Original Article

Vitamin B_{12} Deficiency with Megaloblastic Anemia: An Experience at Tertiary Care Hospital of Sindh

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ABSTRACT

OBJECTIVE: To determine frequency of Vitamin B_{12} deficiency in subjects with anemia and elevated mean corpuscular volume.

STUDY DESIGN: Cross sectional study

PLACE AND DURATION: Department of Medicine, Liaquat University and Isra University Hospital Hyderabad from January 2011 to August 2013.

METHODOLOGY: A sample of 113 subjects was selected according to inclusion and exclusion criteria. Serum vitamin B_{12} deficiency was defined as per standard criteria. Blood samples were analyzed on Sysmex KX 21 hematology analyzer. Continous variables were analyzed by ANOVA and Tukey-Cramer test while categorical variables by Chi-square test. The association between variables was analyzed by Pearson`s correlation. A p-value of ≤ 0.05 was taken statistically significant.

RESULTS: Of 113 subjects, 37 (32.7%) were male and 76 (67.2%) female, mean±SD age was 34.48±6.71 years and 89.3% (n=101) were anemic. Mean hemoglobin, hematocrit and RBC counts were found as 11.3 g/dl, 33.1% and 3.0 million/µL respectively. Of total 113 subjects, vitamin B_{12} deficiency was noted in 65 (57.5%); further subdivided as borderline and definitive deficiency in 19.4% and 37.9% respectively. Mean corpuscular volume as high as 139 fl and vitamin B_{12} levels as low as <30pg/ml were found. Pancytopenia was noted in 11 (9.7%) with severe vitamin B_{12} deficiency (<100pg/ml). Significant negative correlation was found between vitamin B_{12} and mean corpuscular volume (r=-0.79, p=0.0001). Peripheral blood film revealed anisocytosis, poikilocytosis, megaloblasts and hypersegmented neutrophils.

CONCLUSION: Vitamin B_{12} deficiency is prevailing and is a major contributing factor of megaloblastic anemia.

KEY WORDS: Vitamin B_{12} deficiency, Megaloblastic, anemia, Sindh.

INTRODUCTION

Vitamin B_{12} belongs to the family of cobalamins. It is an essential micronutrient required for DNA synthesis and cell proliferation particularly by rapidly proliferating hematopoietic cells of bone marrow. The foods of animal origin are rich in vitamin B_{12}. The daily requirement of vitamin B_{12} is 3 µg and daily absorption is up to 5 µg. The liver stores of vitamin B_{12} are 2000-5000 µg, which are sufficient for 3-5years before manifestations of deficiency do occur.[1] The causes of vitamin B_{12} deficiency are dietary lack, terminal ileum surgery, intrinsic factor deficiency, chronic gastritis, Helicobacter pylori infection, pancreatitis, bacterial overgrowth syndrome, transcobalamin II deficiency, and fish tape worm (Diphyllobothrium latum) infestation. Vitamins B_{12} acts as co-enzyme and catalyzes two important biochemical reactions in human body.[1] The co-enzyme forms include the methylcobalamin and S-adenosylcobalamin. S-adenosylcobalamin is co-enzyme for L-methylmalonyl-CoA– coenzyme A mutase which catalyzes the reaction of conversion of methylmalonyl-CoA to succinyl-CoA and the methylcobalamin is co-enzyme for methionine synthetase, which catalyzes conversion of homocysteine to methionine.[1,2] Clinically, vitamin B_{12} deficiency presents with various hematological, gastrointestinal and neurological manifestations. The most common hematological hallmark is the megaloblastic anemia.[1,3] Vitamin B_{12} deficiency produces anemia of varying severity that on occasion may be very severe. The mean corpuscular volume (MCV) is strikingly elevated; may be between 110-140 fl.[1] However, it is not impossible to have Vitamin B_{12} deficiency with normal MCV. The peripheral blood film shows anisocytosis, poikilocytosis and macro-ovalocytes. Marked erythroid hyperplasia is observed in bone marrow, as a response to defective erythropoiesis, termed as *ineffective erythropoiesis*. Asynchronous nucleus to cytoplasm maturation causes large cell erythroid cells. The hypersegmented neutrophils are one of the characteristic finding, with mean lobe count more than four. Six-lobed neutrophils may be seen on peripheral blood film. Reticulocyte count is diminished. In severe cases, it may present as pancytopenia because vitamin B_{12} defi-

ciency affects bone marrow.[1] The megaloblastic anemia of B_{12} deficiency is frequently observed in clinical practice but remains underestimated.[4-6] The reported studies are a few in number regarding it`s frequency and prevalence.[4,8-11] The present study was conducted to find out the frequency of isolated vitamin B_{12} deficiency in subjects with anemia and elevated mean corpuscular volume in our tertiary care hospital setup.

