Association of Chronic H. pylori infection with Pernicious Anemia in Ibb City – Yemen

Gamal A. Al-Ameri1, Mawhoob N. Alkadasi2, Essam Ali Hassan Al-shuga3, Ali Sallam4, Fahad A.M. Al-zowahi5, E.T. Putaiah6
1 Department of Microbiology, Medical College, Taiz University, Yemen
2 Department of Chemistry, Zabid Education College, Hudaiah University, Yemen
3 Department of Chemistry, Faculty of Science, Ibb University, Yemen
4 Department of Microbiology, Science Collage, Taiz University, Yemen
5 Department of Laboratories, Medical College, National University, Yemen
6 Former vice chancellor Gulbarga University, Gulbarga, Karnataka, India

Abstract

Pernicious anemia (PA) is widely distributed a public health problem with sever life threatening complications. Deficiency of vitamin B12 is the main cause of it. The chronic infection with Helicobacter pylori (H. pylori) is one of the causative agents of both vitamin B12 deficiency and thereby development of PA, and this fact led us to study this relationship here in Ibb city-Yemen. Our study sample size was a total of 60 persons divided equally into two groups (30 healthy control persons and 30 chronic H. pylori infected patients), and each group was divided into 3 age groups (less than 40 years, 40-60 years, and more than 60 years). Also the sex was an important factor in study of such association. Frankly, the standard laboratory investigations used in diagnosis of PA (especially assessment level of serum vitamin B12 and intrinsic factor IF) were totally difficult to be done, especially in the past year due to hard, unusual, and killer conditions as a result of the war here in Yemen, so that, we simply examined the blood film microscopically for confirmation the presence of abnormal macrocytic red blood cells which later was used as a marker of PA. Even though our study sample size was so small in comparison to other studies, but we can say that results of our study surprisingly exhibited a very close agreement with results of other international published studies, since we founded that only 10% of chronic H. pylori infected patients were suffered from PA. Regarding the age we founded that all of the chronic H. pylori infected patients with PA (100%) were in the age group more than 60 years. The female sex, as we founded in our results was the most abundant among chronic H. pylori infected PA patients (11%) versus the male sex (10%). We finally strongly recommend the physicians and the patients themselves to use the typical drugs for eradication the chronic H. pylori infection for prevention of PA development and to avoid it’s sever complications.

Keywords: Association, Chronic H. pylori infection, Pernicious Anemia.

INTRODUCTION

Helicobacter 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]. It has been suggested that H. pylori infection may play an important role in the reduction of acid production, reduced intrinsic factor secretion and therefore the development of vitamin B12 deficiency which causing megaloblastic anemia and other complicated neurological disorders. Kaptan et al., [8] found that H. Pylori seem to be the causative agent in development of vitamin B12 deficiency in adults. De Luca [9]. H. pylori infection might impair the absorption of vitamin B12 from food, leading to PA [10]. Dietary cbl is bound to other proteins, and its release is closely related to the gastric pH status [11]. Food-cbl malabsorption is characterized by the inability to absorb food-bound or protein-bound cbl by subjects normally capable of absorbing free cbl Possibly, antacid drugs used by infected symptomatic subjects and the modification of the intragastric pH [12] caused by H. pylori are the principal factors of malabsorption of vitamin B12 [12]. Annibale et al., [11] described the presence of H. pylori-related gastritis as the unique pathological finding in 57.1% of patients with macrocytic anemia caused by B12 deficiency, the majority (76%) of the patients reported a classic PA due to atrophy of the gastric body, with associated hypergastrinemia and hypo-achlorhydria. H. pylori may also act as a molecular mimicker, as antibodies
directed against the H+, K+-adenosine triphosphate protein may be evoked by a similar antigen expressed by H. pylori. Hyperhomocysteinemia related to vitamin B12 deficiency may constitute a risk for ischemic heart disease and cerebrovascular diseases. This phenomenon would therefore be the link between H. pylori infection and vascular diseases [14].

