

## Evaluation of Serum Vitamin B12 and Ferritin Levels in H. Pylori- Associated Gastritis

Awad-Elkareem Abass<sup>1,2\*</sup>, Khalid O. Mohamed<sup>1</sup>, Alaa A. Yosif<sup>1</sup>, Fatima A. Mohamed<sup>1</sup>, zobaida Mohamed<sup>1</sup>, Marwa Elfadil<sup>1</sup>

<sup>1</sup>(Hematology department, Faculty of Medical Lab. Sciences, University of Khartoum, Khartoum, Sudan)

<sup>2</sup>(Faculty of Medicine and Applied Medical Sciences, Northern Border University, Arar, KSA)

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**Abstract:** *H. Pylori* infection is a major gastric infection worldwide and has been associated with many haematinics deficiencies. The aim of the study was to find out an association between *H. Pylori* associated gastritis, vitamin B12 and iron deficiencies. A prospective case control study was conducted on 60 confirmed *H. Pylori* associated gastritis' patients and 60 non- *H. Pylori* infected controls. The detection of *H. Pylori* was confirmed by endoscopy and either rapid urease test or urea breath test for each patient. A venous blood sample was collected; complete blood count, serum ferritin and vitamin B12 measurement were performed for all cases and controls. Among the total 60 *H. Pylori* associated gastritis there were 32(53.3%) males and 28(46.7%) were females, their ages ranged between 17-80 years. CBC parameters and B12 level were measured in 30 cases and compared to 30 *H. Pylori* non-infected controls; Hb/PCV, RBCs, TWBCs, and platelets count showed significant differences between cases and controls ( $p$ -values:  $<0.05$ ), while MCV, and other RBCs indices were not varied significantly. CBC parameters and ferritin level also were measured in the rest 30 cases and compared to 30 controls; these parameters were not differed significantly ( $p$ -values:  $>0.05$ ). *H. Pylori* infection is the most common cause of gastritis in Sudan. There is an association between *H. Pylori*-induced gastritis and low levels of B12. Therefore, measurement of serum vitamin B12 level might has a clinical value in such patients.

**Keywords:** *Deficiency, Cobalamin, gastritis, H.pylori, Iron*

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### I. Introduction

Helicobacter Pylori (*H. Pylori*) is a gram-negative spiral -microaerophilic commensal- bacterium that colonizes the human stomach [1]. It survives by neutralizing gastric acidity and damages gastric mucosa by producing toxins of Cag A and Vac A [2]. *H. pylori* infection is a major gastric infection worldwide. Approximately more than 50% of the adult population in the developed countries and 90% of those in the developing countries is infected with this bacterium [3-5]. It is well known that *H. Pylori* is involved in gastritis, gastric and duodenal ulcers, and carcinoma of the stomach [6]. *H. Pylori* associated gastritis is reported to result in many extra gastric complications like vitamin B12 and iron deficiency, megaloblastic anemia, and iron deficiency anemia [7].

The association between *H. Pylori* infection and iron deficiency anemia (IDA) is exist in the published literature, five meta-analyses [8], that showed this association with resolution of *H. Pylori* associated disease after eradication in children [9], male and females in puberty [10], [11], prepubertal girls [12], adult men and women [13], [14], older adults [15], pregnant women [12] and non-pregnant women [16].

*H. Pylori* infection can cause mal-absorption of different micronutrients [17] among which included the vitamin B12 [18]. A systematic review and meta-analysis in 17 studies involving 2454 patients showed a significantly reduction of serum levels of vitamin B12 in *H. Pylori* infected patients than in non-infected [19]. Marino et al [20] demonstrated in 62 older patients a relationship between the decrease in serum levels of vitamin B12 and increase of serum homocysteine due to *H. Pylori* infection. Likewise, *H. Pylori* eradication in vitamin B12 deficiency patients is followed by increasing of serum levels of vitamin B12 and decreased serum levels of homocysteine. This study is aimed to evaluate derangement in the serum ferritin and vitamin B12 in patients with *H. Pylori* associated gastritis.

### II. Methods

A prospective case control study was conducted on 60 outpatients referred to gastroscopy clinic - Military Armed Hospital (Umdorman) - who had gastritis diagnosed clinically and confirmed by upper gastrointestinal endoscopic examination which was performed by consultant gastroenterologist. *H. Pylori* was confirmed either by endoscopy and rapid urease test/or urea breath test. Patients with history of chronic autoimmune disease, inflammatory bowel disease, previous GI surgery, strict vegetarian, pregnancy, and patients under *H. Pylori* eradication therapy were excluded. Sixty *H. Pylori* non-infected matched controls were

also enrolled in whom H. Pylori infection was screened by rapid immune chromatographic test (ICT). A written consent form was obtained from all cases and controls; a questionnaire was filled with demographic and clinical data. Venous blood (5 ml) was collected; divided into two parts; one part (2 ml) was collected in EDTA anticoagulated container for complete blood count using automated cells counter (Sysmex KX.21) and peripheral blood picture. Serum was separated from the rest of blood for measurement of ferritin and B12 levels.

