

HEMOGLOBIN, FERRITIN, VITAMIN B12 AND *Helicobacter Pylori* INFECTION: A STUDY IN PATIENTS UNDERWENT UPPER GI ENDOSCOPY AT CIVIL HOSPITAL KARACHI

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ABSTRACT

Objective: To compare hemoglobin, ferritin, and vitamin B12 levels in patients undergoing upper GI endoscopy according to their *Helicobacter pylori* status.

Study Design: Cross-sectional study.

Place and Duration of the Study: Civil Hospital and Lyari General Hospitals, Karachi, from March 2004 to June 2006.

Patients and Methods: All patients undergoing upper GI Endoscopy were selected, while patients of active peptic ulcers, malignancy, varices, malabsorption and recent blood donation were excluded. Blood samples for CBC, serum ferritin and vitamin B12 were collected and *H.pylori* status was ascertained by urease test on gastric biopsy. Differences in mean values of age, ferritin, hemoglobin, MCV and vitamin B12 were done by student's 't-test'. Significant confounding factors were identified on multivariate analysis and were further analyzed by univariate analysis.

Results: Two hundred and eighty-five subjects were studied, including 171 males and 114 females. *H.pylori* was positive in 214 (75.1%) patients. Significantly low levels of hemoglobin ($p=0.0$), ferritin ($p=0.0$) and vitamin B12 ($p=0.0$) were found in patients with *H. pylori* infection. Gender, menopause, contraception and history of peptic ulcer disease were identified as significant confounders.

Conclusions: Significantly low levels of hemoglobin, ferritin and vitamin B12 were found in patients with *H.pylori* infection.

KEY WORDS: *H. pylori*. Vitamin B12 deficiency. Ferritin. Iron deficiency. Anemia.

INTRODUCTION

Anemia is a common disorder in developing countries like Pakistan and the commonest cause is iron deficiency.¹⁻³ There are many causes for high prevalence of this disorder in our society, many are well-known and investigated while some are new and not well-established. The prevalence of *Helicobacter pylori* is high in Pakistan with 80% of infants acquiring this infection within their first month of life.⁴ It can lead to many complications and its role is well-established in peptic ulcer and GI malignancy.^{5,6} It has been reported to be associated with 53% of duodenal ulcers in Pakistan⁷ but its role in causing micronutrient deficiencies in infected subjects is under evaluation. Although *H.pylori* associated peptic ulcers and malignancy can cause bleeding, resulting in iron deficiency, but majority of patients infected with *H. pylori* does not have ulcer or malignancy. They usually have chronic gastritis; that is not associated with GI bleeding.⁸ Serum ferritin relates to mobilizable iron stores of the body and can be used as a marker for iron stores.⁹ Hemoglobin levels are maintained within normal range until the body iron stores are exhausted.⁸ Thus, hemoglobin levels could be within normal ranges with very low/absent iron stores.^{8,9}

Vitamin B12 is essential component for RBC maturation.^{10,11} *H.pylori* could lead to decrease in vitamin B12 absorption leading to its deficiency.^{1,12} As there are no reports of iron and vitamin B12 deficiency associated with *H.pylori* from Pakistani population, we undertook this study to compare hemoglobin, ferritin, and vitamin B12 levels in patients undergoing upper GI endoscopy according to their *H.pylori* status.

PATIENTS AND METHODS

A cross-sectional study was conducted at Civil Hospital and Lyari General Hospital affiliated with Dow University of Health Sciences, Karachi from March 2004 till June 2006. All patients undergoing upper GI endoscopy were selected for evaluation and informed consent was taken. Patients with active peptic ulcer, malignancy, varices, blood donation during last 3 months and malabsorption were excluded. Confounding factors [gender; menopause; contraceptive or NSAID use and history of peptic ulcer disease (PUD)] were noted in the proforma for multivariate analysis.⁸ All patients were subjected to standard four antral biopsies and urease test was done on one of them.¹³ Blood samples for CBC, serum ferritin and vitamin B12 were collected from all patients. Those who tested positive for *H. pylori* were allocated to Group-A and those who tested negative to Group-B. Intra group and inter group comparisons were done in qualitative and quantitative values of hemoglobin, ferritin, vitamin B12 and *H.pylori* status. The

