Section: Biochemistry

Original Research Article

VITAMIN B12 LEVELS AND THEIR CORRELATION WITH HAEMATOLOGICAL PARAMETERS- A HOSPITAL BASED STUDY

Md Shahnawaz Alam[1], Aadhaar Singhal[2], Varun Kumar Kashyap[3], Sabina Khan[4], Sana Alam[1]

[1]Department of Biochemistry, Hamdard Institute of Medical Science and Research, New Delhi, India.
[2]MBBS student, Hamdard Institute of Medical Science and Research, New Delhi, India.
[3]Scientists-C, ICMR National Aids Research Institute, Bhosari Pune, India.
[4]Professor, Department of Pathology, Hamdard Institute of Medical Science and Research, New Delhi, India.

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Corresponding Author:
Dr. Sana Alam,
Email: sana2k2@gmail.com.

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Abstract

Background: Vitamin B12 also known as Cobalamin plays a vital role in hematopoietic, neurological and cardiovascular function. This study aims to look for correlation between Serum Vitamin B12 levels and haematological parameters such as Haemoglobin, Mean Corpuscular Volume(MCV), Platelet count and Total Leucocyte Count(TLC). A correlation of Vitamin B12 levels with dietary habits and gender has also been made. **Material & Methods:** Present study is a cross sectional observational study with a sample size of 606 adults of both genders. Statistical analysis has been performed using SPSS 21. **Results:** The study was carried out on 606 subjects, of these 257 were male and 349 were female. Mean value of vitamin B12 in males and female and their association was found to be non-significant. The difference between vitamin B12 levels in different age groups was found to be statistically significant p=0.005. The difference between vegetarian and non-vegetarian population was also found to be statistically significant p=0.045. Association between haematological parameters (Hb, TLC, MCV and platelets) with normal, deficient and excess vitamin B12 levels was not statistically significant (p>0.05). **Conclusion:** The findings of the present study showed that Vitamin B12 levels vary with age and dietary preferences. Correlation between Vitamin B12 levels and haematological parameters was not found to be statistically significant, although they were broadly in line with what is theoretically expected. This highlights the need for strong clinical suspicion to detect Vitamin B12 deficiency as it can present with varied multi systemic manifestations.

INTRODUCTION

Vitamin B12, often known as cobalamin, is a crucial vitamin that dissolves in water. It is essential for the process of DNA synthesis, cellular metabolism, and the preservation of the integrity of the nervous system. The exclusive source of vitamin B12 for humans is food derived from animals, such as meat, fish, and dairy products. The presence of a Vitamin B12 shortage can mimic other diseases, hence complicating the process of diagnosing the condition. It may manifest as megaloblastic anaemia, cognitive deterioration, peripheral nerve problems such as paraesthesia, and age-related macular degeneration.

Estimating the actual occurrence of cobalamin insufficiency is challenging because of the differences in criteria for inclusion and the specific laboratory methods used for analysis. Dietary inadequacy is the primary source of health issues in the vegetarian and vegan community. Additional factors contributing to this condition include malabsorption resulting from the lack of intrinsic factor in pernicious anaemia or as a result of stomach surgery, chronic gastritis, or exposure to nitrous oxide.

A significant quantity of vitamin B12 is stored in the liver, resulting in a delay of 5-10 years before clinical issues arise due to reduced intake or absorption of vitamin B12. Signs and symptoms of megaloblastic anaemia caused by vitamin B12 deficiency include fatigue, headache, palpitations, dyspnea, and potential neurological problems such dysesthesia and hypoesthesia. Subacute combined degeneration, characterised by ataxia, reduced proprioception, and vibratory feeling, may manifest

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in severe cases. Folate deficiency typically does not manifest with neurologic symptoms. An insufficiency of Vitamin B12 does not inevitably result in anaemia and macrocytosis.[29]

Megaloblastosis is a condition that affects various cells in the body, including non-hematopoietic cells like those found in the gastrointestinal tract and uterine cervix, which can exhibit megaloblastic characteristics. Megaloblastic anaemias have various causes, but a common underlying factor is the hindered synthesis of DNA. Cobalamin (vitamin B12 deficiency) and folate deficits are the primary causes of megaloblastosis. In cases with macrocytic anaemia, when the mean corpuscular volume (MCV) exceeds 100 femtoliters, a slight enlargement of red blood cells (macrocytosis) may indicate the initial stages of a megaloblastic process. However, due to the longer lifespan of red blood cells, there is a progressive change in the average size of red blood cells as they mix with older normal-sized red blood cells. The initial detectable alteration in the red cell indices is the elevation in red cell distribution width.[1]

Multiple hospital-based studies have been conducted to examine B12 deficiency in various countries. However, there is a scarcity of data from Indian research that have evaluated B12 deficiency using large sample sizes. Hence, we have undertaken this study to examine the extent of the occurrence of B12 deficiency and establish a connection with haematological observations such as Hb, TLC, MCV, and platelet counts in the semi-urban population of South Delhi who seek treatment at a specialised hospital.

