"PREVALENCE OF FUNCTIONAL B12 DEFICIENCY AMONGST EXCLUSIVELY BREASTFED INFANTS BETWEEN 1 TO 6 MONTHS OF AGE- A CROSS SECTIONAL STUDY AT KLE'S DR PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI."

BY

REG NO: BM0122004

Dissertation

Submitted to the KLE Academy of Higher Education and Research, Belagavi, Karnataka

In Partial Fulfilment

of the Requirements for the Degree of

M.D. (Doctor of Medicine)

In

PAEDIATRICS

DEPARTMENT OF PAEDIATRICS
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

SEPTEMBER /OCTOBER 2025

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

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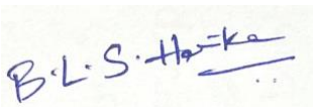

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PREVALENCE OF FUNCTIONAL B12 DEFICIENCY AMONGST EXCLUSIVELY BREASTFED INFANTS BETWEEN 1 TO 6 MONTHS OF AGE- A CROSS SECTIONAL STUDY AT KLE'S DR. PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
Hcy	Homocysteine
MMA	Methylmalonic acid
Cbl	Cobalamin
MCV	Mean corpuscular volume
RBC	Red blood cells
NHANES	National Health and Nutrition Examination Survey
HBA	Hydrogenobyrinic acid
HBAD	Hydrogenobyrinic acid a,c-diamide
AdoCby	Adenosylcobyric acid
APP	(R)-1-Amino-2-propanol O-2-Phosphate
Holo TC	Holotranscobalamin
TC	Transcobalamin
HC	Haptocorrin
IF	Intrinsic factor
WBC	White blood cells
TC II	Transcobalamin II
Me-Cbl	Methylcobalamin
Ado-Cbl	Adenosylcobalamin
NFHS	National Family Health Survey

AdoHcy	S-adenosylhomocysteine
MTHFR	5, 10-methylenetetrahydrofolate reductase
AAG	Autoimmune atrophic gastritis
CBC	Complete blood count
IGS	Imerslund-Gräsbeck syndrome
GERD	Gastro-esophageal reflux disease
ROS	Reactive oxygen species
MCA	Methylcitric acid
ELISA	Enzyme-linked Immunosorbent Assay
PBS	Phosphate Buffered Saline
RDW	Red cell distribution width
TDSC	Trivandrum Developmental Screening Chart
EBF	Exclusively breastfed

ABSTRACT

PREVALENCE OF FUNCTIONAL B12 DEFICIENCY AMONGST EXCLUSIVELY BREASTFED INFANTS BETWEEN 1 TO 6 MONTHS OF AGE: A CROSS SECTIONAL STUDY AT KLE'S DR PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI

BACKGROUND AND AIM

Vitamins and minerals are important micronutrients which are crucial for the synthesis of hormones, enzymes and facilitating growth and development in the human body. One of the important deficiencies of these vitamins is Vitamin B12 deficiency also known as cobalamin deficiency. Vitamin B12 deficiency is considered as endemic in Indian population. The prevalence of B12 deficiency in India ranges from 47 to 59.1% in adults and 63.7% in infants. Hence, Vitamin B12 deficiency is considered as a significant public health issue especially in children as it not only leads to megaloblastic anemia, but various serious non-hematological problems like demyelination, infantile tremor syndrome, etc., Vitamin B12 deficiency is the commonest preventable cause of mental retardation. Vitamin B12 is one of the commonest preventable deficiency disorders and can lead to various health problems in both children and adults. The present study aimed to estimate the prevalence of functional B12 deficiency in exclusively breastfed infants.

METHODOLOGY

The study was carried out in Pediatric Hematology OPD, Pediatric OPD & Well baby clinic and in mothers, who gave consent for the study in Dr. Prabhakar Kore hospital and MRC, Belagavi. The study was conducted as a cross sectional study for a period of 1 year. Infants between 1 to 6 months of age attending pediatric Hematology OPD,

Pediatric OPD & Well baby clinic pediatric OPD for immunization or with mild symptoms were included in the study.

RESULTS

The prevalence of functional vitamin B12 deficiency among exclusively breastfed infants was 47%. In our study, 78.57 % of infants with low vitamin B12 levels had high Homocysteine value. 66.67 % of infants with low-normal vitamin B12 levels, had high Homocysteine value and 45.65 % of infants with high vitamin B12 levels had high Homocysteine values. A statistically significant association was observed between low serum vitamin B12 levels and elevated homocysteine levels ($p=0.018$). Among mothers, there was no statically significant correlation between low serum vitamin B12 levels and elevated MMA. A statistically significant association was found between low serum vitamin B12 and elevated homocysteine levels ($p=0.035$). In exclusively breastfed infants, no statistically significant correlation was found between functional B12 deficiency and elevated MCV ($p=0.276$). However, statistically significant correlation was observed between low serum B12 levels and high MCV ($p<0.001$). In mothers, functional B12 deficiency showed a significant correlation with elevated MCV ($p=0.002$) and even low vitamin B12 levels also showed statistically significant correlation with high MCV ($p=0.010$).

CONCLUSION

In our study, a higher prevalence of Vitamin B12 deficiency was reported (n=39, 47%). Among the functional B12 deficient infants, 38.5% (n=15) had low vitamin B12 levels and 61.5% (n=24) did not have low vitamin B12 levels. Hence, focus on exclusively breastfed mothers for Vitamin B12 should be done and biomarkers like MMA and RDW were reported useful in determining the functional Vitamin B12 status.

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INTRODUCTION

Vitamins and minerals are important micronutrients which are crucial for the synthesis of hormones, enzymes and facilitating growth and development in the human body. One of the important deficiencies of these vitamins is Vitamin B12 deficiency also known as cobalamin deficiency[1]. Vitamin B12 deficiency is considered as endemic in Indian population[2]. The prevalence of B12 deficiency in India ranges from 47 to 59.1%[2, 3] in adults and 63.7% in infants[4]. Hence, Vitamin B12 deficiency is considered as a significant public health issue especially in children as it not only leads to megaloblastic anemia, but various serious non-hematological problems like demyelination, infantile tremor syndrome, etc., Vitamin B12 deficiency is the commonest preventable cause of mental retardation[5]. Vitamin B12 is one of the commonest preventable deficiency disorders and can lead to various health problems in both children and adults[6].

Human body does not synthesize Vitamin B12. The common source of Vitamin B12 are found in non-vegetarian foods like meat, eggs and fish. Vitamin B12 deficiency is very common in exclusively breastfed infants, whose mothers are vegan[7]. Infants of 1 to 6 months are usually exclusively breastfed to obtain good nutrition and immune protection from mothers. However, the B12 deficiency of the mother can impact the infants[4]. Role of Vitamin B12 in infants includes cellular metabolism, cell growth and differentiation by influencing DNA synthesis and epigenetic regulation[8].

Diagnosis of B12 deficiency can be challenging in infants especially if they have received oral supplements (hematinics) or received a blood transfusion as they can artificially increase the levels of Vitamin B12 temporarily and as the assay is very

sensitive to recent intake of Vitamin B12[9]. Serum Vitamin B12 levels are usually used to diagnose the deficiency. However, among 50% of deficient patients were presented in sub-clinical stage have normal B12 levels. Hence, higher sensitive tests to diagnose the deficiency are crucial. Methylmalonic acid (MMA) and Homocysteine (Hcy) are good early indicators for deficiency of Vitamin B12 as they tend to increase during the B12 deficiency[10]. Vitamin B12 acts as a cofactor for two enzymatic reactions. One, the conversion of MMA to succinic acid and another the production of methionine from homocysteine. Eventually the conversion of MMA to succinic acid and production of methionine from Hcy is reduced due to the deficiency of the Vitamin B12. Hence, the MMA and Hcy levels are elevated and can serve as the early biomarker for diagnosis of functional B12 deficiency[11].

Functional B12 deficiency is defined by the presence of elevated levels of MMA and/ or HCy despite serum B12 values well within the normal reference range[12]. However, low B12 levels is considered when an individual's Vitamin B12 levels reduces below 200 pg/mL[13]. The development of functional vitamin B12 deficiency can occur even in the presence of normal serum cobalamin (Cbl) levels, as indicated by the accumulation of cobalamin-dependent metabolites such as methylmalonic acid (MMA) and homocysteine (Hcy). This phenomenon has been termed "subtle" or "subclinical" Cbl deficiency, suggesting a progression in the depletion of vitamin B12 that initially leads to decreased serum levels, followed by metabolite buildup, and ultimately resulting in hematologic or neurocognitive disorders over several years.

Early detection of vitamin B12 deficiency and appropriate treatment to reverse the deficiency in the exclusively breastfed infants is crucial in preventing possible irreversible neurologic damage, megaloblastic anemia and failure to thrive[8].

Another important parameter which can help in identifying B12 deficiency is the mean corpuscular volume (MCV). MCV is a measure of average volume of red blood cells (RBCs) and serves as a useful parameter in evaluating various types of anemia including Vitamin B12 deficiency. MCV levels are usually increased in megaloblastic anemia and non-megaloblastic macrocytic anemia in conditions like chronic hypothyroidism and chronic liver diseases[14]. Higher MCV levels have been reported in early stages of the disease (sub-clinical stage) when the hemoglobin level is still within normal levels[15]. MCV is a useful tool in early diagnosis of functional Vitamin B12 deficiency[16].

Vitamin B12 plays a crucial role in myelination by acting as a co-enzyme in the synthesis of myelin, which is the insulating layer around the nerve fibers and is essential for proper nerve signal transmission. In Vitamin B12 deficiency, disruption of the formation of healthy myelin leads to impaired nerve function due to its involvement in key metabolic pathways related to fatty acid synthesis necessary for myelin production[17]. Inadequate Vitamin B12 can lead to neural damage and atrophy of brain. Vitamin B12 deficiency in mothers can lead to impaired growth, psychomotor function, demyelination and brain development of fetus in utero[6]. Hence it is important to diagnose the infants with Vitamin B12 deficiency early rather than diagnosing after the development of symptoms to reduce the risk of morbidities associated with the disorder and to prevent it by addressing it in the early stages of deficiency.

Importance of the study

This study is vital as it focuses on a critical developmental period when infants are solely dependent on breast milk for nutrition. Identifying the prevalence of

functional B12 deficiency among exclusively breastfed infants will provide essential information for healthcare professionals, allowing for early identification of at-risk infants not only anemia, but also helps in prevention of neurological manifestations like demyelination, ataxia, and gait disturbances. The findings could enhance awareness of the significance of adequate vitamin B12 levels and guide recommendations for supplementation and dietary planning for breastfeeding mothers, ultimately contributing to improved health outcomes for infants.

Study Rationale

The rationale for conducting this study stems from the increasing recognition of vitamin deficiencies as a significant health issue in pediatric group. By focusing on the functional B12 levels in exclusively breastfed infants, the study aims to fill a knowledge gap in the existing literature. Understanding the prevalence of this deficiency will enable to implement necessary interventions and educational programs for the mothers. Furthermore, with breastfeeding as a primary source of infant nutrition, ensuring that breastfeeding practices are paired with adequate maternal nutrition is crucial. This research can pave the way for further investigations into the relationship between maternal dietary habits and infant health, ultimately promoting a more holistic approach to maternal and child health care.

Vitamin B12 plays a crucial role in neurological development and metabolic functions. Traditional serum B12 assays are commonly used to assess B12 levels. However, their accuracy is often compromised due to prior blood transfusions (which may transiently elevate serum B12 levels), autoimmune disease (which may alter B12 metabolism) and variability in B12 binding proteins (leading to misleading serum B12 levels)[13].

Functional B12 deficiency occurs when B12-dependent metabolic pathways are impaired, despite normal or borderline serum B12 levels. Since Vitamin B12 is a co-factor for two critical metabolic reactions including conversion of homocysteine to methionine and conversion of methylmalonyl CoA to succinyl CoA. When B12 levels are insufficient, these pathways become blocked, leading to an accumulation of homocysteine (Hcy) and methylmalonic acid (MMA) in the blood. Elevated homocysteine and methylmalonic acid serve as reliable biomarkers for diagnosing functional B12 deficiency, making them superior to routine serum B12 testing[10].

Given the limitations of conventional serum B12 assays, assessing functional B12 deficiency through metabolic markers (homocysteine and methylmalonic acid) offers a more accurate prevalence estimate in breastfed infants. Early detection is crucial to prevent developmental delays, neurocognitive impairment and growth failure[8].

AIMS AND OBJECTIVES

Primary objectives

To estimate the prevalence of functional B12 deficiency in exclusively breastfed infants.

Secondary objectives:

- To correlate serum B12 levels and serum MMA and Homocysteine levels.
- To correlate MCV and RDW with functional B12 deficiency.
- To correlate with mothers B12 status in infants found to have functional B12 deficiency.
- To do the developmental status screening of infants between 3-6 months who are found to be B12 deficient by TRIVANDRUM DEVELOPMENT SCREENING TEST.

REVIEW OF LITERATURE

Epidemiology of Vitamin B12 deficiency

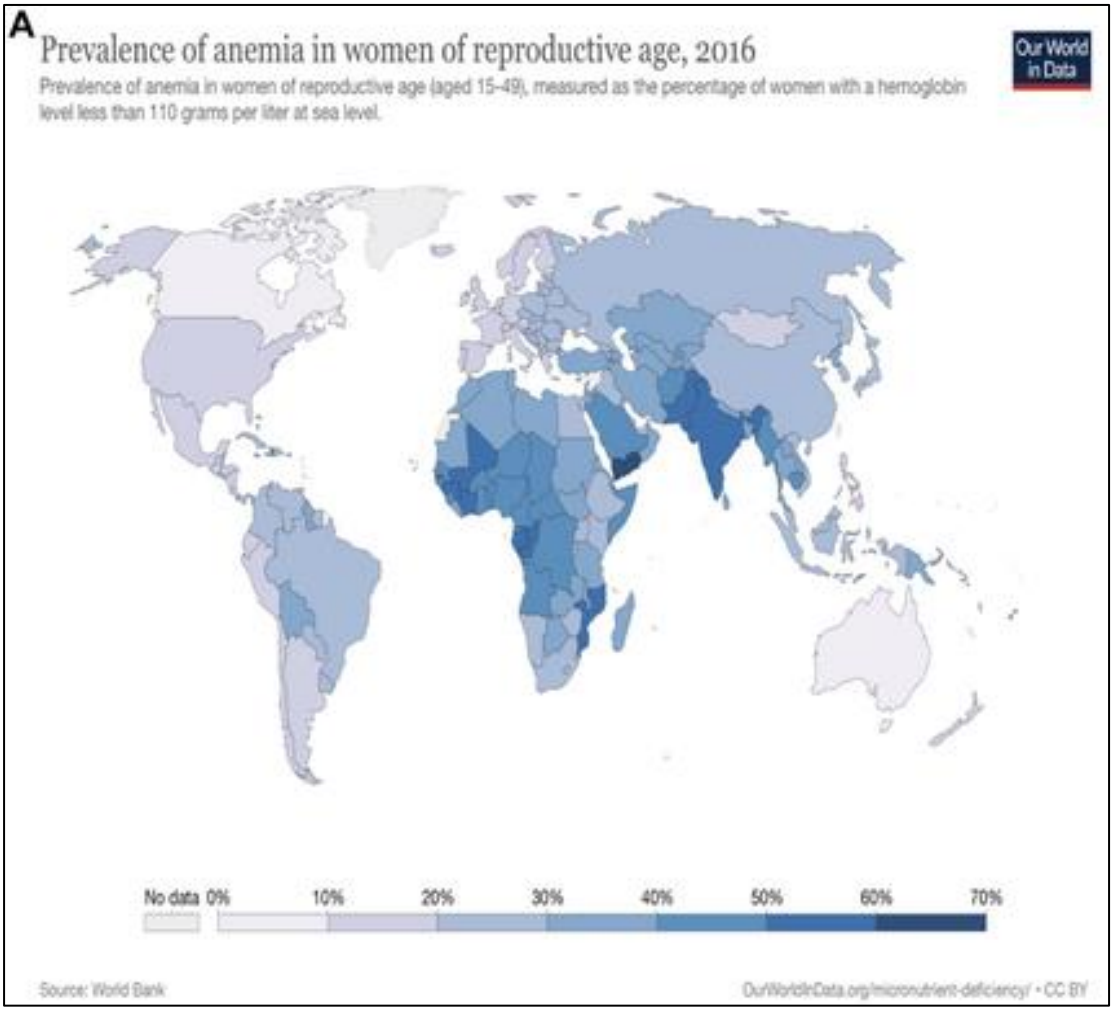


Figure 1: Global prevalence of Vitamin B12 deficiency in women of reproductive age *(Source: Oh S et al[18])*

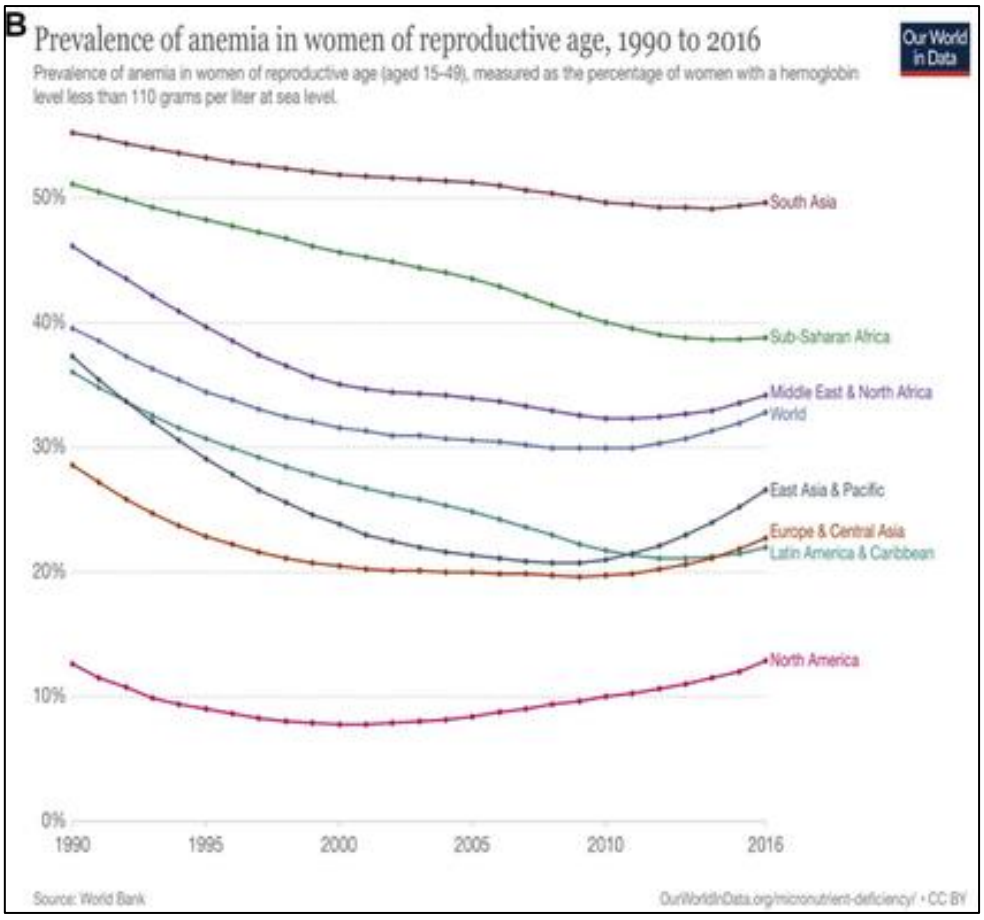


Figure 2: Prevalence of anemia in women in reproductive age in 1990 to 2016

(Source: Oh S et al[18])

Globally, the prevalence of Vitamin B12 deficiency ranges from 2.9% to 35%[19]. In India, prevalence of Vitamin B12 among adolescents were reported as 31%, and among preschool children as 13.8%[20]. In pregnant women, the prevalence of vitamin B12 were reported as 46% - 60%[21, 22]. In infants between the age 0-6 months, the prevalence of B12 was reported as 54% (21) and in newborns the prevalence was reported as 29%[22].

