The Professional Medical Journal
www.theprofesional.com

ORIGINAL PROF-0-4748

https://doi.org/10.29309/TPMJ/2021.28.04.4748

Vitamin Cobalamin deficiency in macrocytic anemia reporting at a Tertiary Care Hospital.

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Article received on:
28/04/2020
Accepted for publication:
02/06/2020

ABSTRACT… Objective: Determine the frequency of vitamin cobalamin deficiency in macrocytic anemia cases reporting at tertiary care hospital. **Study Design:** Cross Sectional study. **Setting:** Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad, Sindh Pakistan. **Period:** January 2017 to October 2018. **Material & Methods:** 450 cases of both genders, diagnosed as macrocytic- megaloblastic anemia were studied for the vitamin Cobalamin levels. Cases were collected through non- probability convenient sampling by inclusion and exclusion criteria. Consenting volunteers were asked for blood sampling. 5 mL blood was taken from ante – cubital fossa. Samples were centrifuged and sera were collected for the estimation of vitamin cobalamin by ELISA – assay kit. Continuous and categorical variables were entered in SPSS (version 21.0) and analyzed by Student t-test and Chi-square test respectively at 95% CI ($P \leq 0.05$). **Results:** Male and female comprised 225 (43.3%) and 294 (56.6%) of 519 subjects. Male to female ratio was noted 1.30:1. MCV, MCH and MCHC show statistically significant difference between male and female ($P<0.05$). MCV in male was 96.8±9.92 fl vs. 105.5±12.04 fl in female ($P=0.0001$). Normal cobalamin was noted in 15.2% (n= 79) and any type of cobalamin deficiency was noted in 84.7% (n= 440) ($P=0.0001$). **Conclusion:** The present study reports frequency of 84.7% Cobalamin deficiency in macrocytic anemia reporting at Indus Medical College Hospital. Further studies are recommended by the treating physicians.

Key words: Cobalamin Deficiency, Macrocytic Anemia, RBC Indices.

Article Citation: Shaikh KR, Shaikh S, Tabassum S, Memon S, Soomro UA, Siddiqui SS, Khoharo HK. Vitamin Cobalamin deficiency in macrocytic anemia reporting at a Tertiary Care Hospital. Professional Med J 2021; 28(4):527-532. https://doi.org/10.29309/TPMJ/2021.28.04.4748

INTRODUCTION

Mean corpuscular volume (MCV) is a measure of red blood cell (RBC) volume. Large MCV is a clinic – hematological indicator of macrocytic anemia. RBC showing volume ≥100 fl is termed as macrocyte that occurs due to the deficiency of folic acid and cobalamin.[1,2] Macrocytes RBC are large in size and are hemolyzed as they pass through microcirculation whose diameter is less than these. Hemolysis is common in the sinusoids of liver and spleen. Other causes of cobalamin deficiency are; antibiotic drugs, proton pump inhibitors, chronic liver and thyroid disorder, etc. Vitamin cobalamin is an essential maturation factor for the nucleus of proliferating cells such as developing erythroblast along with folic acid. Nuclear maturation of RBC is arrested in case of cobalamin deficiency resulting in large MCV – RBC prone to hemolysis resulting in anemia clinically.[3,4] As regards cobalamin deficiency, the RBC appear large in size, are hemolyzed and is termed as macrocytic- megaloblastic anemia.[3,4] Cobalamin functions as one carbon – methyl donor for the nucleotides synthesis in young erythroblasts. Both folic acid and cobalamin are needed for the nuclear maturation of rapidly proliferating cells of bone marrow such as precursors of red and white blood cells. Cobalamin forms 2 co – enzymes called SAM (S- adenosyl cobalamin) and methyl-cobalamin. SAM (S- adenosyl cobalamin) is co – enzyme for the L – methylmalonyl-CoA– coenzyme A mutase that catalyzes the methylmalonyl-CoA to succinyl-CoA. Methyl – cobalamin is co-enzyme for the "methionine synthetase" that catalyzes the biochemical reaction of homocysteine converted to methionine.[4,5] Cobalamin deficiency is manifested earliest in the rapidly proliferating

bone marrow before in others. Cobalamin is a water soluble vitamin. Nutritional deficiency of vitamin cobalamin is very common due to malnourishment; caused by parasite infestation, gut malabsorption, etc. Pregnancy is characterized by an increase demand of folic acid and cobalamin both; similar is the condition for the growing children. Parasite infestation, gut malabsorption, gastric achlorhydria, terminal ileum and pancreatic disorders are some causes of cobalamin deficiency. Fish tape – worm (Diphyllobothrium latum) is an intestinal parasite notoriously causing cobalamin deficiency.[6,7] Prevalence of cobalamin deficiency is very high particularly in the developing countries that is under – reported. Few published studies show high prevalence of cobalamin deficiency[5-8], and there is need for conducting further research. This scenario of neglect compelled to conduct the present study to evaluate the frequency of cobalamin deficiency in macrocytic anemia cases reporting at a tertiary care hospital.