MATERIALS AND METHODS

Type of study

This was a prospective cross sectional study

Methodology

This study is a cross-sectional observational study conducted at the Department of Biochemistry in HIMSR and HAHC Hospital, Jamia Hamdard. Approval was received from the Institutional Ethics Committee. The study cohort comprises individuals of both genders who are seeking medical treatment at HIMSR. Information regarding the levels of Vitamin B12, age, gender, and dietary history was collected. The study population excluded patients undergoing antimetabolite therapy, taking anti-convulsant medications, and using proton pump inhibitors (PPIs). Additionally, individuals with isolated occurrences of iron deficiency anaemia and persistent blood loss (such as from haemorrhoids or gastric ulcers) were also removed. The assessment of vitamin B-12 was conducted using the Abott i 1000 SR Roche electrochemiluminescence analyser. Normal serum vitamin B12 levels range from 200 to 900 pg/ml, whereas levels below 200 pg/ml indicate inadequacy. The haematological parameters of vitamin B-12 insufficiency were assessed using the sysmex (6 part) XN-1000 haematology analyser.

The statistical analysis was conducted using IBM SPSS 21, while data cleaning was carried out using Microsoft Excel. The data will be displayed as the average value plus or minus the standard deviation (SD). A p-value less than 0.05 will be deemed statistically significant at a 95% confidence interval. The relationship between haematological markers and Vitamin-B12 levels was assessed using Pearson's correlation. A p-value of < 0.05 was used as the threshold for statistical significance.

RESULTS

Socio Demographic Profile of Study Subjects

A total of 606 participants were included in the study, consisting of 257 males and 349 females (as shown in Table 1 and Figure 1). A total of 210 participants, accounting for 34.8% of the sample, were found to be deficient in vitamin B12. On the other hand, 289 subjects, representing 47.6% of the sample, had normal levels of vitamin B12. Additionally, 107 subjects, making up 17.7% of the sample, had excessive levels of vitamin B12. These findings are summarised in Table 2 and Figure 2.

Prevalence of vitamin B12 deficiency

The distribution of population by age, along with the number of individuals with deficiencies in each age group, is provided in Table 3 and Figure 3. The prevalence of vitamin B12 deficiency was markedly greater (39.92%) among individuals aged 31-50, in comparison to those under 30 years (20.93%) and beyond 50 years (9.3%).

A total of 210 patients were found to be deficient in vitamin B12. Out of them, 85 were male, accounting for 33% of the total, while 125 were female, accounting for 35.8% (as shown in table 4). The prevalence is higher in females, however the disparity is not statistically significant.

Out of the 606 subjects, 407 followed a vegetarian diet, and 198 of them were found to be deficient, which accounts for 48% of the vegetarian group. On the other hand, 199 subjects followed a non-vegetarian diet, and 52 of them were deficient, making up 26% of the non-vegetarian group (as shown in table 5). Vegetarians have a 22% greater incidence of deficiency. This suggests that nutrition is extremely important in maintaining adequate levels of vitamin B12, and it is necessary to have a strong clinical suspicion in such cases.

Association between vitamin B12 levels with different parameters

The mean concentration of vitamin B12 in males is 522.20 ± 166.48 (pg/ml), whereas in females it is 515.23 ± 160.73 (pg/ml). The statistical analysis revealed that there was no significant link between the two genders, as indicated by a p-value of 0.889. The average vitamin B12 level for persons under 30 is 361 ±166.17 (pg/ml), for adults between 30 and 50 it is 581.48±270.20 (pg/ml), and for individuals

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above 50 it is 595.98 ± 266.96 (pg/ml). The study identified a statistically significant disparity in vitamin B12 levels across different age groups, with a p-value of 0.005.

The mean vitamin B12 concentrations in individuals adhering to a vegetarian diet (n=407) were 325.7 ± 129.93 (pg/ml), whereas those adhering to a non-vegetarian diet (n=199) exhibited mean vitamin B12 concentrations of 448.7 ± 207 (pg/ml). There was a significant statistical distinction observed between persons who follow a vegetarian diet and those who do not, with a p-value of 0.045. The relationship between haematological indices (Hb, TLC, MCV, and platelets) and vitamin B12 levels, classified as normal, inadequate, and excessive, is presented in tables 9 and 10.