A study from south India reported 63.7% of prevalence of Vitamin B12 deficiency in exclusively breastfed infants between the age 1-6 months[4].

In Karnataka, a prevalence of 55% was reported among pregnant women[23].

National Health and Nutrition Examination Survey (NHANES) reported that low levels of serum B12 concentration was associated with moderate increase in all-cause mortality in general population[24].

In developed countries, megaloblastic anaemia is observed in approximately 3-4% of pregnant women with anaemia, whereas in developing nations, the prevalence rises to 25%. Despite advancements in socioeconomic conditions and overall health standards, megaloblastic anaemia remains a significant public health concern in many developing regions[23].

Prevalence of anaemia among pregnant women in India according to the latest National Family Health Survey-5 (NFHS-5) data was 52.2%[25]. Prevalence of anaemia in exclusively breastfed infants between 3 to 6 months was 87.6% in India[26].

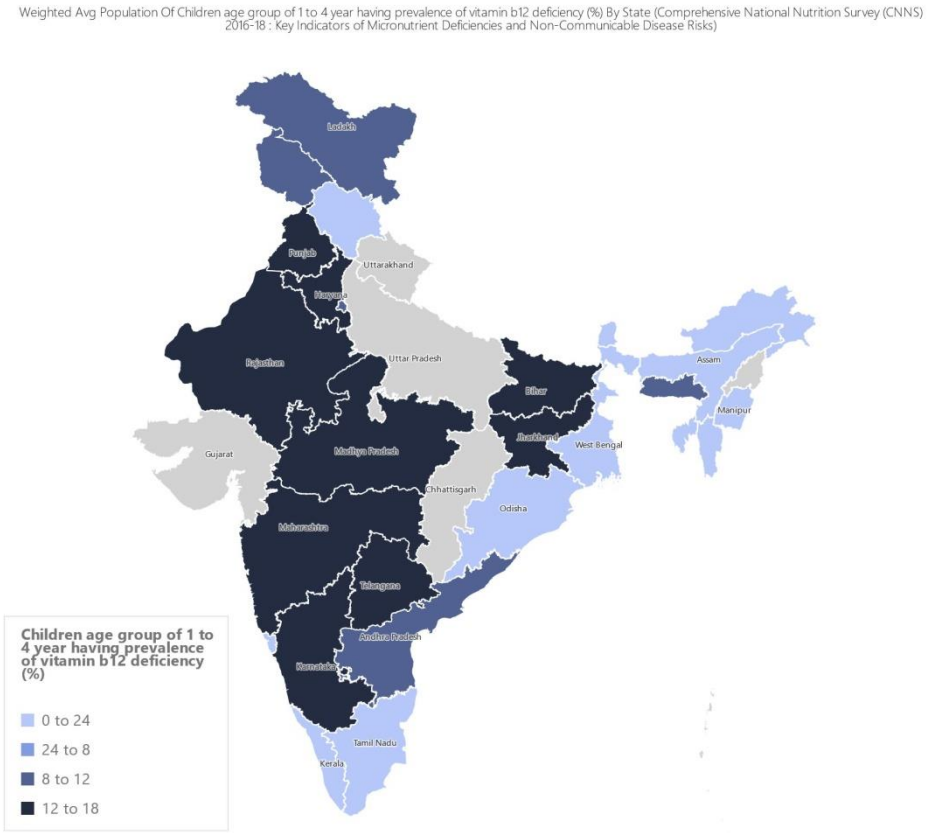


Figure 3: Prevalence of Vitamin B12 deficiency in children in India

Chemistry of cobalamins

Vitamin B12, also known as cobalamin, is an essential nutrient crucial for various bodily functions, including the formation of red blood cells and the maintenance of neurological health. Deficiencies in vitamin B12 can lead to severe health issues, including megaloblastic anemia and neurological disorders. The primary dietary sources of B12 are animal-based products, which poses a risk for individuals following strict vegetarian or vegan diets. Its absorption in the body requires the presence of intrinsic factor, a protein produced in the stomach[27]. The structure of cobalamins which are the class of molecules known as corrins is given in the Figure 4.

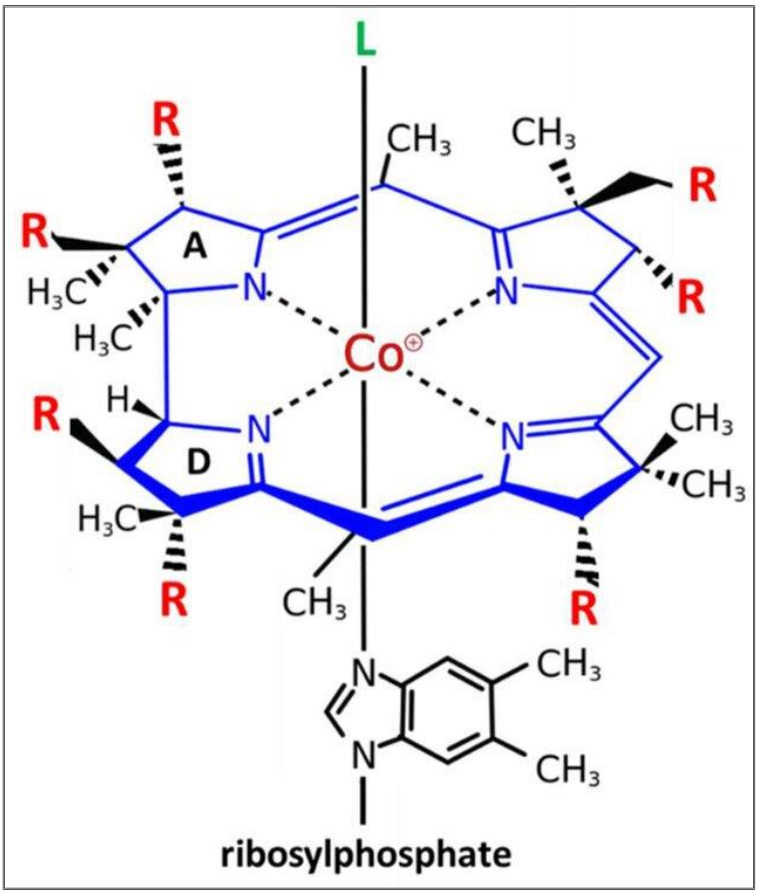


Figure 4: Structure of Cobalamin

(Source: Schelicher E et al 2023[28])

Nutritional sources and requirements

The natural sources of vitamin B12 are animal-based foods including meat, milk, egg, fish and shellfish. However, B12 is also present in supplements and fortified foods[29]. Vitamin B12 is synthesized exclusively by certain bacteria and archaea through two distinct pathways: the aerobic and anaerobic routes. These pathways primarily differ in their requirement for oxygen and the timing of cobalt insertion[30].

Aerobic pathway

In the aerobic pathway, seen in *Pseudomonas denitrificans*, uroporphyrinogen III undergoes methylation, decarboxylation and ring contraction, leading to the formation of hydrogenobyrinic acid (HBA). The CobB enzyme facilitates amidation, converting HBA into hydrogenobyrinic acid a,c-diamide (HBAD). After cobalt insertion, a series of enzymatic reactions including reduction, adenylation and amidation produces adenosylcobyric acid (AdoCby). This further transform incorporating (R)-1-Amino-2-propanoll (AP) to form adenosylcobinamide (AdoCbi), which is subsequently phosphorylated and modified by the CobP enzyme to generate AdoCbi-GDP[30].

Anaerobic pathway

In the anaerobic pathway, as observed in *Salmonella typhimurium*, a similar process occurs but with small differences. Instead of AP, the intermediate (R)-1-Amino-2-propanol O-2-Phosphate (APP) is used, facilitated by CbiB synthase. The enzyme CobU then converts AdoCbi-P into AdoCbi-GDP, after which additional reactions complete the synthesis of adenosylcobalamin (AdoCbl), the bioactive form of vitamin B12[30].

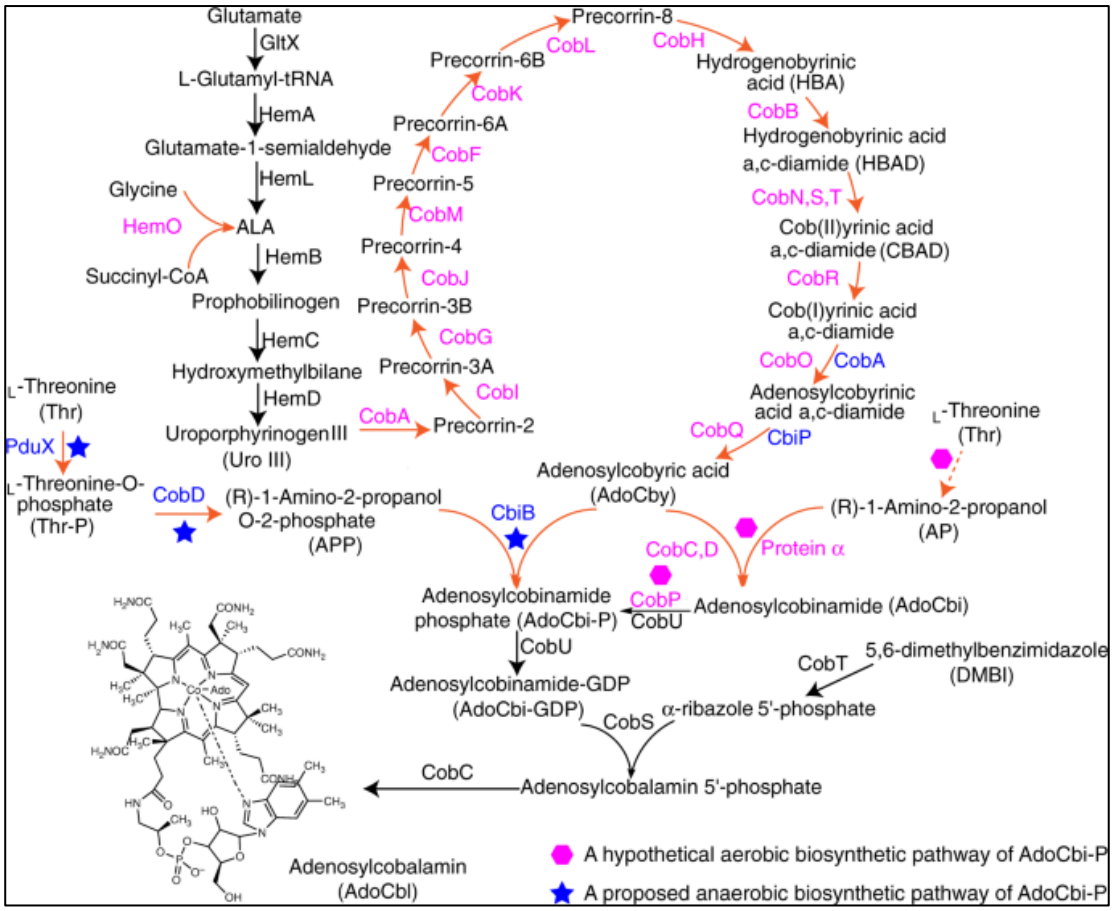


Figure 5: Pathway of adenosylcobalamin

(Source: Fang H et al 2018[30])

Recommended daily requirement of Vitamin B12 in infants less than 6 months is 0.4 µg which is obtained from breastmilk from the exclusively breastfed infants[31].

Table 1: Recommended daily dose of Vitamin B12

Group	Age	Dose (µg)
Infant	< 6 months	0.4 (Breast milk)
	6 months to 1 year	0.5
Children	1-3	0.9
	4-8	1.2
	9-13	1.8
Adolescent	14-18	2.4
Adult	More than 18	2.4
Pregnant women		2.6
Lactating women		2.8

(Source: Bjorke-Monsen AL et al[31])

Cobalamin-binding proteins

Vitamin B12 is transported in the bloodstream by two key carrier proteins:

i. Transcobalamin (TC) and

ii. Haptocorrin (HC).

Transcobalamin, a 43-kDa nonglycoprotein, facilitates the movement of vitamin B12 from the intestines into the bloodstream and subsequently into body cells. When fully saturated with cobalamin, it is referred to as holotranscobalamin (holoTC), which comprises 6% to 20% of the total circulating vitamin B12. The

unsaturated form, also known as apotranscobalamin (apoTC), makes up the majority of transcobalamin (Up to 90%). While primarily synthesized by intestinal cells, other organs can also produce TC.

Haptocorrin (HC), also known as R-binder, is a group of immunologically related proteins, including TCI and TCIII. It is produced by various cells, including white blood cells (WBCs), HC binds 80% to 94% of total plasma cobalamin. Unlike TC, HC is a glycoprotein and remains largely saturated with cobalamin. While TC is crucial for delivering active B12 to cells, HC primarily binds inactive forms of cobalamin, with no clearly defined biological role. Individuals with congenital HC deficiency generally exhibit reduced serum B12 levels but does not develop metabolic complications. However, congenital TC deficiency leads to severe neurological symptoms, accompanied by elevated Hcy and MMA levels.

Cobalamin transport occurs at several key sites in the body. Firstly, in the intestines, intrinsic factor transfers B12 to TC in enterocytes, allowing it to enter circulation. Second, TC-bound vitamin B12 is reabsorbed in the kidneys and reenters the bloodstream, likely bound to newly synthesized TC. The placenta plays a significant role in vitamin B12 transport, as it produces both TC and its receptor, facilitating B12 transfer from mother to fetus.

Measuring TC, HC, and their B12-bound forms (holoTC and holoHC) has been a long-standing challenge. Accurate isolation of these proteins is essential for evaluating total B12 status and its active fraction in the body[32].

Absorption, Digestion and Transportation of dietary cobalamins

Vitamin B12, which is primarily synthesized from animal-based foods and requires a complex absorption process involving multiple proteins and enzymes. After ingestion, cobalamin binds to dietary proteins in food. In the stomach, gastric acid and pepsin break down these proteins, releasing free vitamin B12. However, to prevent degradation by stomach acid, B12 immediately binds to haptocorrin (R-protein), which is secreted by the salivary glands. Meanwhile, the stomach also secretes intrinsic factor (IF), a crucial glycoprotein for Vitamin B12 absorption, though its binding affinity is lower in the presence of haptocorrin.

As the food enters the duodenum, pancreatic enzymes break down the haptocorrin-B12 complex, freeing vitamin B12. This allows vitamin B12 to bind with intrinsic factor, forming the B12-IF complex, which remains stable in the alkaline environment of the small intestine. The complex then travels to the ileum, where it binds to a specialized cubam receptor that facilitates endocytosis. Once inside the enterocyte, intrinsic factor is degraded, and vitamin B12 binds to specific transport proteins: transcobalamin I, II and III. Among these, transcobalamin II (TCII) plays the most significant role in delivering vitamin B12 to all body cells through the portal circulation.

After entering the bloodstream, the TCII-B12 complex is absorbed by endocytosis, and vitamin B12 is enzymatically converted into its active coenzyme forms: methylcobalamin (Me-Cbl) and adenosylcobalamin (Ado-Cbl). The liver serves as the main storage site for vitamin B12, and a portion is excreted in bile, undergoing enterohepatic circulation, where it is reabsorbed to maintain adequate levels[18].

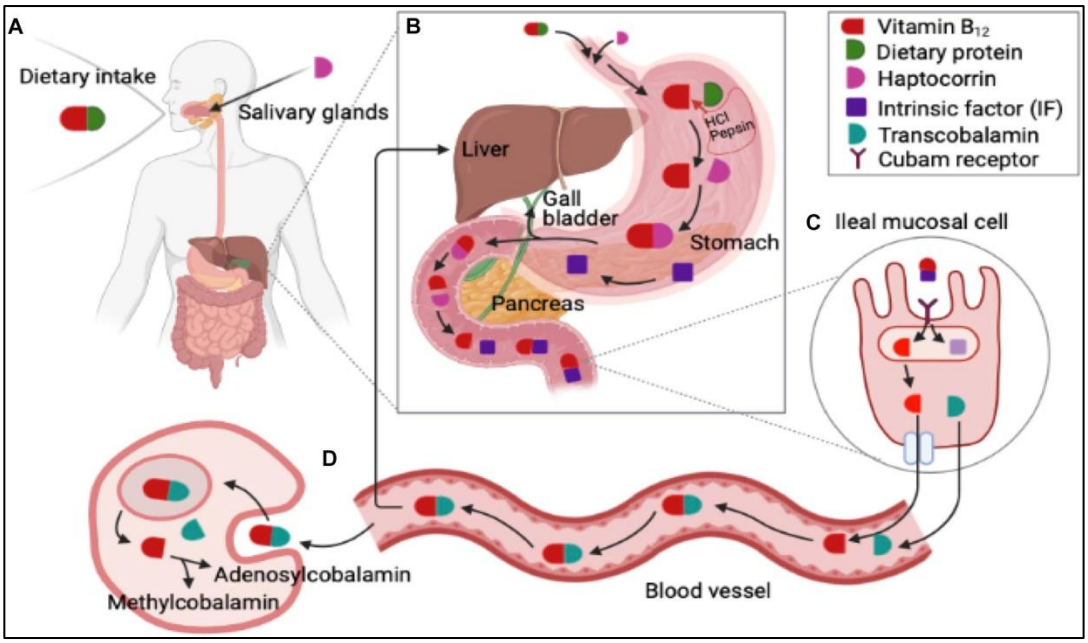


Figure 6: Absorption of dietary Vitamin B12

(Source: Oh S et al[18])

Factors Affecting Vitamin B12 Absorption

Several endogenous and exogenous factors can impact gastrointestinal absorption and enterohepatic circulation of vitamin B12. Achlorhydria, a condition marked by low or absent stomach acid, impairs protein breakdown and disrupts B12 release, leading to malabsorption. Conditions such as chronic gastritis or insufficient intrinsic factor production can contribute to megaloblastic anemia and neurological disorders.

In addition, exocrine pancreatic insufficiency affects B12 absorption by hindering haptocorrin degradation, as pancreatic enzymes play a crucial role in freeing B12 from its bound state. Bacterial overgrowth in the small intestine, particularly Pseudomonas and Klebsiella species, can also interfere with absorption by competing for vitamin B12. This is often seen in individuals with gastrectomy,

ileocolic resection, or impaired gastric acid secretion. Furthermore, genetic disorders affecting vitamin B12 transport and conversion can result in deficiencies, impacting its essential functions in DNA synthesis, red blood cell production, and neurological health[18].

Metabolism of cobalamin

Homocysteine accumulation in various conditions is recognized as a toxic factor affecting multiple systems. It can cause damage either directly or indirectly through its conversion into S-adenosylhomocysteine (AdoHcy). This inhibits crucial methyltransferases. Direct toxicity leads to endothelial dysfunction and neuronal cell death. Disorders affecting re-methylation pathways, such as CblC deficiency and 5, 10-methylenetetrahydrofolate reductase (MTHFR) mutations, not only result in elevated homocysteine levels but also lead to methionine deficiency, which is essential for protein synthesis and the production of S-adenosylmethionine (AdoMet). This reduction in cellular methylation capacity is particularly harmful to the central nervous system.

CblC deficiency is an inherited disorder of intracellular vitamin B12 metabolism, caused by mutations in the MMACHC gene. After being absorbed through the intestines and transported in the bloodstream, cobalamin reaches the cytoplasm, where it normally binds to MMACHC. This protein plays a key role in removing alkyl or cyanide groups from cobalamin, converting it into its active forms: methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl). Without a functional MMACHC protein, the synthesis of MeCbl and AdoCbl is impaired. As a result, methionine synthase activity decreases, leading to a re-methylation defect, while

methylmalonyl-CoA mutase dysfunction causes an accumulation of methylmalonic acid (MMA)[23].