MATERIALS AND METHODS

A cross sectional study was conducted at Department of Medicine, Liaquat University of Medical and Health Sciences, Jamshoro and Isra University Hospital Hyderabad from January 2011 to May 2013. A sample of 113 subjects was selected through convenient sampling technique with exclusion and inclusion criteria. The sample size for the study was calculated by the formula for sampling for proportions. Subjects of > 12 years age of either sex, hemoglobin < 12g/dl for females and <13 g/dl for male or mean corpuscular volume ≥ 96 fl with megaloblasts and hypersegmented neutrophils were included. Very old subjects >80 years age, strict vegetarians, diabetic subjects, hypertension, cardiac disease, pulmonary tuberculosis, gastrointestinal disorders were excluded. The subjects with concomitant folic acid deficiency, dimorphic blood picture and iron deficiency anemia were also excluded. Anemia was defied according to WHO guidelines.[3] Blood samples were collected by venepuncture of antecubital vein. The blood complete picture was performed on Sysmex KX 21 hematology analyzer. Serum vitamin B_{12} level was measured. Vitamin B_{12} levels were defined as; Group I: normal > 240pg/ml, Group II: Borderline 170-240 pg/ml, Group III: Deficiency < 170 pg/ml and Group IV: Severe deficiency as <100 pg/ml.[1] Consent was taken from willing participants. The subjects were registered on a prestructured questionnaire. The subjects were examined by physician. A detailed medical history was sought and complete general and systemic clinical examination was conducted.

The data was entered into Statistical Package for Social Sciences (SPSS) version 17.0. Continous variables were analyzed by ANOVA and Tukey-Cramer test and presented as mean±SD, while categorical variables by Chi-square test and presented as frequencies and percentage. The association between variables was analyzed using Pearson`s correlation e.g. between vitamin B_{12} levels and mean corpuscular volume. Confidence interval was calculated at 95% and p-value of ≤ 0.05 was taken statistically significant.

RESULTS

Out of 113 subjects, 37 (32.7%) were male and 76 (67.2%) were female. The male to female ratio was 1:2.1 approximately. The mean age was 34.48±6.71 years. Majority of study subjects were young with mean±SD age of 34.4±6.7 years. (Table.I). Anemia was observed in 89.3%. Mean hemoglobin, hematocrit and RBC counts were found as11.3 g/dl, 36.1% and 3.0 million/µL respectively (Table I). The demographic characteristics are shown in Table. I. Mean corpuscular volume as high as 139 fl was observed in severely deficient vitamin B_{12} subjects (<100pg/ml) (Table II). Vitamin B_{12} value as low as <30pg/ml were observed. Highly significant vitamin B_{12} differences between and among groups were observed (p=0.0001) (Table III). The details of measured vitamin B_{12} levels are shown in Table. IV. Of total 113 subjects, 48 (42.4%) were found having normal vitamin B_{12} levels and 65 (57.5%) were found vitamin B_{12} deficient. Borderline and definitive vitamin B_{12} deficiencies were noted in 19.4% and 37.9% of subjects respectively (Table. IV). A highly significant negatively correlation was found between vitamin B_{12} and mean corpuscular volume (r=-0.79, p=0.0001) (Table.V).Pancytopenia was observed in 11 (9.7%) on peripheral blood film with reduced reticuocyte counts. Red cell distribution width (RDW) was elevated in majority of subjects. Megaloblasts, oval Macrocytes, anisocytosis, poikilocytosis, and hypersegmented neutrophils were observed on peripheral blood film shown in figures 1 and 2 respectivley.

