Research article

Association of *Helicobacter pylori* infection with anemia in patients presenting with histopathology proven chronic gastritis

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(Received: June 2023 Revised: October 2023 Accepted: December 2023)

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ABSTRACT

Introduction and Aim: *Helicobacter pylori* infection causing anemia has been attributed to impaired iron metabolism due to bleeding peptic ulcers or hypochlorhydria resulting in reduced absorption secondary to atrophic gastritis. Studies have revealed iron and Vitamin B12 deficiency and iron deficiency anemia to be a consequence of *H. pylori* infection and its eradication in improving the anemic status across varied demographic populations. The aim is to find the association of *H. pylori* infection with anemia in histopathology proven samples of chronic gastritis using red cell indices as a marker for anemia.

Materials and Methods: 336 formalin fixed paraffin embedded tissue blocks of histopathology proven chronic gastritis specimens were considered from which details of *H.pylori* infection was obtained. Clinical pathology and biochemical findings were used to identify the anaemic status. Data was tabulated and analyzed using appropriate statistical analysis.

Results: Prevalence of *H.pylori* was found to be 50% and was significantly higher (66.3%) in the age group of 31-45 yrs (p=0.008). There was no statistical significance between gender and *H.pylori* infection. Study showed a clinical difference in the RBC indices between patients with and without *H.pylori* infection but it was not statistically significant.

Conclusion: Though studies in the past have shown association between *H. pylori* infection and anemia, the same might not be applicable in patients with chronic gastritis. They might develop iron deficiency not severe enough to cause anemia, a possible reason behind the current study not proving an association between the two in patients with chronic gastritis.

Keywords: *Helicobacter pylori*; chronic gastritis; iron deficiency.

INTRODUCTION

Helicobacter pylori is a slow growing, microaerophilic organism and an inhabitant of the gastric mucosa (1,2). Apart from being the causative agent of the commonly known peptic ulcer disease and adenocarcinoma, studies have brought to light its notable effect on hematological parameters and thereby causing anemia due to iron and Vitamin B12 deficiencies in children and adults (3-7).

Prevalence of *H. pylori* infection is far more in developing countries than its developed counterparts, very similar to the prevalence of anemia. It is found that people acquire this infection early in age but remain asymptomatic for long years (8) and they present commonly in their thirties. Thus, it becomes important to identify the organism at a younger age for earlier diagnosis, prompt treatment and addressing preventable complications such as anemia, for its importance in affecting the morbidity of an individual.

Different studies have indicated H. pylori infection to be independently associated with vitamin B12 deficiency, iron deficiency and iron- deficiency anemia and its presence a hindrance to oral iron therapy leading to poor response (4). In a study conducted in Alaska, it was found that among children, *H.pylori* infection was an important cause of anemia despite ruling out other important contributors such as inadequate nutritional intake and parasitosis (7). A cross sectional study conducted in Southern Ethiopia showed a statistically significant difference in the mean values of hemoglobin and red cell indices of *H. pylori* infected patients (4).

Development of iron deficiency has been attributed to impaired iron metabolism in the form of occult bleeding from peptic ulcers or reduced absorption due to hypochlorhydria secondary to atrophic gastritis caused by the infection leading to defective formation of ferrous heme from its dietary ferric form (4). Gabriel *et al.*, concluded that there was a decrease in the serum ferritin levels among subjects infected with CagA *H.pylori* strains owing to the uptake of ferritin (9). *H.pylori* as such can affect iron stores by increasing the demand due to the presence of a ferritin like 19-KDa iron binding protein that causes excessive iron intake. As the organisms die, a large amount of iron is lost in the stools due to its high turnover rate (10).

The use of histopathology to identify the presence of *H. pylori* and clinical pathology to assess the anemic

status of the participants respectively have been done in a few previous studies but lack of similar analysis in our part of the world was the stimulus behind conducting this current study. We believe that this may pave way in creating awareness about the various hematological consequences of the infection and iron deficiency anemia being likely one among them.

The aim of the study was to analyze the association of *H.pylori* infection with anemia in histopathology proven samples of chronic gastritis using clinical pathology parameters such as red cell indices as a marker for anemia.

MATERIALS AND METHODS

Study design

The study was initiated after obtaining the Ethical approval and informed consent from the Institutional Ethics Committee (IEC). This study was carried out at the Department of Pathology in a tertiary care centre in the southern part of India. This study was carried out for a period of one year between January 2018 and January 2019.