In MTHFR deficiency, the 5-methyltetrahydrofolate (5-methylTHF) needed for methionine synthase is not produced, disrupting homocysteine re-methylation. While homocysteine build -up and methionine depletion largely explain the effects of MTHFR deficiency, the underlying mechanisms of CblC deficiency remains unclear. One proposed explanation is that due to methionine synthase impairment, 5-methylTHF accumulates, leading to a shortage of other folate derivatives, which are essential for various cellular processes. Additionally, the excess MMA in CblC deficiency may contribute to toxicity.

Beyond MTHFR and CblC deficiencies, homocysteine re-methylation can also be compromised at various stages of cobalamin and folate metabolism. These include issues with dietary intake, intestinal absorption, transport via transcobalamin, cellular uptake, and intracellular processing. Other known intracellular cobalamin metabolism disorders affecting homocysteine re-methylation include CblF, CblJ, CblC, CblD, CblD-HCy, CblE and CblG deficiencies[33].

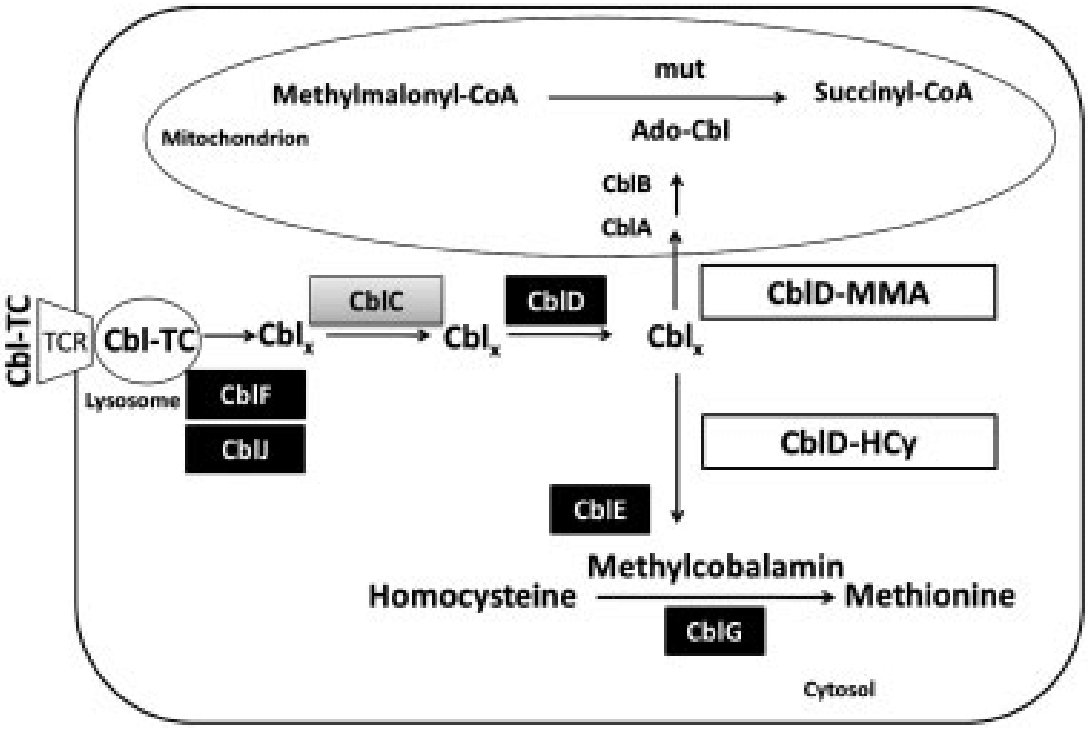


Figure 7: Cobalamin metabolic pathway

(Source: Schiff et al[33])

Development of cobalamin deficiency

Vitamin B12 deficiency arises from a variety of pathophysiological mechanisms that can affect individuals at any stage of life, from infancy to adulthood, impacting both the supply and demand for this essential nutrient. Cellular deficiency may result from inadequate dietary intake, malabsorption issues, chemical inactivation, or genetic factors that disrupt the transport or metabolism of Vitamin B12 within the body.

Inadequate intake of Vitamin B12

As discussed previously, the main source of vitamin B12 are synthesized from bacteria and archaea, and found in animal-based products. The daily requirement for vitamin B12 is approximately 2-3 μg, which can be easily met by those who consume

ample animal-based foods or take supplements. However, vegans may only receive 0–0.25 μg daily, which significantly increases their risk for deficiency of vitamin B12. Reduced intake of animal-derived foods may occur due to limited availability or due to cultural, religious or personal dietary choices. Additionally, the demand for vitamin B12 can vary based on age and physiological states, such as during pregnancy and lactation.

Although dietary recommendations for B12 exist for children and adolescents, adult figures tend to be more general, often applied to pregnant and breastfeeding women and the elderly. Moreover, these recommendations typically do not consider factors affecting bioavailability, which vary significantly among demographic groups, including gastrointestinal health and total B12 consumption. The bioavailability of vitamin B12 is partly dependent on its release from protein carriers found in food. For instance, B12 is more bioavailable from milk than from other animal sources, and B12 derived from cow's milk may be better absorbed compared to that from human breast milk, possibly due to higher concentrations in cow's milk. Furthermore, transcobalamin present in cow's milk has been shown to facilitate the absorption of B12 in the intestines. The role of transcobalamin and other factors in enhancing B12 absorption could be important for both preventing and managing vitamin B12 deficiency[34].

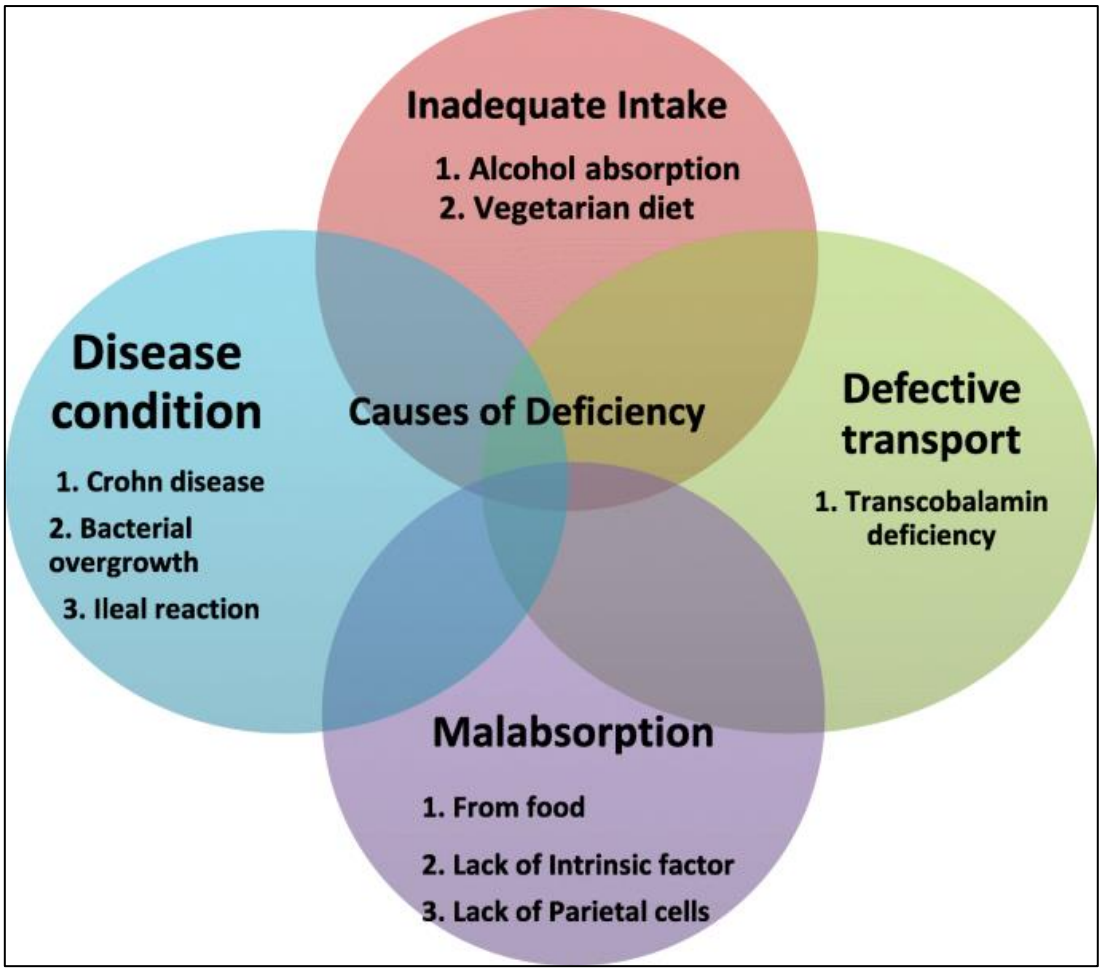


Figure 8: Causes of Vitamin B12 deficiency

(Source: Nawaz A et al 2020[35])

Chemical inactivation of Vitamin B12

The anesthetic gas nitrous oxide causes the chemical inactivation of vitamin B12 by irreversibly oxidizing its coenzyme form, methylcobalamin, at the active site of the methionine synthase reaction that depends on vitamin B12. The severity and rapidity of vitamin B12 deficiency resulting from exposure to this gas can vary based on the individual's existing B12 status, along with the frequency and duration of nitrous oxide usage[34].

Malabsorption of Vitamin B12

The primary factors contributing to vitamin B12 malabsorption includes genetic disorders such as intrinsic factor deficiency, Imerslund-Gräsbeck disease, and pernicious anaemia. Additionally, obesity, bariatric surgery, and gastrectomies can impair the absorption. Other medical conditions associated with B12 malabsorption includes pancreatic insufficiency, obstructive jaundice, tropical sprue, and celiac disease. Furthermore, bacterial overgrowth, parasitic infections, Zollinger-Ellison syndrome, inflammatory bowel diseases, chronic radiation enteritis affecting the distal ileum, and short bowel syndrome can also hinder proper vitamin B12 absorption[36].

Pernicious anaemia

Pernicious anaemia is a type of megaloblastic anaemia caused due to the inability of the body to absorb vitamin B12 effectively. This condition results from an autoimmune response that affects the production of intrinsic factor, a glycoprotein essential for vitamin B12 absorption in the small intestine. Without adequate vitamin B12, the body struggles to produce healthy red blood cells (RBCs), leading to anaemia and various neurological complications[37].

i. Causes and Pathophysiology

The primary cause of pernicious anaemia is an autoimmune disorder that leads to the destruction of parietal cells in the stomach. These cells are responsible for producing intrinsic factor, which binds with vitamin B12 to facilitate its absorption in the ileum. When intrinsic factor is deficient or absent, vitamin B12 is poorly absorbed, resulting in reduced RBC production and subsequent anaemia[38].

ii. Another contributing factor to pernicious anaemia is atrophic gastritis. Atrophic gastritis is a condition where chronic inflammation damages the stomach lining, further impairing intrinsic factor production. Additionally, individuals who have undergone gastric surgery or suffer from malabsorption disorders such as Crohn's disease are at higher risk of developing this condition[39].

iii. Symptoms of Pernicious Anaemia-

The symptoms of pernicious anaemia develop gradually and can be categorized into hematologic, neurological, and gastrointestinal manifestations:

- Hematologic Symptoms: Fatigue, pallor, shortness of breath, rapid heartbeat and dizziness due to inadequate oxygen transport in the blood.

- Neurological Symptoms: Tingling or numbness in the hands and feet, muscle weakness, memory loss, difficulty concentrating, and in severe cases, psychiatric symptoms such as depression and paranoia.

- Gastrointestinal Symptoms: Loss of appetite, weight loss, nausea, diarrhoea and a smooth, swollen, red tongue (glossitis)[40].

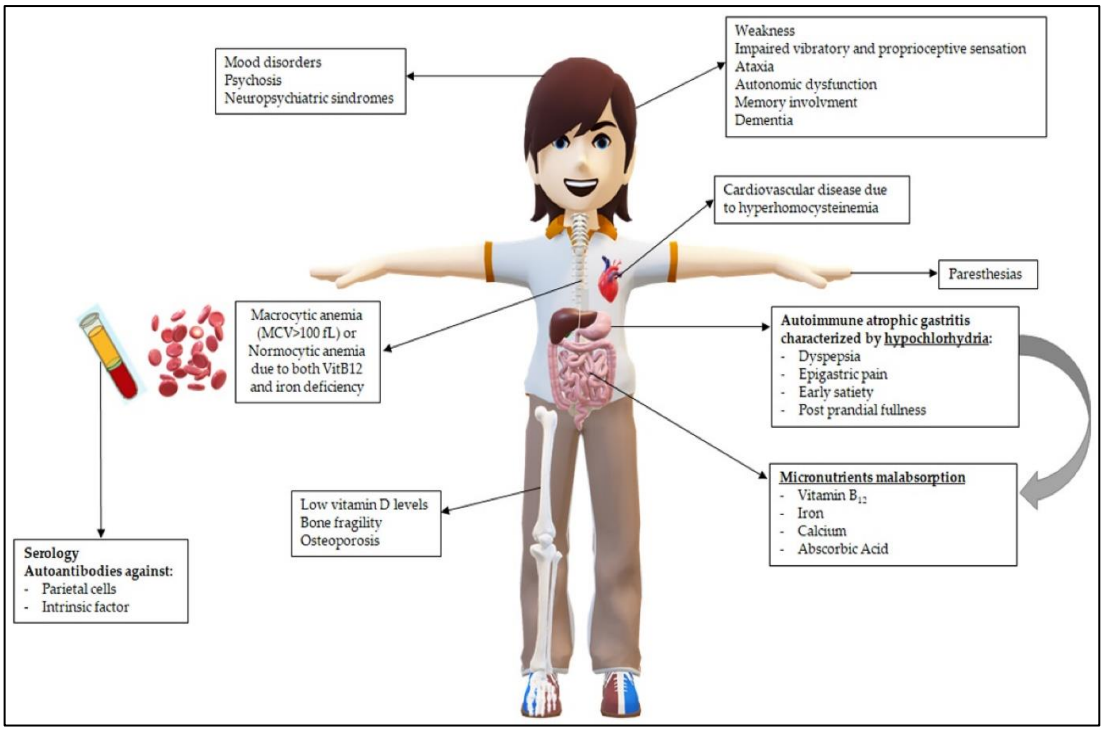


Figure 9: Consequences in the Vitamin B12 deficiency

(Source: Esposito G et al 2022[41])

iv. Diagnosis of Pernicious Anaemia

Diagnosing pernicious anaemia involves a series of laboratory tests to assess vitamin B12 levels and the presence of autoantibodies:

- Complete Blood Count (CBC): Shows macrocytic anaemia with large, immature red blood cells.
- Vitamin B12 Levels: Low serum B12 levels confirm deficiency.
- Intrinsic Factor Antibody Test: Identifies autoimmune-mediated destruction of intrinsic factor.
- Methylmalonic Acid (MMA) and Homocysteine Tests: Elevated levels of these metabolites indicate B12 deficiency.
- Schilling Test (Historical): Previously used to assess B12 absorption but is now largely replaced by antibody tests.

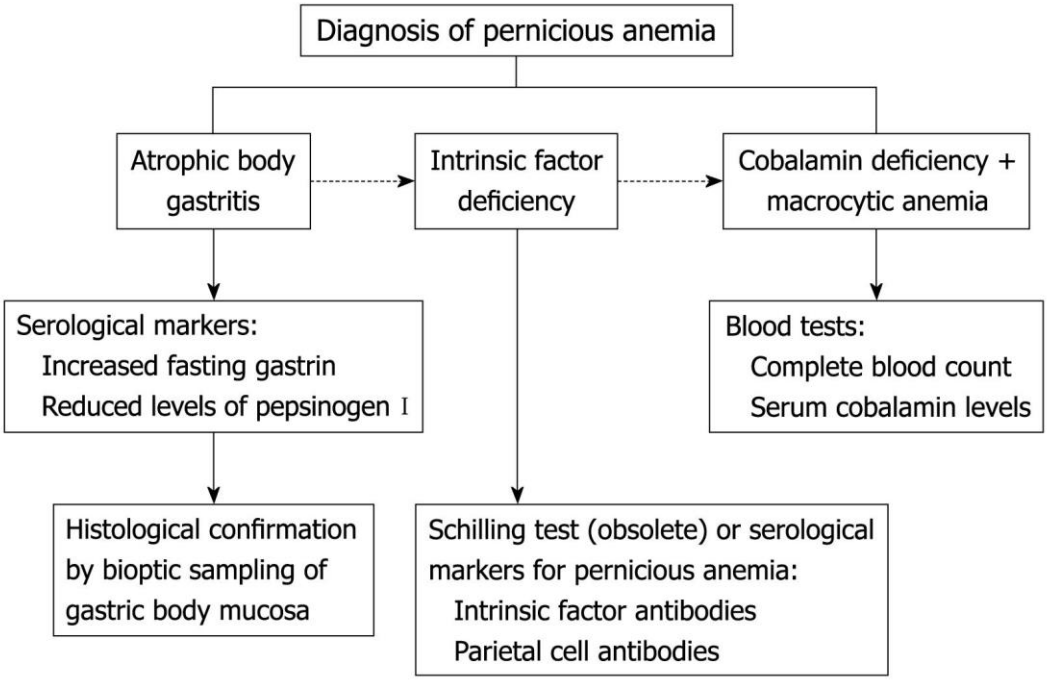


Figure 10: Diagnosis of Pernicious anemia

(Source: Lahner E et al 2009[42])

v. Treatment and Management

The primary treatment for pernicious anaemia is vitamin B12 supplementation, which can be administered through intramuscular injections or high-dose oral supplements. It includes:

- Initial Therapy: Intramuscular B12 injections (1000 mcg) given daily or weekly until deficiency is corrected.
- Maintenance Therapy: Monthly B12 injections or high-dose oral supplementation for lifelong management.

Dietary modifications may also help in maintaining adequate Vitamin B12 levels. Foods rich in vitamin B12 include meat, fish, eggs, dairy products and fortified cereals. However, dietary intake alone is insufficient for individuals with pernicious anaemia due to impaired absorption.

vi. Complications and Prognosis

If left untreated, pernicious anaemia can lead to severe neurological complications, including permanent nerve damage, cognitive impairment, and increased risk of cardiovascular diseases due to elevated homocysteine levels. However, with early diagnosis and lifelong B12 supplementation, individuals can lead normal, healthy lives[37].

Autoimmune atrophic Gastritis

Autoimmune atrophic gastritis (AAG) is a chronic condition in which the immune system mistakenly attacks the oxyntic mucosa of the stomach, leading to its gradual destruction. This process is primarily mediated by the production of autoantibodies against gastric parietal cells and intrinsic factor, key components necessary for digestion and nutrient absorption. The inflammatory response triggered by autoreactive T-cells results in the loss of parietal cells, leading to reduced gastric acid secretion and impaired vitamin B12 absorption.

Notably, AAG exhibits a strong predilection for older adults, particularly postmenopausal women. Additionally, individuals with a personal or family history of autoimmune diseases, such as type 1 diabetes or autoimmune thyroiditis, are at a significantly higher risk of developing AAG[43].

i. Pathogenesis

The underlying mechanisms of AAG involve a complex interplay between genetic and environmental factors. Autoimmune-mediated destruction of gastric parietal cells leads to impaired acid secretion (hypochlorhydria) and intrinsic factor deficiency, resulting in vitamin B12 malabsorption and an increased risk of pernicious

anaemia. In many patients, autoantibodies against the H+/ K+ ATPase enzyme in parietal cells appear long before the onset of clinical symptoms, suggesting a progressive disease course. Emerging evidence also indicates a potential role of vitamin D deficiency in autoimmune diseases, including AAG, due to its influence on T-cell differentiation and immune tolerance.

