

## VITAMIN B<sub>12</sub> LEVELS AMONG HEALTHY ADULT POPULATION- A CROSS SECTIONAL STUDY

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### ABSTRACT

**Objective:** To determine vitamin B<sub>12</sub> levels among healthy adult population. **Study Design:** Cross sectional study. **Place and Duration:** Outpatient Department, Indus Medical College Hospital Tando Muhammad Khan from March 2017 to September 2017. **Methods:** A sample of 147; comprising 95 female and 52 male was selected for study. Volunteer participants were selected according to inclusion and exclusion criteria. Blood samples were collected. Hemoglobin, hematocrit, and RBC counts were detected and vitamin B<sub>12</sub> was detected by ELISA assay kit. Student's t-test and Chi-square test were used on SPSS (ver 22.0) for data at 95% CI (P ≤ 0.05). **Results:** Mean ± SD age was noted as 39.9 ± 5.35 and 40.10 ± 11.24 years in male and female respectively. Of 147 subjects, 95 (64.6%) were female and 52 (35.5%) were male (P=0.0001). Vitamin B<sub>12</sub> was found as 214.78 ± 76.4 and 133.81 ± 35.1 pg/ml in male and female respectively (P=0.0003). Total vitamin B<sub>12</sub> was noted as 166.04 ± 68.84 pg/ml. Of total 147 subjects, normal, borderline deficiency, deficiency and severe deficiency of vitamin B<sub>12</sub> was noted in 20 (13.6%), 27 (18.3%), 75 (51.02%) and 25 (17.01%) respectively (P=0.0001). **Conclusion:** The present study reported frequency of 86.39% of vitamin B<sub>12</sub> deficiency. This shows the gravity of Vitamin B<sub>12</sub> deficiency problem that is prevalent in the community and remains undiagnosed. Early screening and vitamin B<sub>12</sub> supplements campaigns should be launched for prevention of long term irreversible complications. Further large scale studies are recommended.

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### INTRODUCTION

Vitamin B<sub>12</sub> also called cobalamin, acts as co-enzyme for biologically important enzymes. The vitamin B<sub>12</sub> dependent enzymes catalyze essential biochemical reactions in the living organisms.<sup>1</sup> Vitamin B<sub>12</sub> forms two important co-enzymes; the methyl-cobalamin (MC) and the S-adenosyl cobalamin (SAC). Methyl cobalamin is co-enzyme for the methionine synthetase enzyme. This enzyme catalyzes biochemical reaction that converts homocysteine to methionine.<sup>1-3</sup> While SAC is co-enzyme for the enzyme L-methylmalonyl-CoA- coenzyme A mutase. This enzyme catalyses the biochemical reaction of converting the methylmalonyl-CoA to succinyl-CoA. Deficiency of vitamin B<sub>12</sub> causes

methymalonic aciduria. MC acts as one carbon donor essential for the biosynthesis of nucleotides of cells through its interactions with folate. Thus vitamin B<sub>12</sub> is essential for the cell proliferation through nuclear maturation. Rapidly proliferating bone marrow and epithelia are commonly affected in vitamin B<sub>12</sub> deficiency. Animal diet is the sole source of vitamin B<sub>12</sub>. Its daily gut absorption is 5 µg and daily body need is 3 µg. Liver is the major storing organ that stores approximately 2000-5000 µg of vitamin B<sub>12</sub>. Vitamin B<sub>12</sub> stores of 2000-5000 µg are sufficient for years.<sup>1-3</sup> Dietary deficiency is the sole cause of vitamin B<sub>12</sub> deficiency. Other causes of vitamin B<sub>12</sub> deficiency are related to its gut absorption; such as the pancreatic disease, chronic gastritis,

intrinsic factor deficiency, small intestinal bacterial growth, celiac disease, ileal resection, *H. pylori* and worm infestations. *Diphyllobothrium latum* infestation is an important cause of vitamin B<sub>12</sub> deficiency in tropical countries. Vitamin B<sub>12</sub> deficiency causes abnormalities of red and white blood cells and mucosal epithelia. Megaloblastic anemia is a frequently encountered manifestation of vitamin B<sub>12</sub> deficiency.<sup>4-6</sup> Search of medical literature shows only a few studies have been conducted in the country.<sup>4,7-9</sup> The present cross sectional study was designed to determine the vitamin B<sub>12</sub> levels among patients accompanying healthy adults presenting at our tertiary care hospital.

