Original Article

Vitamin B_{12} Deficiency in Megaloblastic Anemia in Rural Population of Tando Muhammad Khan, Sindh

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ABSTRACT

Objective: Determine the vitamin B_{12} deficiency in megaloblastic anemia in rural population of Tando Muhammad Khan, Sindh

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Pathology Department, Indus Medical College Hospital Tando Muhammad Khan from May 2017 to Feb. 2018.

Materials and Methods: A sample of 170 cases of megaloblastic anemia (75 male and 95 female were studied according to inclusion criteria. Volunteers were informed and asked for blood sampling. Blood samples were collected. Vitamin B_{12} was detected by ELISA assay kit. Data was analyzed on SPSS (ver 22.0) at 95% CI ($P \leq 0.05$).

Results: Mean vitamin B_{12} deficiency was noted in both male and female subjects, however, the female subjects were having significantly low levels of 141.75±30.61 pg/ml in contrast to 201.16±36.7 pg/ml in male subjects (P=0.0001). Vitamin B_{12} categorized as normal levels, borderline B_{12} levels, deficient levels and severe deficiency levels were found in 40 (23.52%), 25 (14.70%), 87 (51.17%) and 18 (10.57%) respectively (P=0.0001). Total 130 (76.47%) subjects were having one or other type of vitamin B_{12} deficiency.

Conclusion: Frequency of 76.47% of vitamin B_{12} deficiency in megaloblastic anemia was noted in rural population of Tando Muhammad Khan, Sindh

Key Words: Vitamin B_{12} deficiency, Megaloblastic anemia, Rural, Sindh

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INTRODUCTION

Megaloblastic anemia is one of macrocytic anemia characterized by large-sized red blood cells that are prone to lysis in peripheral circulation. Megaloblastic anemia is caused by delayed nuclear maturation due to deficiency of vitamin B_{12} and folic acid.[1] Vitamin B_{12} is also known as cobalamin. It is needed as co-enzyme for the enzymes involved in nuclear maturation. Its deficiency results in larger red blood cells (RBC). Mean corpuscular volume (MCV) is a measure of mean RBC volume and is a clinical marker of macrocytic megaloblastic anemia. MCV more than 100 femtoliter (fl) is considered as macrocytic RBC. Vitamin B_{12} deficiency is one of cause of macrocytic megaloblastic anemia. Vitamin B_{12} plays essential role in nuclear maturation.[1,2] Vitamin B_{12} forms 2 co-enzymes; the methyl-cobalamin (MC) and the S- adenosyl cobalamin (SAC). Methionine synthetase needs methyl cobalamin as co-enzyme and catalyzes the reaction of conversion of homocysteine to methionine.[1-3] While the L-methylmalonyl-CoA– coenzyme A mutase requires SAC as co-enzyme; this enzyme converts methylmalonyl-CoA to succinyl-CoA. Methyl cobalamin and S-adenosyl cobalamin (SAC) act as one carbon donor for the synthesis of nucleotides of proliferating cells as red blood cells in bone marrow. This shows the essentiality of vitamin B_{12} for nuclear maturation. Bone marrow and epithelial cells are the rapidly proliferating cells of body and vitamin B_{12} deficiency adversely affects at the most. Diet of animal origin is the sole source of vitamin B_{12}. Vitamin B_{12} is absorbed from gut and circulates in blood bound with its carrier proteins. Daily gut absorption approximates 5 µg. While daily body requirement of Vitamin B_{12} is 3 µg. Human liver stores 2000-5000 µg of vitamin B_{12} approximately and these are sufficiency for many years.[3-6] The causes of vitamin B_{12} deficiency include dietary deficiency, malabsorption syndrome and increased body demands as during pregnancy and in growing children. Causes of vitamin B_{12} deficiency include; stomach disease, pancreatic disease, and small intestine disorders; all of these result in its malabsorption. Worm infestation is an important cause of vitamin B_{12} deficiency as Diphyllobothrium latum (fish tape worm) being the cause. Megaloblastic anemia

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is a common manifestation of vitamin B_{12} deficiency.[4,7] True burden of Vitamin B_{12} deficiency is not known for the developing countries, however, available studies show high burden. Search of published studies shows a few studies are available on the topic of frequency of vitamin B_{12} deficiency.[3-7] We planned a prospective cross sectional study to determine the vitamin B_{12} deficiency in megaloblastic anemia in rural population of Tando Muhammad Khan, Sindh.