The association between H. pylori infection and AAG remains controversial. Some studies suggest that H. pylori-related gastritis may trigger autoimmune responses in genetically predisposed individuals, leading to AAG development. Conversely, other research indicates that H. pylori infection may exert a protective effect by modulating immune responses and delaying AAG progression[44].

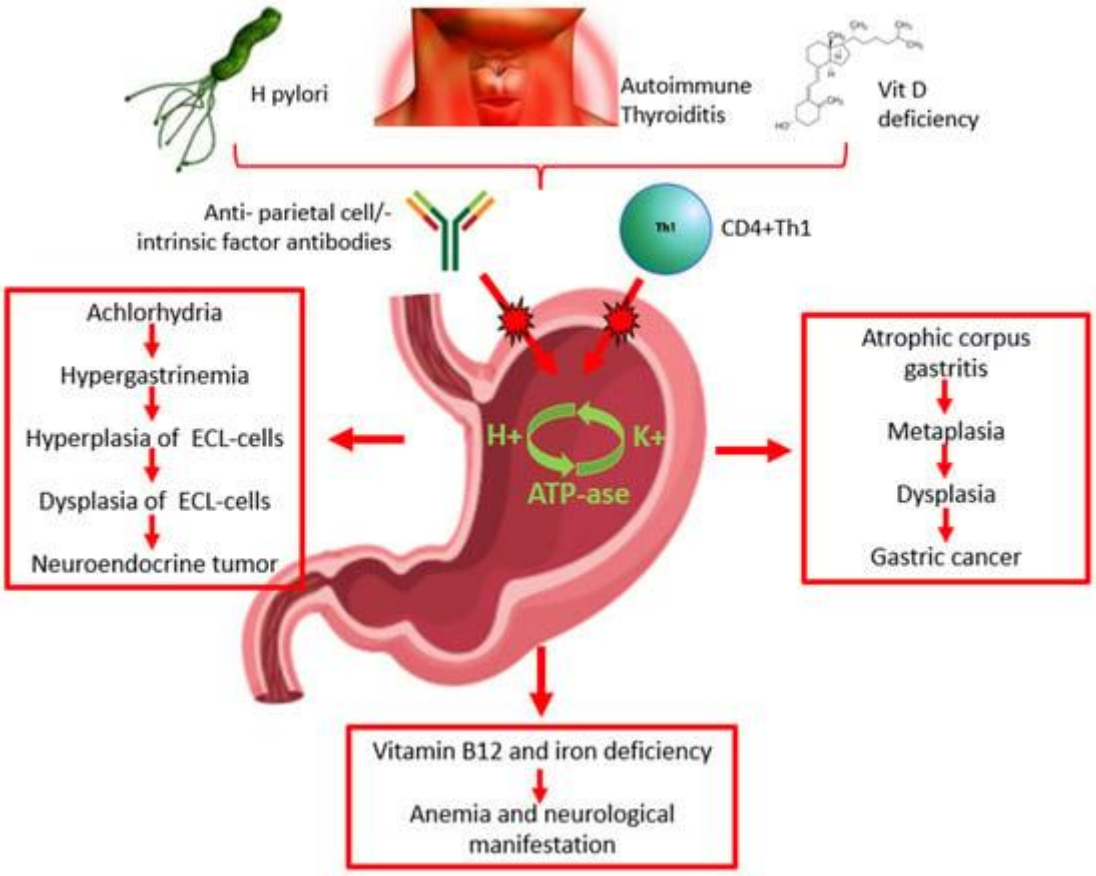


Figure 11: Pathogenesis of Autoimmune atrophic gastritis

(Source: Castellana C et al[43])

ii. Clinical Manifestations

AAG is often asymptomatic in its early stages but can eventually manifest as nonspecific gastrointestinal symptoms, including dyspepsia, bloating and postprandial discomfort. As the disease progresses, patients may develop iron deficiency anaemia due to chronic blood loss and impaired iron absorption, as well as pernicious anaemia resulting from vitamin B12 deficiency. Neurological symptoms, such as paraesthesia, cognitive impairment and ataxia, may occur in advanced cases of pernicious anaemia[45].

iii. Diagnosis

The diagnosis of AAG is based on a combination of serological markers, endoscopic findings, and histopathological examination of gastric biopsies. Key serological markers include anti-parietal cell antibodies and anti-intrinsic factor antibodies, which are present in a majority of AAG patients. Elevated serum gastrin levels and reduced pepsinogen I levels are additional indicators of corpus atrophy. Endoscopic examination may reveal characteristic findings such as gastric mucosal atrophy, nodularity and prominent submucosal vasculature. Histological analysis remains the gold standard for confirming AAG, with findings of mononuclear cell infiltration, oxyntic gland atrophy, and enterochromaffin-like cell hyperplasia[46].

iv. Complications

AAG is associated with an increased risk of gastric malignancies, including type 1 gastric carcinoids and adenocarcinoma. Chronic inflammation and hypergastrinemia contribute to enterochromaffin-like cell proliferation, predisposing patients to neuroendocrine tumours. Regular surveillance with endoscopic evaluation

is recommended for patients with established AAG to detect early neoplastic changes[43].

v. Management and Treatment

Currently, there is no definitive cure for AAG, and treatment is primarily aimed at managing complications and preventing nutritional deficiencies. Lifelong vitamin B12 supplementation is essential for patients with pernicious anaemia to prevent neurological complications. Iron supplementation may be required for individuals with iron deficiency anaemia. Proton pump inhibitors are generally avoided in AAG patients due to their potential to exacerbate hypergastrinemia. In cases where H. pylori infection is detected, eradication therapy may be considered to reduce inflammation and possibly slow disease progression[43].

Imerslund-Gräsbeck disease

Imerslund-Gräsbeck syndrome (IGS) is a rare autosomal recessive disorder resulting in vitamin B12 deficiency due to selective malabsorption, despite normal secretion of intrinsic factor and gastric acid. Before diagnosis, affected individuals may exhibit non-specific symptoms such as poor growth, pallor, fatigue, recurrent viral infections, and mild neurological issues. Laboratory findings typically reveal macrocytic anaemia, sometimes accompanied by proteinuria.

First identified in 1960 by Imerslund and Gräsbeck, IGS is linked to mutations in the CUBN or AMN genes, which encode cubilin and amnionless, respectively. These proteins form the cubam receptor in the ileal mucosa, essential for vitamin B12 absorption and renal protein reabsorption. The condition is managed primarily with intramuscular vitamin B12 injections, which usually alleviate symptoms. The estimated prevalence is fewer than six cases per million individuals[47].

Transcobalamin II deficiency

Transcobalamin II deficiency is an autoimmune recessive disorder with onset in early infant i. stage. Transcobalamin II is the chief transport carrier protein system of cobalamin. The Transcobalamin II gene is situated on chromosome 22. It is inherited as an autosomal recessive trait. This disease can potentially initiate as early as 3-5 weeks of early infancy period (both).

i. Clinical features

This deficiency is characterized by failure to thrive, megaloblastic anaemia, pancytopenia, methylmalonic aciduria, recurrent infections, vomiting and diarrhoea. Improvement is observed in treated individuals, however, if not treated, the disease may progress to mental retardation and neurological abnormalities[48, 49]. Cellular and humoral immunologic deficiency is present. Hyperhomocysteinemia, homocystinuria and methylmalonic aciduria.

ii. Diagnosis

Absence of protein capable of binding radiolabelled cobalamin is the diagnosis. Migration with transcobalamin II on chromatography or gel electrophoresis or by immunological techniques the deficiency can be diagnosed. The protein is synthesized by amniocytes. Hence, testing for the presence of protein is possible as prenatal diagnosis.

iii. Treatment

Treatment of Transcobalamin II deficiency is treated with one thousand µg Vitamin B12 intramuscularly one to two times weekly. The serum cobalamin levels to be maintained at 1000 to 10,000 pg/ml.

Megaloblastic anaemia

Megaloblastic anaemia develops due to the impaired DNA synthesis, which inhibits nuclear division. It is a heterogenous group of macrocytic anaemias characterized by the presence of large red blood cell precursors known as megaloblasts in the bone marrow.

i. Etiology

Vitamin B12 deficiency and folic acid deficiency leads to the development of megaloblastic anaemia. The source of folic acid includes green vegetables, fruits, meat and liver. In new born, the daily requirement of folic acid is 65 mcg and in pregnant women, 600 mcg. It can also occur in alcohol use disorder, malnutrition, malabsorption, hemodialysis, hemolysis, and in some cases while taking medications like anticonvulsants, and anticancer drugs.

ii. Pathophysiology

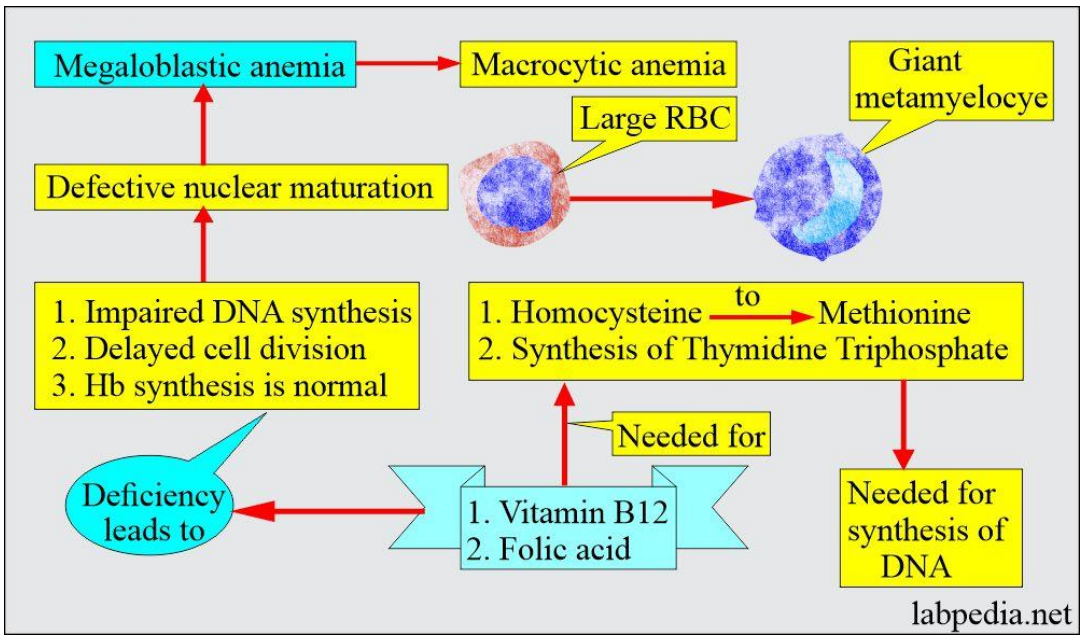


Figure 12: Megaloblastic anemia pathophysiology [50]

The mechanism underlying megaloblastic anaemia is ineffective erythropoiesis due to the premature destruction of hematopoietic cell precursors within the bone marrow, primarily caused by DNA synthesis defects. Both Vitamin B12 and folate deficiencies contribute to the impaired DNA synthesis leading to asynchronous maturation between nucleus and cytoplasm. While haemoglobin synthesis in the cytoplasm proceeds normally, nuclear development is hindered, resulting in cell cycle arrest during the DNA synthesis (S) phase, replication errors and eventual apoptosis.

Folate plays a crucial role in DNA synthesis by donating methyl groups, whereas vitamin B12 is essential for recycling 5-methyl-tetrahydrofolate back to tetrahydrofolate. This process closely linked to the conversion of homocysteine to methionine. A deficiency in vitamin B12 leads to accumulation of 5-methyl-tetrahydrofolate, disrupting the one-carbon metabolism pathway necessary for proper DNA replication.

Since hematopoietic precursor cells undergo rapid division, impaired DNA synthesis disrupts normal blood cell development. This results in abnormal nuclear maturation, enlarged precursor cells in bone marrow, macrocytic red blood cells and hyper segmented neutrophils in peripheral blood. Prolonged deficiency may cause intramedullary haemolysis, leading to bone marrow hypercellularity and peripheral signs of haemolysis, including a low reticulocyte count.

iii. Diagnosis

Megaloblastic anaemia should be suspected in patients with unexplained macrocytosis (MCV >100 fL) or hypersegmented neutrophils on a peripheral smear. An MCV greater than 115 fL is more indicative of vitamin B12 or folate deficiency,

though a normal MCV does not exclude the condition. A reticulocyte count is useful in the diagnostic workup and in cases with characteristic blood smear findings and a low reticulocyte count, testing for serum vitamin B12 and folate levels is typically sufficient. Folate testing may be omitted in individuals with a normal diet but should be included in those with malabsorption disorders or excessive alcohol consumption.

Vitamin B12 levels above 300 pg/mL are considered normal, while levels between 200–300 pg/mL are borderline and require further evaluation. Levels below 200 pg/mL indicate deficiency, and additional testing is necessary only to determine the appropriate method of supplementation. However, serum B12 assays can be inaccurate in patients with intrinsic factor autoantibodies, leading to false-negative results. Additionally, falsely low B12 levels may be observed in conditions such as multiple myeloma, HIV infection, pregnancy, oral contraceptive use, and certain medications, while falsely high levels can occur in myeloproliferative disorders, alcoholic liver disease, and renal disease. Folate deficiency is diagnosed when levels fall below 2 ng/mL, with borderline values ranging from 2 to 4 ng/mL.

For patients with borderline B12 results, methylmalonic acid (MMA) and homocysteine testing can help confirm the diagnosis. MMA is elevated only in B12 deficiency, whereas homocysteine is increased in both B12 and folate deficiencies. However, MMA levels can be falsely elevated in patients with renal insufficiency, making its use unreliable in such cases.

If B12 deficiency is confirmed, intrinsic factor autoantibody testing should be performed to diagnose pernicious anaemia. Though this test has low sensitivity, its high specificity makes a positive result diagnostic. Negative results do not rule out autoimmune causes, as parietal cell antibodies may also be present.

It is crucial to test for both vitamin B12 and folate deficiencies simultaneously, as treating folate deficiency alone without addressing an underlying B12 deficiency can lead to worsening neurological symptoms associated with B12 deficiency.

iv. Treatment

Vitamin B12 and folic acid can be administered either orally or via injection. When malabsorption is not a concern, oral supplementation is preferred. However, oral supplementation requires time to take effect and is not suitable for cases needing urgent treatment. Asymptomatic patients can be managed with oral supplements, while those with neurological symptoms, increased physiological demands (such as during pregnancy or infancy), or malabsorption issues may require parenteral administration. In severe cases of anaemia, blood transfusions may be necessary for immediate symptom relief, as supplementation alone does not correct anaemia quickly. Sublingual vitamin B12 is an alternative for patients with absorption difficulties.

For children, the recommended parenteral dose of vitamin B12 is 50–100 mcg per week until deficiency is corrected, followed by monthly or bimonthly maintenance doses. Adults typically require 1000 mcg weekly via injection until levels normalize, with continued supplementation at monthly or bimonthly intervals. Alternatively, an oral dose of 1000 mcg daily is equally effective, provided there are no absorption issues. A 2018 Cochrane review found that oral supplementation was as effective as intramuscular injections for increasing serum B12 levels and offered a cost-effective alternative. The duration of treatment depends on the underlying cause- if the deficiency is reversible, supplementation can be discontinued once levels normalize. However, lifelong supplementation is necessary for conditions such as pernicious anaemia or post-gastric bypass surgery.

For folic acid deficiency, the standard treatment is 1 mg daily until the deficiency is corrected. If the underlying cause is reversible, supplementation can be stopped after repletion; otherwise, continuous supplementation is required.

With proper treatment, haemolytic markers improve within a week and haemoglobin/haematocrit levels normalize within one to two months. However, neurological symptoms take longer to resolve, ranging from three months to a year and in some cases, they may not fully recover. Studies have shown that early intervention improves neurological outcomes, whereas prolonged or severe deficiency can result in persistent symptoms. Early diagnosis and treatment are crucial in preventing irreversible neurological damage[51].

Understanding functional B12 deficiency

Definition of Functional B12 Deficiency and Low vitamin B12 Levels

Functional B12 deficiency is defined by the presence of elevated levels of MMA and/ or HCys despite serum B12 values well within the normal reference range[12]. However, low vitamin B12 levels is considered when an individual's Vitamin B12 levels reduces below 200 pg/mL[13]. In Functional B12 deficiency, the serum levels of B12 are under the normal range, however, the symptoms of B12 deficiency are present.

Mechanism leading to functional deficiency

Functional vitamin B12 deficiency can develop as a result of oxidative inactivation of vitamin B12, particularly in the presence of conditions associated with heightened oxidative stress.

Interestingly, while vitamin B12 is recognized as a potent antioxidant, its deficiency can still occur despite normal serum levels; this deficiency can be better

indicated by low levels of holotranscobalamin, a marker of active vitamin B12 in the body[12].

The development of functional vitamin B12 deficiency can occur even in the presence of normal serum cobalamin (Cbl) levels, as indicated by the accumulation of cobalamin-dependent metabolites such as methylmalonic acid (MMA) and homocysteine (Hcy). This phenomenon has been termed "subtle" or "subclinical" Cbl deficiency, suggesting a progression in the depletion of vitamin B12 that initially leads to decreased serum levels, followed by metabolite buildup and ultimately resulting in hematologic or neurocognitive disorders over several years.

Maternal and pediatric cobalamin deficiency

Cobalamin deficiency present in the newborn is primarily due to the deficiency of vitamin in the mother. Pregnant women who follow strict vegetarian, vegan or macrobiotic diets are at higher risk of development of Vitamin B12 deficiency as they lack in the vitamin. Another important factor is the role of socioeconomic factor and malnutrition. When the mother is severely malnourished, the risk of deficiency is increased.

Severe deficiency of Vitamin B12 can cause infertility in women. However, when a moderate level of deficiency is present in the mother, it affects the supply of vitamin to the newborn and hence causing the deficiency in the infant. In few cases, maternal antibodies against intrinsic factor crosses the placenta and hinder the infant's cobalamin absorption in early weeks of life.

Apart from diet, certain medical conditions can impair cobalamin absorption in the mother. Gastric surgeries which affect the production of intrinsic factors fish tapeworm infections, bacterial overgrowth and gastrointestinal diseases like Crohn's disease and celiac disease can significantly reduce the absorption of vitamin B12.

Exposure to nitrous oxide which inactivates methionine synthase can also develop deficiency in the mothers.

Newborn evaluating the mother's dietary intake and cobalamin status is crucial as maternal deficiency is most common cause of neonatal cobalamin deficiency[52].

Signs and symptoms

Table 2: Signs, symptoms and risk factors of Vitamin B12 deficiency

Signs of Vitamin B12 deficiency 1. Anemia (typically megaloblastic anemia, but because Vitamin B12 deficiency and iron deficiency often occur together, patients can develop anemias with a range of red cell sizes) 2. Paranoia, hallucinations, delusions (megaloblastic anemia) 3. Decreased bone mineral density (osteoporosis) 4. Orthostatic hypotension 5. Delirium
Symptoms 1. Fatigue 2. Leg weakness or paresthesia, unsteady gait and falls 3. Swallowing difficulties, sore tongue, weight loss and diarrhea 4. Severe depression 5. Excessive sleepiness 6. Incontinence
Risk factors 1. Age 2. Long-term use of proton pump inhibitors (usually prescribed for gastro-esophageal reflux disease or GERD) 3. Diabetes (especially patients taking metformin) 4. H. pylori infection 5. Opioid use 6. Other conditions that slow gastric emptying or bowel transit 7. Untreated celiac disease (gluten intolerance) 8. Gastric or small bowel surgery 9. Tea and toast diet, vegan diet 10. Surgery with nitrous oxide anesthesia or exposure to nitrous oxide 11. Cigarette smoking as well as those exposed to cigarette

(Source: Olders H 2011[53])

The common signs and symptoms presented in the deficiency of Vitamin B12 is tabulated in Table 2.

