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COBALAMIN DEFICIENCY;
HELICOBACTER PYLORI INFECTED PATIENTS: A MYTH OR REALITY

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ABSTRACT... Objectives: The present study aims to evaluate the serum cobalamin in Helicobacter pylori (H. pylori) infected patients. **Study Design:** Case control study. **Place and Duration:** Department of Medicine, Isra University Hospital Hyderabad from March 2013- April 2014. **Methodology:** A sample of 109 subjects including Helicobacter pylori positive subjects and controls were selected according to study criteria. Centrifugation of blood was performed at 4000 rpm for 10 minutes and sera were stored at -20°C. Blood sera were used for H. pylori serological testing. Blood counting was performed on hematoanalyzer. Cobas e411 analyzer was used for detection of cobalamin. 64 kD H. pylori antigens was detected by ELISA. The data was entered into SPSS version 21.0. (IBM, Incorporation, USA) A 2-tailed p-value of ≤0.05 was considered significant for statistical analysis. **Results:** Of total 109, 54.1% (n=59) were H. pylori seropositive cases and others were controls i.e. 45.9% (n=50). Cases and controls showed cobalamin levels of 290±49.3 vs. 351±32.9 pg/ml respectively (p=0.0001). Red blood cell indices were found to show statistically significant difference between cases and controls (p=0.001). **Conclusion:** Serum cobalamin deficiency was noted in both Helicobacter pylori positive and controls, however deficiency was more pronounced in Helicobacter pylori positive subjects.

Key words: Cobalamin Helicobacter pylori Isra University.

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INTRODUCTION

Helicobacter pylori (H. pylori) are microaerophilic spirochete and stain as gram negative. H. pylori colonize the inner mucosal lining of stomach and duodenum exclusively and is capable of surviving in acidic medium while invading the host.[1,2] H. pylori has emerged as a major health problem the World over. It is prevalent in developing than developed countries because of unknown reason; hence it's likely associated with socioeconomic status rather than ethnicity.[3-5] Cobalamin, being vitamin, is one of the "essential nutrient" similar to other vitamins. Cobalamin is essential because of being required for the DNA synthesis and cell division particularly in tissues with high cell turnover such as epithelial cells, bone marrow cells, etc. The exclusive source of cobalamin is the food of animal origin as vegetables are absolutely lacking this vitamin.[3-6]

Daily gut absorption is approximately 5 μg while daily requirement is 3 μg. Liver normally stores 2000-5000 μg of cobalamin, this much vitamin is sufficient to meet body demands for duration of 5 years before symptoms and signs of deficiency manifest clinically.[6] Diet deficient in vitamins is a major cause of deficiency.

Other causes include the small intestine diseases particularly the distal part of ileum, Diphyllobothrium latum, antibiotic therapy, intestinal surgery, chronic pancreatic disorders, transcobalamin deficiency intrinsic factor (IF) deficiency or receptor defects. Intestinal bacterial colonization is also a cause of cobalamin deficiency.[6]

Cobalamin is required as a co-enzyme for various cellular enzymes for catalysis of cellular reactions.[6] Cobalamin forms 2 essential co-enzymes; the methylcobalamin (MetC) and S-adenosylcobalamin (SAC). The SAC is required for the "L-methylmalonyl-CoA–coenzyme A mutase" which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA. On the other hand, MetC is needed for the "Methionine synthetase" which catalyzes resynthesis of methionine from homocysteine.[6,7] Previous studies had reported link of H. pylori infection and cobalamin. As majority of our population is infected with H. pylori; hence this link might be existing, causing the vitamin deficiencies. Eradication of H. pylori, once completed by drugs, increases the serum cobalamin back to normal.[8] It is suggested that the H. pylori causes gastritis and gastric ulcerations which disturb the stomach milieu and may be interfering with the cobalamin absorption.[9]

The primary objective of present study was to evaluate cobalamin in H. pylori infected subjects compared to controls and secondary objective was to determine if the H. pylori are linked to cobalamin deficiency or not.

SUBJECTS AND METHODS

A sample of 109 subjects; 59 H. pylori infected subjects and 50 normal controls, was selected. The study was carried out at the Department of Medicine, Isra University Hospital Hyderabad from March 2013 - April 2014. Clinical symptoms of pointing to gastric problems were asked and physical examination was performed. Aim of study was explained to volunteer participants and asked for consent if willing. Data was gathered on pre-structured profroma. H. pylori positive without associated co-morbidities were included for study purpose. Following were excluded form study protocol; strict vegans, worm infestation, anti H.pylori drug therapy, taking multi-vitamin pills and taking proton pump inhibitors. Subjects with concomitant chronic liver disease were excluded from study.

