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A Study on Vitamin B12 Deficiency and Its Haematological Manifestations

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Abstract

Background: Identify the incidence of vitamin B12 insufficiency in those who have anaemia and a high mean corpuscular volume. **Materials and Methods:** During the nine-month period from April to December 2020, a cross-sectional study was carried out in a private tertiary care hospital. Based on inclusion and exclusion criteria, 200 patients' blood samples were chosen for the study. Subjects with severe anaemia (Haemoglobin 7 g/dl and mean corpuscular volume 96 fl) in any age group and sex were included. On a Sysmex KX 21 haematology analyzer, blood samples were analysed. On a peripheral blood smear, the morphology of the blood cells was assessed. Tests on serum vitamin B12 were conducted. Utilizing statistical techniques, both qualitative and quantitative characteristics were examined. **Results:** There were 200 participants in the study population. 78 (39%) of them were men, and 122 (61%) were women. The majority of study participants had an average age of 48.8 ± 20.2. A total of 132 individuals (66%) were found to have vitamin B12 deficiency, with 32 (16%) showing borderline, 56 (28%) deficient, and 44 (22%) severely deficient levels. 68 (34%) people had vitamin B12 levels that were normal. Red blood cell indices, such as MCV, MCH, and MCHC, are divided into groups based on the levels of serum vitamin B12. The severely deficient group has the highest mean corpuscular volume (MCV), 142 fl, and mean corpuscular haemoglobin (MCH), 52.2 pg. Vitamin B12 levels and mean corpuscular volume were shown to be significantly inversely correlated ($r = -0.218$, $p = 0.012$). **Conclusion:** In India, vitamin B12 deficiency is not unusual. It is frequently identified while performing tests for a haematological problem or neurological symptoms. The supposed diagnosis of macrocytic anaemia, which is linked to vitamin B12 deficiency, cannot be reliably made using MCV as a screening criterion.

Keywords: Vitamin B 12 deficiency, prevalence, Megaloblastic anaemia, Pancytopenia, Mean corpuscular volume.

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Introduction

Even though cobalamin (vitamin B12) deficiency has been known about for more than a century, it can still be challenging to diagnose and treat. The effects of a vitamin B12 shortage might range from neurologic to mental symptoms. Many people who don't get enough vitamin B12 may exhibit the typical megaloblastic anaemia. In clinical practise, many instances of vitamin B12 insufficiency go unnoticed or are even incorrectly diagnosed.[1]

The wide range of illnesses that can result from a vitamin B12 deficiency, with diagnoses made on the basis of either low serum B12 levels, elevated biomarkers like methylmalonic acid and/or homocysteine, or the improvement of clinical symptoms following the initiation of parenteral vitamin B12 therapy.[2,3,4]

Many generations of clinicians have been trained with the belief that vitamin B12 insufficiency only manifests itself with megaloblastic anaemia because this is the classic

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presentation of Addison-Biermer disease. There have been more cases recorded in which the primary presenting symptom was a neurologic abnormality, with subacute combined degeneration of the spinal cord being one of the most dreaded forms and frequently resulting in lifelong impairment. A large series of 40 patients with neurologic symptoms or psychiatric illnesses brought on by vitamin B12 insufficiency but no anaemia or macrocytosis was described by Lindenbaum et al.[2] From depression to mania, psychosis, and perhaps suicidal thoughts, psychiatric symptoms can range widely. It is still unclear why some patients primarily appear with megaloblastic anaemia whereas others come with neurologic symptoms. The cornerstone of diagnosis relies on laboratory tests that show low serum B12 levels and high levels of methylmalonic acid (MMA), although normal serum B12 and MMA levels do not rule out symptomatic B12 deficiency. Because of misunderstandings and false assumptions among medical professionals, many cases of B12 deficiency are missed in clinical practise and occasionally even misdiagnosed. For the synthesis of DNA and the growth of haematopoietic cells in the bone marrow, gastrointestinal cells, epithelial cells, cervico-vaginal cells, and testicular germ cells, vitamin B12 is a crucial micronutrient.[5]

Vitamin B12 assimilation Animal proteins are the main source of the vitamin B12 in the body. Vitamin B12's release from animal sources is the first step in its metabolism, and this process is governed by the actions of pepsin and stomach acid. The R-protein released by the salivary glands is then bound by dietary vitamin B12 following release. The R- protein is hydrolyzed to liberate vitamin B12 in the duodenum in the presence of alkaline medium and pancreatic proteases, where it then binds with the intrinsic factor (IF) released by the stomach parietal cells. It is very difficult to break down the vitamin B12 -IF complex through proteolysis.[6]