Figure 13: Knuckle hyperpigmentation in Vitamin B12 deficiency

(Source: Srivastava A et al 2020[54])

Clinical features like knuckle hyperpigmentation, vitiligo, angular cheilitis, and hair & nail changes are important in diagnosing Vitamin B12 deficiency[55].

Significance of Vitamin B12 deficiency in mother and baby

Vitamin B12 plays a pivotal role in DNA synthesis and metabolism of fatty acids and amino acids. As a cofactor for enzymatic reactions, it is involved in converting homocysteine to methionine and in the metabolism of methylmalonyl-CoA. Given its significance, maintaining adequate levels of vitamin B12 is vital for overall health, especially in populations at risk for deficiency, such as the elderly or

those with absorption issues. Health interventions to ensure sufficient intake and management of vitamin B12 deficiency include dietary adjustments, supplementation, and monitoring for potential complications. Understanding the importance of vitamin B12 is essential for the prevention and treatment of associated health conditions[27].

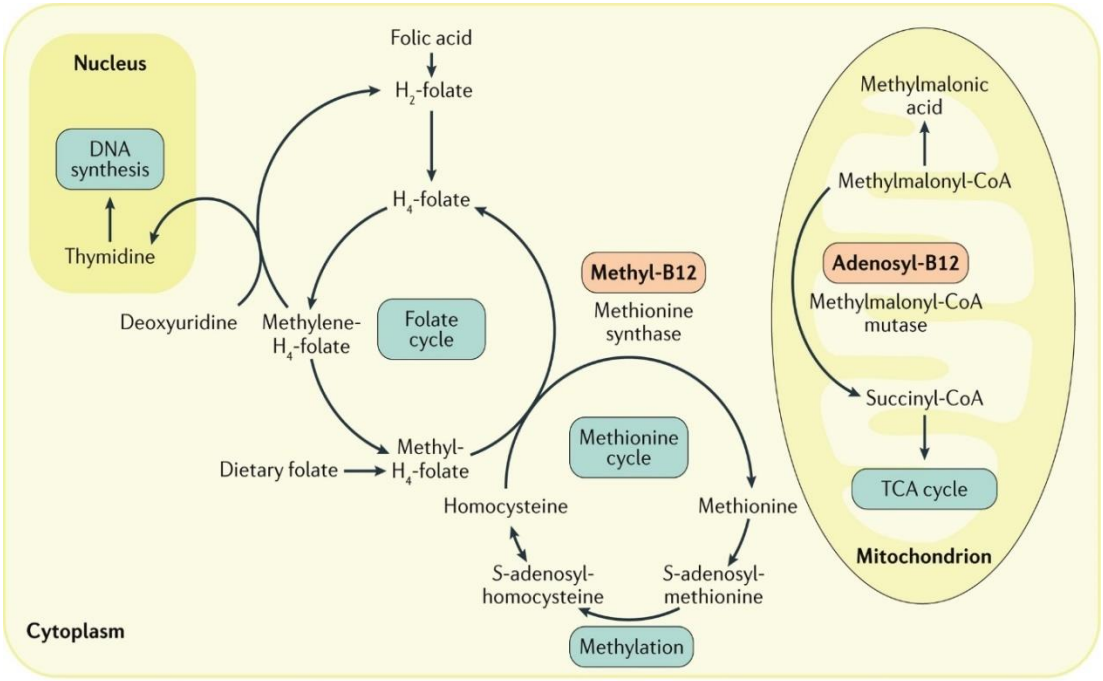


Figure 14: Vitamin B12 and folate metabolism & function

(Source: Green R et al[34])

Vitamin B12 plays a crucial role in DNA synthesis and genome stability by serving as a cofactor for methionine synthase (MS), an enzyme that converts homocysteine into methionine. Methionine is further processed into S-adenosyl-L-methionine (SAM), a key methyl donor involved in DNA methylation. Proper DNA methylation is essential for gene regulation, genomic imprinting, and the suppression of transposable elements. Disruptions in this process, caused by Vitamin B12 deficiency, can result in abnormal methylation patterns, leading to diseases such as rheumatoid arthritis, type 1 diabetes and metabolic syndrome.

Beyond its role in methylation, Vitamin B12 also supports redox balance by functioning as an antioxidant, helping to neutralize reactive oxygen species (ROS) and reduce oxidative stress. This protective function minimizes DNA damage, prevents apoptosis and maintains glutathione levels which is an important cellular antioxidant. However, excessive or insufficient Vitamin B12 levels can lead to DNA hypomethylation, which is linked to various disorders, including cancer. Vitamin B12-deficient individuals may be at a higher risk of developing stomach and other cancers due to increased DNA damage and impaired methylation.

Furthermore, Vitamin B12 influences nucleotide metabolism by regulating the availability of 5,10-methylene tetrahydrofolate (THF), a key component in thymidine synthesis. A deficiency in Vitamin B12 can lead to reduced levels of thymidine monophosphate (dTMP), causing DNA strand breaks and chromosomal instability. This functional folate deficiency increases the risk of carcinogenesis and other genetic disorders.

Overall, Vitamin B12 is essential for maintaining DNA integrity through its roles in methylation, oxidative stress management and nucleotide metabolism. Adequate intake of Vitamin B12 is vital for preventing genomic instability, reducing cancer risk and supporting overall cellular function[52].

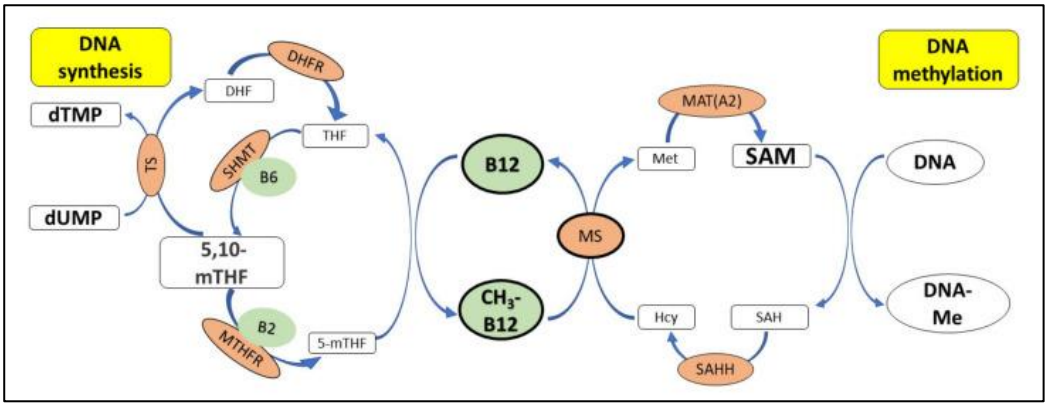


Figure 15: Role of Vitamin B12 in synthesis and methylation of DNA

(Source: Halczuk K et al[52])

Complications of Vitamin B12 deficiency

Cellular and molecular consequences

A common outcome of vitamin B12 deficiency and genetic disorders affecting its metabolism is a cellular shortage of one or both active coenzyme forms of B12, specifically adenosyl-B12 and methyl-B12. At the molecular level, a lack of vitamin B12 disrupts methylation processes and impairs the metabolism of methylmalonate, which is produced during the breakdown of certain amino acids and fatty acids. Methyl-B12 deficiency leads to the accumulation of homocysteine and reduces the synthesis of methionine and S-adenosylmethionine, resulting in cellular stress, apoptosis and the homocysteinylation of functional proteins within blood and tissues. S-adenosylmethionine acts as a methyl donor necessary for epigenetic modifications, including the methylation of DNA and histones that regulate gene expression. On the other hand, adenosyl-B12 deficiency results in the buildup of MMA, although the consequences of this accumulation are not well understood.

Recent evidence indicates a strong relationship between vitamin B12 deficiency and cellular stress, characterized by decreased expression of NAD+-dependent protein deacetylase sirtuin 1 (hSIRT1), which leads to increased acetylation of heat shock factor 1 (HSF1) and impaired synthesis of heat shock proteins. Additionally, fibroblasts from individuals with cobalamin C deficiency, caused by mutations in the MMACHC gene, showed altered expression of genes linked to oxidative stress, including those coding for heat shock proteins and proteins involved in the glutathione pathway. The heightened production of reactive oxygen species (ROS) contributes to endoplasmic reticulum stress and cell death.

The specific causes of neurological complications associated with vitamin B12 deficiency are still under investigation. The decreased production of succinyl-CoA and the accumulation of MMA might theoretically contribute to neurological symptoms through the formation of odd-chain and methyl-branched fatty acids; however, there is limited evidence to substantiate this theory. Research on inherited defects suggests that a deficiency in methyl-B12 or inhibition of methionine synthase is primarily responsible for the neurological damage linked to B12 deficiency. Furthermore, inflammation, oxidative stress and microvascular disease related to hyperhomocysteinemia could also play a role in these complications.

Low levels of vitamin B12 and elevated homocysteine concentrations are associated with reduced methylation of gene promoters linked to amyloid precursor protein and γ-secretases, leading to increased production of amyloid-β. Administration of S-adenosylmethionine has been shown to reverse these effects and improve spatial memory performance. Vitamin B12 deficiency is also linked to increased expression of protein phosphatase 2A, nerve growth factor, and tumor necrosis factor, along with decreased expression of epidermal growth factor in cell and animal models. These altered expressions are consistent with vitamin B12's influence on myelin homeostasis and the amyloid and tau pathways in neurodegenerative diseases[34].

Table 3: Complications of Vitamin B12 deficiency

Consequences of Vitamin B12 deficiency in infants 1. Feeding difficulties 2. Regurgitation 3. Constipation 4. Apathy 5. Irritability 6. Twitching, tremors and myoclonic jerks 7. Slow growth, small head circumference and brain lesions 8. Developmental delay, including reduced gross motor development, smiling and babbling 9. Pancytopenia
Consequences of low maternal B12 status or deficiency in infants 1. Neural tube defects reported in infants of mothers with low B12 status in folic acid-fortified and non-fortified populations. 2. Effects on infant development, including stunting, cerebral atrophy, hypotonia, lethargy, development delays and abnormal electroencephalogram 3. Contribution to adult cardiovascular disease, neurodegenerative disorders and psychiatric illness through increased levels of circulating homocysteine Contribution to the development of diabetes mellitus through effects on insulin and lipid metabolism.
Consequences of low B12 status or deficiency in adults 1. Megaloblastic anemia or macrocytic anemia 2. Subacute combined degeneration of the spinal cord 3. Impaired sensory and peripheral nerve function 4. Cognitive impairment 5. Depression 6. Bone disease 7. Hearing loss Macular degeneration

(Source: Green R et al[34])

Neurological manifestations

Vitamin B12 deficiency impacts the nervous system by causing demyelination of both peripheral and central neurons, which is believed to be the underlying mechanism of the characteristic myeloneuropathy associated with this deficiency. The long white matter tracts in the spinal cord, particularly in the posterior and lateral columns that contain sensory neurons responsible for transmitting vibration and positional information, are especially vulnerable to demyelination, although motor neuron myelination can also be compromised. Neurological symptoms may emerge before any hematological changes, and can even arise without hematological complications.

Maintaining adequate vitamin B12 levels is essential for proper neurodevelopment, as evidenced by the clinical manifestations seen in children with inherited B12 metabolism disorders. The first few months after birth represent a critical and sensitive phase for brain development. The specific signs and symptoms of vitamin B12 deficiency in children vary based on age, severity and duration of the deficiency. A double-blind randomized intervention study demonstrated functional motor impairments in infants displaying biochemical markers of moderate B12 deficiency. Administering a single intramuscular dose of 400 μg of hydroxycobalamin resulted in notable biochemical repletion and improvements in motor function and regurgitation, highlighting the importance of sufficient B12 for developing nervous systems.

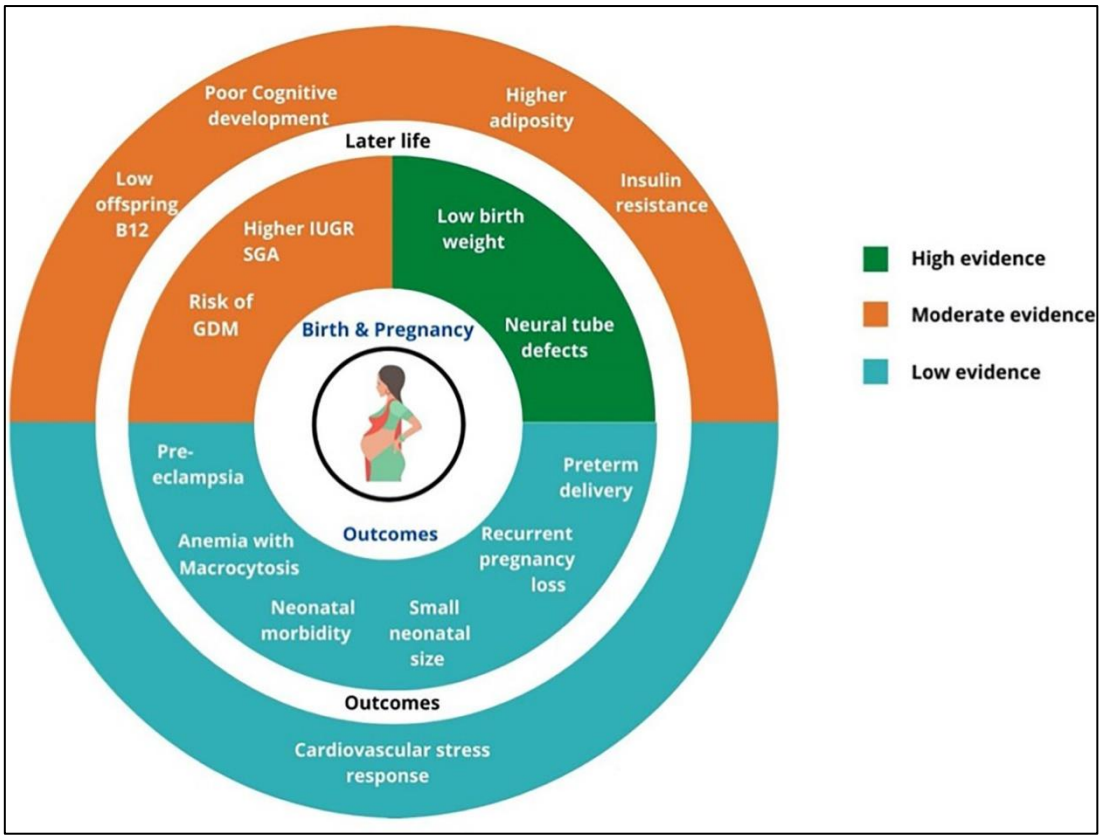


Figure 16: Neonatal outcomes of pregnant women with Vitamin B12 deficiency

(Source: Behere R V et al 2021 [56])

Although treatment for deficient infants and toddlers usually leads to rapid improvements in motor development and clinical symptoms, prolonged deficiency can cause lasting developmental disabilities, even with optimal intervention. Similar to folate deficiency, low maternal B12 levels during pregnancy and lactation can adversely affect offspring, potentially leading to conditions such as neural tube defects. While the effects of subclinical low B12 status in adults are not entirely understood, evidence suggests that persistent deficiency may elevate the risk of several age-related chronic diseases. Low B12 levels are a primary modifiable factor contributing to increased plasma homocysteine levels in populations supplemented with folic acid. This is particularly relevant for older adults, as higher homocysteine levels linked to low B12 status are associated with accelerated brain atrophy and

increased risk of cognitive impairment. Analysis of NHANES data indicates a greater likelihood of anemia and cognitive issues in older adults with low serum B12 levels and elevated serum folate compared to those with low B12 without increased folate levels. Furthermore, while folic acid supplementation can partially alleviate hematological complications due to impaired DNA synthesis in B12-deficient patients, it does not address or may even worsen neurological complications[34].

Hematological manifestations

The hematological consequence of vitamin B12 deficiency is megaloblastic anemia, which arises from the disruption of DNA synthesis. A deficiency in B12 leads to impaired synthesis of H4 folate, which restricts the availability of the necessary form of folate required for producing thymidylate and DNA. This shortage results in the incorporation of dUTP instead of thymidine triphosphate during nucleic acid synthesis. Tissues that undergo rapid cellular turnover, particularly the hematopoietic system, are especially impacted. The unregulated growth of dividing bone marrow cells results in the formation of unusually large cells with immature nuclear chromatin. This primarily affects erythroid precursors, leading to anemia characterized by the presence of large red blood cells known as macrocytes. Other hematopoietic cells may also demonstrate abnormal development, resulting in oversized granulocyte precursors in the bone marrow and neutrophils in the blood with an excessive number of nuclear lobes, referred to as hyper segmented neutrophils. Furthermore, in addition to anemia, there may also be a reduction in the overall number of blood cells, resulting in pancytopenia[34].

Diagnosis of cobalamin deficiency

Complete blood count

When a vitamin B12 deficiency is suspected, initial diagnostic tests typically include a complete blood count (CBC) with a peripheral blood smear, along with measurements of serum B12 and folate levels. If the initial findings are inconclusive, additional tests such as methylmalonic acid (MMA) and homocysteine levels may be necessary.

A CBC often reveals anaemia in individuals with Vitamin B12 deficiency, characterized by reduced haemoglobin and haematocrit levels. Additionally, the mean corpuscular volume (MCV), which indicates red blood cell size, is usually elevated above 100 fL, signifying macrocytic anaemia. A peripheral blood smear can further support the diagnosis by showing hyper segmented neutrophils, where some neutrophils have five or more lobes.

Since folate deficiency can also cause macrocytic anaemia, testing both serum B12 and folate levels helps distinguish between the two conditions. A serum B12 level above 300 pg/mL is considered normal, while levels between 200 and 300 pg/mL are borderline, often requiring further enzymatic testing. A B12 level below 200 pg/mL confirms deficiency, but additional tests may be needed to identify the underlying cause.

Biomarkers

To further differentiate between B12 and folate deficiencies, MMA and homocysteine levels are assessed. Both markers are typically elevated in B12 deficiency, whereas in folate deficiency, only homocysteine levels increase while MMA levels remain normal.

Once B12 deficiency is confirmed, identifying its cause is essential. A detailed medical history, including prior gastrointestinal surgeries such as gastrectomy, ileal resection or gastric bypass, can indicate malabsorption as the underlying issue. Conditions like Crohn's disease and celiac disease should also be considered. In cases where dietary intake is suspected, strict veganism may be a contributing factor.

If neither gastrointestinal issues nor dietary insufficiency explain the deficiency, an autoimmune origin is likely. Testing for anti-intrinsic factor antibodies can help diagnose pernicious anaemia, an autoimmune condition that impairs B12 absorption. Previously, the Schilling test was used for this diagnosis, but it is no longer in practice. This test involved administering radio labelled B12 and measuring its urinary excretion to assess absorption efficiency.

Peripheral smear

Through a combination of blood tests, peripheral smears and a thorough medical history, healthcare providers can accurately diagnose vitamin B12 deficiency and determine its root cause, ensuring appropriate treatment and management[13].