The obtained results data were collected formulated in tables and figures using Microsoft office. Simple T-test was used to test the differences in CBC parameters, B12 and ferritin levels between cases and controls. The level of significance was set at ( $p \leq 0.05$ ).

#### 2.1. Serum vitamin B12

Vitamin B12 level was measured using Electroluminescence (ECL) technique. Procedure followed as the manufacture's sheet instructions using competitive B12 assay method (Elecys 2010, vitamin B12 Test System, and Roche Diagnostics). Vitamin B12 level was assessed in only 30 cases.

#### 2.2. Serum Ferritin:

Ferritin was assessed using Turbidimetric latex immunoassay. Ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies, the agglutination of the latex particles is proportional to the ferritin concentration and can be measured by turbidimetry, Procedure followed as the manufacture's sheet instructions.

### III. Results

The present study included 60 H. Pylori associated gastritis patients, 32 (53.3) males and 28(46.7%) females, their ages ranged between 17-80 years. 60 H. Pylori non-infected- controls were included.

Based on the endoscopy results; antral gastritis had the highest frequency (38.7%), gastritis (25.8%), pangastritis (22.6%), severe gastritis (9.7%) and the lowest frequency (3.2%) in patients with gastric carcinoma, Figure: 1.

#### 3.1. Comparison of CBC parameters and B12 in cases and controls:

CBC parameters and B12 level were measured in 30 cases and compared to 30 controls; Hb/PCV, RBCs, TWBCs, and platelets count showed significant differences between cases and controls ( $p$ -values:  $<0.05$ ), while MCV, and other RBCs indices were not varied significantly, table 1.

#### 3.2. Comparison of CBC parameters and Ferritin in cases and controls:

CBC parameters and ferritin level also were measured in the rest 30 cases and compared to 30 controls; these parameters were not differed significantly ( $p$ -values:  $>0.05$ ), table 2.

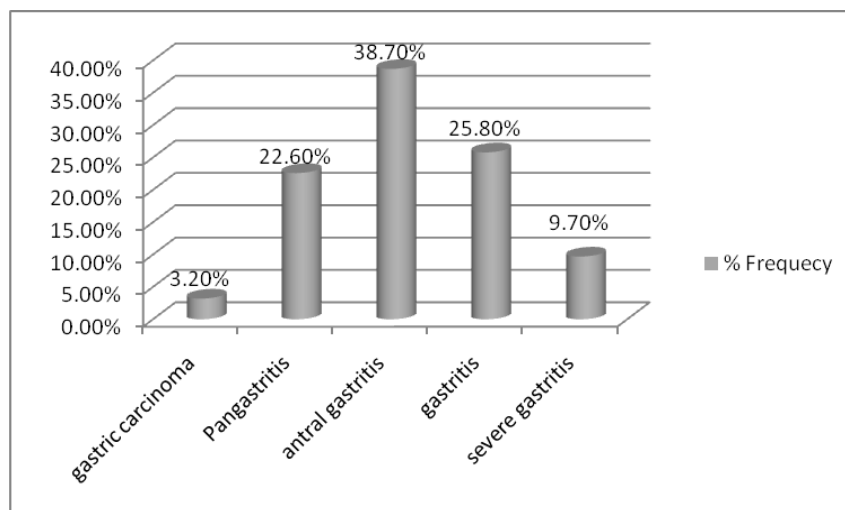


FIG 1. ENDOSCOPY RESULTS IN H.PYLORI ASSOCIATED GASTRITIS

**TABLE 1. SERUM B12 AND CBC PARAMETERS IN STUDY GROUPS (N/ 30)**

Parameters	H. Pylori-gastritis(30) Mean ±STD	Healthy Controls(30) Mean ±STD	p-value
Sex	M:16 F:14	M:16 F:14	
Age/year	37.6±290.18	36.1±134.67	0.69
Hb g/dl	12.07±4.73	13.30±2.41	0.01
RBCs ×10 <sup>9</sup> / µl	4.46±0.49	4.80±0.25	0.03
PCV%	36.57±39.88	39.66±21.06	0.03
MCV fl	81.57±40.78	83.24±22.19	0.25
MCH pg	27.07±7.98	27.72±1.94	0.26
MCHC %	32.24±2.65	32.90±1.27	0.07
WBCs ×10 <sup>3</sup> / µl	5.82±1.78	6.72±3.64	0.03
Platelets ×10 <sup>3</sup> /µl	250±7956	294±5751	0.04
Serum B12 pg/ml	278±110	590±128	0.0002

**TABLE 2. SERUM FERRITIN AND CBC PARAMETERS IN STUDY GROUPS (N/30)**

Parameters	H. Pylori-gastritis(30) Mean ±STD	Healthy controls(30) Mean ±STD	p-value
Sex	M:16 F:14	M:16 F:14	
Age/year	39.9±1.57	39.1±1.46	0.80
Hb g/dl	13.9±2.15	13.5±1.31	0.13
RBCs ×10 <sup>9</sup> / µl	5.07±0.67	4.84±0.41	0.13
PCV%	42.83±5.82	40.97±3.46	0.13
MCV fl	84.31±2.94	84.18±3.85	0.88
MCH pg	27.56±1.43	28.20±1.22	0.88
MCHC %	32.68±1.29	32.39±0.84	0.29
WBCs ×10 <sup>3</sup> / µl	5.47±2.31	6.59±1.87	0.04
Platelets ×10 <sup>3</sup> /µl	237±117.07	250 ±66.61	0.61
Ferritin ng/ml	140±7.10	186±7.80	0.13