The complex binds to its particular receptors on the mucosa of the terminal ileum, which is also where it is absorbed. Calcium mediates this stage of vitamin B12 absorption. After IF breakdown, the intracellular vitamin B12 is released. This free vitamin B12 binds to transcobalamin-II (TC-II), another protein carrier, and is subsequently released into the bloodstream. The liver, bone marrow, and other essential bodily cells then aggressively absorb this vitamin B12-TC-II complex, also known as holo TC-II. Up to 90% of the body's total vitamin B12 is mostly stored in the liver.[7] Clinical or biochemical vitamin B12 shortage will result from a disruption in any of the aforementioned stages that have been discussed. This includes inadequate food intake, particularly among vegetarians and alcoholics, and malabsorption caused by a number of illnesses and medications, such as metformin and proton pump inhibitors, chronic atrophic gastritis, pernicious anaemia, celiac disease, and chronic pancreatitis (PPIs).[8,9] 5 to 30 µg of vitamin B12 must be consumed daily, and only 1 to 5 µg are actually absorbed. 2000–5000 µg of B12 are stored in one liver and last for three to five years. In India, nutritional cobalamin insufficiency is widespread. This can be the result of an improper diet or malabsorptive conditions. Additionally, chronic gastritis, H. Pylori infection, blind loop syndrome, transcobalamin II deficiency, and fish tape worm infestation are causes of vitamin B12 deficiency. Multiple symptoms of vitamin B12 insufficiency might appear, ranging from haematological manifestations to brain disorders.[10] There are also recognised manifestations affecting the skeletal, cutaneous, and cardiac systems. Megaloblastic anaemia is the hematologic sign of B12 deficiency that is most frequently observed. In clinical practise, megaloblastic anaemia due to B12 insufficiency is frequently seen, yet it is still underdiagnosed. In our outpatient department, we saw numerous people with severe anaemia and increased MCV. Pancytopenia was prevalent among them. Subsequent testing revealed that these people were vitamin B12 deficient. This led us to conduct a study in our tertiary care hospital setup to determine the incidence of vitamin B12 insufficiency in people with severe anaemia and increased MCV.[11]

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Physiological roles of vitamin B12

The function of vitamin B12 in our bodies can be used to explain how these indicators may show cobalamin insufficiency. In two enzymatic processes, vitamin B12 is a crucial cofactor. Its lack will prevent these enzymes from working properly and cause the substrate to build up. One of the vitamin B12-dependent enzymes, methylmalonylecoenzyme A (CoA) mutase, catalyses the isomerization of methylmalonyl-CoA to succinyl-CoA. When function is compromised, elevated levels of methylmalonyl-CoA and consequently elevated levels of MMA will result from the cleavage of CoA.[4] A co-factor called vitamin B12 makes it easier for homocysteine to be converted to methionine, which is then activated into S-adenosyl-methionine and gives its methyl group to methyl acceptors such myelin, neurotransmitters, and membrane phospholipids. Methionine synthase is the other vitamin B12-dependent enzyme; it converts homocysteine into methionine and as a result of reduced activity brought on by vitamin B12 shortage, homocysteine will build up in the body.[12] This process explains how low tissue levels of cobalamin will raise levels of MMA and homocysteine, and the National Health and Nutrition Examination Survey population has shown a clear correlation between serum vitamin B12, MMA, and homocysteine.[7] However, it is unknown whether high MMA and/or homocysteine levels in patients with symptoms of vitamin B12 deficiency are sensitive or specific. Additionally, it has been shown that individuals with severely compromised renal function have increased MMA levels.Similar to low folate or vitamin B6, poor renal function, hypothyroidism, and some medications, high homocysteine levels can also result from these conditions. Because of this, a metabolically significant vitamin B12 deficit will cause the methylation process to be disrupted and will cause an accumulation of intracellular and serum homocysteine. Neurones and the vascular endothelium have been found to be potentially harmed by hyperhomocysteinemia.[12] The transformation of dietary folate (methyl-tetrahydrofolate) into its active metabolic form, tetrahydrofolate, depends on this process as well. The co-factor vitamin B12 promotes the transformation of methylmalonyl coenzyme A (CoA) to succinyl-CoA in another crucial enzymatic pathway. This conversion route is impaired in vitamin B12 deficiency, which leads to an increase in serum methylmalonic acid (MMA). The fatty acid production of the neuronal membranes then deviates from what it should. Monoamines, or neurotransmitters like serotonin and dopamine, are synthesised with the help of vitamin B12. Vitamin B12 deficiency impairs this production. The resulting neurocognitive or psychiatric signs of vitamin B12 insufficiency are explained by all of the aforementioned factors taken together. Vitamin B12 deficiency-induced neuronal damage is characterised by axonal demyelination, degeneration, and eventually death. These symptoms include severe peripheral or autonomic neuropathy, subacute mixed spinal cord degeneration, delirium, and dementia. Because of the cellular and vasculo-toxic effects of hyperhomocysteinemia, there is compelling evidence that it is also linked to a higher risk of cardiovascular events. The clinical value of holoTC measurement as a B12 vitamin status assessment has come to light in recent years. The physiologically active form of vitamin B12 in plasma is called holoTc. According to some research, holoTC is more accurate at making diagnoses than total serum vitamin B12 levels, and reference values are highly dependent on the assay technique. Patients with holoTC levels between 23 and 75 pmol/L may need additional MMA testing to confirm or rule out a real vitamin B12 deficiency.[13,14] Moreover, 63% of individuals had holoTc levels below the threshold (300 nmol/L). This problem suggests that MMA is a poor indication of vitamin B12 insufficiency and calls into question whether holoTC testing is actually preferable than measurement of total serum vitamin B12 + MMA for detecting vitamin B12 deficiency41. In fact, serum vitamin B12 and holoTC levels were only marginally predictive of aberrant MMA levels in a different investigation.[15,16,17]