H. pylori has been suggested as an important agent in the aetiology of vitamin B12 deficiency and PA [15]. A result of H. pylori-induced gastritis and ulcers is destruction of the parietal cells which are important for the production of IF which is essential for vitamin B12 absorption. H. pylori were isolated in 56% of patients with PA and eradication of the infection has been shown to result in improved blood levels of vitamin B12 in 40% patients [16]. However, H. pylori-induced vitamin B12 deficiency has been shown to occur in the absence of atrrophic gastric mucosa [17]. The aim of this study is to determine the association of H. Pylori infection among patients with pernicious anemia in Ibb city- Yemen.

MATERIALS AND METHODS

1- Study population

This study was performed on 60 Yemeni persons, 30 with chronic H. pylori infected patients (proved by rapid anti- H. pylori test), and 30 control healthy persons. Regarding the sex of the studied sample, 34 were males and 26 females aged form (< 40 years, 40 – 60, and >60 years), who were enrolled on Family Health Center, and other hospitals in IBB city such as Al-Thawrah hospital, ALKhansa hospital for follow-up of their infection, over a 6 months period (March’ 2016 to August’ 2016). Our study persons were asked with previously predisposed questionnaire including: age, sex, residence, marital status.

2- Sample collection and processing

Venous blood samples 2 ml were collected from each person and it was divided equally into two separated plain tubes, plasma specimen was separated from the first plain tube by centrifugation within 5 minutes of collection at 3500 rpm for 5 minutes after addition of Ethylene demine tetra acetic acid (EDTA) anticoagulant, while preparation of blood film was done from whole blood specimen.

Rapid Anti-H. Pylori Test

For test cards

1 drop (10μl) of serum or plasma was dispensed to the circular sample well of the test card using the plastic dropper, immediately two drops of sample diluent were added to the sample well, then results were interpreted within 15 minutes.

Hemoglobin drabkin Colorimetric

Mixed venous blood 20 μl (0.02 ml) was dispensed into 5 ml Drabkin’s neutral diluting fluid.

The diluted blood was mixed and lift at room temperature, protected from sunlight for 4–5 minutes. The spectrophotometer was adjusted to a wavelength of 540 nm. Adjustment of colorimeter to zero value was done by using Drabkin’s fluid, and the absorbance of the patients’ sample was recorded. The patients’ haemoglobin value was read according to the table prepared from the calibration graph [18].

White Blood Cell Solution

Procedure

Diluted fluid 0.38 ml (400) was dispensed in to a small tube, then 20μl (0.02 ml, 20 of well-mixed EDTA anticoagulated venous blood was added and mixed well.

The counting chamber was assembled and the well mixed sample was loaded on the grids of the chamber. The central grid areas of the chamber and the special haemocytometer cover glass should be completely clean and dry.

- The cover glass was placed into position over the grid areas and pressed down from each side until rainbow colors (Newton’s rings) are seen. Prior moistening of the chamber surface on each side of the grid areas will help the cover glass to adhere to the chamber.

The diluted blood sample was re-mixed. Using a plastic bulb pipet fill one of the grids of the chamber with the sample, taking care not to over fill the area. The chamber was lift undisturbed for 2 minutes to allow time for the white cells to settle. The underside of the chamber was dried and placed on the microscope stage and observed using the 10 X objective with the condenser iris closed sufficiently to give good contrast. The cells in the four large corner squares and the cells lying on the lines of two sides of each large square were counted. The number of white cells per liter of blood was reported using the simple calculation.

(WBC count X 100)

STATISTICAL ANALYSIS

1. Data analyzed by SPSS –version -22
2. By crosstabs and compare mean methods

RESULT

This study included sixty cases where 30 cases have chronic H. Pylori infection, and 30 cases chosen as a control, from the infected cases 34 (56.7 %) were males and 26 (43.3 %) were females table 1.

