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Prevalence of helicobacter pylori infection in children with Vitamin B12 Deficiency - A cross-sectional observational study

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ABSTRACT

Background: Vitamin B_{12} deficiency is common in children but under diagnosed disorder. Helicobacter pylori infection plays an important role in the development of atrophic gastritis and related malabsorption. There may be a relationship between H.Pylori infection and vitamin B12 deficiency. **Aims and Objective:** To find out prevalence of *Helicobacter pylori* infection by endoscopic and histopathological findings, in children aged 2-18 years, with vitamin B_{12} deficiency. **Materials and Methods:** Seventy-eight patients with deficient serum vitamin B_{12} levels were evaluated. Upper GI Endoscopy was performed in all cases and gastric biopsies were obtained for histopathological examination and evidence of H. pylori infection. **Results:** Tissue biopsy revealed chronic atrophic gastritis in 09 patients and chronic antral gastritis in 52 patients. H. pylori infection by histology was positive in 45(57.70%) patients. We found significant correlation between atrophic gastritis and H. pylori infection, as well as between H. pylori infection and B_{12} deficiency. **Conclusion:** H.pylori has an effect on gastric mucosa, which affects the absorption of vitamin B_{12}. Thus individuals with B_{12} deficiency should be subjected for diagnostic evaluation of H.pylori infection so that appropriate therapy can be initiated.

Key words: H.pylori; Vitamin B_{12} deficiency; Chronic gastritis; Malnutrition

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INTRODUCTION

Vitamin B_{12} deficiency is common in children which produces megaloblastic anemia and neurological disorder. It is characterized by immature large erythrocytes in peripheral smear and cluster of neurological manifestations in children, which respond very rapidly to Vitamin B_{12} supplementation.[1] Its prevalence is ranging from 7% to 50% in paediatricpopulation.[2-4]

Helicobacter pylori is one of the commonest bacterial pathogens in human. Prevalence of H. pylori infection in India is 22%, 56% and 87% in 0-4, 5-9 and 10-19 years age group respectively.[5] H. pylori has been associated with suppression of the gastric acid barrier, allowing enteropathogens ingested from contaminated foods to gain access to the small intestine. This can cause childhood diarrhoea, malabsorption of essential micronutrients and growth failure in childhood.[6,7]

H. pylori infection is acquired early in life and grows up and once it is established, persists into adulthood.[8] H. pylori infection is the principal factor for malabsorption of vitamin B_{12} and play an important role in the development of megaloblastic anemia.[9-11] Untreated H. pylori infection can lead to several complications like gastric ulcers, atrophic gastritis, duodenal ulcer, megaloblastic anemia, cardiovascular and cerebrovascular diseases.[12] So early detection and eradication of Helicobacter pylori can prevent these complications.

We have planned this study to find out prevalence of Helicobacter pylori infection in children aged 2-18 years

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with vitamin B_{12} deficiency by doing upper GI endoscopy and histopathological examination of gastric mucosa.

MATERIALS AND METHODS

The Present cross sectional observational study was conducted over a period of one year at a tertiary care teaching hospital of Western India after approval of institute's ethical committee (ref no- SNMC/IEC/2019/42, dated 09-03-2019). A total of 78 children, aged between 2 to 18 years with a serum vitamin B_{12} level less than 200 pg/dl were included in the study.

Inclusion criteria

Children aged 2-18 years attending OPD/IPD with Vitamin B_{12} deficiency (Serum Vitamin B_{12} level <200pg/ml) [13,14] and given the consent for endoscopy and histopathological study (gastric biopsy).

Exclusion criteria

- Diagnosed case of pernicious anemia or post gastrectomy.
- Evidence of renal failure or liver disease.
- Received prior H pylori eradication therapy or cyanocobalamin treatment parenterally.

Detailed history from parents of patients was recorded on a predesigned pro-forma.

Under all aseptic precautions blood samples were collected and transported within one hour to laboratory.

All enrolled patients were subjected to upper gastrointestinal endoscopy and two biopsy samples were taken from each antrum and corpus of stomach. The biopsy samples were preserved in formalin and sent to department of pathology, Dr. S.N. Medical College, Jodhpur for histopathological examination (Hematoxylin-Eosin and Giemsa stain) and two biopsy samples from each part use for Rapid Urease card test for H. pylori. The density of helicobacter pylori i.e., chronic inflammatory infiltrate, inflammatory activity (polymorphonuclear cells), glandular atrophy and intestinal metaplasia were score (0; none, 1; mild, 2; moderate, or 3: severe) according to the Sydney system.[15]

Statistical analysis

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS). All quantitative variables were estimated using measures of central location (mean, median) and measures of dispersion (standard deviation). For normally distributed data means were compared using student's t-test. Proportions were compared using Chi square or Fisher's exact test whichever was applicable. All statistical tests were performed at a significance level of $P<0.05$.