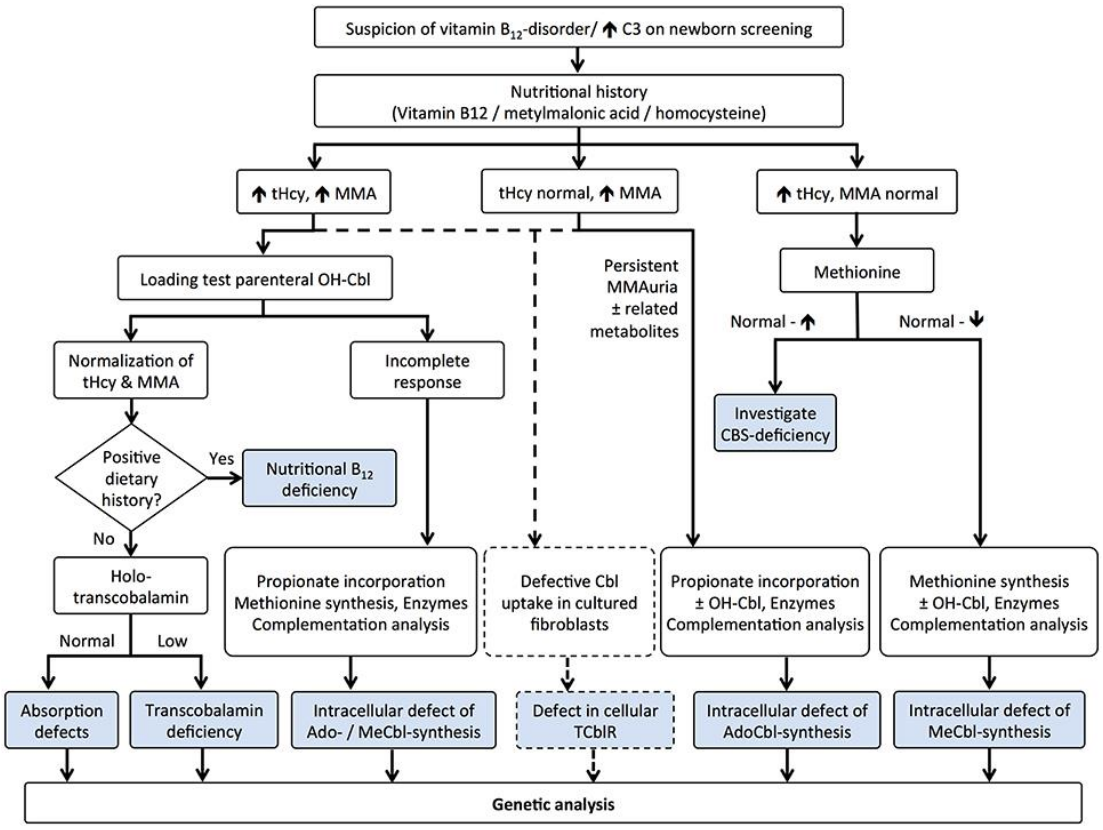


Figure 17: Diagnosis of Vitamin B12 deficiency in newborns (Source: Hannibal L et al 2016 (57))

Pitfalls on diagnosis based on Vitamin B12 levels

Serum vitamin B12 measurement is the most commonly used diagnostic test, but it has significant limitations. While a low serum B12 level (<200 pg/mL) is generally indicative of deficiency, levels in the borderline range (200–300 pg/mL) may not provide a definitive diagnosis. Additionally, serum B12 levels do not always correlate with functional deficiency, as they do not assess whether the vitamin is biologically active or effectively utilized at the cellular level. Several conditions can interfere with serum B12 levels, leading to misinterpretation:

- **False negatives (normal B12 despite deficiency)**: Liver disease, renal failure, pregnancy and autoimmune disorders can cause falsely normal or elevated

B12 levels despite an underlying deficiency.

- **False positives (low B12 without deficiency)**: Conditions like folate deficiency and oral contraceptive use may lower serum B12 levels without true deficiency.

To improve diagnostic accuracy, additional tests such as holotranscobalamin (holoTC), methylmalonic acid (MMA), and homocysteine levels are used. However, these tests also have limitations: Holotranscobalamin (holoTC) measures the active form of B12, but lacks widespread availability and standardization. Methylmalonic Acid (MMA) measures elevated levels indicate functional B12 deficiency, but MMA is also increased in renal disease, reducing its specificity. Homocysteine measures elevated homocysteine levels suggest B12 or folate deficiency, but they can also rise due to other factors such as kidney disease and vitamin B6 deficiency[58].

Metabolic markers such as MMA and total homocysteine (tHcy) serve as more reliable indicators of B12 deficiency. These compounds accumulate in the bloodstream when B12-dependent enzymatic processes are impaired. Specifically, MMA increases due to disruptions in methylmalonyl-CoA metabolism, while elevated tHcy indicates a defect in the methionine cycle, which depends on methylcobalamin. These markers can detect early functional B12 deficiency, even before serum B12 levels drop below the normal range, making them valuable diagnostic tools.

Additionally, methylcitric acid (MCA) has been investigated as a potential biomarker for B12 deficiency. MCA accumulates due to impaired conversion of propionyl-CoA in the Krebs cycle. However, its clinical use remains limited due to complex analytical challenges, such as its high polarity and difficulty in detection[59].

Treatment of cobalamin deficiency

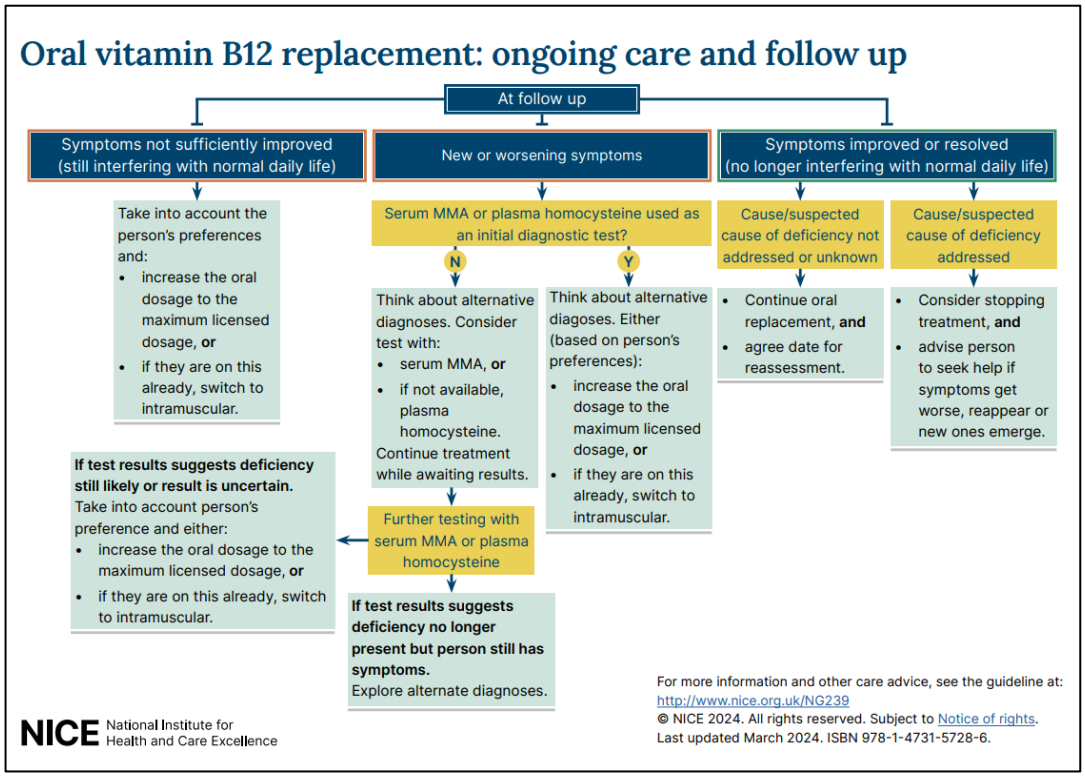


Figure 18: Treatment for Vitamin B12 deficiency

(Source: NICE)

Preventive strategies for managing B12 deficiency primarily focus on promoting the intake of B12-rich foods. Vegetarians may struggle to meet their B12 needs since the primary sources are animal-derived; however, dairy products and eggs can provide adequate B12. The use of fortified foods, such as wheat flour, bread, milk, and breakfast cereals, is also a potential strategy for improving B12 intake across populations. The introduction of probiotics has shown limited success in boosting B12 status. It is crucial to conduct trials in regions where B12 deficiency poses a significant public health concern, particularly as high levels of folate, commonly consumed through supplementation, can exacerbate B12 deficiency among individuals with low dietary intake of B12-rich foods. The mechanisms behind this

adverse interaction between high folate levels and B12 status require further investigation[34].

Severe Deficiency

Severe anemia can lead to heart failure due to sodium retention, myocardial hypoxia, and reduced oxygen delivery. Initial management includes oxygen therapy, diuretics, and slow red blood cell (RBC) transfusions to prevent circulatory overload. Historically, a significant mortality rate was associated with the early treatment of severe pernicious anemia, but recent studies do not confirm these findings. Life-threatening complications such as hypokalemia, strokes, and thrombotic events can occur during recovery. To mitigate these risks, an initial subcutaneous dose of 10 μg cyanocobalamin (CNCbl) is recommended for two days in adults, while children require 0.2 μg/kg/day. This regimen helps stabilize metabolic markers but does not fully restore vitamin B12 stores.

Hypokalemia in these cases results from potassium shifting intracellularly and delayed renal retention. Clinicians should anticipate and manage this with potassium supplementation. The role of hyperhomocysteinemia in thrombosis risk during recovery remains uncertain. Children with severe cobalamin deficiency may experience similar complications.

Correction of megaloblastosis requires 15–150 μg of CNCbl. Standard treatment includes daily injections of 1000 μg of CNCbl or hydroxocobalamin (OHCbl) for one week, followed by weekly 100 μg doses for a month, then monthly maintenance. This regimen restores and maintains cobalamin levels effectively. Recent comparisons between oral and injectable therapy show similar outcomes in correcting anemia. Oral therapy, with 1–2 mg of cobalamin daily, is a cost-effective

and well-tolerated option used in many countries. However, its effectiveness in resolving neurological symptoms as quickly as injections remains unclear.

Some slow-release cobalamin tablets have dissolution times of 3–6 hours, but they lack sufficient study to confirm efficacy. Patients with neurological involvement should initially receive an injection to ensure prompt delivery to the central nervous system. Dementia and depression often improve quickly with treatment, while other neurological symptoms may take six months or longer to resolve. In some cases, complete neurological recovery has been observed within weeks, though severe symptoms may persist despite treatment.

Patients with pernicious anemia require lifelong therapy. A monthly subcutaneous dose of 100 μg CNCbl is standard, but some patients may need higher doses. OHCbl provides sustained tissue binding, allowing for 1000 μg injections every three months or a 14-day course every 6–12 months. Oral supplementation of 1–2 mg daily is also an option, with lower doses for children. Some patients can maintain normal serum cobalamin levels by taking 50–200 μg of CNCbl daily, particularly when consumed away from meals.

Rarely, antibodies may develop against the transcobalamin-cobalamin complex, leading to falsely elevated plasma cobalamin levels without adverse health effects. Cobalamin resistance, where high-dose supplementation is needed despite normal serum levels, has been observed in diabetes, renal failure and aging.

For dietary-related malabsorption, daily doses of 10–25 μg CNCbl can maintain normal cobalamin levels, with periodic monitoring of serum cobalamin, methylmalonic acid and homocysteine. Children with inherited cobalamin metabolism disorders require OHCbl injections (1000 μg) two to three times per week, with

effectiveness monitored through serum homocysteine, methylmalonic acid and methionine levels[60].

Trivandrum Development Screening Test

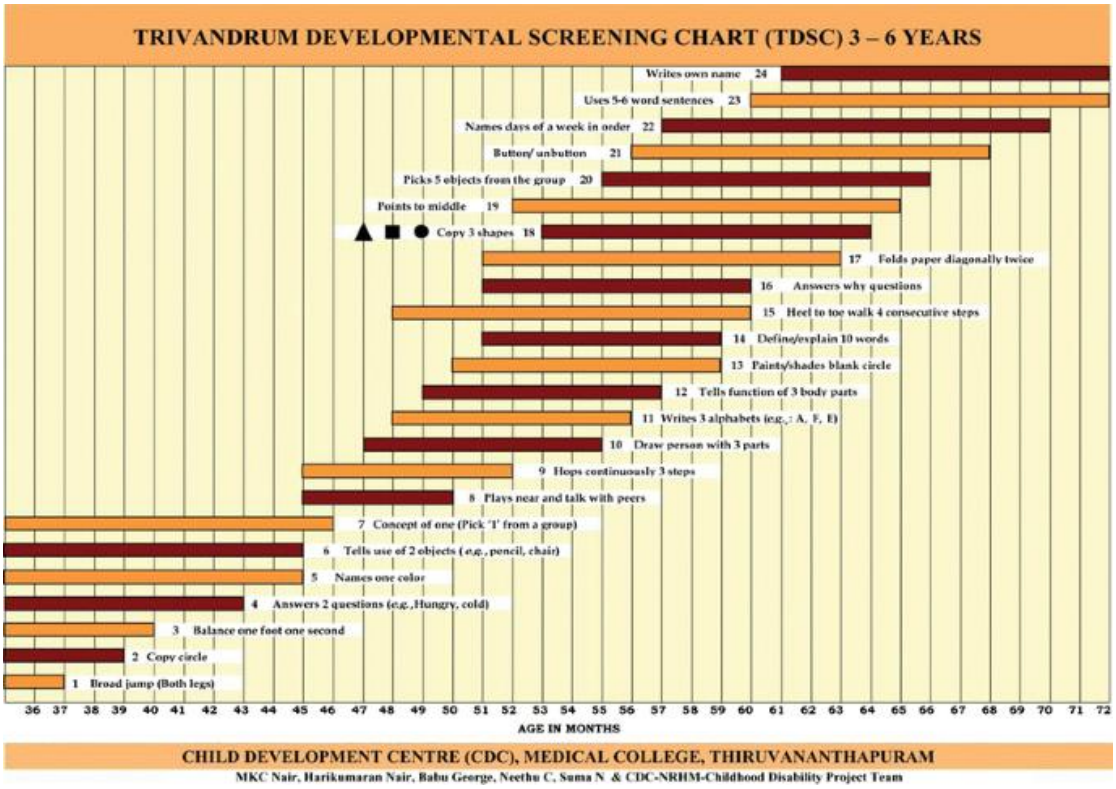


Figure 19: Trivandrum Development screening test for children from 3-6 years

The Trivandrum Developmental Screening Chart (TDSC) for children aged 0–6 years was developed at the Child Development Centre, Government Medical College, Thiruvananthapuram. It consists of 51 key developmental milestones selected after thorough discussions with experts, including pediatric neurologists, child psychiatrists, developmental pediatricians, speech therapists and epidemiologists. The chart covers cognitive and motor milestones and includes assessments for hearing, vision, speech, and language. Whenever possible, items were chosen from standardized tools with normative validation.

The age range for each milestone is represented by a horizontal dark line, based on norms from established developmental assessment tools such as the Bayley Scale of Infant Development (Baroda norms), Developmental Assessment Scale for Indian Infants (DASSII) and Jamaica Portage Guide to Early Education. Some additional items were included from the Denver Developmental Screening Test II (DDST-II) and the Nursery Evaluation Scale Trivandrum (NEST).

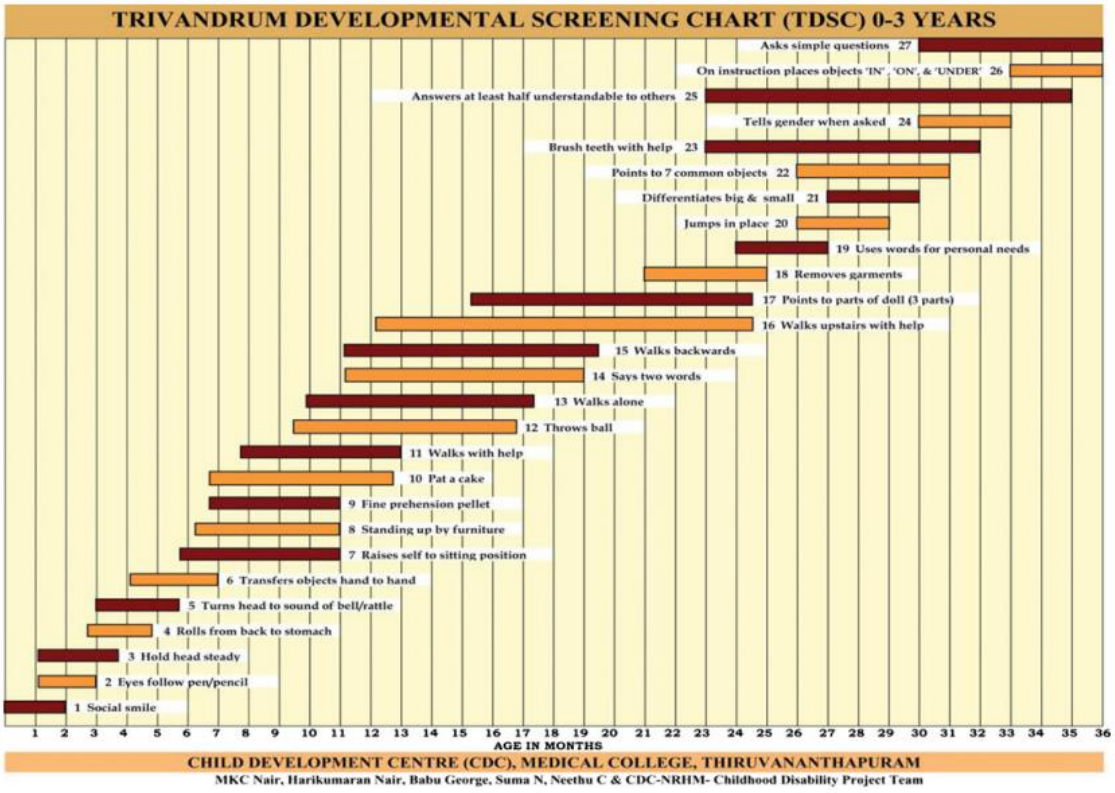


Figure 20: Trivandrum Development screening test for children from 0-3 years

To use the tool, the child's chronological age is determined and a vertical line is drawn at that point on the chart. Milestones with an upper age limit ending to the left of this line should typically be achieved by the child. If a child has not attained a milestone by the expected age, developmental delay is suspected. The tool is designed for ease of use and does not require specialized training, making it more accessible compared to DDST.

Prematurity corrections are not included in the chronological age calculation to maintain simplicity. While adjusting for prematurity could enhance specificity and predictive value, the developers opted against it to ensure the tool remains user-friendly, even for Anganwadi workers and community health workers[61].

Relevant studies

Study conducted by Kadiyala A et al 2021 aimed to examine serum vitamin B12 levels in exclusively breastfed (EBF) infants aged 1 to 6 months and to identify potential risk factors for deficiency. Vitamin B12 is not synthesized in the body and is primarily obtained from non-vegetarian dietary sources. Mothers in resource-limited countries who follow mainly vegetarian diets often produce breast milk that lacks sufficient vitamin B12, which can lead to deficiencies in their infants and negatively impact neurodevelopmental outcomes. Conducted as a cross-sectional study in a well-baby clinic, the research enrolled 149 otherwise healthy EBF infants, with an average age of 3.1 months. Blood samples were collected to measure serum vitamin B12 levels and complete blood counts. The findings revealed that 63.7% of the infants had low serum vitamin B12 levels, defined as below 200 pg/ml, with an average serum level of 199.91 pg/ml. The multivariate analysis indicated that no significant risk factors were identified for B12 deficiency among the infants studied. The study highlights a concerning prevalence of vitamin B12 deficiency in EBF infants, emphasizing the necessity to address this issue to support proper neurological development while promoting the benefits of exclusive breastfeeding[4].