Subject Groups

Subjects were divided into two groups; Cases: H. pylori seropositive subjects and Controls: H. pylori seronegative subjects.

Blood sampling

5 ml of venous blood was drawn from ante cubital fossa after skin was sterilized. Blood samples centrifugation was performed at 4000 rpm for 10 minutes and sera were stored at -20°C. Blood sera were used for H. pylori serological testing.

Complete blood counts

Complete blood counts were detected on "Sysmex-KX 21" hematolyzer. Blood counts and red blood cell parameters such as MCV, MCH, MCHC and RDW were noted. White cells and platelets were also computed.

Cobalamin detection

Cobalamin was detected by Cobas e411 analyzer; Roche Diagnosis GmbH, Mannheim, Germany. Cobalamin levels were defined as; Group I: ≥ 240pg/ml for the - controls, Group II- 170 to 240 pg/ml for – the vitamin borderline deficiency, Group III: < 170 pg/ml for the – vitamin cobalamin deficiency and Group IV: <100 pg/ml for the - severe vitamin cobalamin deficiency.[6]

Helicobacter pylori serological test

H. pylori's was detected by the enzyme linked immunosorbent assay (ELISA). The ELISA is based on a monoclonal antibody against 64 kD H. pylori antigen.[10] 100 µl of undiluted serum sample and calibrators were added to micro wells coated with a solicited H. pylori preparation. 100 µl of peroxidase conjugated HpN45 were added to all wells. 1 hour incubation, the plate was washed; color was developed with a TMB solution. Computed absorbance was counted at the 450nm; negative values were considered below this. Anti H. pylori antibody concentrations were expressed as units/ml. Sensitivity and specificity were 100% and 90% respectively.[10]

Statistical Analysis

Data typed on SPSS 21.0 (IBM, incorporation, USA). Student t-test (independent samples)

was used for continuous variables. Analysis of variance and Bonferroni test were applied for more than 2 group's analysis. Significant results were defined as p ≤0.05.

RESULTS

Of total 109, 59 (54.02%) were Helicobacter pylori positive and remaining 50 subjects (45.80%) were healthy normal controls. Study population baseline characteristics, cobalamin levels and red blood cells indices are shown as in table I. In present study, the male population predominated. Hemoglobin and red blood cell counts differed much between cases and controls (p=0.03). Differences were not found for the white blood cells and platelets. Cobalamin showed significantly different values between cases and controls (p=0.0001). However, results were non-significant for the borderline cobalamin deficiency in cases and controls as shown in table II & III. Serum cobalamin was found as 290±49.3 vs. 351±32.9 pg/ml with significant p-value (p=0.0001) between groups. The red blood cell indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) showed significant differences in cases and controls (p=0.001) as shown in table IV.

	Cases (n=59)	Controls (n=50)	p-value
Age (years)	39±6.3	40±8.4	0.08
Male	38(64.4%)	27 (54 %)	0.10
Female	21(35.5%)	23 (46%)	0.11
Obesity	22(37.3%)	19(32%)	0.045
BMI	27±3.9	28±2.7	0.09
Smokers	19 (38%)	23(38.9%)	0.043
Hemoglobin (g/dl)	11±3.9	12±2.9	0.031
WBC counts (μl^{-1})	7193±178	7178±156	0.10
Platelets (x10^9 μl^{-1})	3.6±1.1	3.8±1.3	0.09
RBC counts (x109 μl-1)	3.9±2.1	4.1±3.9	0.035

Table-I. Baseline characteristics of cases and controls (n=109)

Cobalamin (pg/ml)	Cases (n=59) N (%)	Controls(n=50) N (%)	p-value
Normal (>240pg/ml)	22(37.2%)	26 (52%)	0.01
Borderline (170-240 pg/ml)	16 (27.1%)	18(36%)	0.07
Deficiency (<170 pg/ml)	11 (18.6%)	04 (8%)	0.0001
Severe deficiency (<100pg/m)	10 (16.8%)	02 (4%)	0.002