MATERIAL & METHODS

Ethical approval was taken from the ERC (Ethical review committee) Faculty of Medicine and Allied Medical Sciences, Isra University, Hyderabad, Sindh Pakistan. Study covered duration from January 2017 to October 2018. Cases were selected through non – probability convenient sampling according to inclusion and exclusion criteria. A sample of 450 cases of macrocytic anemia was calculated 'sampling for proportions' and included in study protocol. RBC showing raised mean corpuscular volume (MCV) ≥100 fl, termed as macrocyte was inclusion criteria for analyzing blood cobalamin levels. Other inclusion criteria were; both male and female gender, age 20 – 50 years and hyper segmented neutrophils (>5 lobes).[9] Strict vegetarians, microcytic, normocytic and normochromic RBC were exclude. Patients using proton pump inhibitors and multivitamins were strictly excluded. Liver, thyroid, lung and cardiac diseases were excluded on clinical history. Diabetics and those suffering from chronic diarrhea was exclusion. Subjects with history of meat and liver diet intake recently were also excluded. Clinical history of consenting volunteers was taken for ensuring inclusion and exclusion criteria. Volunteers were given proforma to sign for consent. Advantages and dis – advantages of study were briefed during the clinical history taking. Biodata and findings of blood testing were kept confidential and record was maintained strictly during the study duration. Volunteers were informed of no loss; no expenses of cobalamin testing and data will never ever be publicized. Medical officers were asked to help for screening the subjects. MCV ≥100 fl was defined as macrocytic anemia.[1,2] Diagnosis was made by consultant hematologist. Blood samples were collected under aseptic measures from ante – cubital fossa after a tourniquet was tightly tied. Ante – cubital fossa was sterilized with alcohol – swab. 5 ml of blood sample was taken by venesection with a 5 ml BD Disposable syringe (BD, USA). 2 ml was centrifuged to get sera and 3 ml was put in NaF containing tubes for complete blood counts. Sera were collected for the estimation of vitamin cobalamin by ELISA – assay kit. Vitamin cobalamin levels were defined as normal ≥240pg/ml, borderline cobalamin deficiency – 170-240 pg/ml, cobalamin deficiency <170 pg/ml and severe cobalamin deficiency <100 pg/ml.[10] Continuous and categorical variables were entered in SPSS (version 21.0) and analyzed by Student t-test and Chi-square test respectively at 95% CI ($P \leq 0.05$).

RESULTS

Male and female comprised 225 (43.3%) and 294 (56.6%) of 519 subjects (P=0.9) (Table-I and Figure-1). Age (mean± SD) of male and female was 47.5±8.06 and 46.7±7.93 years respectively (P=0.30). Male to female ratio was noted 1.30:1. Hemoglobin and Hct (%) shows statistical difference between male and female gender (P=0.0001). Male and female shows hemoglobin and Hct (%) 13.42±1.37 and 11.5±1.07 g/dl (P=0.0001) & 43.0±6.07 and 36.1±3.81% (P=0.047) respectively. RBC counts were noted as $3.5\pm1.31 \times 10^9/\mu L$ and $3.8\pm1.35 \times 10^9/\mu L$ in male and female respectively. MCV, MCH and MCHC show statistically significant difference between male and female (P<0.05) (Table-I). MCV in male was 96.8±9.92 fl vs. 105.5±12.04 fl in female (P=0.0001). Serum Cobalamin level in female was low 187.1±73.41 pg/ml compared to 245.3±63.0

pg/ml in male (P=0.0001) (Table-I). Table-II shows the mean +/- SD cobalamin levels in total study subjects. Table-III shows the frequency of cobalamin level – normal, borderline, deficiency and severe deficiency in male and female subjects. Normal cobalamin was noted in 15.2% (n= 79) and any type of cobalamin deficiency was noted in 84.7% (n= 440) (P=0.0001).

	Male (n=225)	Female (n=294)	P-Value
Age (years)	47.5±8.06	46.7±7.93	0.30
Gender	225 (43.3%)	294 (56.6%)	0.09
Hemoglobin (g/dl)	13.42±1.37	11.5±1.07	0.0001
Hematocrit (Hct.) (%)	43.0±6.07	36.1±3.81	0.0001
RBC counts (x10^9/μL)	3.5±1.31	3.8±1.35	0.09
MCV (fl)	96.8±9.92	105.5±12.04	0.0001
MCH (pg)	27.8±4.22	30.1±3.67	0.0001
MCHC (%)	34.3±2.14	35.7±2.29	0.0001
Cobalamin (pg/ml)	245.3±63.0	187.1±73.41	0.0001

Table-I. Findings of male and female study subjects (n=519)

Vitamin B_{12} categories	Mean	SD	P-Value
Normal levels (>240 pg/ml)	280.18	26.85	0.0001
Borderline deficiency (170-240 pg/dl)	203.09	22.09	
Deficiency (<170 pg/dl)	139.85	24.17	
Severe deficiency (<100 pg/dl)	73.23	20.33	
Total	212.34	74.82	

Table-II. Cobalamin levels in study subjects (n=300)