RESULTS

In our study we enrolled 78 patients with proven Vitamin B_{12} deficiency. The mean age for male patients was 8.72 ± 4.2 years, while in female patients it was8.66 ± 4.71 years (M: F= 1.17:1). 52.57% patients were from urban area, while 47.43% from rural area. Out of total 78 patients, 44.87% had serum Vitamin B_{12} levels between 100-150 pg/dl while 19.23% cases had <100 pg/dl and remaining 35.90% cases had 150- 200 pg/dl. Majority (46.67%) of patients with vitamin B_{12} level < 100 pg/dl were in age group 11-14 years, while 45.71% patients with levels100-150 pg/dl were in age group 6-10 years and 39.29% with levels 150-200 pg/dl were in age group 2-5 years.

On upper gastrointestinal endoscopic examination, we found gastric pallor as the commonest abnormality (75.64%) followed by oesophageal mucosal pallor (67.95%) and duodenal villous atrophy (42.30%) while 21.79% patients had normal gastroesophageal endoscopic finding. All Patients with Vitamin B_{12} levels<100 pg/dl had gastric pallor while it is seen in 71.42% cases with vitamin B_{12} level 100-150 pg/dl.

In our study histopathological examination shown that 43.59% patients had mild chronic gastritis followed by moderate chronic antral gastritis in 14.10% and atrophic gastritis in 10.25% cases while normal gastric mucosa seen in 33.33% cases (Table 1).

Prevalence of H. Pylori in our study was 57.69% (Table 2). Cases of severe chronic antral gastritis have mean age 9.55 ± 4.14 years, mean MCV 110.41 ± 6.33 fl ($P<0.0001$), mean vitamin B_{12} level 119.0 ± 53.69 pg/dl and mean haemoglobin 4.97 ± 2.20 gm/L and have 100% H. pylori positivity. We found significant correlation between severity of the chronic antral gastritis and mean corpuscular volume (p value <0.0001) (Table 2). All cases with severe chronic antral gastritis depicted H. pylori followed by moderate chronic antral gastritis 81.82% and their correlation found to be statistically significant ($P<0.0001$) i.e., more the

Table 1: Histopathological findings (HPE) of gastric biopsy in study population

Biopsy finding	Sex		Number (%)
	Male	Female	
Mild Chronic antral gastritis	15	19	34 (43.59%)
Moderate Chronic antral gastritis	7	4	11 (14.10%)
Severe Chronic antral gastritis	4	3	07 (8.97%)
Atrophic gastritis	4	5	09 (10.25%)
Normal	16	10	26 (33.33%)

[x^2=2.649; d.f.=4; p=0.618]

Table 2: Co-relation between Vitamin B_{12}, Hemoglobin and H. Pylori status with histopathological examination (HPE) finding

Parameter (mean)	HPE findings (Chronic antral gastritis)				Total	P value
	Mild	Moderate	Severe	Normal		
Age (years)	7.49±4.86	11.45±2.87	9.55±4.14	8.88±4.13	8.70±4.46	0.069
MCV (fl)	88.72±16.44	94.10±18.67	110.41±6.33	73.63±16.49	86.37±19.22	<0.0001
Vitamin B_{12} (pg/dl)	128.73±41.90	126.72±36.67	119.0±53.69	142.46±46.14	132.15±43.63	0.489
H. pylori (+)	26 (76.47%)	09 (81.82%)	07 (100%)	03 (11.54%)	45 (57.69%)	<0.0001
H. pylori (-)	08 (23.53%)	02 (18.18%)	00	23 (88.46%)	33 (42.31%)	
Hemoglobin (gm/L)	5.87±2.25	6.70±2.66	4.97±2.20	5.82±2.38	5.89±2.34	0.498

abnormal changes in gastric biopsy findings more chances of detecting positive H. pylori infection (Table 2).

In our study, 45 (57.70%) cases were positive for H. pylori infection by Giemsa stain while 38(48.71%) cases were positive by Rapid urease test (Table 3).

When we compared the H. Pylori positivity with Giemsa and Rapid urease test, we found all cases with severely density were also positive with Rapid urease test (P value-<0.0001) while positivity with moderate and mild density was 94.73% and 70% respectively (Table 4).

In patients with severe density of H. pylori (83.33%) there is more macrocytosis in PBF, and their correlation was found to be statistically significant (P value <0.05) (Table 5).

65.71% cases were with vitamin B_{12} levels between 100-150pg/dl had H. pylori followed by 53.33% cases with levels <100pg/dl and 50% cases with levels 150-200 pg/dl.(P-0.423) (Table 6).