Study conducted by Mittal M et al 2017 aimed to evaluate the vitamin B12 status of healthy exclusively breastfed Indian infants aged 1 to 6 months, along with their mothers. A total of 100 term infants in this age group visiting the pediatric

outpatient department were included in the research. Blood samples were taken from both infants and their mothers to measure hemogram parameters, serum B12, folate and ferritin levels. The results indicated that 57% of the infants were vitamin B12 deficient, with 46% of mothers also showing deficiency. Additionally, a positive correlation was observed between the B12 levels of infants and their mothers (r = 0.23). The findings highlight a significant prevalence of vitamin B12 deficiency among Indian infants and their mothers, underscoring the urgent need for targeted supplementation. Antenatal supplementation is suggested as the optimal time for addressing this deficiency to ensure better health outcomes for both mothers and their infants[22].

Study conducted by Tangeraas T et al (2023) conducted a retrospective study which focused on newborns identified with confirmed B12 deficiency from NBS records between 2012 and 2021. Out of 552,970 screenings, 31 newborns were diagnosed with B12 deficiency, with 25 cases identified from 61 false-positive results for methylmalonic acidemia and propionic acidemia (PA), and six infants screened positive for other metabolic disorders with normal propionylcarnitine (C3) levels. In the original DBS samples, a considerable percentage of B12-deficient newborns with false-positive results for methylmalonic acidemia and PA had MMA and tHcy levels above the 99th percentile. The study highlights that B12 deficiency often serves as a significant differential diagnosis in screenings that indicate methylmalonic acidemia and PA. However, the C3 test did not adequately identify a group of newborns with B12 deficiency. Additionally, the analyses of MMA and tHcy in DBS exhibited limited sensitivity in detecting B12 deficiency. The researchers emphasize the need to recognize the limitations of NBS when addressing B12 deficiency as a key focus of screening panels[62].

Lipari Pinto P et al in 2022 conducted a study, which evaluates the effectiveness of early detection of asymptomatic B12 deficiency associated with acquired conditions and underscores the importance of monitoring serum B12 levels during pregnancy. The research involved 12 exclusively breastfed newborns (5 males), aged 1 to 2 months, who were asymptomatic but referred to a metabolic unit due to abnormal results in expanded newborn screening, specifically high levels of methylmalonic acid and/or total homocysteine (tHcy). All mothers followed a vegetarian diet, except for three who had issues with B12 absorption, and all exhibited low or borderline serum B12 levels along with elevated plasma tHcy. Oral supplementation with vitamin B12 effectively restored metabolic balance in the mothers. For the infants, an intramuscular injection of 1.0 mg hydroxocobalamin resulted in rapid normalization of metabolic patterns, leading to healthy outcomes. The study suggests that acquired B12 deficiency should be considered in the differential diagnosis of conditions like cobalamin metabolism deficits, methylmalonic acidemia and homocystinuria before concluding a definitive diagnosis[8].

A study focuses on the maternal characteristics related to vitamin B12 deficiency identified through newborn screening. Conducted as a prospective single-center study, it employed a systematic screening approach using a combination of two second-tier strategies to detect vitamin B12 deficiency. A total of 121 mother-infant pairs were evaluated, along with assessments of factors such as the mother's ethnic background, dietary habits and vitamin supplementation during pregnancy. Remarkably, 66% of mothers reported following a balanced diet that included meat. In cases of maternal vitamin B12 deficiency, the cause was undetermined in 56% of instances, with dietary factors accounting for 32% and organic causes for 8%. Most mothers on a vegan diet and many on a vegetarian diet, took vitamin supplements,

while only 55.8% of those on a balanced diet reported taking folic acid or other vitamins. Significant correlations were found between maternal vitamin B12, folic acid, and homocysteine levels with the corresponding levels in infant's blood samples from the first and second newborn screenings. Most child participants exhibited normal blood counts with normocytosis, although 36.7% of mothers showed signs of anemia and only one case of macrocytosis was recorded. The study highlights the low adherence to vitamin supplementation during pregnancy, despite recommendations for folic acid intake. It suggests that evaluating maternal vitamin B12 levels and providing appropriate treatment should begin early in pregnancy. A multidisciplinary approach to assess and treat both mothers and infants identified through newborn screening is strongly recommended[63].

MATERIALS AND METHODS

Source of Data

The study was carried out in Pediatric Hematology OPD, Pediatric OPD & Well baby clinic and in mothers, who gave consent for the study in Dr. Prabhakar Kore hospital and MRC, Belagavi.

Study Design

Cross sectional study

Study Period

1year

Sample Size

Sample size calculation

Sample size was calculated assuming the proportion of B12 Deficiency as 57% as per the study by Mittal M et al[22].

The other parameters considered for sample size calculation were 5% absolute precision and 95% confidence level. The following formula was used for sample size calculation. Based on the previous hospital records, the approximate number of potential Eligible subjects to be attending the study setting during the data collection period were considered as 100. Hence a finite population correction was applied for 100.

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where *n'* = Sample size

N= Population Size= 100

Z= Z statistic for a level of confidence level= 1.960

P = Expected prevalence/proportion of outcome= 0.57

d = Precision= 0.05

The required sample size as per the above-mentioned calculation was 79. To account for a non-participation rate/ loss to follow up rate of about 5%, another 4 subjects were added to the sample size. Hence the final required sample size was 83.

Inclusion Criteria

- Infants between 1 to 6 months of age attending pediatric Hematology OPD, Pediatric OPD & Well baby clinic pediatric OPD for immunization or with mild symptoms.
- Patients who gave consent to participate including their baby in the study.

Exclusion Criteria

- Those with severe illness at the time of presentation.
- Those on oral hematinics.
- Those diagnosed with Inborn Errors of Metabolism.
- Those who had received blood transfusion in the past 3 months.
- Parents not consenting for the study.

Study protocol

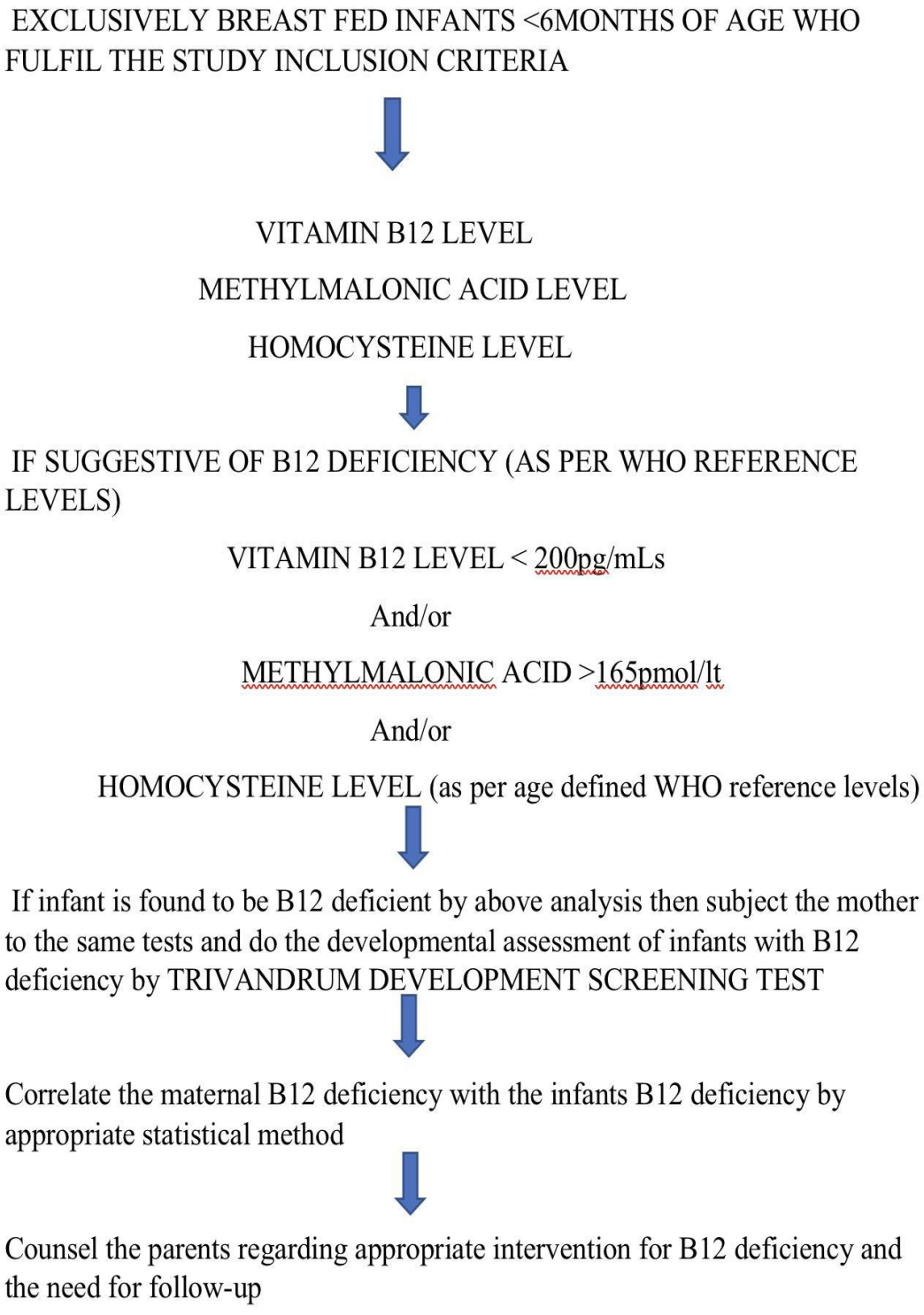


Figure 21: Flowchart of study methodology

Methodology

ELISA (Enzyme-linked Immunosorbent Assay)

ELISA kit was used to assess the Vitamin B12 levels in sample analytes either by using serum, plasma or cell culture supernatant.

Principle of ELISA for measuring Vitamin B12

The kit used in the present study to quantify the Vitamin B12 levels used Sandwich ELISA technique. Human Vitamin B12 monoclonal antibodies were pre-coated onto microwells. Samples and standards were pipetted into microwells and Human Vitamin B12 present in the sample are bound by the antibodies. Biotin labelled Vitamin B12 antibody was added and followed by Streptavidin-HRP was pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) was added to microwells and color develops proportionally to the amount of Human Vitamin B12 in the sample. Color development was then stopped by addition of stop solution. Absorbance was measured at 450 nm.

Materials and Reagents

- Human Vitamin B12 antibody coated microtiter plate (8*12 wells)
- Standard, Human Vitamin B12 (concentrated 1.6mmol)
- Biotinylated Vitamin B12 antibody
- Streptavidin: HRP Conjugate
- Standard diluent
- Sample Diluent
- Wash buffer

- TMB substrate
- Stop solution
- Microtiter plate reader able to measure absorbance at 450 nm.
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 to 1000 µl
- Deionized water
- Wash bottle or automated microplate washer
- Graph paper
- Timer
- Absorbent paper

Handling and storage

- All reagents were stored and indicated on the component label.
- All the reagents and wash solutions were used within 12 months from manufacturing date.
- Before using, all the components were brought to the room temperate (18 to 25 °C).

Sample preparation

- Specimens used were clear and non-hemolyzed.
- Samples were run at a number of dilutions to ensure accurate quantitation.
- Extracted as soon as possible after specimen collection was done. The samples were tested as soon as possible after extraction. Alternatively, the extracted samples were kept in -80°C. Avoided repeated freeze-thaw cycles.
- Serum-Coagulated at room temperature for 10 to 20 minutes, centrifuged for

20 minutes at 2000 to 3000 rpm. Removed the supernatant. If precipitation appeared, recentrifuged.

- Plasma-Used EDTA or citrate plasma as an anticoagulant, mixed for 10 to 20 minutes, centrifuged for 15 minutes at 2000 to 3000 rpm. Removed supernatant. If precipitation appeared, recentrifuged.

Reagent preparation

- All reagents were brought to room temperature before use.
- To make wash buffer, dilute 25 ml of 20 x wash buffer in 475 ml of diluted water.
- Standard preparation: Diluted 120 µl of original standard 1.6 pmol/ml with 120 µl of standard diluent to generate a 0.8 pmol/ml standard stock solution. Kept the standard for 15 minutes with gentle agitation before making further dilutions.

ELISA Procedure

- All the standards and samples were run in duplicates.
- Added 50 µl standard diluent to respective blank wells.
- Added 50 µl prepared standards to respective standard wells.
- Added 40 µl samples to respective sample wells.
- Pipetted 10 µl biotinylated vitamin B12 antibody to respective sample wells.
- Pipetted 50 µl streptavidin: HRP conjugate to all wells. Mix well.
- Covered the plate with a sealer and incubated for 60 minutes at 37°C.
- Aspirated and washed plate 4 times with diluted wash buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wiped off any liquid from the bottom outside of the microtiter wells as any residue can

interfere in the reading step.

- Pipette 100µl TMB substrate to all wells.
- Incubated the plate at 37°C for 10 minutes. Positive wells turned bluish in color.
- Pipetted 100 µl of stop solution to all wells. The wells turned from blue to yellow in color.
- Read the absorbance at 450nm with a microplate within 10 to 15 minutes after addition of stop solution.

Calculation of results

Determined the mean absorbance for each set of duplicate standards and samples. using graph paper, plotted the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Drawn the best fit curve through the standard points. To determine the unknown human Vitamin B12 concentrations, findings the unknown's mean absorbance value on the Y-axis and a horizontal line was drawn to the standard curve. At the point of intersection, a vertical line was drawn to the X-axis and read the human vitamin B12 concentrations.

If samples were diluted, it was multiplied by the appropriate dilution factor. Software which was able to generate the cubic-spline curve-fit or -PL is best recommended for automated results.

Figure 22: ELISA Kit

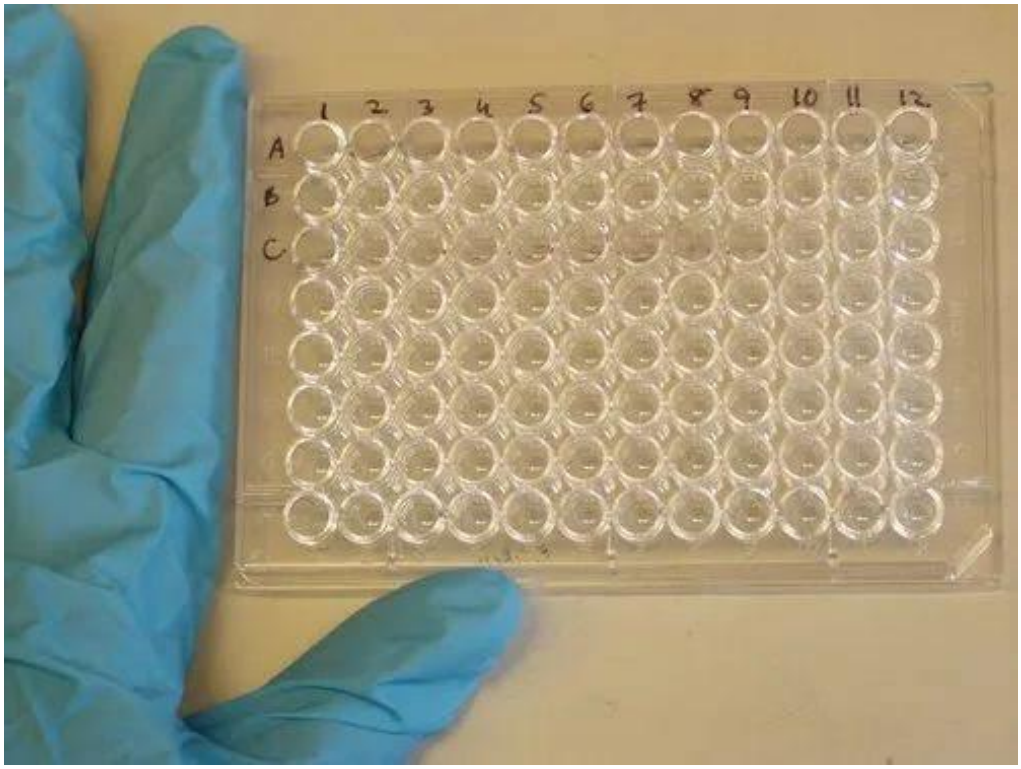


Figure 23: ELISA Kit wells

Hcy (Homocysteine) ELISA Kit

1. Principle of Homocysteine Measurement

The Enzyme-Linked Immunosorbent Assay (ELISA) for homocysteine detection was based on competitive binding, where homocysteine in the sample competed with a fixed amount of enzyme-labeled homocysteine for binding to specific antibodies coated on a microplate. The intensity of the developed color was inversely proportional to the homocysteine concentration in the sample, which was measured spectrophotometrically.

Materials and Reagents

- Homocysteine ELISA Kit (included pre-coated microtiter plate, enzyme conjugate, standards, substrate solution, and stop solution)
- Phosphate Buffered Saline (PBS)
- Wash buffer
- Microplate reader (450 nm wavelength)
- Pipettes and micropipette tips
- Deionized or distilled water
- Absorbent paper
- Tubes for sample preparation

Sample Collection and Preparation

i. Sample Type: Serum or plasma (EDTA or heparinized plasma recommended)
ii. Collection: Blood was drawn by venipuncture into anticoagulant-treated tubes.
iii. Centrifugation: Samples were centrifuged at 2,000-3,000 rpm for 10-15 minutes to separate plasma or serum.

iv. Storage: Samples were stored at 2-8°C for short-term use (within 24 hours) or frozen at -80°C for long-term storage.

v. Dilution: If the sample concentration exceeded the highest standard, appropriate dilution with sample diluent was done.

ELISA Procedure

i. Plate Preparation: All the reagents and samples were brought to the room temperature before use.

ii. Standard and Sample Addition: Added 50-100 µL of standards, controls and diluted samples to the wells.

iii. Enzyme Conjugate Addition: Added 50-100 µL of enzyme-labeled homocysteine conjugate to each well.

iv. Incubation: Incubated the plate at 37°C for 30-60 minutes to allow binding.

v. Washing: Washed the wells 3-5 times with wash buffer to remove unbound substances.

vi. Substrate Addition: Added 50-100 µL of chromogenic substrate (TMB solution) to each well and incubate at room temperature in the dark for 10-15 minutes.

vii. Stopping Reaction: Added 50-100 µL of stop solution to halt the enzymatic reaction.

viii. Reading Absorbance: Measured absorbance at 450 nm using a microplate reader.

Calculation of Results

A standard curve was constructed by plotting the absorbance values of standards against their known concentrations. Homocysteine concentration was determined in

samples by interpolating their absorbance values on the standard curve. If the sample dilution had been performed, the calculated concentration was multiplied by the dilution factor. Results are typically expressed in µmol/L or ng/mL, depending on the assay kit specifications.

Figure 24: Homocysteine ELISA Kit

Methylmalonic acid

Principle of MMA Measurement

The Enzyme-Linked Immunosorbent Assay (ELISA) for MMA detection was based on competitive binding, where MMA in the sample competed with a fixed amount of enzyme-labeled MMA for binding to specific antibodies coated on a microplate. The intensity of the developed color was inversely proportional to the MMA concentration in the sample, which was measured spectrophotometrically.

Materials and Reagents

- MMA ELISA Kit (included pre-coated microtiter plate, enzyme conjugate, standards, substrate solution, and stop solution)
- Phosphate Buffered Saline (PBS)
- Wash buffer
- Microplate reader (450 nm wavelength)
- Pipettes and micropipette tips
- Deionized or distilled water
- Absorbent paper
- Tubes for sample preparation

3. Sample Collection and Preparation

- Sample Type: Serum or plasma (EDTA or heparinized plasma was recommended)
- Collection: Blood was drawn by venipuncture into anticoagulant-treated tubes.
- Centrifugation: Samples were centrifuged at 2,000-3,000 rpm for 10-15 minutes to separate plasma or serum.
- **Storage:** Samples could be stored at 2-8°C for short-term use (within 24 hours) or frozen at -80°C for long-term storage.
- **Dilution:** If the sample concentration exceeded the highest standard, appropriate dilution with sample diluent was required.