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Material and Methods

During the nine-month period from April to December 2020, a cross-sectional study was carried out in a private tertiary care hospital. Based on inclusion and exclusion criteria, 200 patients' blood samples were chosen for the study. Subjects with severe anaemia (Haemoglobin 7 g/dl and mean corpuscular volume 96 fl) in any age group and sex were included. Blood samples were obtained by venepuncture of the anti-cubital vein while patients receiving antimetabolite treatment, anticonvulsants, and proton pump inhibitor drug use were excluded. Serum vitamin B12 levels were measured in picograms, and the WHO Scientific Group on Nutritional Anemia states that the normal value range for serum vitamin B12 level is between 210 and 900 pg/ml (Organization, 1968, 2009). The Bekman Coulter Access-2 fully automated immunoassay was used to determine vitamin B12 using the immunometric assay method (competitive principle) (Fernandez, Wang, Chao, & Guignon, 1990). A fully automated, five-part haematology analyzer was used to perform the whole blood count (Mindray BC -5150). Using the Sysmex Kx21 haematology analyzer, complete blood counts, a differential count, and red blood cell indices were estimated. For each patient, blood smears were obtained, stained with giemsa stain, and examined for macrocytosis and hypersegmented neutrophils in the red blood cells. Based on symptomatic manifestations, serum vitamin B 12 levels were tested and divided into four groups: Group I: Normal > 240 pg/ml, Group II: Borderline 170- 240 pg/ml, Group III: Deficient 170 pg/ml, and Group IV: Severe deficiency 100 pg/m. Clinical examinations were performed on the subjects, and a thorough history was obtained.

Statistical Analysis: Calculated descriptive statistics include frequencies and proportions. Chi-square test was used to compare pancytopenia and a B12 deficiency. Pearson's correlation test was used to determine the relationship between serum vitamin B12 levels and other indicators, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leukocyte counts, and platelet counts. P value less than 0.05 was deemed significant.

Results

There were 200 participants in the study population. 78 (39%) of them were men, and 122 (61%) were women. The majority of study participants had an average age of 48.8 ± 20.2.

A total of 132 individuals (66%) were found to have vitamin B12 deficiency, with 32 (16%) showing borderline, 56 (28%) deficient, and 44 (22%) severely deficient levels. 68 (34%) people had vitamin B12 levels that were normal. Red blood cell indices, such as MCV, MCH, and MCHC, are divided into groups based on the levels of serum vitamin B12.

The severely deficient group has the highest mean corpuscular volume (MCV), 142 fl, and mean corpuscular haemoglobin (MCH), 52.2 pg. Vitamin B12 levels and mean corpuscular volume were shown to be significantly inversely correlated (r = -0.218, p = 0.012).

CBP	**Correlation coefficient (r-value)**	**p-value**
MCV	-0.218	0.012
MCH	-0.176	0.043
MCHC	-0.104	0.211
TLC	0.566	<0.001
Platelets	0.763	<0.001

Even though the correlation was statistically insignificant, it was discovered that mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were negatively associated. The amount of platelets and vitamin B12 were discovered to be positively correlated. It was determined that this link was statistically significant (p= 0.001).

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Additionally, it was discovered that leukocyte counts and vitamin B12 had a strong positive link with a p value of 0.001. The majority of B12-deficient people (68%), who had pancytopenia, were determined to be statistically significant (p = 0.001).
On evaluation of peripheral blood films, 112 smears (56%) showed macrocytes, while 120 smears (60%) showed hypersegmented neutrophils.

Table 1: Gender distribution in study population (n = 200)

Gender	No. of Cases	Cases (%)
Male	78	39.0%
Female	122	61.0%
Total	200	100.0%

Out of the 200 subjects, therer was 78 (39%) males and 122 (61%) females.

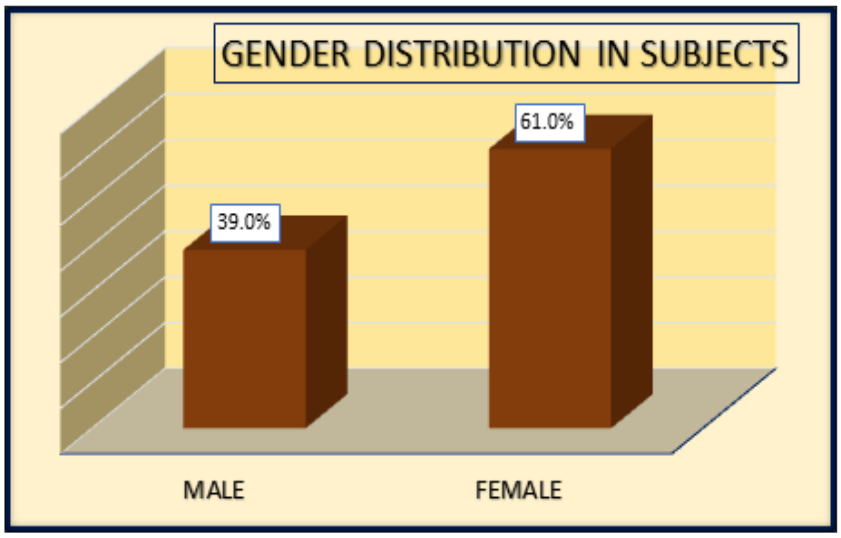

Figure 1: Gender distribution in the study subjects

Table 2: Vitamin B12 levels measured in study population (n = 200)