**SUBJECTS AND METHODS**

A cross sectional study was conducted at Indus Medical College Hospital Tando Muhammad Khan from March 2017 to September 2017. Ethical approval was taken from institute`s review committee. A sample of 147; comprising 95 female and 52 male was selected for study. Volunteer participants were selected according to inclusion and exclusion criteria. Sample size was estimated by formula for sampling for proportions. Normal healthy adults accompanying the patients at our tertiary care hospital were facilitated for study protocol. Inclusion criteria were age >20 years of both genders were included. Apparently healthy adults were examined for any disease. An interview was arranged for exclusion of major systemic disease. Dietary vegetarians, old age (>60 years), and those suffering from Diabetes mellitus, cardiac failure, ischemic heart disease, chronic urinary tract infection, sexually transmitted disease, pulmonary tuberculosis, malabsorption syndromes, etc were excluded. Normal subjects were asked for intake of any multi vitamin multi mineral pill intake. Any subjects with positive history of multivitamin pill intake were excluded. Volunteers were interviewed for the purpose of study. They were informed of no loss or harm to them and laboratory investigations will not be charged. Volunteers were asked to sign consent

form who gave enthusiastic consent. Venous blood samples were collected in EDTA tubes and gel tubes. EDTA blood was used for the detection of hemoglobin, hematocrit and red blood cell counts on Sysmex KX 21 hematology analyzer. Vitamin B<sub>12</sub> was detected by ELISA assay kit. Vitamin B<sub>12</sub> levels were categorized as; normal vitamin B<sub>12</sub> levels >240pg/ml, borderline deficiency - 170-240 pg/ml, Deficiency <170 pg/ml and Severe deficiency was defined as Vitamin B<sub>12</sub> <100 pg/ml.<sup>1</sup> Biodata was noted on a pre-structured designed proforma. Consent form was also designed for signing by volunteers. Data was analyzed on SPSS (ver 22.0) software. Student`s t-test (continuous variables) and Chi-square test (categorical variables) were used. Level of significance was taken at 95% CI (P ≤ 0.05).

**RESULTS**

Mean± SD age was noted as 39.9±5.35 and 40.10±11.24 years in male and female respectively. Age, body weight, systemic blood pressure, hemoglobin, red blood cells and hematocrit are shown in table 1. Of 147 subjects, 95 (64.6%) were female and 52 (35.5%) were male (P=0.0001). Female to male ratio was 1.81:1 approximately (table 2). Vitamin B<sub>12</sub> (mean± SD) was found as 214.78±76.4 and 133.81±35.1 pg/ml in male and female respectively (P=0.0003). Table 3 shows the total (mean± SD) vitamin B<sub>12</sub> was noted as 166.04 ± 68.84 pg/ml. Of total 147 subjects, normal, borderline deficiency, deficiency and severe deficiency of vitamin B<sub>12</sub> was noted in 20 (13.6%), 27 (18.3%), 75 (51.02%) and 25 (17.01%) respectively (P=0.0001) (table 4)

**Table 1: Characteristics findings of study subjects (n=147)**

	Male	Female	P-value
Age (years)	39.9±5.35	40.10±11.24	0.17
Body weight (Kg)	80.73±10.53	86.28±10.15	0.049
Systolic BP (mmHg)	120.6±5.21	121.5±7.01	0.96
Diastolic BP (mmHg)	68.58±4.77	69.56±7.27	0.47
Hemoglobin (g/dl)	14.5±3.49	12.81±4.62	0.0001
Hematocrit (Hct.) (%)	40.92±5.26	39.5±8.40	0.0001
RBC (million/μL)	3.25±0.22	3.5±0.49	0.0001
Vitamin B12 (pg/dl)	214.78±76.4	133.81±35.1	0.0003

**Table 2: Gender distribution of study subjects (n=147)**

	No.	Percentage	P-value
Male	52	35.5	0.0001
Female	95	64.6	

**Table 3: Vitamin B<sub>12</sub> distribution of study subjects (n=100)**

Category	Mean	SD	P-value
Normal >240 (pg/ml)	307.35	55.29	0.0001
Borderline deficiency (170-240 pg/dl)	174.25	26.37	
Deficiency <170 (pg/dl)	154.78	7.38	
Severe deficiency <100 (pg/dl)	77.81	16.87	
Total	166.04	68.84	

**Table 4: Frequency of Vitamin B<sub>12</sub> in study subjects (n=147)**

Category	No.	Per (%)	P-value
Normal >240 (pg/ml)	20	13.6	0.0001
Borderline deficiency (170-240 pg/dl)	27	18.3	
Deficiency (<170 pg/dl)	75	51.02	
Severe deficiency (<100 pg/dl)	25	17.01	
Total	147	100	