MATERIALS AND METHODS

The present cross sectional study was conducted at Indus Medical College Hospital Tando Muhammad Khan from May 2017 to Feb. 2018. Prior approval was taken from the institute`s ethical review committee. A sample of 170 cases of megaloblastic anemia (75 male and 95 female) were studied according to inclusion criteria. Sample size was calculated by sampling for proportions of sample calculation. Inclusion criteria were; age 20- 40 years, both gender, MCV > 100 fl, and peripheral blood film showing hyper segmented neutrophils.[8] Exclusion criteria were old age, normocytic and normochromic anemia, microcytic anemia and concomitant major systemic disease. Strict vegetarians, diabetics, major cardiac disorders, chronic inflammatory diseases, pulmonary tuberculosis, etc were excluded. Also the subjects taking multi vitamins were strict exclusion criteria. Medical officers and physicians were requested communicated to help in proper screening of patients and provision of complete patient's biodata. Patient's biodata, presenting problems were collected on a pre-designed clinical proforma. These were provided to the medical officers. Medical officers were requested to help in screening of patients according to the inclusion and exclusion criteria and to fill the proforma properly. Only Properly labelled blood samples were screened for study. Volunteers were asked for signing the consent form. They were informed about the objective of study. They were assured that the personal information will be confidential and there will be no harm to them and no extra financial burden. Volunteers were asked for blood sample. Prominent vein in ante cubital fossa was marked. Area was cleaned with alcohol swab. 5 ml blood was withdrawn in disposable syringe and collected in EDTA tubes. Red blood cells, hematocrit and hemoglobin were analysed on hematology analyzer (Sysmex, KX 21). Serum vitamin B_{12} was estimated by ELISA method. Normal, borderline, deficiency and severe vitamin B_{12} levels were defined as >240pg/ml, 170-240 pg/ml, <170 pg/ml and <100 pg/ml respectively.[9] Data was kept confidential and consent forms were secured. Data was analyzed on SPSS (ver 22.0) at 95% CI ($P \leq 0.05$). Student`s t-test analysed the continuous variables (age, vitamin B12 level, etc) and Chi-square test analysed the categorical variables (gender and vitamin B12 categories).

RESULTS

Of total 170 study subjects, male and female were noted as 75 (44.17%) and 95 (55.82%) respectively (P=0.001). Female to male ratio was 1.26:1 approximately (table 1).

Table No.1: Descriptive findings of study subjects (n=170)

	Male	Female	P-value
Gender	75 (44.17%)	95 (55.82%)	0.001
Age (years)	32.95±8.43	32.35±5.73	0.17
Hemoglobin (g/dl)	13.97 ±1.45	13.06±3.13	0.0001
Hematocrit (Hct.) (%)	43.10±3.75	41.06±7.08	0.0001
RBC (million/µL)	4.23±0.23	4.08±0.43	0.0001
Vitamin B12 (pg/dl)	201.16±36.7	141.75±30.61	0.0001

Table No.2: Vitamin B_{12} distribution of study subjects (n=170)