Vitamin B 12 Levels	No.	Frequency	Mean	Std. Dev
Normal (> 240pg/ml)	68	34.0%	644.22	512.2
Borderline deficiency (170-240 pg/ml)	32	16.0%	190.33	24.22
Vitamin B12 deficiency (< 170pg/ml)	56	28.0%	124.82	20.98
Severe Vit.B12 deficiency (<100pg/ml)	44	22.0%	54.2	23.86
Total	200	100.0%		

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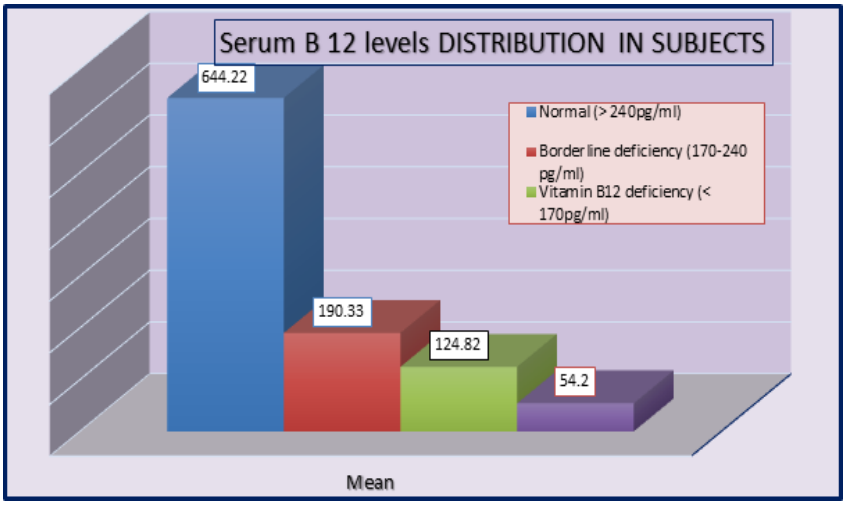

Figure 2: Serum B 12 levels Distribution in Subjects

Table 3: Red Blood cell indices in different groups

Vitamin B 12 levels	MCV	MCH	MCHC
Normal (> 240pg/ml)	102.28 ± 10.8	32.82 ± 4.4	30.20 ± 2.80
Borderline deficiency (170-240 pg/ml)	108.48 ± 9.10	33.60 ±4.02	30.28 ± 2.4
Vitamin B12 deficiency (< 170pg/ml)	107.22 ± 12.0	31.2 ± 5.8	30.0 ±2.88
Severe Vit.B12 deficiency (<100pg/ml)	110.8 ± 10.12	33.20 ± 3.8	31.10 ±2.88

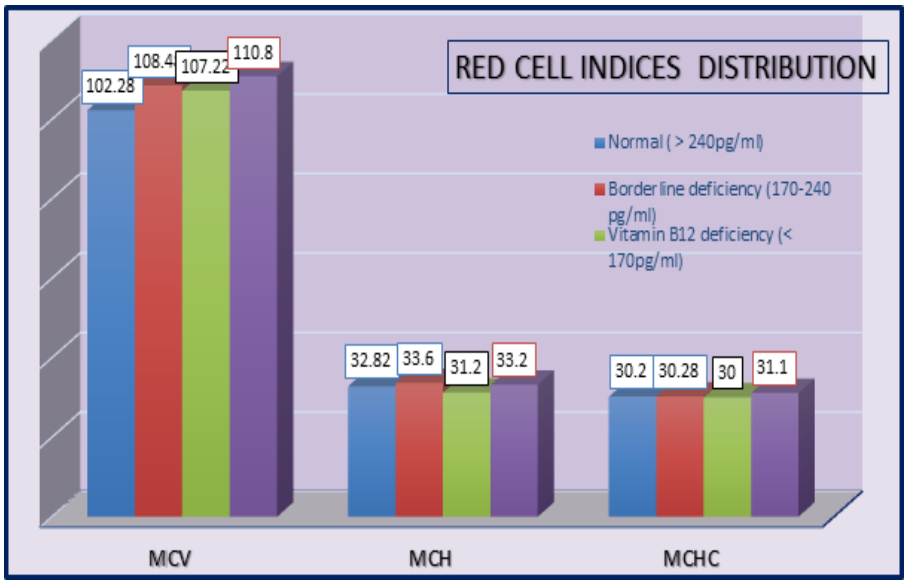

Figure 3: Red Cell Indices Distribution in Subjects

Table 3: CBP features in the study group

Parameters	Mean± SD
Hb (g/dl)	5.2 ± 2.8
Total leukocyte count(cells/cmm)	4.8 ± 2.2
Platelet count (per cmm)	72 ± 28

