Association of helicobacter Pylori Infection and Vitamin B₁₂ Level among Sudanese Patients

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Abstract
Background: Helicobacter Pylori (H. Pylori) infection is a major gastric infection worldwide and has been associated with many hematologic disorders.

Objective: The purpose of this study was to determine the association between H. Pylori infection and vitamin B₁₂ levels among Sudanese population.

Materials and Methods: 120 samples were collected for this study (60 samples from H. Pylori positive patients and 60 samples from apparently healthy individuals as controls). All samples were assessed for hematological parameters and vitamin B₁₂ using Electrochemiluminescence immune assay.

Results: Vitamin B₁₂ levels mean was significantly lower among H. Pylori patients (207.56±184.51) than control group (494.45±181.47) (P-value 0.000), lymphocyte count mean was higher in H. Pylori positive patients (37.33±9.53) was significantly higher than the controls (32.28±8.72) (P-value 0.003), Granulocyte count mean was lower among H. Pylori positive patients (5.13±9.91) than control patients (5.70±8.43) (P-value 0.001). Other hematological parameters showed no significant difference between H. Pylori positive patients and control subjects.

Conclusion: This study has concluded that vitamin B₁₂ level is lower in H. Pylori infected Sudanese individuals.

Keywords: CBC, H. Pylori, Sudanese, Vitamin B₁₂.

I. Introduction

Helicobacter pylori (H. pylori) is a type of bacteria responsible for wide spread infection with more than 50% of the world’s population infected, even though 80% of them have no symptoms. Infection with H. Pylori has been recognized as a public health problem worldwide and more prevalent in developing than the developed countries [1]. The disease is most often acquired in childhood [2-6].

Asymptomatic carrier state is common in H. Pylori infection [7], and if left untreated H. pylori infection is lifelong [8]. It has been suggested that H. pylori infection may play an important role in impairment of vitamin B₁₂ absorption owing to diminished acid secretion, lower ascorbic acid levels in gastric juice and reduced secretion of intrinsic factor [9].

Infection with H. pylori is a co-factor in the development of duodenal or gastric ulcers (reported to develop in 1 to 10% of infected patients), gastric cancer (in 0.1 to 3%) and gastric mucosa associated lymphoid tissue (gMALT) lymphoma (in <0.1%) [10].

Absorption of dietary cobalam (vitamin B₁₂; vB₁₂) depends on several factors, including acid-dependent deproteinization of vB₁₂. Only free vB₁₂ can form a complex with the intrinsic factor (IF). The vB₁₂–IF complex is then absorbed by mucosal cells via its specific receptor (cubilin) in a calcium-dependent fashion. Gastric parietal cells are responsible for production of both hydrochloric acid and IF. Absorbed vB₁₂ is then stored in the liver. Some H. pylori patients develop auto antibodies directed against gastric parietal H+/K+-ATPase cells (APCAs), resulting in achlorhydria and increased infection with H. pylori, which in turn contributes to gastric damage and atrophy of the corpus [11].

Infection with H. pylori can lead to inflammation of the gastric mucosa with subsequent ulceration [12,13]. Infection is a major cause of chronic gastritis, a condition that initiates the pathogenic sequence of events leading to atrophic gastritis, intestinal metaplasia, dysplasia and subsequently cancer [12,13]. While the bacterium is not a direct cause of cancer, its presence and resultant reduction in acid production are necessary factors in causation [13]. The risk of these disease outcomes in infected patients varies widely among different populations and the major portion of patients with H. pylori will not have any clinically significant complications [10,11]. The purpose of this study was to determine the association between H. Pylori infection and vitamin B₁₂ levels among Sudanese population.
II. Materials and Methods

This study is a case control study, conducted in Khartoum, Sudan. One hundred and twenty samples were included in this study (60 samples from H. pylori positive patients and 60 samples from healthy individuals as controls). All of them were evaluated to determine the association of H. pylori infection and vitamin B12 levels among Sudanese population. Diagnosis of H. pylori infection was based on the detection of H. pylori Antigen in stool (HanzouAllTest Biotech Co., Ltd, Germany).

Five ml of venous blood was collected from each subject: 2.5 ml in EDTA for the blood count, and 2.5 ml in plain container for the estimation of vitamin B12 levels. Laboratory analysis was performed at the Department of Haematology, Faculty of Medical Laboratory Sciences, Alneelain University. Hematological parameters were determined using automated hematological analyzer (electronic impedance principle). Vitamin B12 levels were determined using Electrochemiluminescence immune assay with a competitive test principle using intrinsic factor (IF) specific for Vitamin B12. Vitamin B12 in the sample competes with the added Vitamin B12 labeled with biotin for the binding sites on the ruthenium-labeled IF complex. The test procedure consists of three phases of incubation. In the first incubation phase, the sample is incubated with dithiothreitol, sodium hydroxide, and sodium cyanide. In the second incubation phase, the pretreated sample is incubated with ruthenium-labeled IF, and in the third incubation phase, sites on the ruthenium-labeled IF become occupied by Vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via the interaction of biotin and streptavidin, which is later aspirated to the measuring cell wherein the micro particles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces the chemiluminescent emission which is measured by a photomultiplier. The measuring range of Vitamin B12 assay is 30–2000 pg/ml Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient’s data was performed using the t-test and P value less than 0.05 have been considered as statistically significant.