Table 3: Prevalence of H. Pylori infection in study population

Helicobacter pylori (Giemsa Stain)	Sex		P value	Number (%)
	Male (n=42)	Female (n=36)		
Mild density	09	11	x^2=0.975; d.f.=3; P=0.807	20 (25.64%)
Moderate density	11	08		19 (24.36%)
Severe density	03	03		06 (7.69%)
Negative	19	14		33 (42.30%)
Helicobacter Pylori (Rapid Urease Test)	**Male (n=42)**	**Female (n=36)**		
Positive	19	19	x^2=0.190; d.f.=1; P=0.662	38 (48.71%)
Negative	23	17		40 (51.28%)

Table 4: Comparison of Helicobacter Pylori infection by Giemsa stain and rapid urease test

Helicobacter pylori (Giemsa Stain)	Helicobacter Pylori (Rapid Urease test)		P value	Total
	Positive	Negative		
Mild density	14 (70%)	06 (40.0%)	<0.0001	20
Moderate density	18 (94.73%)	01 (2.50%)		19
Severe density	06 (100%)	00 (0.0%)		06
Negative	00	33 (82.50%)		33
Total	38	40		78

DISCUSSION

In our study we have found prevalence of H. Pylori was 57.69% in vitamin B12 deficiency children, similarly Kaptan et al[11] showed presence of H. pylori in 56% of the 138 patients with Vitamin B12 deficiency, and eradication treatment for H. pylori resulted in improvement of Vitamin B12 level. In our study majority of cases with vitamin B_{12} level < 100 pg/dl were in age group 11-14 years. In a similar study Ramani et al[16] found megaloblastic anemia was more common in the age group of 11-15 years. Shuval-Sudai et al[17] found significant association of H.pylori infection and low prevalence of cobalamin and folate levels. Ravi K et al[18] found significant association between the H. pylori status and serum B12 level, in their study more than 60% of H. pylori negative cases had serum B_{12} value greater than 100, whereas among those patients with positive H. pylori status, majority (58%) had vitamin B_{12} value less than 100, while we have not found any statistically significant correlation between level of vitamin B_{12} deficiency and H. pylori infection as a cause, which may be due to small sample size of our study, but we found high prevalence of H. pylori infection in Vitamin B_{12} deficiency patients. Although Akcam Met al[13] showed that H.pylori infection has a negative effect on serum vitamin B_{12} level. Our study shown Geimsa stain of gastric biopsy has more sensitivity to detect H. pylori infection as compare to rapid urease card test. We found significant association between chronic antral gastritis and H. pylori infection, similar results also seen by Dholakia et al[19] and Ravi et al in their study. Ravi K et al[18] showed a high prevalence of chronic atrophic gastritis in patients with cobalamin deficiency (54.1%) and 32.5% of them had evidence of chronic antral gastritis,

Table 5: Comparison of peripheral blood film findings with Helicobacter pylori infection (Giemsa stain)

Hemogram (PBF)	Helicobacter Pylori (Giemsa stain)				Total
	Mild	Moderate	Severe	Normal	
Macrocytic cells	06 (30%)	08 (42.11%)	05 (83.33%)	06	26
Microcytic cells	04 (25%)	01 (05.26%)	00	14 (42.42%)	19
Normocytic cells	03 (15%)	01 (05.26%)	00	04 (12.12%)	08
Microcytes with Macrocytes	07 (35%)	06 (31.58%)	01 (16.67%)	06 (18.18%)	19
Normocytes with Macrocytes	00	03 (15.79%)	00	03 (09.09%)	06
Total	20	19	06	33	78

[P value- 0.023]

Table 6: Correlation of Vitamin B12 levels with H. Pylori bacteria [Giemsa stain]

H. Pylori status	Vitamin B_{12} level (pg/dl)			Total	P value
	Less than 100	100 – 150	150-200		
Present	08 (53.33%)	23 (65.71%)	14 (50%)	45	x^2=1.71;
Absent	07 (46.67%)	12 (34.29%)	14 (50%)	33	d.f.=2;
Total	15	35	28	78	P=0.423

majority (56.6%) of them also showed evidence of H. pylori infection on biopsy.

CONCLUSION

In our study we have found prevalence of having H. pylori infection in children with vitamin B_{12} deficiency was 57.70%. In paediatric patients with vitamin B_{12} deficiency usually manifest in older age group but whenever we come across the cases with vitamin B_{12}deficiency, apart from nutritional cause we should also keep H. pylori infection as one of cause of vitamin B_{12}deficiency. Although we found significant correlation between severity of the chronic antral gastritis with H. pylori infection, yet we have not found any statistically significant correlation between H. pylori infection and level of vitamin B_{12} deficiency, which may be due to small sample size of our study.

We recommend that further studies should be done with large sample size to find out H. pylori infection as a definitive cause of vitamin B_{12} deficiency.

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Author's Contribution:
RJ-Concept and design of the study; prepared first draft of manuscript; **RD-** Interpreted the results; reviewed the literature and manuscript preparation; **SV-** Concept, coordination, review of literature and manuscript preparation; **SC-** Statistically analysed and interpreted, preparation of manuscript and revision of the manuscript.

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