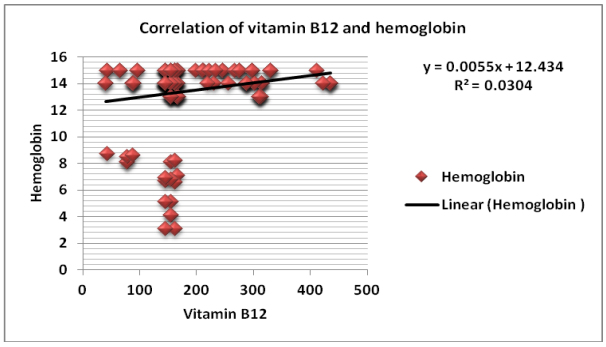
Vitamin B_{12} levels	Mean	SD	P-value
Normal B_{12} level (>240 pg/ml)	312.43	55.57	0.0001
Borderline B_{12} deficiency (170-240 pg/dl)	175.68	26.73	
B_{12} Deficiency (<170 pg/dl)	154.59	7.27	
Severe B_{12} deficiency (<100 pg/dl)	72.19	21.11	
Total	186.10	81.07	

Table No.3: Frequency of Vitamin B_{12} in study subjects (n=170)

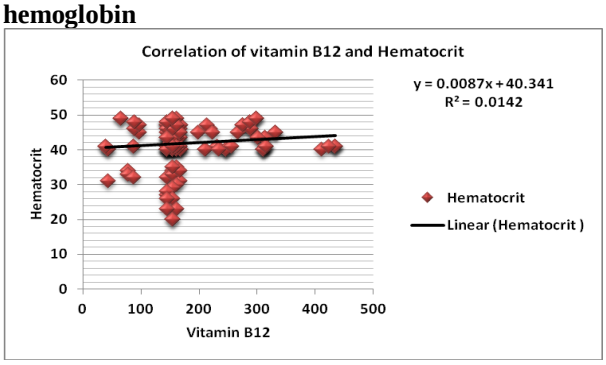
Vitamin B_{12} levels	No.	%	P-value
Normal B_{12} level (>240 pg/ml)	40	23.52	0.0001
Borderline B_{12} deficiency (170-240 pg/dl)	25	14.70	
B_{12} Deficiency (<170 pg/dl)	87	51.17	
Severe B_{12} deficiency (<100 pg/dl)	18	10.57	
Total	170	100	

Age (mean± SD) of male and female was 32.95±8.43 and 32.35±5.75 years respectively (P=0.17). Hemoglobin, **hematocrit** and RBC counts were low in female subjects compare to male (P<0.05) as shown in table 1. Mean vitamin B_{12} deficiency was noted low in both male and female subjects, however, the female subjects were having significantly low levels of 141.75±30.61 pg/ml in contrast to 201.16±36.7 pg/ml in male subjects (P=0.0001). Vitamin B_{12} categorized as

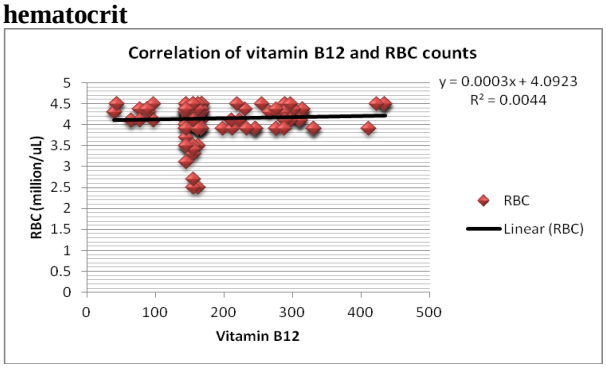
normal levels, borderline B_{12} levels, deficient levels and severe deficiency levels were found in 40 (23.52%), 25 (14.70%), 87 (51.17%) and 18 (10.57%) respectively (P=0.0001). Total 130 (76.47%) subjects were having one or other type of vitamin B_{12} deficiency. Graph 1-3 shows the correlation of vitamin B_{12}, hemoglobin, hematocrit and RBC counts.



