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**ORIGINAL ARTICLE****Comparison Of Vitamin B<sub>12</sub> Levels In Gastritis With And Without H.Pylori.**

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**Abstract:**

**Background:** *H. pylori* infection is widespread in developing nations, prevalence is more than 80% among middle-aged adults. It may play an important role in impairment of vitamin B<sub>12</sub> absorption. It is almost invariably associated with the presence of gastritis in India. The classical sign of vitamin B<sub>12</sub> deficiency is megaloblastic anemia which, occurs in only 50 % of vitamin B<sub>12</sub>-deficient subjects. Other signs are psychiatric and neurodegenerative changes.

**Aim:** To study the status of vitamin B<sub>12</sub> in gastritis with and without *H.pylori*.

**Methods and Material :** Prospective study carried out at tertiary care hospital in Mumbai between June to December 2013. Ninety gastritis suspected patients who underwent gastroscopy were enrolled. Rapid urease test was used to diagnose *H.pylori* infection. Chemiluminescent immunoassay based Immulite 1000 analyzer was used for analysis of vitamin B<sub>12</sub>.

**Statistical analysis used:**

The mean serum levels of vitamin B<sub>12</sub> in *H.pylori*-positive and *H.pylori*-negative gastritis groups of patients were compared by independent sample 't' test.

**Results:**

Serum vitamin B<sub>12</sub> levels were significantly lower in patients with *H.pylori* positive gastritis than in those with *H.pylori* negative gastritis ( 261.2 ±89.2 ; 382.7 ± 164.9 respectively, p = 0.0001 )

**Discussion:**

The study shows serum vitamin B<sub>12</sub> levels to be lower in *H.pylori* positive as compared to *H.pylori* negative gastritis.

**Key-words:** *H.pylori* gastritis, vitamin B<sub>12</sub>, gastroscopy, rapid urease test, chemiluminescence

**Introduction:**

The epidemiology of *H. pylori* infection in developing countries, such as India is characterized by a rapid rate of acquisition of the infection such that approximately 80% of the population is infected by the age of 20 yrs<sup>1,2,3</sup> because the disease is most often acquired in childhood.<sup>4-8</sup> In developing countries the prevalence of infection peaks in the 20 to 30 year old age group. *H.pylori* is recognized as a major etiologic agent for chronic active gastritis.<sup>9</sup> Asymptomatic carrier state is

common in *H.pylori* infection<sup>10</sup> and if left untreated *H. pylori* infection is lifelong.<sup>11</sup> It has been suggested that *H. pylori* infection may play an important role in impairment of folate and vitamin B<sub>12</sub> absorption owing to diminished acid secretion, lower ascorbic acid levels in gastric juice and reduced secretion of intrinsic factor.<sup>12</sup> Studies have been published where Vitamin B<sub>12</sub> was compared between *H.pylori* positive and *H.pylori* negative gastritis.<sup>13</sup>

### Subjects and Methods:

We conducted our study at Biochemistry laboratory in collaboration with Department of Surgery, at a tertiary care hospital in Mumbai. It was conducted over a period of six months from June 2013 to December 2013. A complete medical history and informed consent was obtained from all participants included in the study.

Ninety symptomatic patients, in the age group 20-60 yrs of either sex, suspected of gastritis were subjected to upper gastrointestinal endoscopy and enrolled in the study. On confirmation of gastritis by endoscopy, biopsy was taken from the gastric antrum to diagnose the presence of *H.pylori* infection with Rapid Urease Test.

### Inclusion criteria:

- Patients of age group 20-60 yrs.
- Patients of either sex.
- Patients diagnosed as gastritis with or without *H.pylori* by rapid urease test on gastric antral biopsy specimen taken during endoscopy.

### Exclusion criteria:

- Patients with previous *H.pylori* eradication therapy in last 6 months.
- Renal failure.
- Liver diseases.

- Use of drugs affecting plasma vitamin B<sub>12</sub> and folic acid levels.
- Patients with history or presence of other causes of vitamin malabsorption.
- Pregnant women.

Of the Ninety patients ten were excluded on the basis of exclusion criteria.

### Cases:

Forty patients in the age group 20-60 yrs of either sex diagnosed as *H.pylori* associated gastritis by positive rapid urease test on gastric antral biopsy specimen taken during endoscopy.

### Control:

Forty patients in the age group 20-60 yrs of either sex diagnosed as gastritis other than *H.pylori* associated gastritis, by negative rapid urease test on gastric antral biopsy specimen taken during endoscopy.

All the patients enrolled in the study, who underwent upper gastrointestinal endoscopy were subjected to gastric antral biopsy for diagnosis of *H.pylori* infection so as to categorize them into gastritis with and without *H.pylori* infection.