#### IV. Discussion

H. Pylori infection is one of the dominant health problems of the stomach that leads to development of gastritis especially in developing countries. H. Pylori is a causative agent for iron deficiency anemia (IDA), although the mechanisms remain unclear, clinico- epidemiologic studies suggest that H. pylori infection is linked with IDA [21]. A randomized controlled trial study by Valiyaveetil et al [22] revealed evidence that supports a role of treating H. Pylori infection for patients with H. pylori gastritis and unexplained IDA. In other randomized controlled trial by Choe et al showed a benefit of H. Pylori treatment in patients with unexplained IDA who have H. Pylori-gastritis. They found that patients who underwent H. Pylori treatment, with or without iron, had a significantly greater rise in hemoglobin level at 8 weeks after therapy compared with those who received only iron supplementation [23]. Valiyaveetil et al found that iron deficient –infected H. pylori patients who underwent anti-H. Pylori treatment had a significantly larger increase in hemoglobin concentration one month after H. Pylori treatment compared to iron deficient –infected H. pylori patients who did not receive H. Pylori treatment (3.6 g/dl vs. 1.1 g/dl, p=0.025) [22]. Many other recent studies have shown an association between H. pylori and IDA [24].

Ferritin is measurable in plasma, where it correlates closely with bone marrow iron stores in the steady state. In this study, we investigated the ferritin levels and full blood count parameters among H. Pylori gastritis patients and the results was compared with normal controls groups who were H. Pylori negative, our results revealed no significant differences in these parameters between the two studied groups. However, it has been shown in the literature that two different subsets of H. Pylori strains exist, one causes iron deficiency and the other one doesn't. This suggests that polymorphism of H. Pylori strains could be a factor determining the prevalence of H. Pylori associated iron deficiency anemia. [25] Our findings revealed normal ferritin levels in H. Pylori gastritis patients. This could be justified by our small sample size and most of the studied patients who were assessed for ferritin were newly diagnosed. Furthermore, etiology of IDA as consequence of H. Pylori infection is a strain dependent. In addition, our study methodology doesn't include polymorphism genotyping for H. Pylori strain.

Patients with food-B12 mal-absorption cannot absorb food-bound or protein-bound B12 even if they absorb free B12 normally and this results in low serum levels. It is indicated that this event is associated with gastric dysfunction [26].

Clinical and histopathological features of chronic gastritis almost link to H. Pylori colonization of the stomach [27]. However, in comparing H. Pylori gastritis, gastritis in non-infected mucosa has no inflammatory infiltrates and shows mild histological grade [28].

Our results revealed an association between H. Pylori -gastritis and low B12 levels. More frequent low levels of B12 were observed in infected gastritis patients. Nonetheless mean of vitamin B12 values in colonized patients was lower than that seen in non-infected controls. The concordance results support an existing role of H. pylori associated gastritis in predisposing to vitamin B12 deficiency. The pathological mechanism in gastric mucosa seems to be responsible for the vitamin mal-absorption, as an induced gastritis leads to destruction or partial loss of parietal cells that produce IF; a substance important for B12 absorption. If left untreated for many years, H. Pylori - induced gastritis and related pathological changes in gastric cells might leads to complete depletion of body's B12 store; causing megaloblastic anemia and other complicated neurological disorders. Kaptan et al [17] found that H. Pylori seem to be the causative agent in development of vitamin B12 deficiency in adults. De Luca [29] in his study concluded that the pernicious anemia may represent a destination of a process that begins with H. Pylori gastritis.

In the present study, H. Pylori -induced gastritis patients were mild anemic, had low levels of B12, and blood cells were significantly decreased. However, MCV and other RBCs indices were normal and no evidences of macrocytosis in the peripheral blood pictures. In fact, signs and features of B12 deficient- macrocytic anemia takes many years to be more obvious; till most of the body vitamin is being depleted; that why such laboratory tests were normal in this study. It would have been more valuable if we have investigated the gastric acid, IF levels and IF/gastric parietal cells antibodies. However, we performed these measurements in small sample size due to limitation of the personal fund.

## V. Conclusion

H. Pylori infection is the most common cause of gastritis in Sudan. There is an association between H. Pylori-associated gastritis and low levels of B12, there may be a clinical value to evaluate the serum vitamin B12 in patients infected with such bacteria. Molecular genotyping study of H. Pylori strains must be performed in Sudan.

## Acknowledgements

Authors are grateful to staff of Gastroscopy Unit and staff of Medical Laboratory in Omdurman Military Hospital and Suba University Hospital for their cooperation, and Mr. Mario Sulhi for his technical support.

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