TABLE I: DEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION (n=113)

	Mean	Std. Dev	Range
Age (years)	34.48	6.71	24-29
Hemoglobin (g/dl)	11.32	2.15	4-16
Hematocrit (Hct)(%)	36.16	8.04	19-47
RBC (million/µL)	3.0	0.57	2.3-4.0
Mean corpuscular volume (fl)	100.64	19.27	70-134
Mean corpuscular hemoglobin (pg/dl)	31.92	4.50	24-39
Mean corpuscular hemoglobin concentration(%)	29.38	4.62	23-38
RDW*(%)	12.5	2.5	11.5-15.5
Vitamin B12 (pg/dl)	171.18	82.64	27-298

* red cell distribution width

TABLE II: RED BLOOD CELL INDICES IN DIFFERENT GROUPS (n=113)

	MCV	MCH	MCHC
Normal (>240pg/ml)	84.7±5.3	29.2±3.1	27.5±3.6
Borderline deficiency (170-240 pg/ml)	97.3±4.8	28.9±3.5	26.6±4.0
Vitamin B12 deficiency (<170 pg/ml)	119±5.8	36.4±2.9	32.3±2.8
Severe Vit. B12 deficiency (<100pg/m)	121.8±8.5	35.1±3.0	32.2±4.5

TABLE III: ANALYSIS OF VARIANCE OF VITAMIN B_{12} BETWEEN AND AMONG GROUPS

	Sum of Squares	df	Mean Square	F-value	p=value
Between Groups	556048.468	3	185349.489	148.042	0.0001
Within Groups	120192.292	96	1252.003		
Total	676240.760	99			

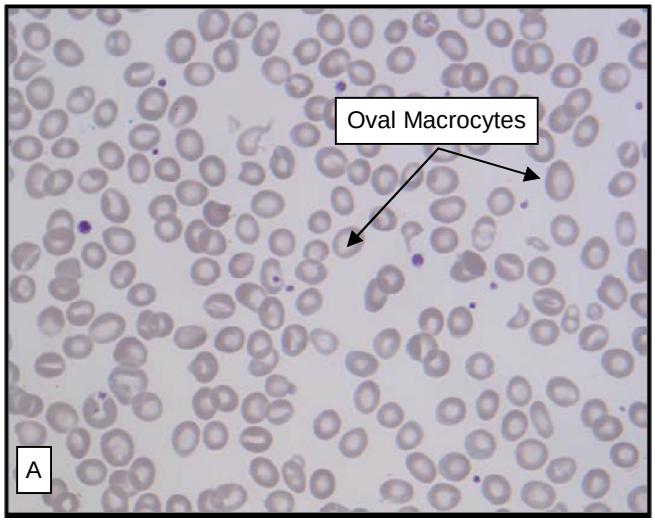
TABLE IV: VITAMIN B12 LEVELS MEASURED IN STUDY POPULATION (n=113)

	n (%)	Mean	Std. Dev	p=value
Group I. Normal (> 240pg/ml)	48 (42.4%)	245.22	50.3	p<0.002 (for all groups)
Group II. Borderline deficiency (170-240 pg/ml)	22 (19.4%)	210.22	31.5	
Group III. Vit. B12 deficiency (<170 pg/ml)	15 (13.2%)	139.20	22.1	
Group IV. Severe Vit. B12 deficiency (<100pg/ml)	28 (24.7%)	65.07	15.3	

TABLE V: PEARSON`S CORRELATION OF VITAMIN B_{12} (n=113)

	MCV	MCH	MCHC
Correlation coefficient (r-value)	-0.79	-0.43	-0.58
p=value	0.0001	0.001	0.001

FIGURE I: OVAL MACROCYTES (a) AND MEGALOBLASTIC CHANGES (b)



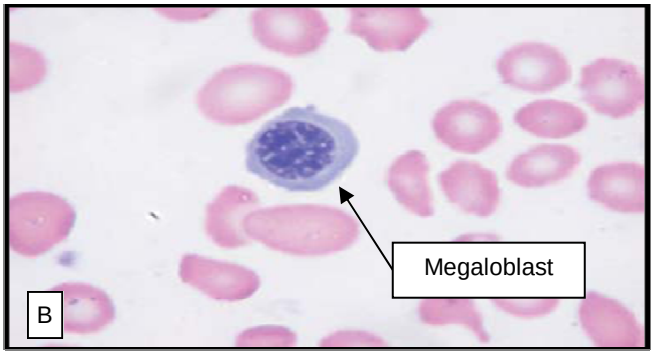
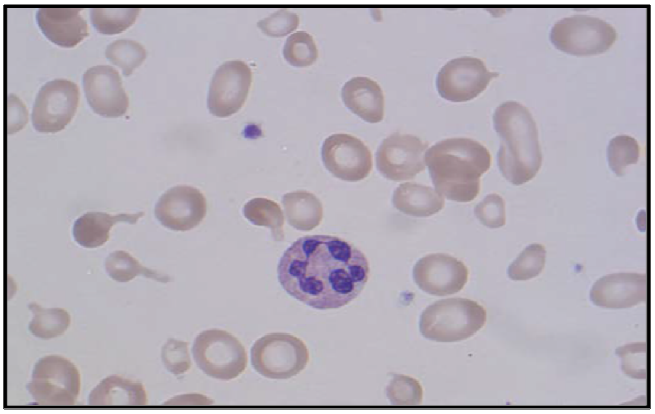