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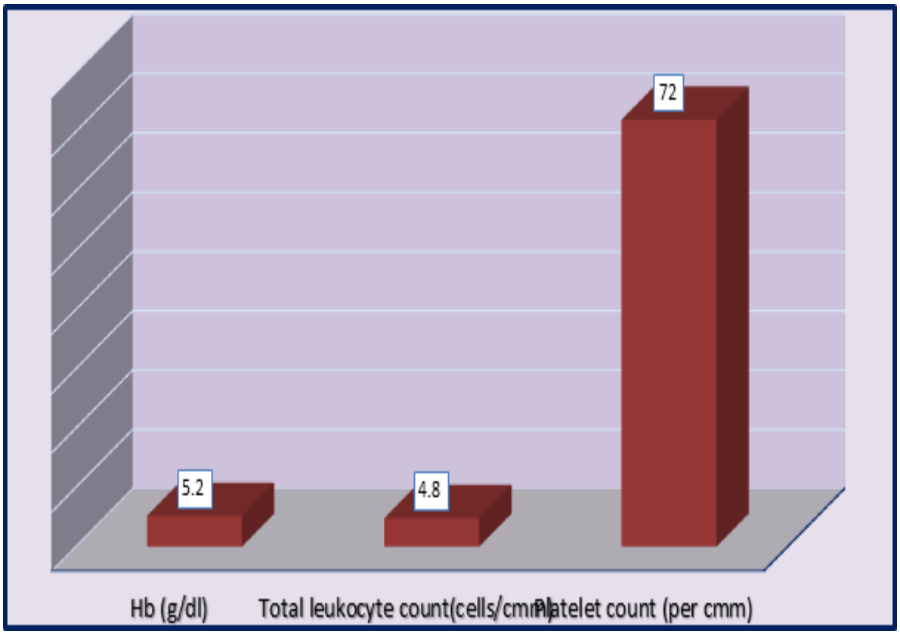

Figure 4: Red Cell Indices Distribution in Subjects

Table 4: Pearson's correlation of the study parameters with serum Vitamin B 12 (n =200)

CBP	**Correlation coefficient (r-value)**	**p-value**
MCV	-0.218	0.012
MCH	-0.176	0.043
MCHC	-0.104	0.211
TLC	0.566	<0.001
Platelets	0.763	<0.001

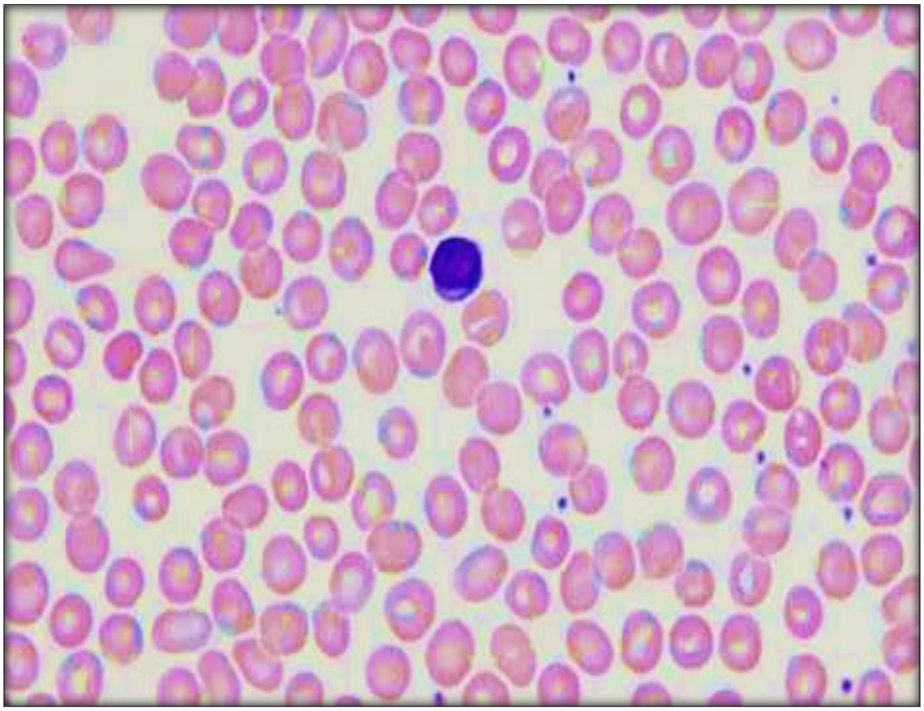

Figure 5: Dimorphic picture of RBCs in B12 deficiency

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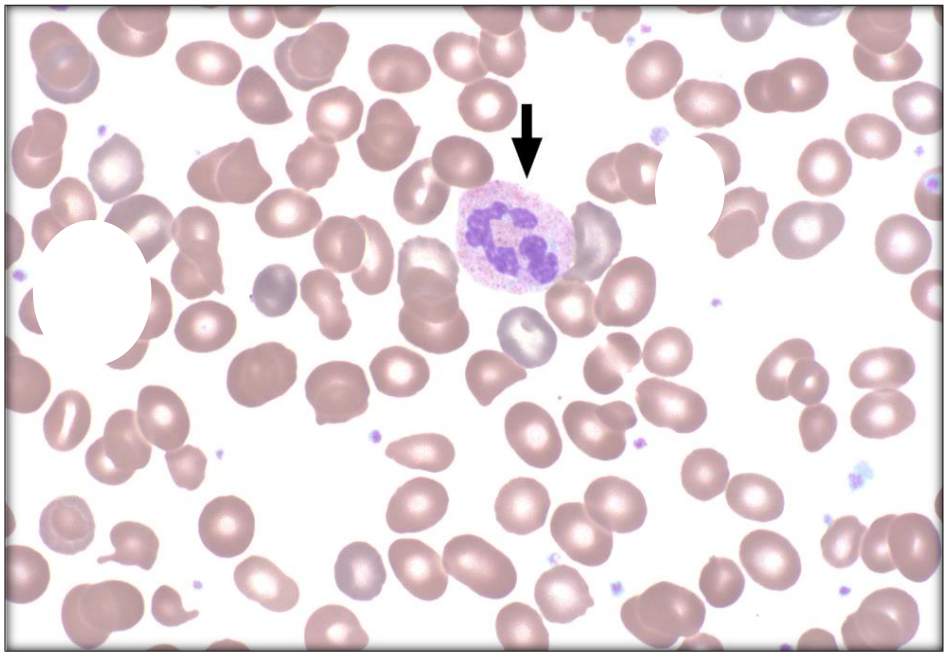