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CL-8/5,

Received October 30, 2006; accepted July 31, 2007.

status of iron and vitamin B12 were defined as under: 9,14-16

- Adequate iron stores: Ferritin > 30 µg/L
- Low iron stores: Ferritin 15-30 µg/L
- Depleted iron stores: Ferritin < 15 µg/L
- Iron deficiency anemia males: Ferritin < 15 µg/L + hemoglobin < 13.0 g/dL
- Iron deficiency anemia females: Ferritin < 15 µg/L + hemoglobin < 11.5 g/dL
- Vitamin B12 deficiency: B12 levels < 200 µg/L

The SPSS version 15.0 statistical analysis software was used for analysis. Difference between means was calculated for age, hemoglobin, MCV, ferritin and vitamin B12 with *H.pylori* status by 'Independent Samples t-test'. The significance level was set at • 0.05. Multivariate analysis was done for significance for gender; menopause; contraceptive or NSAID use and history of peptic ulcer disease. The values of serum ferritin were dichotomized to separate variable to reflect the iron status, as low iron stores (serum ferritin 15-30 µg/L), iron deficiency (serum ferritin < 15 µg/L). Similarly, the hemoglobin levels were dichotomized for iron deficiency anemia (serum ferritin <12 µg/L; hemoglobin < 13.0 g/dL in males and < 11.5 g/dL in females) into a separate variable for analysis. GLM Multivariate analysis was done to see the relative effect of gender, menopause, and history of peptic ulcer disease, NSAID and contraceptive usage (fixed factors) on hemoglobin, ferritin, vitamin B12 and MCV (dependent variables). The factors found significant on multivariate analysis were re-examined by the univariate analysis for their individual effect on studied variables.

Table I: Independent samples t-test on age, MCV, ferritin, vitamin B12 and hemoglobin according to *H.pylori* status.

status	H. pylori	Mean ± SD 2-tailed)	Sig. Lower	95% Confidence interval	
				Upper	
Age (years)	Group-A Group-B	33.0 ± 9.9 30.1 ± 9.3	0.03*	-5.5456	-2.857
MCV (fl)	Group-A Group-B	85.3 ± 10.4 85.4 ± 5.5	0.912	-2.3920	2.6764
Ferritin (µg/L)	Group-A Group-B	48.1 ± 46.7 66.4 ± 62.6	0.0*	-57.2295	-35.1039
Vitamin B12 (pg/L)	Group-A Group-B	233.0 ± 44.7 267.2 ± 42.6	0.0*	22.3647	46.1796
Hemoglobin (g/dl)	Group-A Group-B	11.7 ± 2.6 13.5 ± 1.9	0.0*	1.1749	2.4963

¹Significance ≤ 0.05

RESULTS

A total to 285 patients fulfilling the inclusion criteria were inducted. These included 171 males and 114 females. A total of 214 (75.1%) tested positive to *H.pylori* and were allocated to Group-A while 71 (24.9%) tested negative to *H.pylori* and were allocated to Group-B. The results of independent samples t-test on age, MCV, ferritin, vitamin B12 and hemoglobin according to *H.pylori* status are given in Table I. It showed statistically significant lower values in Group-A in all variables except that in MCV.

Frequency of iron deficiency anemia as defined earlier in Group-A was 26 as compared to 8 in Group-B. Frequency of depleted iron stores without anemia was 15 in Group-A and 13 in Group-B. Low vitamin B12 levels were present in 32 patients in Group-A while it was low in 3 patients in Group-B. Details are given in Table II.