Table 1: Distribution of H. pylori based on the sex of patients

| cases          | Sex | Total | P. value |
|---------------|-----|-------|----------|
|               | male| Female|          |
| H.pylori      | 21  | 9     | 30       |
| positive      |     |       | 0.310    |
| negative      | 13  | 17    | 30       |
| Total         | 34  | 26    | 60       |

Among the 30 patients who completed data, the highest positive result was found among 21 (70%) males followed by 9 (30%) female, while the highest negative results was found among female 17 (56.7%), followed by male 13 (43.3%) p. value is significant at < 0.05 as shown in table 1.

Table 2: Distribution of H. pylori on the basis of the age

| cases          | Age                  | Total | P. value |
|---------------|----------------------|-------|----------|
| H. pylori     | <40 years            | 24    | 30       |
|               | 40-60 years          | 5     |          |
|               | more 60 years        | 1     |          |
| positive      | 30                   |       | 0.310    |
| negative      | 21                   | 9     | 30       |
| Total         | 45                   | 14    | 60       |

In this study, it was found that the highest group who has H. pylori infection found within the age group less than 40 which recorded 24 (80%) result shown in Table-2.
Regarding to relationship between chronic *H. pylori* infection and RBC's size as indicator of pernicious anemia by using a blood smear were measured in 30 cases and compared to 30 controls found that among 30 patients who have chronic *H. Pylori* infection there were 3 cases which represented (10%) have macrocytic anemia (pernicious anemia), and 3 (10%) were suffering from microcytic hypochromic anemia, and the most of the patients group were normally regarding RBCs size. In the present study found that 83% of the healthy control group were normally regarding RBC size, and only 17% were suffered from microcytic hypochromic anemia and no macrocytic anemia have been observed and p.value is significant at < 0.05 to both cases as shown in table 3.

In the present study using concentration of RBC’s hemoglobin as a confirmatory marker of pernicious anemia diagnosis so we found that there was a minor difference in hemoglobin concentration between chronic H. Pylori infected patients and healthy control persons (13.1 and 13.9 respectively) as shown in table 4.

### Table 3: The relationship between Chronic *H. pylori* infection and RBC’s size

| Blood Film | Normal cells | Microcytic hypochromic | Macrocytic | Total | p.value |
|------------|--------------|------------------------|------------|-------|---------|
| *H. pylori* positive | 24 | 3 | 3 | 30 | 0.172 |
| negative  | 25 | 5 | 0 | 30 | |
| Total     | 49 | 8 | 3 | 60 | |

### Table 4: the relationship between Chronic *H. pylori* infection and Hemoglobin concentration (pernicious anemia)

| cases | N | Mean | p.value |
|-------|---|------|---------|
| positive | 30 | 13.1 ± 7.1 | 0.083 |
| negative | 30 | 13.9 ± 2.0 | |
| Total   | 60 | 13.5 ± 1.9 | |

### Table 5: Association between age and pernicious anemia development

| Age       | Blood Film | Normal | Microcytic| Macrocyclic | Total  |
|-----------|------------|--------|----------|------------|--------|
| <40 years | Positive   | 20     | 18       | 2          | 3      | 0      | P=24 |
|           | Negative   | 2      | 18       | 2          | 3      | 0      |      |
| 40-60 years | Positive  | 4      | 7        | 1          | 2      | 0      | N=9  |
|           | Negative   | 1      | 7        | 1          | 2      | 0      |      |
| More 60 years | Positive | 0      | 0        | 0          | 1      | 0      | N=0  |
|           | Negative   | 0      | 0        | 0          | 1      | 0      |      |
| Total     | 24         | 25     | 3        | 5          | 3      | 0      | 60   |

### Table 6: The relationship between sex and pernicious anemia development.

| Blood Film | Normal | Microcytic| Macrocyclic | Total  |
|------------|--------|----------|------------|--------|
| sex        | Positive | Negative | Positive | Negative |       |
| Male       | 18     | 11       | 1         | 2       | 2      | 0      | P=21 |
| Female     | 6      | 14       | 2         | 3       | 1      | 0      | N=13 |
| Total      | 24     | 25       | 3         | 5       | 3      | 0      | N=17 |

The relationship between age and sex as risk factors and pernicious anemia development among chronic H. pylori infected patients have been investigated and found that development of pernicious anemia was observed in 2 from 24 patients (8%) in age group less than 40 years, there was no pernicious anemia (0%) seen in the age group 40-60 years, while the highest percentage observed in age group more than 60 with 1 (100%) and 2 from 21 patients (10%) in the males, while observed in 1 from 9 patients (11%) in the females as shown in table 5 and 6.

