ORIGINAL ARTICLE

Vitamin B_{12} Deficiency in Macrocytic-Megaloblastic Anemia Reporting at A Tertiary Care Hospital of Sindh

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ABSTRACT

Objective: To determine the frequency of vitamin B_{12} deficiency in macrocytic- megaloblastic anemia cases reporting at a tertiary care hospital of Sindh

Methods: This cross sectional study was conducted at Isra University Hospital, Hyderabad from January 2016 to September 2017. 300 cases of macrocytic- megaloblastic anemia were selected according to criteria and vitamin B_{12} was estimated from the blood sera. 5 ml blood samples were centrifuged to separate sera for the vitamin B_{12} estimation by ELISA assay kit. Result variables were analyzed on SPSS (version 21.0) at 95% CI ($P \leq 0.05$) by Student t-test and Chi-square test for the continuous and categorical variables respectively.

Results: Age was noted as 49.5±4.59 and 47.5±3.57 years in male and female subjects respectively (P=0.71). Of 300 subjects; male and female were noted as 153 (51%) and 147 (49%) respectively (P=0.076). MCV in male and female was noted as 110.5±5.31 and 113.91±7.35 fl (P=0.005). Mean vitamin B_{12} level in female subjects was noted as 135.35±15.51 pg/ml compared to 211.6±16.7 pg/ml in male subjects (P=0.0001). 73 (24.33%) subjects revealed normal vitamin B_{12} (>240 pg/ml) levels. Remaining 227 (75.66%) subjects revealed vitamin B_{12} borderline, deficiency and severe deficiency levels among 119 (39.67%), 56 (18.66%) and 52 (17.33%) respectively (P=0.0001).

Conclusion: The present study reports vitamin B_{12} deficiency of 75.66% among macrocytic-megaloblastic anemia reporting at Isra University Hospital.

Key Words: Macrocytic- Megaloblastic anemia, MCV, Vitamin B_{12} deficiency

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INTRODUCTION:

Red blood cells (RBC) with large mean corpuscular volume (MCV) are termed as the macrocytes. MCV is a measure of mean red blood cell volume and is a hematological indicator of macrocytic- megaloblastic anemia.[1,2] An MCV of more than100 fl is termed as macrocyte RBC. Macrocytes are prone to hemolysis in the small size capillaries resulting in hemolytic anemia. Macrocytosis is caused by drugs, thyroid and liver disorders, and vitamin deficiencies such as the vitamin B_{12} and folate. Deficiency of vitamin B12 and folic acid cause macrocytic- megaloblastic anemia. Vitamin B_{12} and folate are essential for the nuclear maturation of red blood cells. In case of deficiency of vitamin B_{12} and folate, the red blood cells nuclear maturation is delayed resulting in large MCV.[3,4] Vitamin B_{12} participates in the biochemical synthesis of nucleotides through donating one carbon (methyl) group. Vitamin B_{12} functions along with folic acid in the maturation of nucleus of rapidly proliferating cells as those of bone marrow. Deficiency of vitamin B_{12} causes macrocytic- megaloblastic anemia.[3,4] Vitamin B_{12} helps in nuclear maturation as 2 co-enzymes that include the "methyl-cobalamin" and "S- adenosyl cobalamin". These co-enzymes function as 1-carbon donor for the biosynthesis of nucleotides and nuclear maturation. Methyl- cobalamin is

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co-enzyme for the methionine synthetase and S-adenosyl cobalamin for the L-methylmalonyl-CoA coenzyme A mutase. Former enzyme catalyzes the conversion of homocysteine to methionine,[3-5] while the later catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA. Vitamin B_{12} is required for the nuclear maturation of all of body cells including rapidly bone marrow. Hence the Vitamin B_{12} is manifested in the bone marrow before in other tissues. Vitamin B_{12} is a water soluble vitamin of animal origin that belongs to the B-complex family. Vitamin B_{12} binds to the intrinsic factor (IF) produced by stomach cells. Vitamin B_{12}- IF complex binds to the receptors in the terminal ileum from where it is absorbed into the blood capillaries. In the blood, vitamin B_{12} is protein bound. Daily requirement of vitamin B_{12} is 3µg and daily absorption is 5 µg approximately. Liver is capable of storing the Vitamin B_{12}. Liver stores of vitamin B_{12} approximate 2000-5000 µg that are sufficient for years before manifest defiency.[5-8] Dietary deficiency is the most common cause of vitamin B_{12}; followed by parasite infestation, gut malabsorption, etc. Vitamin B_{12} demands are increased during pregnancy and in growing children. Gastric problems, intestinal and pancreatic disorders cause vitamin B_{12} deficiency. Intestinal infestation of Diphyllobothrium *latum* is notorious cause. Macrocytic- megaloblastic anemia is common manifestation of vitamin B_{12} deficiency.[6,9] A few studies are published with small sample size and other confounding factors. Hence, the prevalence of vitamin B_{12} deficiency in developing countries is never estimated. However, published literature shows high prevalence of deficiency.[5-9] Keeping in view this scenario of neglect, the present study was conducted prospectively to determine the frequency of vitamin B_{12} deficiency in macrocytic-megaloblastic anemia at tertiary care hospital of Isra university.