Figure 6: Macro ovalocytes & dimorphic RBCs scattered in peripheral blood smear

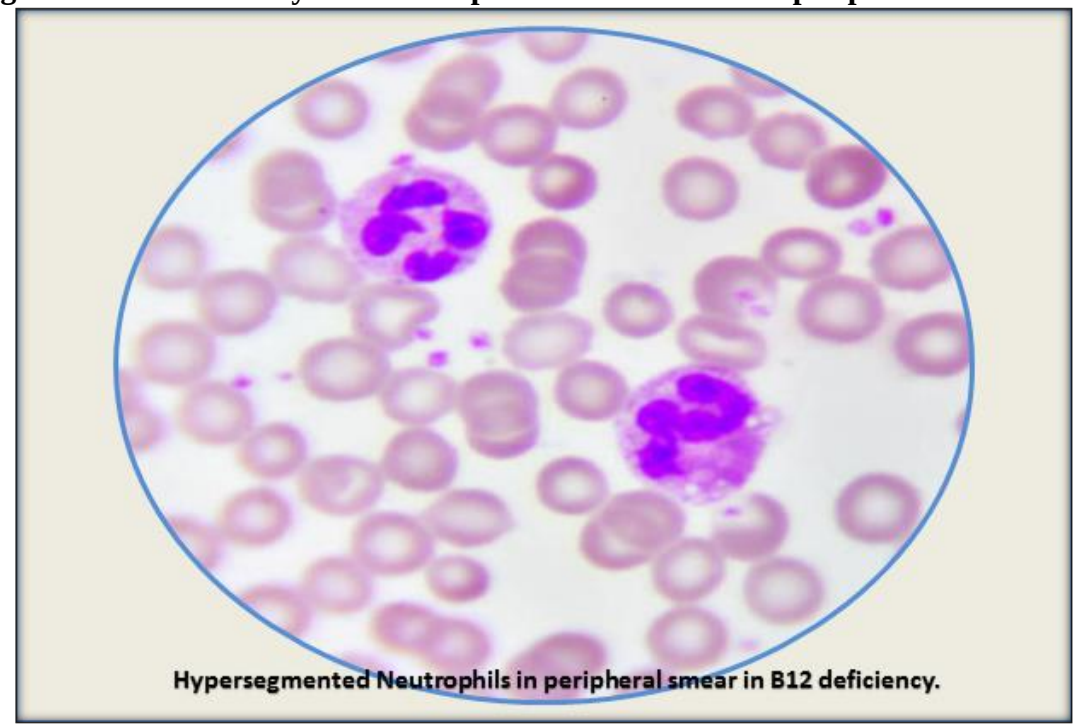

Figure 7: Hyper segmented Neutrophils in peripheral smear in B12 deficiency

Discussion

Clinical signs of vitamin B12 insufficiency include multisystem disorders. It frequently manifests neurological problems and megaloblastic anaemia haematologically. Megaloblastic anaemia is primarily brought on by folate deficiency in India, although new research has highlighted the significance of vitamin B12 insufficiency.

According to studies by Khanduri et al, subnormal levels of B12 alone or a combined folate-B12 insufficiency affect 46.9% of non-anemic adult participants. This occurs more frequently than only a folate deficit does.[18]

Other clinical characteristics include fatigue, psychological symptoms like sadness, and macrocytic anaemia, as well as neurologic symptoms like paresthesia in the hands and feet,

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muscle cramps, dizziness, cognitive problems, ataxia, and erectile dysfunction. Less than 20% of those with demonstrably low serum vitamin B12 levels in both China and the West, it appears, also have macrocytic anaemia.

The presence of low serum vitamin B12 levels is regarded as diagnostic, however there is little connection between these levels and symptoms—even those with levels below 140 pmol/L of vitamin B12 may not experience any. This aspect casts new light on the debate over suitable cutoff values for serum vitamin B12 and associated measures. According to some researchers, it is required to define various reference cutoffs depending on the age group and the chosen analytic technique. The fact that many persons with symptoms of cobalamin insufficiency may have serum vitamin B12 levels above the lower reference threshold of 140 pmol/L, however, means that serum vitamin B12 tests also run the risk of failing. The usage of oral multivitamin supplements or high-dose oral vitamin B12 preparations in the past may have contributed to this problem in many instances, notwithstanding the possibility of other causes.