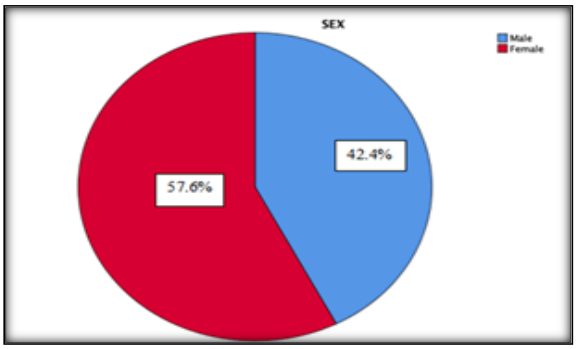

Figure 1: Sex wise distribution (%) of vitamin B12 groups in our study

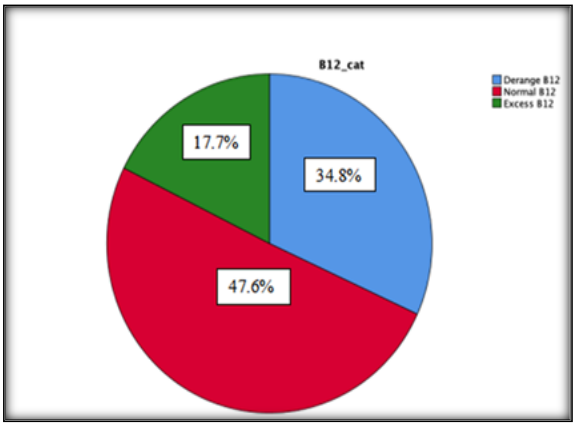

Figure 2: Prevalence of subjects with deficient, normal and excess level of vitamin B12

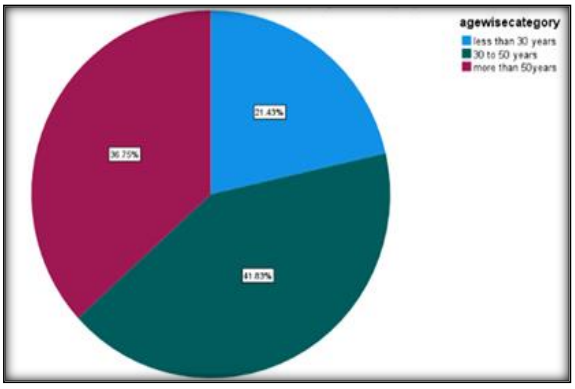

Figure 3: Age wise distribution of vitamin B 12 levels

Table 1: Age and sex distribution of study population

Gender	Vitamin B12 percentage (%)
Male	42.4%
Female	57.6%

Table 2: Prevalence of subjects with deficient, normal and excess level of vitamin B12

Categories	Vitamin B12 Percentage (%)
Deficient levels of vitamin B12	34.8%
Normal levels of vitamin B12	47.6 %
Excess levels of vitamin B 12	17.7%

Table 3: Age wise distribution of Vitamin B12 levels

Age – group	Total subjects percentage (%)	Vitamin B12 deficient subject (%)
<30 year	21.43 %	20.93 %
31 - 50 year	41.83 %	39.92 %
>50 year	36.75 %	9.3%

Table 4: Prevalence of Vitamin B12 deficiency based on gender

Gender	Total subjects	Vitamin B12 deficient subjects
Male	257	85
Female	347	125

Table 5: Prevalence of vitamin B12 deficiency according to dietary preference

Diet	Total Vitamin B12 subjects	Deficient Vitamin B12 subjects
Vegetarian	407	198 (48%)
Non-vegetarian	199	52 (26%)

Table 6: Levels of Vitamin B12 in males and females

Gender	Mean ± S.D	p-value
Male (257)	522.20±165.48	0.889
Female (347)	515.23±160.73	

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Table 7: Levels of Vitamin B12 in different age group

Age Group	Mean ± S.D	p-value
<30 year	361.93±166.17 (129)	0.005
30-50 year	581.48±270.20 (253)	
>50 year	595.18±266. 96 (224)	

Table 8: Levels of vitamin B12 in vegetarian and non-vegetarian

Diet	Mean ± S.D	p-value
Veg	325.7 ± 129 .93 (407)	0.045
Non-veg	448.7 ± 207 (199)	

