# Association of helicobacter Pylori Infection and Vitamin B<sub>12</sub> 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  $B_{12}$  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  $B_{12}$  using Electrochemiluminescence immune assay.

**Results:** Vitamin  $B_{12}$  levels mean was significantly lower among H. Pylori patients (207.56±184.51) than controlgroup (494.45±181.47)(P-value0.000),lymphocyte count mean 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 meanwas lower among H.Pylori positive Subjects (51.53±9.91) than control subjects (57.10±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  $B_{12}$  level is lower in H.Pylori infected Sudanese individuals. *Keywords*: CBC, H.Pylori, Sudanese, Vitamin  $B_{12}$ .

### 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<sup>[1]</sup>. The disease is most often acquired in childhood <sup>[2-6]</sup>.

Asymptomatic carrier state is common in H.Pyloriinfection <sup>[7]</sup>, and if left untreated H. pylori infection is lifelong <sup>[8]</sup>. It has been suggested that H. pylori infection may play an important role in impairment of vitamin  $B_{12}$  absorption owing to diminished acid secretion, lower ascorbic acid levels in gastric juice and reduced secretion of intrinsic factor. <sup>[9]</sup>.

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 <0.01%)<sup>[12].</sup>

Absorption of dietary cobalamin (vitamin  $B_{12}$ ;  $vB_{12}$ ) depends on several factors, including aciddependent deproteinization of  $vB_{12}$ . Only free  $vB_{12}$  can form a complex with the intrinsic factor (IF). The  $vB_{12}$ -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  $vB_{12}$  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 <sup>[13].</sup>

Infection with H. pylori can lead to inflammation of the gastric mucosa with subsequent ulceration<sup>[12,11]</sup>. 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<sup>[12,11]</sup>. While the bacterium is not a direct cause of cancer, its presence and resultant reduction in acid production are necessary factors in causation<sup>[11]</sup>. The risk of these disease outcomes in infected patients varies widely among different populations and the great majority of patients with H. pylori will not have any clinically significant complications<sup>[10,11]</sup>. The purpose of this study was to determine the association between H.Pylori infection and vitamin B<sub>12</sub> 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 fromH.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  $B_{12}$  levels among Sudanese population. Diagnosis of H.pylori infection was based on the detection ofH.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 2.5ml inplain container for the estimation of vitamin  $B_{12}$  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  $B_{12}$  levels were determined using Electrochemiluminescence immune assay with a competitive test principle using intrinsic factor (IF) specific for Vitamin B<sub>12</sub>. Vitamin B<sub>12</sub> 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 ruthenium-labeled IF - Vitamin B<sub>12</sub> 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 B<sub>12</sub> assay is 30–2000 pg/mlStatistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient's data was performed using the t-test and Pvalue less than 0.05 have been considered as statistically significant.

# III. Results

Patients included 31 males and 29 females; their mean age was  $35.9\pm12.7$  years. All patients were tested for the blood cell countand vitamin B<sub>12</sub>level.Vitamin B<sub>12</sub> levels mean wassignificantly lower amongH.pyloripositive 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

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Parameters	Control (Mean±SD)	Case	P-value
		(Mean±SD)	
Vitamin B12	494.45±181.47	207.56±184.51	0.000
Hb%	13.40±0.90	13.11±1.58	0.220
HCT	40.52±3.08	40.915.57	0.636
RBCs	4.77±0.44	4.73±0.71	0.707
MCV	86.62±4.35	87.78±5.35	0.195
MCH	28.55±1.58	28.38±3.09	0.710
MCHC	37.95±9.67	31.87±3.28	0.239
WBCs	6.54±1.78	6.25±1.81	0.836
Lymphocyte	32.28±8.72	37.33±9.53	0.003
Granulocyte	57.10±8.43	51.53±9.91	0.001
Mix	10.67±3.17	11.15±3.12	0.402
Platelet	275.75±72.04	272.20±76.24	0.794

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

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 <sup>[2]</sup>.Gastric parietal cells produce both hydrochloric acid and IF.Vitamin  $B_{12}$  ( $B_{12}$ /IF Complex) is absorbed by the mucosal cells of the stomach.Our study revealed that Vitamin  $B_{12}$  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  $B_{12}$  deficiency is megaloblastic anemia which, however, occurs in only 50 % of vitamin  $B_{12}$ -deficient subjects<sup>[13]</sup>. No evidence of megaloblastic anaemia was determined among the study group, according to the haematological values, this may be due to the infection period orto the severity of the infection. Our study highlighted the need for screening system that may predict

Vitamin  $B_{12}$  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 byBikha Ram Devrajani, Shaikh Muhammad Zaman,SyedZulfiquar Ali Shah, TarachandDevrajani, Raj Kumar Lohana and Thanwar Das<sup>[14]</sup> in Pakistan 2011 in that vitamin  $B_{12}$  level was lower in H.Pylori infected individuals.And also agrees with the study done by Shrikant C. Rauta ,Rittu S. Chande<sup>[15]</sup> in India 2014 in that vitamin  $B_{12}$  level was also lower in H.Pylori infected individuals.

#### V. Conclusion

Our study has concluded that vitamin B<sub>12</sub>level is lower in H.Pylori infected Sudaneseindividuals.

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