Even a dose of 10 mg/d has been shown to raise vitamin B12 levels in older people (those over 65) to more than 200 pmol/L. Unless very large dosages (1000–2000 mg/d) of vitamin B12 are utilised, oral supplementation may raise the serum vitamin B12 level but frequently not enough to refill the vitamin B12 levels in the tissues. Table 1's summary of the study's findings indicates that 66% of participants had vitamin B12 deficiencies. Khanduri & Sharma (65%) reported findings that were similar.[18] 72.6% of the study group had vitamin B12 deficiencies, according to Ahmed et al.[19] In their research, Sarode et al.[21] Kaushik Sen et al,[22] and Hashim et al.[23] similarly discovered a prevalence of 76% for B 12 deficiency. A prevalence of 57.5% was found in a different hospital-based study conducted at a Tertiary care hospital in Sindh by Gulam et al.[20] According to research carried out in Delhi by Garewal et al,[24] and Premkumar et al.,[25] the prevalence has climbed to 88% and 81%, respectively. On the other hand, the Gilgit Agency of Pakistan, Puneeta et al,[26] of Karnataka, and Rohit et al,[27] of Jaipur found low prevalences of Vitamin B12 insufficiency as 31.8%, 33.9%, and 36.5%, respectively. These variances in the research may be the result of dietary habits followed for a variety of cultural, religious, and geographic reasons. The majority of people in India practise vegetarianism. The intake of nonvegetarian cuisine is observed to be modest, even among nonvegetarians. According to population-based research conducted in West Bengal and Karachi, 79% and 85% of non-vegetarians, respectively, were found to be vitamin B12 deficient. Therefore, it needs to be stressed that B12 insufficiency affects non-vegetarians as well. Malabsorptive conditions like Tropical sprue, Giardiasis, gastrointestinal infections caused by H. pylori, elderly gastric atrophy, autoimmune gastritis, gastric surgeries, and nitrous oxide anaesthesia during surgery, and drug-induced deficiencies from antimetabolites, anti-convulsants, Metformins, and proton pump inhibitors are other factors that contribute to B12 deficiency. According to investigations by Summer et al,[28] and other studies, the incidence of B 12 deficiency is 1-2% in the younger population and 10–15% in the elderly. In contrast, the bulk of participants in Kaushik Sen et al,[22] studies' are in their 30s. 52 of the study subjects in our investigation were younger people. 48 of who had low serum levels of vitamin B 12. We have discovered an intriguing example of Imerslund Grasbeck disease in a young female patient who has congenital megaloblastic anaemia. This condition is a hereditary defect in the ileum's ability to transport the R-Cobalamin complex, which leads to selective cobalamin malabsorption.

Proteinuria is typically present in addition to it. Families from Finland and Norway have been found to have mutations in the cubilin and amnionless genes on chromosomes 10 and 14, respectively. In the Mediterranean region, a few additional mutations have also been described; nonetheless, this case of congenital megaloblastic anaemia is rare in our area of the world. In their analysis of red cell macrocytosis, Pappo et al,[29] in a paediatric centre

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discovered no cases of cobalamin deficiency; nevertheless, one case has been described in our study.

In our analysis, there was a little male preponderance in terms of sex incidence. There were 122 (61%) ladies and 78 (39%) males among the 200 participants. This is consistent with the majority of females found in earlier studies.[22,24,30]

In our study, there were 79.4% (27) of childbearing women (18–35 years) who did not have enough vitamin B12, compared to 39% (18) in Puneeta et al study.[26]

Because low maternal vitamin B12 status is linked to an increased risk for neural tube abnormalities, intrauterine development retardation, and low birth weight, a low level of vitamin B12 in the reproductive age group is cause for concern. Prior to becoming pregnant, a woman should maintain a serum B 12 value of 300 pg/ml. Serum vitamin B 12 levels often decline during pregnancy due to physiological changes. India's majority population practises vegetarianism for cultural and religious reasons. However, this diet may be lacking in some nutrients. A vegetarian diet is thought to increase health and longevity by warding off diseases like cancer and cardiovascular disease (CVD). A rigorous vegetarian diet in particular has been linked to a higher risk of vitamin B12 insufficiency.

Therefore, biochemical markers reveal a genuine shortage (raised serum homocysteine levels and urine methyl melonic acid). However, given the state of the economy, serum B12 test results could only be interpreted in line with clinical observations. Lack of vitamin B12 primarily affects the neurological, gastrointestinal, and hematologic systems. Anaemia, leukopenia, thrombocytopenia, pancytopenia, macrocytosis, and hypersegmented neutrophils in peripheral smear, as well as megaloblastosis in the bone marrow, are examples of haematological symptoms. In a study of people who lack B12, Seref et al. reported that 96% of the population had haemophilia.[30] Patients who are anaemic are initially evaluated using erythrocyte indices. Antibodies to the gastric parietal cells and intrinsic factor, decreased oral intake, dyserythropoesis due to thyroid hormone deficiency, and defective absorption due to decreased bowel motility, bowel wall oedema, and bacterial overgrowth may all contribute to vitamin B12 deficiency among patients with autoimmune hypothyroidism.[31]

High Mean corpuscular volume (MCV) values in erythrocyte indices are the traditional indicator of B12 and folate insufficiency. According to Wheeler et al., patients with anaemia and MCV levels more than 100 fl should have their vitamin B 12 levels checked. Many of the patients who came to our hospital had clinical symptoms and peripheral smear morphology that pointed to vitamin B 12 deficiencies even if their MCV was below 96 fl.[32]

So, for the purpose of selecting samples for our investigation, we decided to use the inclusion criterion of MCV 96 fl. In the study conducted in Sindh by Gulam et al,[20] erythrocyte indices were classified according to vitamin B 12 levels, and the highest mean values of MCV and MCH were found in the severely deficient group (B 12 100pg/ml).