Table 9: Analysis of haematological parameters in Vitamin B12 study subjects

Parameters	NORMAL (Mean ± S.D)	Deficient (Mean ± S.D)	Excess (Mean ± S.D)	p-value
Hb	10.04 ± 2.96	10 ± 2.99	10.1 ± 3.1	0.946
TLC	9.58 ± 1.36	8.23 ± 6.35	7.6 ± 4.1	0.504
MCV	93.9 ± 54.4	93.7 ± 19.2	91.1 ± 16.2	0.820
Platelets	192.02 ± 10.3	201.4 ± 115.05	194.9 ± 104.4	0.203

Table 10: Correlation of haematological parameters with vitamin B12 levels

Parameters	r-value	p-value
Hb	0.020	0.622
TLC	-0.029	0.481
MCV	-0.028	0.496
Platelets	-0.069	0.091

DISCUSSION

Vitamin B12 is classified as an indispensable vitamin. Despite its ability to be stored for up to 5 years, deficiencies in B12 are not prevalent due to insufficient dietary consumption and/or impaired absorption. A significant proportion of cases of vitamin B12 insufficiency go unnoticed and are not recognised. At the initial phase, vitamin B12 deficiency may manifest with inconspicuous deficits. Therefore, early identification is crucial in order to prevent permanent harm. The study was conducted on a sample of 606 individuals, and their vitamin B12 levels were evaluated. The current investigation revealed a prevalence rate of 34.8% for B12. The findings of our study were consistent with the results of previous research conducted by Puneeta et al from Karnataka and Rohit et al from Jaipur, which reported a prevalence rate of 33.9% and 36.5% respectively.[2,3]

Various investigations have yielded inconsistent findings about the incidence of vitamin B12 insufficiency.[4] The observed differences may be attributed to the dietary practises prevalent in various regions of India. Malabsorptive conditions, such as Tropical sprue, Giardiasis, gastrointestinal infections, Autoimmune gastritis, and Gastric operations, are among the reasons that contribute to B12 deficiency.

The vegetarian diet also played a role in the development of B12 insufficiency. It is evident that following a vegetarian diet significantly increases the chance of acquiring B12 deficiency, with rates of 48% and 26% observed in individuals following vegetarian and non-vegetarian diets, respectively. This is in accordance with multiple research.[5,6]

The average vitamin B12 levels in persons following a vegetarian diet (n=407) were 325.7 ± 129.93 (pg/ml), while individuals following a non-vegetarian diet (n=199) had average vitamin B12 levels of 448.7 ± 207.4 (pg/ml). The observed discrepancy was found to be statistically significant. In their study, Singh et al. found that 107 individuals (58.2%) were vegetarians, while 77 individuals (41.8%) were non-vegetarians, yielding comparable results.[6]

A study conducted in West Bengal revealed that 79% of the population, including non-vegetarians, experienced vitamin B12 insufficiency. The variations observed in different regions may be attributed to diverse dietary practices influenced by cultural, religious, and geographical distinctions.[7]

The study population was categorised into three age groups: those under 30 years, those between 31 and 50 years, and those over 50 years. Our investigation revealed that B12 deficiency was more prevalent among those in the middle age group, however, no particular age range could be determined. In the medium age range (31-50), the prevalence of vitamin B12 insufficiency was 39%. In the age group of over 50 years, 9.3% of the population was deficient in B12. In the younger age group of under 30 years, 20.93% of individuals were deficient in B12.

The findings of our study were consistent with earlier research, which also demonstrated a higher occurrence of B12 insufficiency in this particular age demographic.[8]

Upon comparing the average levels of vitamin B12 in three different age groups, we observed the following results: individuals under the age of 30 (group 1) had an average vitamin B12 level of 361.93 ± 166.17 pg/ml, individuals between the ages of 30 and 50 (group 2) had an average vitamin