*H.pylori* infection was diagnosed by Pylo-dry test, ( manufactured and marketed by Halifax Research Laboratories, Kolkata, India ) which is a rapid urease test.

Procedure For Rapid Urease Test : Written and informed consent was taken for the procedure

Patients subjected to upper gastrointestinal endoscopy were nil by mouth for 8 hours. Local anaesthesia with Lidocaine Topical Aerosol ( LOX 10% spray ) was given. A flexible, fiber-optic, endoscope (PENTAX EG – 2770K (2.8)) was manoeuvred into the stomach. Patients with

gastritis were subjected to a biopsy from the pyloric antrum. The biopsy specimen was transferred from the biopsy forceps onto the exposed yellow media of the Pylo-dry test kit. One drop of distilled water was added onto the yellow media containing the biopsy specimen. Urease enzyme of *H.pylori*, if present, reacts with urea of the media and changes the colour from yellow to red or pink altering the pH to make it alkaline.

The change in the colour of the media from yellow to red or pink was taken as a positive test, thus the patients were categorized as cases and controls.

#### Analysis of Vitamin B12

A fully automated enzyme amplified chemiluminescent immuno assay based Immulite 1000 analyzer was used for quantification of vitamin B<sub>12</sub>. Commercial kits from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA were used.

The reference serum level of :

Vitamin B<sub>12</sub> = 160 – 800 pg/ml

Independent sample t-test was used to compare the difference of means. In this analysis, variables showing p-value less than 0.05 were considered to be statistically significant.

#### Results:

Serum vitamin B<sub>12</sub> levels were significantly lower in patients with *H.pylori* positive gastritis than in those with *H.pylori* negative gastritis

**Table 1** Vitamin B<sub>12</sub> levels in *H.pylori* positive and *H.pylori* negative gastritis

Parameters	<i>H.pylori</i> positive gastritis	<i>H.pylori</i> negative gastritis	p value
	Mean ± SD	Mean ± SD	
Vitamin B <sub>12</sub> (pg/ml)	261.2 ± 89.2	382.7 ± 164.9	0.0001

Discussion: Vitamin B<sub>12</sub> is a water-soluble molecule that functions as an essential coenzyme for two enzymes in the human body : cytoplasmic methionine synthase which catalyzes methylation of homocysteine to methionine; and methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in the mitochondrion. The methionine synthase reaction, which also involves folate is essential for a high number of methyl-transfer reactions and is also, therefore, indirectly involved in nucleotide synthesis. The methylmalonyl-CoA mutase reactions are involved in digestion of different organic compounds, including branched amino acids and odd-chain fatty acids. Once referred to as “nature’s most beautiful cofactor”<sup>14</sup> the red-coloured B<sub>12</sub> is a tetrapyrrole that occurs in several active and inactive forms.<sup>15-20</sup> A complex 30-step pathway of vitamin B<sub>12</sub> biosynthesis is confined to certain prokaryotes, humans are completely dependent upon a dietary source of the vitamin.<sup>21</sup> 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 B<sub>12</sub> deficiency. However, development of vitamin B<sub>12</sub> deficiency occurs slowly due to the low requirement ( 2 µg/day ), the enterohepatic

cycle of cobalamin and the liver stores of the vitamin that have been built up during life and that are about 2–3 mg of cobalamin by the age of 60 years.<sup>22</sup> The classical sign of vitamin B<sub>12</sub> deficiency is megaloblastic anemia which, however, occurs in only 50 % of vitamin B<sub>12</sub>-deficient subjects. Other signs of vitamin B<sub>12</sub> deficiency which are often overlooked are psychiatric and neurodegenerative changes.<sup>23</sup>

Shuval-Sudai and Granot<sup>24</sup> investigated 133 patients in Israel for *H.pylori* infection and cobalamin and folate status and reported a significant association of *H.pylori* infection and prevalence of low cobalamin and folate concentrations.

In the present study, serum vitamin B<sub>12</sub> levels were significantly lower in patients with *H.pylori* positive gastritis as compared to those without. The mechanisms of vitamin B<sub>12</sub> and folic acid malabsorption by *H.pylori* infection are unclear, but the following explanations are possible. First, hypochlorhydria associated with atrophic gastritis may lead to failure in splitting of vitamin B<sub>12</sub> from food binders and its subsequent transfer to R-binder (haptocorrin) in the stomach.<sup>12</sup> Second, decreased secretion of ascorbic acid and secretory dysfunction of the intrinsic factor in the backdrop of *H.pylori* infection could possibly lead to a decrease in vitamin B<sub>12</sub> and folate absorption.<sup>24,25</sup>

### Conclusion:

In our study serum levels of vitamin B<sub>12</sub> were significantly lower in *H.pylori* positive gastritis as compared to *H.pylori* negative gastritis.

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