Study population

A total of 340 samples were received during the study period and after applying exclusion criteria, 336 samples were obtained.

Inclusion criteria

All patients who underwent endoscopic biopsy for chronic gastritis during the period of January 2018 to January 2019

Exclusion criteria

Patients with known history of menorrhagia, prior chemotherapeutic treatment, use of antibiotics and steroids were excluded from the study.

Data collection tools

Demographic details of age, sex, past medical history was obtained from medical records. The microscopic features of *H. pylori* infection, presence of atrophic gastritis and lymphoid aggregates were obtained retrospectively from biopsy reports of formalin fixed, paraffin embedded tissue blocks of histopathology proven chronic gastritis specimens. Clinical pathology and biochemical findings such as serum lactate dehydrogenase, serum folate and serum vitamin B12 levels to identify the anemic status of patients were obtained from respective medical records, laboratory information system and were tabulated as shown in Table 1.

Table 1: Data collection table proforma

GROSS FEATURES				MICROSCOPIC FEATURES						
In patient number	Age and Sex	Biopsy site	No. Of biopsies	Endoscopy features	Diagnosis	Chronic Active gastritis	Presence of H.pylori	Atrophic gastritis	Lymphoid aggregates	Other histopathology features

Statistical analysis

SPSS Version 16- software was used for statistical analysis of the study. Pearson Chi-square test was used to determine the significant relation between *H. pylori* infection and presence of anemia. Independent t test was applied to find the RBC indices in the groups with and without *H. pylori* infection.

RESULTS

Description of the study population

Out of the 336-histopathology proven chronic gastritis specimens, 168 were found to have *H. pylori* infection on histopathology. Table 2 is a descriptive table of the age, gender and status of *H. pylori* infection of the study population. Number of participants was higher in the age category of 46-60 years (38.69%). 66.36% of the study population were males and 50% were found positive for *H. pylori* infection.

Table 2: Descriptive proportion of study population

Variables	Number	Percentage
Age		
19-30yrs	35	10.41%
31-45yrs	83	24.70%
46-60yrs	130	38.69%
>61yrs	88	26.19%
Gender		
Male	223	66.36%
Female	113	33.63%
***H. pylori* status**		
Present	168	50%
Absent	168	50%

Prevalence of *Helicobacter pylori* infection

Fig. 1 shows the histopathological images of specimens showing chronic gastritis and presence of *H. pylori* infection on hematoxylin and eosin staining and special stains such as Giemsa stain. The prevalence percentage of *H.pylori* infection among patients presenting with chronic gastritis was 50% (CI of 95%).

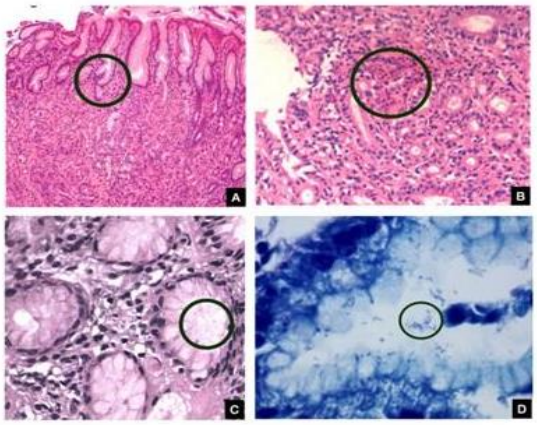

Fig. 1: A. Hematoxylin and Eosin (H & E) staining of gastric tissue showing chronic gastritis X 100, B. Chronic gastritis with activity X 400, C. H & E staining of gastric tissue showing *H. pylori* infection X 400, D. Giemsa staining of gastric tissue showing *H. pylori* infection X 1000 (Oil immersion)

Age and *Helicobacter pylori* infection

The proportion of *H. pylori* infection was high in the age group of 31-45 years (66.3%) and is statistically significant with a p value of 0.008 which is graphically represented in Fig. 2.

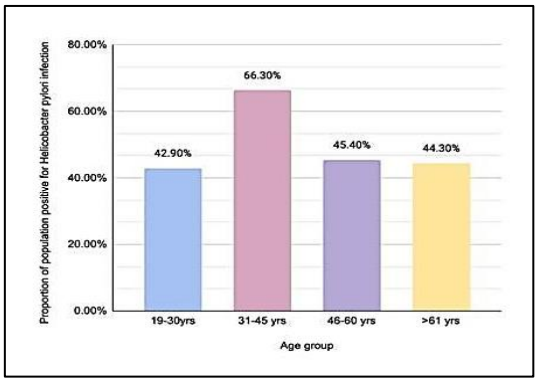

Fig. 2: Graphical representation of association of age and *H. pylori* infection

Sex and *Helicobacter pylori* infection

Fig. 3 shows the proportion of *H. pylori* infection higher in females as compared to males with a p value of 0.193 (not statistically significant).