Table-II. Frequency of Cobalamin deficiency in cases and controls

Cobalamin (pg/ml)	Cases (n=59) mean±S.D	Controls (n=50) mean±S.D	p-value
Normal (>240pg/ml)	301±31.5	349±49.5	0.02
Borderline (170-240 pg/ml)	179.1±19.08	203±20.21	0.01
Deficiency (<170 pg/ml)	101±17.8	152±15.9	0.001
Severe deficiency (<100pg/ml)	67±18.1	87±10.7	0.01
Total	290±49.3	351±32.9	0.0001

Table-III. Cobalamin levels in cases and controls (n=109)

	MCV (fl)	MCH(pg/dl)	MCHC (%)	RDW (%)	p-value
Cases (n=59)	97.2±3.8	27.5±2.5	26.3±3.3	11.1±1.9	<0.001
Controls (n=50)	76.5±4.5	29.1±2.1	27.6±3.5	9.8±1.5	

Table-IV. Red blood cell indices in cases and controls (n=109)

DISCUSSION

To the best of our knowledge, the present study is the first being reported from Isra University hospital. The present study was conducted to determine serum cobalamin levels in the light of previous studies which had reported the linkage of cobalamin deficiency with H. Pylori. Cobalamin deficiency, in a hospital based study, of 76% has been reported.[11] The findings of present study parallel to above study. 65% of study population belonged to urban areas infected with H. Pylori. The cobalamin deficiency is surprisingly high considering that most of our population is non-vegans.[12]

H. pylori are most common infection the World over. It is estimated that approximately 40% of World population is infected by H. pylori, and most affected population belongs to underprivileged social class. As regards the H. pylori, its transmission is uncertain and debatable. Suggested mode of H. pylori transmission is the oro-oral or oro-fecal route. This mode of transmission is supported by the fact that the H.pylori has been isolated from the dental plaque, salivary secretions and feces. The H. pylori as an etiological agent for the chronic gastritis, gastric ulcer disease, gastric malignancy and primary lymphoma of stomach is already established.[13,14]

Chronic persistent irritation of stomach by H. pylori causes the chronic gastritis which becomes manifest clinically. Colonization of gastric mucosa by H. pylori is almost always culminates in a chronic inflammatory response of gastric mucosa resulting in chronic gastritis. H. pylori produce both local as well as systemic immune responses.[15]

Linkage of cobalamin deficiency and H. pylori is an argumentative enigma. Karnes et al[16] and Varis et al[17] reported the causal association of H. pylori with atrophic gastritis, which causes defects in production of intrinsic factor synthesis and secretions culminating eventually into cobalamin deficiency. It is stated that the H. pylori is now an established causative agent of cobalamin deficiency.[5,12-16]

Prevalence of cobalamin deficiency is reported very high in H. pylori infected populations.[15,16] In the present study, the borderline value for low Cobalamin status (<190 pg/ml) was used and markedly prevalence (58%) was found which is higher than that reported by Gumurdulu et al,[18] Tucker et al[19] and Devrajani et al.[12]

In present study, sera were used to detect cobalamin levels because the assessment of serum cobalamin levels is the standard ideal test; used for the diagnosis of cobalamin deficiency.[20] The present study reports the strong correlation of cobalamin deficiency and mean corpuscular volume which has already been established in previous reports.[21] In a previous study, cobalamin deficiency was a prominent finding in H. pylori infected subjects. H. pylori infected subjects had significantly low hemoglobin and hematocrits compared to normal subjects. [21-22]

In present study, the prevalence of cobalamin deficiency in H. pylori positive subjects was 58%, which is consistent to previous studies reported from Italy 60%[22] but higher than other studies; 11% in United States[23] and 21.4% in U.K.[24] Correlation of cobalamin deficiency showed no correlation with age, is a findings consistent to previous studies.[12,25]

Cobalamin deficiency was noticeable in males compared to female counterparts ($p = 0.01$), the results are similar to Devrajani et al[12] but contrary to Gumurdulu et al[18] which has reported female gender. In conclusion, frequency of cobalamin deficiency was observed in H. pylori infected subjects; hence a causal relationship

is suggested. The present study has some limitations like; first: a proper history of diet was not available because, any effect of diet remained uncertain. However, study has internal validity of case control study design and study population selection according to inclusion and exclusion criteria.

CONCLUSION

Cobalamin deficiency was noted in Helicobacter pylori positive cases and control subjects; however, the deficiency was significant in the H.pylori positive cases. It is concluded that the H.pylori may be an etiological agent of cobalamin deficiency.

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