The GLM multivariate analysis for the confounding factors of gender, menopause, and history of peptic ulcer disease, NSAID and contraceptive usage was done. The analysis showed that apart from NSAID use, all confounders had significant impact; details are given in Table III. Following this, univariate analysis was run for menopause, contraception, h/o PUD and gender. On univariate analysis, menopause did not have any significant effect on any of the studied variables. Contraception had significant effect on hemoglobin levels (p = 0.0), h/o PUD had significant effect on hemoglobin (p = 0.028) and MCV (p = 0.001) and gender had significant effect on all studied variables, details are given in Table IV.

Table III: Multivariate analysis.

	Value	F	Hypothesis df	Error df	P-value ²
Menopause	0.085	5.011	5.0	269.0	0.0*
Contraception	0.077	4.472	5.0	269.0	0.001*
NSAIDs	0.02	1.125	5.0	269.0	0.348
H/o PUD	0.061	3.511	5.0	269.0	0.004*
Gender	0.134	8.342	5.0	269.0	0.0*

²Significance ≤ 0.05

Table IV: Significance values on univariate analysis.

	P-values ³			
	Menopause	Contraception	H/o PUD	Gender
Hemoglobin	0.820	0.0*	0.028*	0.002*
MCV	0.072	0.162	0.001*	0.002*
Ferritin	0.819	0.620	0.620	0.0*
Vitamin B12	0.824	0.286	0.073	0.001*

³Significance ≤ 0.05

Table II: Frequency of iron deficiency, iron deficiency anemia and low vitamin B12 according to *H.pylori* status.

<i>H.pylori</i> status	Hemoglobin						Vit B12	
	Low Hb			Normal Hb			Low B 12	Normal B 12
	Ferritin			Ferritin				
	Depleted iron stores	Low iron stores	Adequate iron stores	Depleted iron stores	Low iron stores	Adequate iron stores		
	Count	Count	Count	Count	Count	Count	Count	Count
Absent (Group B)	8	4	0	13	15	31	3	68
Present (Group A)	26	35	42	15	45	51	32	182

DISCUSSION

H. pylori infection is widespread and it is not clear why only some of the infected patients develop complications.¹⁷ In this study, we examined differences in means of ferritin, vitamin B12, hemoglobin and MCV according to *H. pylori* status. We report significant differences in means of vitamin B12, ferritin, hemoglobin and MCV. On multivariate analysis menopause, contraception, h/o PUD and gender were identified as significant confounding factors.

Many recent studies have shown *H. pylori* association with Iron deficiency anemia.^{18,19} Some other independent risk factors including cow/evaporated milk consumption were also identified, which were not studied in our study.²⁰ The iron stores in these patients normalize after successful eradication of *H. pylori*.²¹ It has also been discovered that two different subsets of *H. pylori* strains exist, one that causes iron deficiency and the one that doesn't. These could be recognized by their protein expression profiles. This suggests that polymorphism of *H. pylori* strains could be a factor determining the prevalence of *H. pylori* associated iron deficiency anemia.²² At present, these tests are not available in Pakistan so the polymorphism of *H. pylori* could not be studied.

H. pylori is identified as an independent risk factor for vitamin B12 deficiency.^{23, 24} In this study, it is found significantly lower levels of vitamin B12 in patients with *H. pylori*. The values of MCV were not different between two groups due to concomitant iron deficiency. We also found the significant co-existing ferritin deficiency in patients who were vitamin B12 deficient too. Thus, the combined vitamin B12 and ferritin deficiency was also seen in our studied patients.

Eradication of *H. pylori* infection is recommended in (a) patients with gastroduodenal diseases such as peptic ulcer disease and low grade gastric, mucosa associated lymphoid tissue (MALT) lymphoma; (b) patients with atrophic gastritis; (c) first degree relatives of patients with gastric cancer; (d) patients with unexplained iron deficiency anaemia; and (e) patients with chronic idiopathic thrombocytopenic purpura.²⁵

CONCLUSION

From the results of this study, it is concluded that a significantly low level of ferritin, hemoglobin and vitamin B12 are present in patients with *H. pylori* infection.

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