III. Results

Patients included 31 males and 29 females; their mean age was 35.9±12.7 years. All patients were tested for the blood count and vitamin B12 levels. Vitamin B12 levels mean was significantly lower among H. pylori positive patients (207.56±184.51) than the controls (494.45±181.47)(P-value 0.000). TABLE 1 showed the comparison of the haematological values among the study group and controls.

Table 1. Distribution of vitamin B12 level and hematological parameters in H. pylori infected subjects and test subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Mean±SD)</th>
<th>Case (Mean±SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>494.45±181.47</td>
<td>207.56±184.51</td>
<td>0.000</td>
</tr>
<tr>
<td>Hb%</td>
<td>13.40±0.90</td>
<td>13.11±1.58</td>
<td>0.220</td>
</tr>
<tr>
<td>HCT</td>
<td>40.52±3.08</td>
<td>40.91±5.57</td>
<td>0.636</td>
</tr>
<tr>
<td>RBCs</td>
<td>4.77±0.44</td>
<td>4.73±0.71</td>
<td>0.707</td>
</tr>
<tr>
<td>MCV</td>
<td>86.62±4.35</td>
<td>87.78±5.35</td>
<td>0.195</td>
</tr>
<tr>
<td>MCHC</td>
<td>28.55±2.58</td>
<td>28.38±3.09</td>
<td>0.710</td>
</tr>
<tr>
<td>MCH</td>
<td>37.95±9.67</td>
<td>31.87±3.28</td>
<td>0.259</td>
</tr>
<tr>
<td>WBCs</td>
<td>6.54±1.78</td>
<td>6.25±1.81</td>
<td>0.836</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>32.28±8.72</td>
<td>37.33±9.53</td>
<td>0.003</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>57.10±8.43</td>
<td>51.53±9.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Mix</td>
<td>10.67±3.17</td>
<td>11.15±3.12</td>
<td>0.402</td>
</tr>
<tr>
<td>Platelet</td>
<td>275.75±72.04</td>
<td>272.20±76.24</td>
<td>0.794</td>
</tr>
</tbody>
</table>

The mean difference is significant at the 0.05 level.

IV. Discussion

H. pylori infection can cause duodenal or gastric ulcers and gastric mucosa inflammation and can also cause gastric parietal cells inflammation and ulceration [2]. Gastric parietal cells produce both hydrochloric acid and IF. Vitamin B12 (B12/IF Complex) is absorbed by the mucosal cells of the stomach. Our study revealed that Vitamin B12 levels were lower in H. pylori infected patients comparing to healthy individuals. Lymphocyte count was higher and the granulocyte count was lower in H. pylori infected individuals comparing to healthy individuals. Other hematological parameters showed no significant difference between the H. pylori positive subjects and the control subjects. The classical sign of vitamin B12 deficiency is megaloblastic anemia which, however, occurs in only 50% of vitamin B12-deficient subjects [3]. No evidence of megaloblastic anemia was determined among the study group, according to the haematological values, this may be due to the infection period or the severity of the infection. Our study highlighted the need for screening system that may predict
Vitamin B₁₂ deficiency before its actual manifestation. If not treated it can lead to macrocytic, megaloblastic, pernicious anemia’s and other clinical abnormalities.

These results agrees with study done by Bikha Ram Devrajani, Shaikh Muhammad Zaman, Syed Zulfiqar Ali Shah, Tarachand Devrajani, Raj Kumar Lohana and Thanwar Das[14] in Pakistan 2011 in that vitamin B₁₂ level was lower in H.Pylori infected individuals. And also agrees with the study done by Shrikant C. Rauta, Rittu S. Chande[15] in India 2014 in that vitamin B₁₂ level was also lower in H.Pylori infected individuals.

V. Conclusion
Our study has concluded that vitamin B₁₂ level is lower in H.Pylori infected Sudanese individuals.

Acknowledgement
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References
[16]. Shrikant C. Rauta, Rittu S. Chande, a,b-Postgraduate resident Department of Biochemistry, Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai, Maharashtra, India , Comparison Of Vitamin B₁₂ Levels In Gastritis With And Without H.Pylori. ISSN 2349-2910 :WIMJOURNAL, 2014; Volume No. 1, Issue No. 1.