Graph No.1: Correlation of vitamin B_{12} and hemoglobin



Graph No.2: Correlation of vitamin B_{12} and hematocrit



Graph No.3: Correlation of vitamin B_{12} and RBC counts

DISCUSSION

The present is a small scale study reporting on the vitamin B_{12} levels in healthy adults. Age (mean± SD) of male and female was 32.95±8.43 and 32.35±5.75 years respectively (P=0.17). Of total 170 study subjects, male and female were noted as 75 (41.17%) and 95 (55.82%) respectively (P=0.001). Female to male ratio was 1.26:1 approximately. The mean age of study subjects shows young population that is in contrast to previous studies.[10,11] Role of Vitamin B_{12} lies in its nucleotide biosynthesis one carbon donation through folic acid. Both Vitamin B_{12} and folic acid are essential for the proliferating cells in particular the bone marrow where millions of cells are proliferating each second. The bone marrow produces and supplies millions of blood cells to the peripheral circulation each moment. Thus bone marrow is affected earlier in cases of Vitamin B_{12} deficiency. In present study, total 130 (76.47%) subjects were having one or other type of vitamin B_{12} deficiency. A previous study[11] reported vitamin B_{12} deficiency of 72.6%.[11] Our finding of 76.47% vitamin B12 deficiency is consistent with above study. Normal, borderline deficiency, deficiency and severe vitamin B_{12} deficiency were noted in 40 (23.52%), 25 (14.70%), 87 (51.17%) and 18 (10.57%) respectively (P=0.0001). Our findings are in agreement with previous reported studies.[11,16-19] Vitamin B_{12} deficiency impairs capacity of bone marrow stem cell proliferation resulting in abnormal red blood cell production that are larger than normal in size and prone to destruction in peripheral circulation resulting in anemia. Both erythroid and myeloid series of bone marrow are adversely affected by vitamin B_{12} deficiency. Bone marrow releases immature red blood cells having large mean corpuscular volume. Also the white blood cells are abnormal showing hyper segmented polymorphs. Hypersegemnted neutrophils is a reliable clinical marker of vitamin B_{12} deficiency.[10,11] In present study we found high frequency of vitamin B_{12} deficiency that is prevalent in the rural population of Tando Muhammad Khan, Sindh. Most probable cause of this is the nutritional deficiency. The findings are in agreement with a previous study.[12] Vitamin B_{12} deficiency of 76.47% of present study is consistent with a previous national study.[13] This previous study[13] reported frequency of deficiency of 85% and 78.5% in vegetarians and non- vegetarians respectively. A previous national study reported frequency of 65% vitamin B_{12} deficiency, that is low and in disagreement with 76.47% noted in the present study. This discrepancy could be due to the different sample size and population. Frequency of 76.47% vitamin B_{12} deficiency of present study is in full agreement with previous studies.[11-15] Our findings are also supported by previous studies[16-19] that noted 76% vitamin B_{12} deficiency. Evidence based frequency of 76.47% vitamin B_{12} deficiency of present study is an important clinical finding and shows the prevalent vitamins deficiencies. Present study has certain limitations; first- folic acid was not estimated, second- sample size was small, third- rural population was studied, hence the results are not valid to generalize as representative of total population. However, the vitamin B12 deficiency in diagnosed cases of megaloblastic anemia is important findign. The present study reports vitamin B12 deficiency is prevalent in the rural population. This needs large scale studies to cover the total population of

the area for concluding proper burden of vitamin B^{12} deficiency.

CONCLUSION

The present study reports frequency of 76.47% vitamin B_{12} deficiency in megaloblastic anemia in rural population of Tando Muhammad Khan, Sindh. This shows the severity of unnoticed vitamin B_{12} deficiency in those presenting with megaloblastic anemia. Vitamin B_{12} screening is mandatory for those presenting with high mean corpuscular volume. Vitamin B_{12} supplements should be given to patients for prevention of long term irreversible complications beside anemia. Further studies are recommended with large population sample.

Author's Contribution:

Concept & Design of Study:	Inayatullah Memon
Drafting:	Attiya Memon
Data Analysis:	Attiya Memon
Revisiting Critically:	Inayatullah Memon, Attiya Memon
Final Approval of version:	Inayatullah Memon

Conflict of Interest: The study has no conflict of interest to declare by any author.

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