METHODS

300 cases of macrocytic- megaloblastic anemia were selected according to criteria of inclusion and exclusion. Age 20- 50 years, both male and female, mean corpuscular volume (MCV) >100 fl, and hyper segmented neutrophils (>5 lobes) were inlcuded.[10] Strict vegan, age >50 years, normochromic and normocytic RBCs, microcytic RBCs, proton pump inhibitors, thyroid and liver disease were excluded. Subjects with history of meat and liver rich diet intake and those suffering from concomitant cardiac disorders, chronic inflammatory disease, chronic granulomatous lesions of lungs, and diabetics were excluded. Subjects taking multi- vitamin pills were excluded strictly. Proforma were filled by the researcher after meeting the criteria of inclusion was met. Volunteers were asked for the blood sample. Complete blood counts were got by hematology analyzer. 2 ml blood was centrifuged to separate sera for the vitamin B_{12} estimation. Vitamin B_{12} was estimated from the blood sera by ELISA assay kit. Vitamin B_{12} was categorized as normal (>240pg/ml), borderline deficiency (170-240 pg/ml), deficiency (<170 pg/ml) and severe deficiency (<100 pg/ml).[11] Result variables were analyzed statistically.

RESULTS

Demography and laboratory findings are shown in the table 1. Age distribution (mean± SD) of male and female subjects was noted as 49.5±4.59 and 47.5±3.57 years respectively (P=0.71). Gender distribution shows male and female were noted as 153 (51%) and 147 (49%) out of total 300 subjects (P=0.076). Male to female ratio was noted 1.046:1. Male and female shows hemoglobin 11.7 ±2.15 and 10.6±2.15 g/dL (P=0.003), hematocrit 37.5±4.59 and 35.6±5.07% (P=0.047) and RBC counts 3.11±1.31 and 3.81±1.35 (P=0.078) respectively. MCV in male and female was noted as 110.5±5.31 and 113.91±7.35 fl (P=0.005). Mean vitamin B_{12} level in female subjects was noted as 135.35±15.51 pg/ml compared to 211.6±16.7 pg/ml in male subjects, the difference was statistically significant (P=0.0001). Only 73 (24.33%) subjects revealed normal vitamin B_{12} (>240 pg/ml) levels. Remaining 227 (75.66%) subjects revealed vitamin B_{12} borderline, deficiency and severe deficiency levels among 119 (39.67%), 56 (18.66%) and 52 (17.33%) respectively

Table No: I Demography and Laboratory findings

Variable	Male	Female	P-value
Age (years)	49.5±4.59	47.5±3.57	0.71
Gender	153 (51%)	147 (49%)	0.076
Hematocrit (Hct.) (%)	37.5±4.59	35.6±5.07	0.047
Hemoglobin (g/dl)	11.7 ±2.15	10.6±2.15	0.003
RBC counts ($x10^9/\mu L$)	3.11±1.31	3.81±1.35	0.078
MCV (fl)	110.5±5.31	113.91±7.35	0.005
Vitamin B_{12} (pg/dl)	211.6±16.7	135.35±15.51	0.0001

Table No: II. Vitamin B12 Levels in Study Subjects

Vitamin B_{12} categories	Mean	SD	P-value
Normal levels (>240 pg/ml)	425.13	35.17	0.0001
Borderline deficiency (170-240 pg/dl)	181.83	16.35	
Deficiency (<170 pg/dl)	134.91	5.27	
Severe deficiency (<100 pg/dl)	71.85	12.51	
Total	176.50	73.54	