Cobalamin deficient individuals rarely have low MCV levels. This could be brought on by concurrent iron shortage, thalassemia carrier status, or chronic disease-related anaemia. Therefore, while assessing blood Vitamin B12 levels, preference must be given to changes in MCV and MCH from existing levels to higher values while still falling within normal reference limits (MCV of 90 fl replacing one of 85 fl). In contrast to our study, which found that cobalamin deficiency accounts for 66% of macrocytosis cases, a hospital-based study on the aetiology of macrocytosis by Savage et al,[33] found that cobalamin deficiency accounts for 6% of high MCV values and chemotherapy, antiviral drugs, or alcohol abuse accounts for 64%. On evaluation of peripheral blood films, 112 smears (56%) showed macrocytes, while 120 smears (60%) showed hypersegmented neutrophils. In several other investigations, macrocytosis has been estimated to be 29.8%, 16.1%, and 43%.[26,30,34]

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This discrepancy is likely caused by the fact that our study is prospective and that subjects were chosen based on elevated MCV levels, whereas the studies described above chose their study populations based solely on vitamin B12 levels, regardless of MCV values.
Inflammation and concurrent iron shortage can contribute to disguising macrocytosis. In 60% (120) of our B 12 deficient patients, hypersegmented neutrophils—an early marker of megaloblastosis in nutritional megaloblastic anemia—were seen.
In other prospective datasets, patients with ≥ 5% hypersegmented neutrophils ranged from 25.5% to 100%.[35]

Table 4: Comparison of haematological profiles in various studies

Parameters	Akash et al,[35]	Premkumar et al,[25]	Summer et al,[28]	Kaushik et al,[22]	Present study
Hb (g/dl)	6.39 ±2.7	5.2 ± 1.6	4.8 ± 1.8	5.0 ± 1.2	4.64 ± 1.3
Total leukocyte count(cells/cmm)	4.5 ± 2.3	2.63 ±0.88	4.0 ± 1.12	3.1 ±1.2	4.2 ± 1.6
Platelet count (per cmm)	1.33 ±1.1	82 ± 35	80 ± 30	86± 30	76 ± 34

When compared to Kaushik et al. and Premkumar et al., our study's mean value of haemoglobin and platelet count was found to be lower. Different studies have found varying frequencies of haematological disorders and B12 deficiency. Each level of cobalamin deficiency does not cause anaemia in every patient to the same extent. Anaemia might be absent or unexpectedly low in the severely deficient. Sometimes the cause of a muted haematological response is not immediately apparent. In certain investigations, the prevalence of neutropenia and thrombocytopenia was between 20 and 25 percent. Neutropenia and thrombocytopenia are only frequently seen in conditions of severe anaemia. When there is a cobalamin shortage, platelet production is only 10% of what would be predicted based on the megakaryocyte mass, which may indicate that thrombopoiesis is not functioning properly. Comparing our study to other studies, we found that B 12 deficient individuals had an increased incidence of neutropenia and practically all of them had thrombocytopenia since we only included people with severe anaemia (Hb 7 gm/dl) in our study. In our investigation, we found a significant positive association between vitamin B12 levels and platelet counts and leukocyte counts (p 0.001).
When all three haematopoetic cell lines are reduced, pancytopenia is identified. Haemoglobin levels below 12 g/dl, a total leukocyte count below 4000/mm3, and a platelet count above 150,000/mm3 are the criteria for diagnosis.
As found in our study, megaloblastic anaemia of B 12 deficiency is reported in several Indian studies to be a prominent cause of pancytopenia. On the other hand, Santra and Das' research in a tertiary care facility in Kolkata indicates that it makes the least contribution to pancytopenia.
Pancytopenia is frequently brought on by vitamin shortages and illnesses like HIV and tuberculosis in developing nations around the world.
In contrast, the majority of sickness in industrialised nations is caused by malignancy and marrow aplasia. It is possible to link an inadequate diet to the cobalamin shortage that affects our patient population, which is primarily of lower socioeconomic position. Increased phagocytic trapping of haematopoetic cell lines is caused by nutritional cirrhosis those progresses to hypersplenism. Concomitant tuberculosis, chronic liver illness, and alcoholism all worsen pancytopenia-causing marrow failure. Different pancytopenia etiologies reflect population traits, dietary habits, cultural norms, socioeconomic level, and geographic factors.

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The main effects of B12 deficiency include abnormalities in haematological markers as well as a variety of clinical symptoms. Blood levels of B 12 should be checked if there is anaemia with increased MCV, especially if multiple cell line deficiency is present. Early detection of problems prevents irreversible late complications and identifies a curable cause.

Conclusion

The majority of the participants in our study showed megaloblastic anaemia and pancytopenia, which are symptoms of vitamin B 12 deficiency. When instances appear with these symptoms, doctors should remember to examine the B 12 status. Only iron and folate supplements are currently part of India's prenatal and teen health programmes. In such programmes, vitamin B12 supplements are worth taking into consideration. Following the conclusion of more population-based research, the option of vitamin B12 fortification of food may also be advised.

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