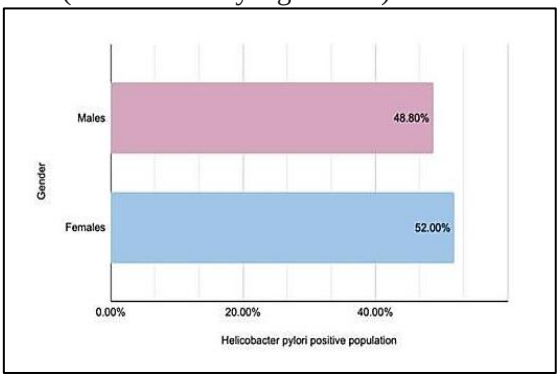

Fig. 3: Graphical representation of association of Sex and *H. pylori* infection

Age, sex, *Helicobacter pylori* infection and anemia

Out of the 336 participants of the study, 200 of them were anemic. The relation between age, sex and *H. pylori* infection to anemia has been tabulated in Table 3. The proportion of anemia was higher in the age group of >61 years (71.6%) and in females (63.8%) but not statistically significant. Among participants with *H.pylori* infection, 59.1% had anemia and among those without the infection, 59.5% had anemia. There was no clinical significance between anemia and Helicobacter pylori from the study.

Table 3: Association of sex, age, *H. pylori* and anaemia

Variables	Anemia (n)	p value
Age		0.039
19-30 years	18 (51.4%)	
31-45years	43 (51.8%)	
46-60years	76 (58.5%)	
>61 years	63 (71.6%)	
Sex		0.305
Males	119 (56.9%)	
Females	81 (63.8%)	
H. pylori		0.931
Present	59.1.%	
Absent	59.5 %	

RBC indices of the study population

The RBC indices of 200 participants with anemia was as follows, mean RBC count of 3.83 cells/mm3, mean hemoglobin of 9.76 g/dl, mean MCV of 76.64fl, mean MCH of 78.64 pg and mean MCHC of 32.22 g/dl and the RBC indices in 136 participants without anemia was, mean RBC of 4.68 cells/mm^3, mean hemoglobin of 14.06g/dL, mean MCV of 87.48 fl, mean MCH of 29.58 pg and mean MCHC of 33.38 g/dl. Though there was a clinical difference, there was no statistical significance in an independent t test.

DISCUSSION

The global prevalence of anemia is approximately 32.9% and is considered a major health problem as of 2010 (11) *Helicobacter pylori* remains the most common infection worldwide and studies done estimates its presence to be more in developing countries like India than the developed counterparts (10).

In the current study, the prevalence of *H. pylori* infection was found to be 50% which is far lesser than other studies that have stated a prevalence of 80% in India owing to lower socioeconomic status, poor education and overcrowding (1). Our finding is similar to a prospective study conducted in Bagalkot, Karnataka, which reported a prevalence of 41.9%

among patients with dyspepsia. Both these studies have been conducted in parallel geographic populations and thus, these areas might have lower prevalence percentages (12, 13) and recent studies attribute it to improved sanitation and wide spread use of antibiotics. In the current study, presence of *H. pylori* was identified solely based on histopathology which could also be a contributor for the variation.

H. pylori infection is believed to be acquired anytime throughout one's life and age is a risk factor for its occurrence and colonization (1) In this study, the proportion of *H. pylori* infection was high in the age group 31-45 years (66.3%) and there was statistical significance between age and *H. pylori* infection which is comparable to a study done in Ethiopia where the relative frequency of infection was higher (50.6%) in the 39-48 years age group (10).

The T-helper 1, T-helper 17 response and high levels of interferon-gamma in adults is associated with damage to the gastric epithelium. Though the colonization of the organism mediated by transforming growth factor-beta and interleukin-10 occurs early in age, due to the presence of milder inflammation, the gastric mucosa remains as a home for the organism to grow and thrive without undergoing any damage and higher inflammatory activity in adults predisposes them to *H. pylori* related gastric damage (1). This study identified the presence of the organism from histopathological specimens of chronic gastritis and thus, could be the probable reason for the increased prevalence of the organism in the adult age group with gastric damage.

