Table 3: Comparison between the studied groups as regards activity of gastritis diagnosed by histopathology.

<table>
<thead>
<tr>
<th>Activity of gastritis</th>
<th>Group IV N = 24</th>
<th>Group II N = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historypathology of gastric mucosa</td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td>0</td>
<td>20.8</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>58.3</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>20.8</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
<td>8.3</td>
</tr>
</tbody>
</table>

### Table 4: Serum levels (pg/ml) of IL-4 in Schistosomiasis, *H.pylori* infected patients and control

<table>
<thead>
<tr>
<th>P value</th>
<th>Test of significance</th>
<th>The studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group IV N = 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.94</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>1</td>
<td>5.94</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>2</td>
<td>0.24</td>
</tr>
<tr>
<td>0.03</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>4</td>
<td>0.01</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1 = Comparison between group I and group II. 2 = Comparison between group I and group III.
3 = Comparison between group I and group IV. 4 = Comparison between group II and group III.
5 = Comparison between group II and group IV. 6 = Comparison between group III and group IV.
infected patients may have a less severe form of the infection and that helminthes infection may be protective against schistosomiasis. Some studies were in agreement with our immunopathogenesis and outcome of concurrent helminthes infection on the inside the colonic mucosa.

Evidence are conflicting on the effect of helminthes infection in most parasitological examination identified in only 19 of the samples by 80 cases to be seroreactive schistosomiasis. Diagnosis of the earliest case reported that prevalence of infection estimated by parasitological examination, stool examination and finding of El Ridi (2013), who reported that immunodiagnostic test led to the diagnosis of the earliest cases of human schistosomiasis. Carneiro et al., (2013) also found 80 cases to be seroreactive while eggs were identified in only 19 of the samples by parasitological examination. This may be also attributed to closed infection in most chronic schistosomiasis cases where the eggs are trapped inside the colonic mucosa.

Evidences are conflicting on the effect of concurrent helminthes infection on the immunopathogenesis and outcome of H. pylori infection. Some studies were in agreement with our study and have shown that helminthes infection may be protective against H. pylori infection and that infected patients may have a less severe form of the disease as our study (Fox et al., 2000; Elshal et al., 2004).

The impact of concomitant S. mansoni infection on H. pylori induced gastritis was studied in twenty patients infected exclusively with H. pylori. The patients were compared with twenty patients coinfected with the bacteria and S. mansoni and twelve patients with schistosomiasis alone. The results revealed that severe gastritis was significantly more common in the patients infected exclusively with H. pylori (Abou Holw et al., 2008).

In contrast, C. rodentium-associated colonic pathology in coinfected BALB/c mice with H. polygyrus which was significantly enhanced (Chen et al., 2005).

In comparison to histopathology finding, it was noticed that high levels of IgG levels were observed in group IV than in group I, which is coincident with the study made by Manojlovic et al. (2008) who concluded that low IgG is more frequent in the patients with advanced gastric pathology than in the patients with simple gastritis.

The current study centered on determining pattern of serum IL-4 in patients with schistosomiasis and its role in disease progression.

The results of this work was consistent with those of other studies which reported high level of IL-4 in patients infected with schistosomiasis as IL-4 has a fundamental role in pathogenesis of schistosomiasis (El-Kady et al., 2005). There was a significant increase in IL-4 levels in studied cases compared to controls and this in agreement with previous studies by (Kamal et al., 2001 and Emam et al., 2006). Schistosomiasis appears to induce a Th2 cytokine profile, with increase in serum levels of IL-4 even in the presence of HCV co-infection (El-Kady et al., 2005).

A significant increase in serum level of INF γ was found in studied cases infected with H. pylori alone in comparison with controls, schistosomiasis alone and coinfected individuals (p <0.001).

Table 5: Serum levels (pg/ml) of INF-γ in Schistosomiasis, H.pylori infected patients and control.

<table>
<thead>
<tr>
<th>P value</th>
<th>Mann Whitney U test</th>
<th>The studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group IV N = 24</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>5.94</td>
<td>3.28±1.66</td>
</tr>
<tr>
<td>0.19</td>
<td>1.31</td>
<td>0.3 – 5.6</td>
</tr>
<tr>
<td>0.001</td>
<td>3.48</td>
<td>2.15±1.85</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>5.94</td>
<td>1.54±1.54</td>
</tr>
<tr>
<td>&lt;0.001</td>
<td>5.94</td>
<td>0.1 – 5</td>
</tr>
<tr>
<td>0.03</td>
<td>2.20</td>
<td>INF γ</td>
</tr>
</tbody>
</table>

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4. Discussion

Approximately 50% of humanity is infected with H. pylori. H. pylori gastritis may progress to atrophic gastritis and lead to the development of intestinal metaplasia, dysplasia and eventually gastric cancer. Fortunately, only a small percentage of the population developed serious disease due to H. pylori infection (Lee et al., 2013).

Host and environmental factors as well as the virulence properties of particular strains of H. pylori probably influence disease outcome in infected individuals. Individuals living in countries with low socioeconomic conditions suffers from prevalence rates of H. pylori acquired at an early age (Salih, 2009). Some of these countries have high rates of gastric cancer, whereas some African countries with equally high prevalence rates of H. pylori have much lower gastric cancer, this paradox has been referred to as the African enigma (Ghoshal et al., 2010).

Diagnosis of schistosomiasis by IHA test was higher than other tests used in diagnosis. This finding is coincident with study of Coulibaly et al., (2013), who found that the prevalence of anti S. mansoni antibodies was more three times than the prevalence of infection estimated by stool examination and finding of El Ridi (2013), who reported that immunodiagnostic test led to the diagnosis of the diagnosis of the earliest cases of human schistosomiasis. Carneiro et al., (2013) also found 80 cases to be seroreactive while eggs were identified in only 19 of the samples by parasitological examination. This may be also attributed to closed infection in most chronic schistosomiasis cases where the eggs are trapped inside the colonic mucosa.

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A significant increase in serum level of INF γ was found in studied cases infected with H. pylori alone in comparison with controls, schistosomiasis alone and coinfected individuals (p <0.001).
These data were consistent with results of Eltayeb et al. (2013) study which was conducted on population suffering from schistosomiasis, and there was very significant difference between IFN-γ levels between patients and control group. The endemic controls also, showed significantly higher IFN-γ concentration than patients (P < 0.001).

Abdollahi et al. (2011) found that the mean of TNF-α and IFN-γ levels in the infected group with H. pylori were significantly higher than that of uninfected patients. Increased serum level of IFN-γ indicates the activation of circulating-T cells against infection. Therefore, they concluded that, H. pylori by inducing certain inflammatory cytokines may contribute the process of disease development.

Many studies reported that both IL4 and IFN-γ appeared to have mutually antagonistic effects which were consistent with the present study. Furthermore, IL4 also inhibited the effect of IFN-γ on immunoglobulin production by B cells and vice versa (Yazdanbakhsh et al., 2001).

These findings were in contrast to Eppllein et al. (2013), where both IFN-γ and IL4 were increased in H. pylori infected patients. However, no significant correlation was detected between serum levels of both cytokines.

References