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B12 level of 581.48 ± 270.20 pg/ml, and individuals over the age of 50 (group 3) had an average vitamin B12 level of 595.18 ± 266.96 pg/ml. There was a notable disparity observed when comparing group 1 with group 2 and group 1 with group 3. However, no statistically significant difference was found when comparing the middle age group with the elder age group. Several studies have demonstrated a notable frequency of vitamin B12 insufficiency across different age groups.[9] Research has indicated that the primary reason for b12 shortage at one age is likely the inadequate absorption of vitamin B12 from meals. However, the precise magnitude of this issue remains uncertain. Furthermore, the concentration of vitamin B12 declines as individuals age, resulting in an increased prevalence of vitamin B12 deficiency as they get older. Research has indicated that the occurrence of vitamin B12 deficiency in older individuals might vary from 5% to 40%, depending on the specific criteria employed to define vitamin B12 deficiency.[10] The lower incidence of b12 insufficiency among the elderly within our study group, in comparison to the middle-aged and younger age groups, is likely due to the limited size of our sample. Furthermore, it has been shown that if the study sample consists of healthier elderly individuals in comparison to older individuals with underlying health conditions, the prevalence of b12 insufficiency may be lower. In addition, we conducted a comparison of haematological parameters across the three groups: B12 deficiency, B12 NORMAL, and B12 Excess groups. There was no discernible disparity in the haematological measures. The levels of haemoglobin (Hb), total leukocyte count (TLC), mean corpuscular volume (MCV), and platelets were compared across the three groups.

The study revealed a drop in haemoglobin (Hb) levels in the group with vitamin B12 deficiency. However, when comparing the average Hb levels between the group with normal B12 levels and the group with excessive B12 levels, there was no statistically significant difference. In addition, there was a slight positive connection between Hb levels and b12 levels ($r = 0.020$, $p = 0.622$). Prior research has demonstrated that a significant deficiency in vitamin B12 might result in either extremely moderate or no anaemia. Research has indicated that not all patients exhibit the same level of anaemia for each degree of B12 deficiency. Patients with severe deficiencies may exhibit mild anaemia or perhaps be completely devoid of it. The cause for such diminished haematological reaction is often ambiguous.[11] A study conducted by Shobha et al. found no significant association between haemoglobin (Hb), mean corpuscular volume (MCV), and plasma vitamin B12 levels.[12]

The MCV values were within the normal range in all three groups, namely the B12 deficient, B12 normal, and B12 excess groups. We noticed a slight negative connection between MCV and B12 levels in our study. The results were determined to be statistically insignificant. The correlation coefficient is -0.028 and the p-value is 0.496. Our findings exhibited a negative connection, consistent with a limited number of earlier research.[13]

Individuals suffering from vitamin B12 deficiency exhibit elevated mean corpuscular volume (MCV) levels. This may be attributed to either concurrent iron shortage or chronic disease-related anaemia. Hence, when MCV readings increase to higher levels that are still within the normal reference range, they must be taken into account when assessing b12 levels [93.94]. The findings of our study were consistent with previous results, as they provided evidence that presentation of symptoms can be sudden in cases of vitamin B12 deficiency. Additionally, it is important to note that having normal levels of haemoglobin (Hb) and mean corpuscular volume (MCV) does not always rule out the possibility of a B12 deficit. In addition, we conducted a comparison of the average TLC levels across three groups: those with B12 deficiency, those with normal B12 levels, and those with excessive B12 levels.

A weak negative connection was seen between TLC levels and the B12 deficient group ($r = -0.029$, $p = 0.481$). Furthermore, the platelet count exhibited a negative connection with a correlation coefficient of -0.069 and a p-value of 0.091. Neither of these outcomes exhibited statistical significance. Our findings contradicted previous studies that demonstrated a favourable connection between the count of white blood cells and platelets with levels of vitamin B12.[13] The discrepancy may be attributed to the variation in the sample group. Our study only examined individuals who were relatively healthy, whereas the aforementioned study exclusively included patients with a vitamin B12 deficiency. It is crucial to conduct extensive multicentric investigations in order to establish a robust correlation between haematological parameters and vitamin B12 levels. This will enable the timely initiation of suitable B12 therapy to prevent the advancement of irreversible brain damage.

CONCLUSION

The frequency of Vitamin B12 insufficiency was 34%. The deficit exhibited a greater prevalence among adults aged 31-50 and was determined to have a statistically significant association. The disparity in average vitamin B12 levels between vegetarian and non-vegetarian individuals was also observed to be statistically significant, with a higher occurrence in vegetarian patients. While the statistical significance was not observed, there was a positive association between Hb and Vitamin B12 levels. Conversely, TLC exhibited a negative correlation, as did MCV. Additionally, platelet levels displayed a strong negative correlation with Vitamin B12 levels. This study indicates that the

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occurrence of Vitamin B12 insufficiency is greater in urban populations than previously assumed. Additionally, it emphasises the dietary foundation for the insufficiency and the specific age group (reproductive) that it predominantly impacts. Although the associations with haematological measures were not statistically significant, they generally aligned with the expected theoretical outcomes.

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