### DISCUSSION

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 [19]. The most common causes of pernicious anemia and food-B12 malabsorption are associated with chronic gastritis, and recently they have been linked to *H. pylori* infection [20]. In present study, development of pernicious anemia as diagnosed only from RBC size without use standard tests which are more accurate and more precise in diagnosis of pernicious anemia among chronic H. pylori infected patients especially assessment level of blood vitamin B12 and IF because of the bad, hard, and the difficult conditions present here in Yemen in this period of time due to the war and other inhibiting factors) was observed in only (10%) of our patients, while most of our chronic H. pylori infected patients (80%) were normal regarding RBC size, and we think that period of the infection was the main factor playing the major role in development of this disease, these results were nearly in agreement with several published studies which strongly suggested an involvement of long-standing H pylori infection in the pathogenesis of ABG and pernicious anemia, but it is still under...
debate whether PA may be included among the long-term consequences of H pylori gastritis [21].

In present study found that microcytic hypochromic anemia was observed in (10%) of our patients group and in (17%) of the healthy control group, and these finding may be due to deficiency of vitamin C or other unknown causes.

Concentration of hemoglobin can also be used as a confirmatory marker in diagnosis of pernicious anemia, and unfortunately there was a minor difference in Hb concentration between our patients and healthy control subjects (13.1 mg/dl and 13.9 mg/dl respectively).

In chronic H. pylori infected patients, development of pernicious anemia was observed in 2 from 24 patients (8%) in the age group number one (less than 40 years), there was no pernicious anemia (0%) seen in the age group number 2 (40-60 years), while there was only one pernicious anemia case (100%) in the age group number 3 (more than 60 years). Our results were in agreement with several published studies which exhibited that pernicious anemia is frequently described as a disease of adults > 60 years of age [19,20]. Among unpublished series of 177 pernicious anemia patients, about one half were < 60 years of age; in particular, 4% of patients were < 30 years and 10% were 30-40 years of age and the mean age of PA patients in published studies ranges from 59 to 62 years. These data challenge the common notion that pernicious anemia is an exclusive disease of the elderly, and suggest that, in clinical practice, pernicious anemia is probably under-diagnosed in elderly and younger patients [25].

Stratification by age cohorts (< 20 years to > 60 years) of ABG patients identified by hypergastrinemia and positive parietal cell antibodies has shown a regular and progressive increase in MCV and levels of ferritin and gastrin, and a decrease in vitamin B12 levels. However, the prevalence of H. pylori infection has decreased from > 80% at age < 20 years to 12.5% at > 60 years [20]. This reminds us that: (1) iron deficiency is a complication of achlorhydria and may precede the development of pernicious anemia [27]; (2) ABG patients frequently present with iron deficiency anemia [28-30]; and (3) iron deficiency may be present concomitantly with pernicious anemia [31]. These findings further support the idea that pernicious anemia seems to be a long-duration disease that is related to H pylori, gastric achlorhydria and atrophy, which begins many years before the establishment of clinical vitamin B12 deficiency.

In our study, pernicious anemia was observed in 2 from 21 (10%) males chronic H. pylori infected patients, while observed in 1 from 9 patients (11%) in the females, and this result agree with other study that reported that a female preponderance ranging from 1.7 to 2.0:1 has been reported in white subjects [32]. This sex distribution has been confirmed in the more recent population survey of persons > 60 years of age. Although males were more than females, the percentage of female patients who had PA was more than male patients. Regarding to BRCs size we can conclude that 10% were suffered from microcytic hypochromic anemia, and most of the patients group were normally regarding RBC size.

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