Table No: III. Frequency of Vitamin B12 in Study Subject

Vitamin B_{12}	No.	%	P-value
Normal levels (>240 pg/ml)	73	24.33	0.0001
Borderline deficiency (170-240 pg/dl)	119	39.67	
Deficiency (<170 pg/dl)	56	18.66	
Severe deficiency (<100 pg/dl)	52	17.33	
Total	300	100	

DISCUSSION

The present is a prospective cross sectional study that was conducted in the adults with macrocytic- megaloblastic anemia to determine the frequency of vitamin B_{12} deficiency. We found vitamin B12 deficiency in 227 (75.66%) out of 300 total subjects. The finding is consistent with previous studies.[12,13] Of total 300 study subjects male and female were noted as 153 (51%) and 147 (49%) (P=0.076) and male to female ratio was noted 1.046:1. Findings are in agreement with previous studies.[14,15] Age of male and female subjects was noted as 49.5±4.59 and 47.5±3.57 years respectively (P=0.71). Our study shows mean young age population that is inconsistent to previous studies.[16,17] In present study, 73 (24.33%) subjects revealed normal vitamin B_{12} (>240 pg/ml) levels. Remaining 227 (75.66%) subjects revealed vitamin B_{12} borderline, deficiency and severe deficiency levels among 119 (39.67%), 56 (18.66%) and 52 (17.33%) respectively (P=0.0001). The findings are inconsistent to previous study[17] that 72.6% vitamin B_{12} deficiency. Finding of 75.66% vitamin B12 deficiency is consistent with previous studies.[18,19] In present study, 73 (24.33%) subjects revealed normal vitamin B_{12} (>240 pg/ml) levels. Remaining 227 (75.66%) subjects revealed vitamin B_{12} borderline, deficiency and severe deficiency levels among 119 (39.67%), 56 (18.66%) and 52 (17.33%) respectively (P=0.0001). Above findings are in agreement with previous studies.[17,20-23] In present study the MCV was found raised in majority of study subjects. MCV in male and female was noted as 110.5±5.31 and 113.91±7.35 fl (P=0.005). Elevated MCV indicates delayed nuclear maturation, through delayed nucleotide bio-synthesis caused by vitamin B_{12} deficiency. As the Bone marrow is rapidly proliferating tissue, hence the findings of vitamin B_{12} and folic acid are observed in the erythroid and myeloid cell series at the earliest. Bone marrow generates millions of blood cells and releases in the circulation. Delayed nuclear maturation caused by vitamin B_{12} and folic acid deficiency is prominently manifested in the bone marrow where millions of cells are proliferating each second. Hence, the bone marrow cells are affected at the earliest in vitamin B_{12} deficiency. Vitamin B_{12} deficiency impairs proliferation of both the erythroid and myeloid series of bone marrow. The delayed nuclear maturation leads to large mean corpuscular volume (MCV). In present study the MCV was elevated in majority of subjects. MCV in male and female was noted as 110.5±5.31 and 113.91±7.35 fl. Other previous studies[16,17] reported

the hypersegmented neutrophils as a reliable hematological marker of vitamin B_{12} deficiency. Vitamin B_{12} deficiency of 75.66% of present study is consistent with a previous national study.[24,25] A previous study[25] reported vitamin B_{12} deficiency in vegans and non- vegans in 85% and 78.5%. Although the frequency is high but concordant with the present study. The differences might be due to different study subjects of vegans and non-vegans. Frequency of 75.66% vitamin B_{12} deficiency of present study is in agreement with previous studies.[17,24-27] Finding of present study is also in agreement with previous studies[20-23] that reported frequency of 76% vitamin B_{12} deficiency. 75.66% vitamin B_{12} deficiency of present study is a clinically important finding being reported. Limitations of present study include 1st small number of study subjects, 2nd serum folate was noted detected due to cost constraints, and 3rd sample size not representative of population of the study area, hence results cannot be generalized to total population and for other geographical areas. However, strength of study lies in its prospective study design and vitamin B12 deficiency in diagnosed cases of macrocytic-megaloblastic anemia is an important contribution for the clinicians.

CONCLUSION

The present hospital based study reports frequency of 75.66% vitamin B_{12} deficiency in macrocytic- megaloblastic anemia reporting at Isra University Hospital. Vitamin B_{12} screening should be mandatory for macrocytic-megaloblastic anemia cases. Vitamin B_{12} supplements may prevent losses and irreversible neurological complications beside anemia.

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