Though the proportion of *H. pylori* infection was higher in females as compared to males, it was not statistically significant (p=0.193) which is consistent with other studies where *H. pylori* infection was found to be independent of gender (10,14). However, these results are contrary to the meta-analysis study that confirmed a male predominance in adults as a global and homogeneous phenomenon. This meta-analysis also explains that studies done in populations with higher prevalence of *H. pylori* infection tend to find a stronger male gender association (15). The difference in our findings to previous studies can be explained due to the lower prevalence of the infection that was reported in this study. However, studies with prevalence of around 80% also did not establish an association between gender and *H. pylori* (16).

This study showed a clinical difference in the RBC indices between patients with and without *H. pylori* infection but it was not statistically significant and there was no clinical difference between anemia in patients with and without *H. pylori*. This is similar to a study among Danish adults that showed no correlation between *H. pylori* infection and hemoglobin levels (17). This finding is contrary to studies that have proved positive association between RBC indices and *H. pylori* infection (4,10) and is in par with a few other studies that did not prove an association (18,8). The clinical difference in the RBC indices could be due to various other factors such as nutritional deficiency. A meta-analysis study showed no association between *H. pylori* infection and anemia in adults and suggested that *H. pylori* played a small part in the etiology of Iron deficiency anemia in adults. In a randomized controlled trial subgroup analysis, the eradication therapy for *H. pylori* in adults did not demonstrate the same curative effect on Iron deficiency anemia as in children (19).

The mechanism of development of anemia, particularly iron deficiency, in patients with *H. pylori* infection has been attributed to defective iron absorption due to hypochlorhydria caused in the presence of chronic gastritis and an increased demand for iron by the colonizing organism (17). In the setting of *H. pylori* related peptic ulcer, iron deficiency anemia was initially attributed to occult blood loss from the lesions. On the contrary, the infection is thought to commonly cause chronic gastritis which is not associated with gastrointestinal bleeding (20,18) and in this study, the presence of *H. pylori* was identified among specimens with chronic gastritis which causes decrease in Serum Ferritin and defective iron absorption leading to Iron deficiency that is not sufficient enough to cause a decrease in the RBC indices leading to anemia (9,17).

The current study could not establish a true causal relation between anemia and *H. pylori* infection in patients with chronic gastritis which could be due to the use of RBC indices to assess anemia while chronic gastritis is commonly thought to unalter the RBC indices. However, this study adds to the literature and emphasizes on the probable mechanism of development of anemia in patients with *H. pylori* induced chronic gastritis to be predominantly due to decrease in serum ferritin due to its reduced absorption and increased demand by the organism. Considering the region where this study was conducted, the prevalence of anemia can be attributed to multiple reasons such as dietary deficiency, malnutrition, lifestyle habits (1), a possible reason behind the clinical difference in RBC indices. To our knowledge this is the first of the kind to find association between *H. pylori* infection and anemia in a subcategory of patients such as those with *H. pylori* related chronic gastritis. This study tried to find an association between anemia using simple clinical laboratory tests such as RBC indices.

CONCLUSION

Though studies in the past have shown association between *H. pylori* infection and anemia, the same might not be applicable in patients with chronic gastritis for the reasons stated above. They might develop iron deficiency which is not severe enough to cause anemia and presence of biochemical details such as serum ferritin, total iron binding capacity and

vitamin B12 levels would have helped in establishing iron deficiency. However, due to the retrospective nature of the study, these additional details could not be obtained for all patients, posing a major limitation to the current study. Thus, the authors would like to further extend this study by using serum ferritin levels as the marker for body iron stores.