**DISCUSSION**

The present is a small scale study reporting on the vitamin B<sub>12</sub> levels in healthy adults. Vitamin B<sub>12</sub> plays role in the nuclear maturation of body cells through nucleotide biosynthesis. Most proliferating cells of body are affected such as the epithelia, bone marrow, etc. Vitamin B<sub>12</sub> deficiency disturbs cell proliferation resulting in epithelial defects as oral ulcers and low red blood cell counts in the circulation. Vitamin B<sub>12</sub> deficiency disturbs the bone marrow first because of rapidly proliferating myeloid cells in particular the erythroid series. Bone marrow releases large immature red blood cells and hyper segmented polymorphs in the circulation, these both are important clinical signs of vitamin B<sub>12</sub> deficiency.<sup>3,10</sup> A previous study<sup>11</sup> reported vitamin B<sub>12</sub> deficiency is common nutritional deficiency in the populace of the country.<sup>11</sup> In present study, of total 147 subjects, normal, borderline deficiency, deficiency and severe deficiency of vitamin B<sub>12</sub> was noted in 20 (13.6%), 27 (18.3%), 75 (51.02%) and 25 (17.01%) respectively (P=0.0001) (table 3). Borderline, deficiency and severe deficiency vitamin B<sub>12</sub> deficiency was noted in 127 (86.39%). These findings are in agreement with previous

studies.<sup>3,9-12</sup> A previous study<sup>3</sup> reported 72.6% vitamin B<sub>12</sub> deficiency in their small scale study comprising 95 participant's only.<sup>3</sup> Vitamin B<sub>12</sub> deficiency of 86.39% of present study is in agreement with a previous Pakistani study,<sup>4</sup> as they reported deficiency of 78.5% and 85% in non-vegans and vegans respectively. Nizamani et al<sup>1</sup> evaluated 113 subjects and vitamin B<sub>12</sub> deficiency was reported in 65% of their study subjects. These findings disagree the 86.39% of present study.

The reasons could be different social class, small sample size, and research bias. However, frequency of 86.39% vitamin B<sub>12</sub> deficiency of present study is supported by other studies.<sup>3-11-13</sup> A previous study<sup>6</sup> reported frequency of 76% vitamin B<sub>12</sub> deficiency. These results are consistent with present study. However, this previous study<sup>6</sup> included a small sample size of 50 subjects that could be reason of inconsistent results. Evidence based findings of 86.39% vitamin B<sub>12</sub> deficiency indicates alarming situation of nutritional deficiencies. This frequency is also in agreement with a previous study.<sup>7</sup> A previous study<sup>9</sup> reported 76% frequency of vitamin B<sub>12</sub> deficiency in their study that included and studied 102 cases of in a hospital-based study. Another previous study<sup>8</sup> reported high frequency of vitamin B<sub>12</sub> deficiency of 85% that is consistent with the present study.<sup>8</sup>

The high frequency of vitamin B<sub>12</sub> deficiency of present study is in contrast to a study reported from Gilgit agency of Northern Pakistan. That previous study<sup>12</sup> reported very low frequency of 31.8% vitamin B<sub>12</sub> deficiency.<sup>12</sup> This is inconsistent to present and previous studies.<sup>7-10</sup> A previous study<sup>12</sup> reported the high frequency of vitamin B<sub>12</sub> deficiency due to the G. lamblia infestation that eats vitamin and casues malabsorption syndrome.<sup>12</sup> Previous studies<sup>15-19</sup> reported that the vitamin B<sub>12</sub> deficiency presents with different clinical symptoms such as the neuropsychiatric symptoms, and these may be present in the absence of red blood cell changes. Hence previous studies<sup>15,16</sup> recommended early screening of vitamin B<sub>12</sub> levels, because the late

complications are often irreversible,<sup>16</sup> this shows the gravity of the vitamin B<sub>12</sub> deficiency. Limitations of present study are first- small sample size, second- concomitant nutritional deficiencies of folate, iron, etc were not analyzed due to economical issues and third- complete blood counts and red blood cell indices were not observed concomitantly. However, the strength of study is evident from its prospective study design. From the evidence based findings of present study, it is concluded that the vitamin B<sub>12</sub> deficiency is highly prevalent in the common asymptomatic people who remain undiagnosed. This needs urgent large scale nationwide studies to be conducted.

### CONCLUSION

The present study reported frequency of 86.39% of vitamin B<sub>12</sub> deficiency. This shows the gravity of Vitamin B<sub>12</sub> deficiency problem that is prevalent in the community and remains undiagnosed. Early screening and vitamin B<sub>12</sub> supplements campaigns should be launched for prevention of long term irreversible complications. Further large scale studies are recommended.

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