Category	Male	Female	Total	P-Value
Normal (>240 pg/ml)	51	28	79	0.0001
Borderline (170-240 pg/dl)	11	52	63	
Deficiency (<170 pg/dl)	78	124	202	
Severe deficiency (<100 pg/dl)	85	90	175	
Total	225	294	519	

Table-III. Frequency of Cobalamin levels in study subjects (n=519)

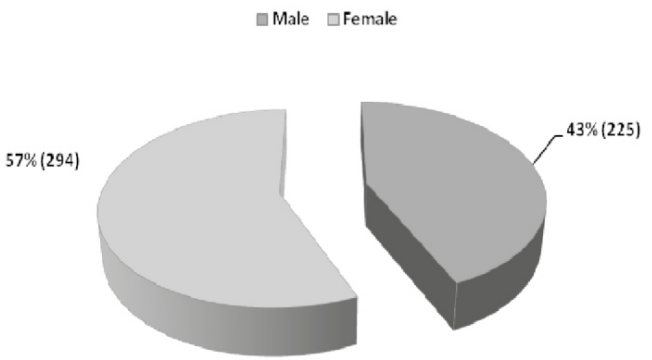

Figure-1. Frequency of male and female subjects

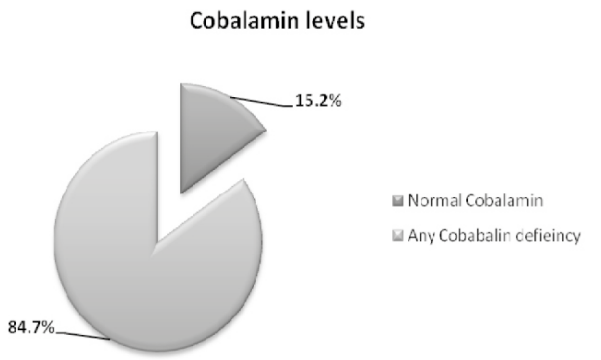

Figure-2. Frequency of cobalamin deficiency in study subjects

DISCUSSION

The present is the first cross – sectional study from a tertiary care hospital that reports on the frequency of vitamin Cobalamin deficiency in macrocytic anemia reporting at our tertiary care hospital. The present study noted normal cobalamin in 15.2% (n= 79) and any type of cobalamin deficiency was noted in 84.7% (n= 440) (P=0.0001). The findings are consistent with recent studies from Pakistan.[11-13] Frequency of Cobalamin deficiency is also consistent with previous studies.[14,15] Of total 519 study subjects, the male and female comprised 225 (43.3%) and 294 (56.6%) respectively (P=0.9). Male to female ratio was noted 1.30:1. Gender distribution of present study is in keeping with previous studies.[16,17] Age (mean± SD) of male and female was 47.5±8.06 and 46.7±7.93 years respectively (P=0.30). In present study, the age recognizes young study subjects. The findings corroborate with previous studies.[15-17] In present study, normal cobalamin was noted in 15.2% (n= 79) and any type of cobalamin deficiency was noted in 84.7% (n= 440) (P=0.0001). Findings are consistent with recent studies reported from Pakistan.[11-13]

A previous study[17] reported cobalamin deficiency of 72.6% that is inconsistent findings. The reason is sample size of previous study was low and different study populations. In present study, any type of cobalamin deficiency was noted in 84.7% (n= 440) (P=0.0001) (Figure-2) that is consistent with previous studies.[18,19] In present study, only 15.2% (n= 79) subjects show normal cobalamin (>240 pg/ml) levels that also corroborate with previous studies.[17,20,21] In present study, the MCV in male was 96.8±9.92 fl vs. 105.5±12.04 fl in female (P=0.0001) that shows raised values. Raised RBC – MCV points to the late nuclear maturation caused by cobalamin deficiency, because vitamin cobalamin is needed for the nucleotide (DNA) biosynthesis. Late nuclear maturation leads to elevated mean corpuscular volume (MCV). In present study the MCV was elevated in majority of subjects. MCV in male was 96.8±9.92 fl vs. 105.5±12.04 fl in female (P=0.0001).

Other previous studies[16,17] reported the hypersegmented neutrophils being reliable clinic – hematological marker of cobalamin deficiency. Cobalamin deficiency of 85% and 78.5% has been reported in vegans and non – vegans.[21] It is consistent with cobalamin deficiency of 84.7% of present study. However, other previous studies[21-23] reported low frequency of cobalamin deficiency that is inconsistent findings. The 84.7% cobalamin deficiency of present study is a clinically important finding that must be reported for positive clinical outcome by doing cobalamin screening. Few limitations of the present study include 1st: – sample size is small number, 2nd:– serum folate was not measured due to laboratory expense, and 3rd:– sample size not representative of population of the study area. It is clear that the generalizability of findings is not possible for whole population and for other geographical countries.

CONCLUSION

The present study reports frequency of 84.7% Cobalamin deficiency in macrocytic anemia reporting at Isra University Hospital. Further studies are recommended by the treating physicians. Cobalamin screening programs be launched for its earlier deficiency detection particularly in anemic subjects. Cobalamin supplementation may prevent hematological complication.

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