A pilot study was conducted initially and presented at the International Conference on Esophagus and Stomach 2018 (ICES INDIA) and won "The Best Research Paper Award". Hence the period of study was extended by 9 months, up to January 2019.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Araujo, G., Marques, H., Santos, M., da Silva, F., da Brito, B., Santos, G., *et al*., *Helicobacter pylori* infection: How does age influence the inflammatory pattern? World J Gastroenterol. 2022; 28:402-411.
2. Hachim, S.K., Ali, A.S. The role of Helicobacter pylori in gastric cancer: A review. Biomedicine. 2020 Nov 9;40(3):267-271.
3. Baysoy, G., Ertem, D., Ademoglu, E., Kotiloglu, E., Keskin, S., Pehlivanoglu, E. Gastric Histopathology, Iron Status and Iron Deficiency Anemia in Children with Helicobacter pylori Infection. J Pediatr Gastroenterol Nutr. 2004;38:146-151.
4. Haile, K., Timerga, A. Evaluation of Hematological Parameters of *Helicobacter pylori*-Infected Adult Patients at Southern Ethiopia: A Comparative Cross-Sectional Study. J Blood Med. 2021;12:77-84.
5. Kaptan, K., Beyan, C., Ural, A.U., Cetin, T., Avcu, F., Gülşen, M., *et al., Helicobacter pylori*--is it a novel causative agent in Vitamin B12 deficiency? Arch Intern Med.2000;160(9): 1349-1353.
6. Agin, M., Kayar, Y., Dertli, R. The relationship between mean platelet volume and platelet levels of children with *Helicobacter pylori* and gastritis. Prz Gastroenterol. 2019; 14(3):198-201.
7. Baggett, H.C., Parkinson, A.J, Muth, P.T. Endemic iron deficiency associated with *Helicobacter pylori* infection among school-aged children in Alaska. Pediatrics. 2006; 117: 396-404.
8. Araf, L. N., Pereira, C.A., Machado, R. S., Raguza, D., Kawakami, E. *Helicobacter pylori* and iron-deficiency anemia in adolescents in Brazil. J Pediatr Gastroenterol Nutr. 2010; 51:477-480**.**
9. Berg, G., Bode, G., Blettner, M., Boeing, H., Brenner, H. *Helicobacter pylori* infection and serum ferritin: A population-based study among 1806 adults in Germany. Am J Gastroenterol. 2001; 96:1014-1018.
10. Kibru, D., Gelaw, B., Alemu, A., Addis, Z. *Helicobacter pylori* infection and its association with anemia among adult dyspeptic patients attending Butajira Hospital, Ethiopia. BMC Infect Dis. 2014; 14, 656.
11. Little, M., Zivot, C., Humphries, S., Dodd, W., Patel, K., Dewey, C. Burden and determinants of anemia in a rural population in South India: A cross-sectional study. Anemia. July 2018: 7123976.
12. Kuntoji, S.B., Naik, A.A., Suhas, D.S. A prospective study of proportion of *Helicobacter pylori* infection in patients presenting with dyspeptic symptoms in S. Nijalingappa medical college and H.S.K. Hospital, Bagalkot. Int Surg J. 2020; 7: 2685-2690.
13. Ganesh, P., Ramya, R., Sundaram, S. Prevalence of Helicobacter pylori infection in dyspeptic patients undergoing upper gastrointestinal endoscopy in a tertiary-care teaching hospital. Int Surg J. 2020;7:3009-3011.
14. Adlekha, S., Chadha, T., Krishnan, P., Sumangala, B. Prevalence of *Helicobacter pylori* infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in Kerala, India. Ann Med Health Sci Res. 2013;3:559-563.
15. de Martel, C., Parsonnet, J. *Helicobacter pylori* infection and gender: A meta-analysis of population-based prevalence surveys. Dig Dis Sci. 2006; 51:2292–2301.
16. Shokrzadeh, L., Baghaei, K., Yamaoka, Y., Shiota, S., Mirsattari, D., Porhoseingholi, A., *et al.*, Prevalence of *Helicobacter pylori* infection in dyspeptic patients in Iran. Gastroenterol. Insights.2012;28;4(1): 8.
17. Milman, N., Rosenstock, S., Andersen, L., Bonnevie, O. Serum ferritin, hemoglobin, and *Helicobacter pylori* infection: A seroepidemiologic survey comprising 2794 Danish adults. Gastroenterology. 1998;115:268-274.
18. Rahman, A., Raihan, A., Ahmed, D.S., Karim, M., Saeed, A., Siddique, A, *et al.*, Association between *Helicobacter Pylori* Infection and iron deficiency anemia: A cross sectional study. J. Bangladesh Coll. Phys. Surg. 2020;38:68-78.
19. Qu, X.H., Huang, X.L., Xiong, P., Zhu, C., Huang, Y., Lu, N., *et al.*, Does *Helicobacter pylori* infection play a role in iron deficiency anemia? A meta-analysis. World J Gastroenterol. 2010;16:886-896.
20. Annibale, B., Marignani, M., Monarca, B., Antonelli, G., Marcheggiano, A., Martino, G., *et al.*, Reversal of iron deficiency anemia after *Helicobacter pylori* eradication in patients with asymptomatic gastritis. Ann Intern Med. 1999; 131: 668-672.