FIGURE II: NEUTROPHIL SHOWING HYPERSEGMENTED NUCLEUS AS SEEN IN PERIPHERAL BLOOD FILM



DISCUSSION

Vitamin B_{12} is essential for nucleotides synthesis, the DNA and RNA of body cells. Deficiency of vitamin B_{12} disturbs the rapidly proliferating cells of bone marrow and the resultant ineffective erythropoiesis causes formation of large immature red blood cells known as megaloblasts.[3,10] Vitamin B_{12} deficiency is quite common among Pakistani population.[11] In one recent study conducted on 95 subjects; the vitamin B_{12} deficiency was reported in 72.6% of study population.[3] In a retrospective study conducted by Iqbal SP et al.[4] at Agha Khan Hospital Karachi, reported frequency of vitamin B_{12} deficiency in vegetarians and non-vegetarian was 78.5% and 85% respectivley. The results of this present study reveal vitamin B_{12} deficiency in 57%, divided into borderline and definitive deficiency in 19.4% and 37.9% respectivley. (Table IV). The present study reveals comparatively less frequency of vitamin B_{12} deficiency compared with previous studies.[3,4] Another hospital based study reported 76% frequency of folate and vitamin B_{12} deficiencies. The results are not consistent with present study. The difference most probably is because of inclusion of both folate and vitamin B_{12} and small sample size (n=50).[6] The results of our present study show that vitamin B_{12} deficiency is not less common. 57% of study population proved to be vitamin B12 deficient. Therefore vitamin B_{12} deficiency should be considered in clinical cases having high mean corpuscular volume more than 96 fl. The 57% frequency of vitamin B_{12} deficiency is surprisingly high because our study has excluded the strict vegetarians. Though the results pertaining to high frequency of vitamin B12 deficiency in megaloblastic anemia represent a patient population largely belonging to Sindh, yet a similar trend has been reported previously from other parts of Pakistan.[7] Sarode et al [9] have shown prevalence of vitamin B12 deficiency to be 76% in 102 cases of nutritional megaloblastic anemia in a hospital-based study. Vitamin B_{12} deficiency of present study is comparable to a hospital based study from India conducted by Khunduri and Sharma who have reported vitamin B12 deficiency of 65%.[8] Naeem et al. from Gilgit agency of Northern Pakistan reported Vitamin B_{12} deficiency prevalence of 31.8%.[12] The results this study is comparatively higher. The previous study further reported the chronic giardiasis and dietary insufficiency as major causes for increased incidence of megaloblastic anaemia.[12] As concomitant iron deficiency impairs identification of several cases of vitamin B12 on the basis of macrocytosis only[13], therefore iron deficient subjects were excluded; hence present study findings are more reliable and authentic. Approximately 89.3% (n=101) of subjects were found anaemic according to WHO criteria. The finding proves that majority of patients were already vitamin B_{12} deficient, sufficient to produce blood changes. Vitamin B_{12} deficiency does not cause only megaloblastic anemia, but it may present with other symptoms,[14] like neuropsychiatric symptoms, even in the absence of anemia or megaloblastosis.[15,16] Reduced reticuocyte count and major differences in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed in present study and findings are consistent with previous studies.[15-17] It is suggested by previous studies[15,16] that vitamin B_{12} screening should be seriously considered for individuals showing even minor symptoms of deficiency as early as possible, because late complications may be irreversible.[16] We are of opinion that nationwide population based in-depth studies should be conducted to determine the proper estimate of vitamin B_{12} deficiency in order to prevent the disease and diability of a treatable cause.

CONCLUSION

Vitamin B_{12} deficiency is prevailing and is a major contributing factor of megaloblastic anemia. Early screening must be considered by medical practitioners in selected patients. Further studies are recommended to evaluate causes of deficiency in the community.

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