ELISA Procedure

1. Plate Preparation: All the reagents and samples were brought to the room temperature before use.
2. Standard and Sample Addition: Added 50-100 µL of standards, controls, and diluted samples to the wells.

3. Enzyme Conjugate Addition: Added 50-100 µL of enzyme-labeled MMA conjugate to each well.
4. Incubation: Incubated the plate at 37°C for 30-60 minutes to allow binding.
5. Washing: Washed the wells 3-5 times with wash buffer to remove unbound substances.
6. Substrate Addition: Added 50-100 µL of chromogenic substrate (TMB solution) to each well and incubated at room temperature in the dark for 10-15 minutes.
7. Stopping Reaction: Added 50-100 µL of stop solution to halt the enzymatic reaction.
8. Reading Absorbance: Measured absorbance at 450 nm using a microplate reader.

5. Calculation of Results

- A standard curve was constructed by plotting the absorbance values of standards against their known concentrations.
- MMA concentration was determined in samples by interpolating their absorbance values on the standard curve.
- If the sample dilution had been performed, the calculated concentration was multiplied by the dilution factor.
- Results were typically expressed in µmol/L or ng/mL, depending on the assay kit specifications.

Figure 25: Methylmalonic acid ELISA Kit

Study variables

In our study, Functional B12 deficiency was defined by the presence of elevated levels of MMA and/ or Hcy despite serum B12 values well within the normal reference range[12]. The criteria for low vitamin B12 levels in both infants and mothers is a concentration below 200 pg/mL[13]. In our study we classified serum vitamin B12 levels as low, low normal and high normal if the value was below 200 pg/ml, between 200 to 300 pg/ml and above 300 pg/ml respectively. Vitamin B12 levels are classified as low (<200 pg/mL), normal (200–300 pg/mL) and high normal (>300 pg/mL). Methylmalonic acid (MMA) levels were considered as normal if the level was less than 271 pmol/L and high if the levels was more than 271 pmol/L[64]. Similarly,

homocysteine (Hcy) levels were considered as normal if the level was less than 15 µmol/L and high if the level was greater than 15 µmol/L[65,66]. The red cell distribution width (RDW) was considered normal, if it was below 15 fL and high, if it was above 15 fL[67]. For infants, mean corpuscular volume (MCV) values, we referred to the standard reference range based on the different age groups. For the age group below 1 month, a value above 116 fL was considered high, for the age group of 1 to 3 months a value above 103fL and for the age group of 3 to 6 months, a value above 84 fL was considered high[68]. In mothers, MCV values are classified as normal (<100 fL) or high (>100 fL)[67].

Data processing and analysis/statistical analysis:

- Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. Data was also represented using appropriate diagrams like bar diagram, pie diagram and box plots.
- The association between explanatory variables and categorical outcomes was assessed by cross tabulation and comparison of percentages. Chi square test was used to test statistical significance.
- The association between quantitative explanatory variables and categorical outcomes was assessed by independent sample t-test (2 groups) will be used to assess statistical significance.
- P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis[69].

RESULTS

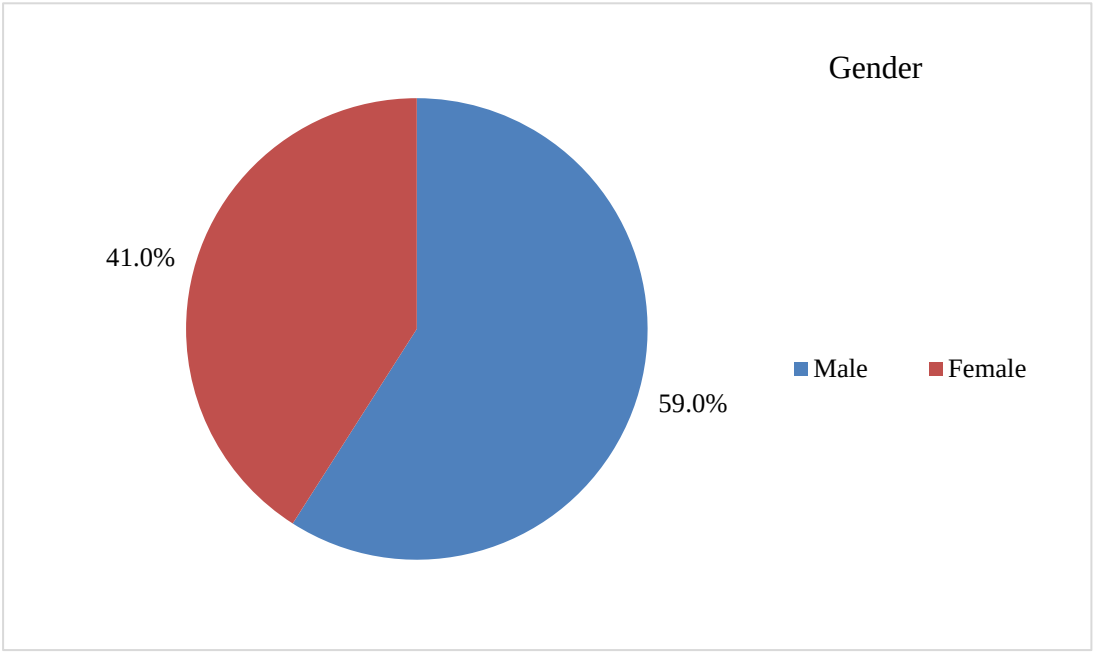
Our study aimed to find out the prevalence of functional Vitamin B12 deficiency amongst exclusively breastfed infants and to study the vitamin B12 status of the mothers of the infants who had Functional vitamin B12 deficiency. A total of 83 exclusively breastfed infants were recruited in our study, based on the inclusion and exclusion criteria. The mean age of the study participants was 3.96 ± 1.72 months. A total of 27.7% (n=23) infants were below 3 months and 72.3% (n=60) infants were above 3 months. The male: female ratio was 1.44. In the present study, the mean hemoglobin level among infants with functional B12 deficiency and low vitamin B12 levels was 10.97 ± 1.54 and 10.96 ± 2.16 respectively.

Table 4: Descriptive analysis of gender in the study population (N=83)

Gender	Frequency	Percentages
Male	49	59.04%
Female	34	40.96%

In the study population of 83 infants, 49 (59.04%) were male and 34 (40.96%) were female. This indicates a slightly higher proportion of male infants in the study.

Figure 26: Pie chart of gender in the study population (N=83)



The following flowchart depicts the vitamin B12 status (functional vitamin B12 deficiency and low vitamin B12 levels) of infants and their mothers.

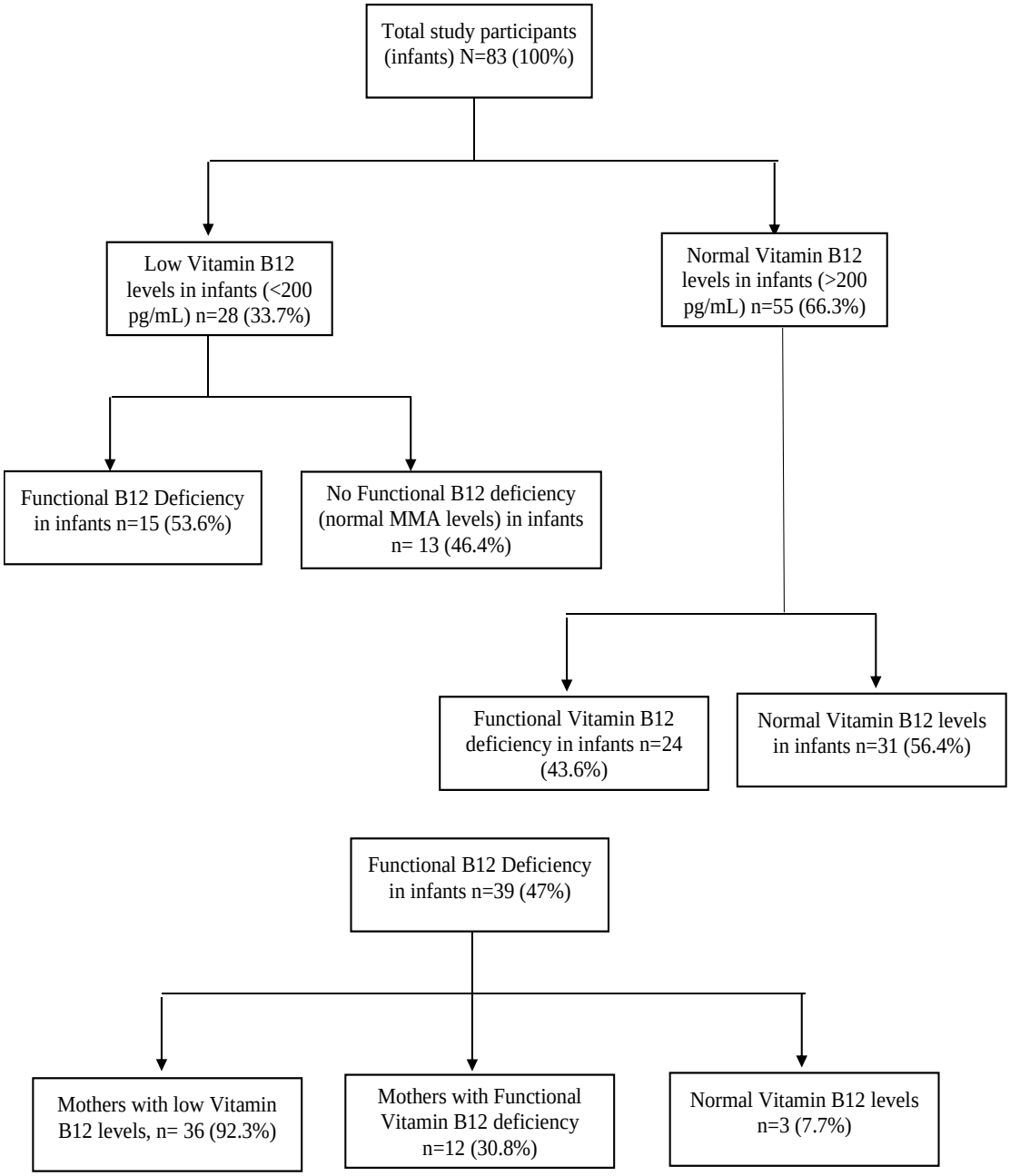


Figure 27: Consort diagram

The prevalence of functional B12 deficiency in exclusively breastfed infants was 47% (n=39). However, low vitamin B12 levels in exclusively breastfed infants was 33.7% (n=28).

Among the functional B12 deficient infants, 38.5% (n=15) had low vitamin B12 levels and 61.5% (n=24) had normal vitamin B12 levels. When we studied the B12 status of the mothers of infants with functional vitamin B12 deficiency, we found that 30.8% (n=12) had functional B12 deficiency, 92.3% (n=36) had low vitamin B12 levels and only 7.7% (n=3) had normal vitamin B12 levels.

Table 5: Descriptive analysis of functional B12 deficiency in the study population (N=83)

Functional B12 Deficiency (MMA)	Frequency	Percentages
Present	39	46.99%
Absent	44	53.01%

Figure 28: Pie chart of functional B12 deficiency in the study population (N=83)

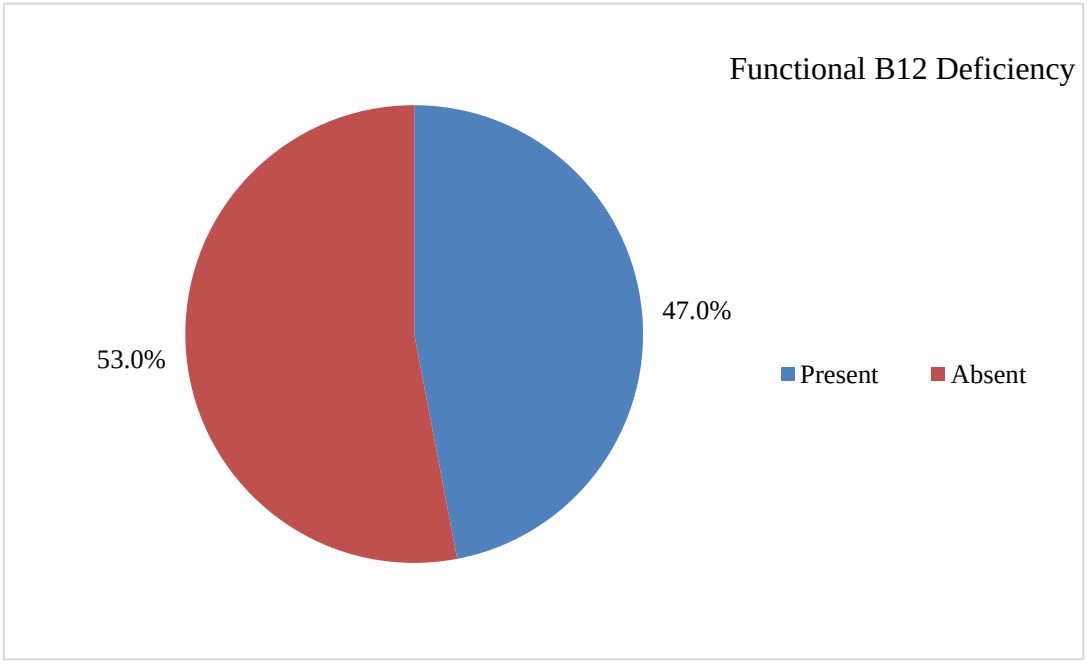
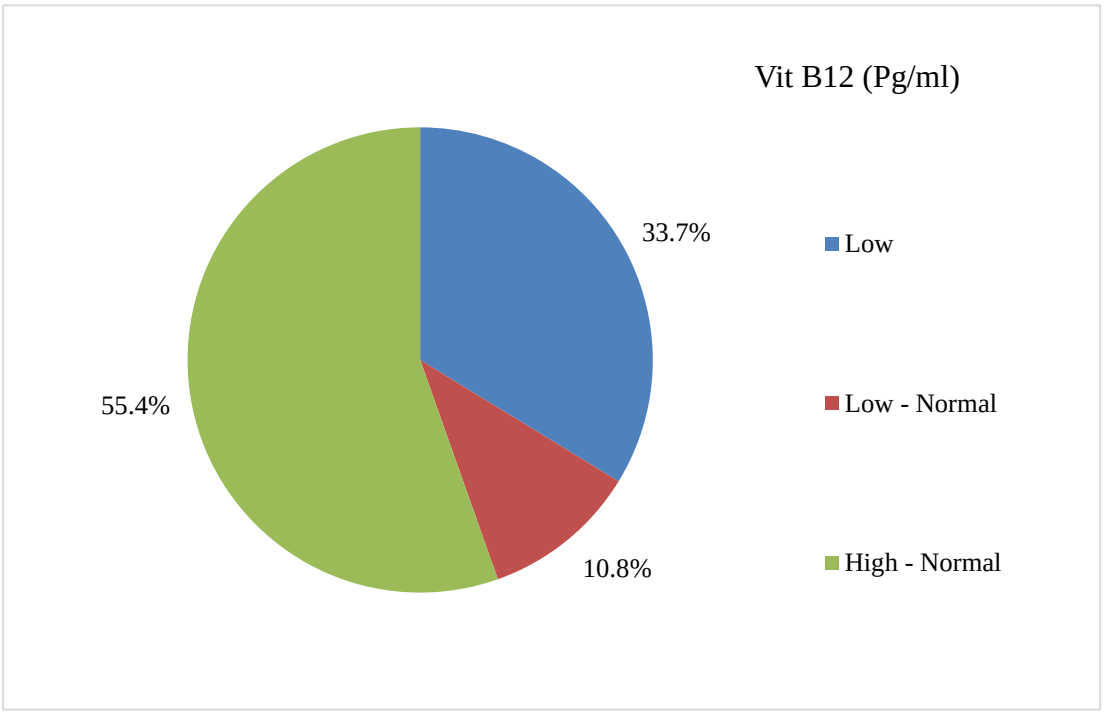


Table 6: Descriptive analysis of vit B12 (pg/ml) in the study population (N=83)

Vit B12 (Pg/ml)	Frequency	Percentages
Low	28	33.73%
Low – Normal	9	10.84%
High - Normal	46	55.42%

Figure 29: Pie chart of vit B12 (pg/ml) in the study population (N=83)



The study found that 47% of exclusively breastfed infants exhibited functional B12 deficiency, as indicated by elevated MMA levels. Additionally, 33.7% of these infants had low vitamin B12 levels based on serum B12 measurements.

Table 7: Comparison of MMA and Homocysteine across vit B12 in infants (pg./ml) (N=83)

Infants	Vit B12 (Pg/ml)			Chi square	P value
	Low (N=28)	Low - Normal (N=9)	High - Normal (N=46)		
MMA					
High	15 (53.57%)	3 (33.33%)	21 (45.65%)	1.19	0.519
Normal	13 (46.43%)	6 (66.67%)	25 (54.35%)		
Homocysteine					
High	22 (78.57%)	6 (66.67%)	21 (45.65%)	8.043	0.018
Normal	6 (21.43%)	3 (33.33%)	25 (54.35%)		

Figure 30: Cluster bar chart of comparison of MMA across vit B12 (pg /ml) (N=83)

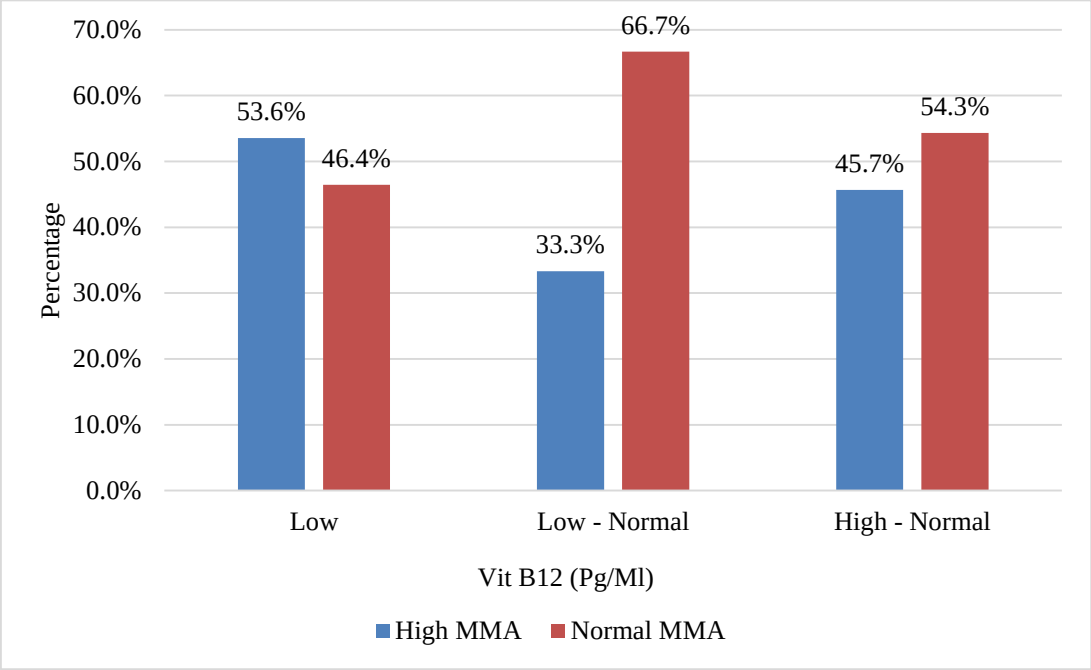


Figure 31: Cluster bar chart of comparison of Homocysteine (mcmol/l) across vit B12 (pg/ml) (N=83)

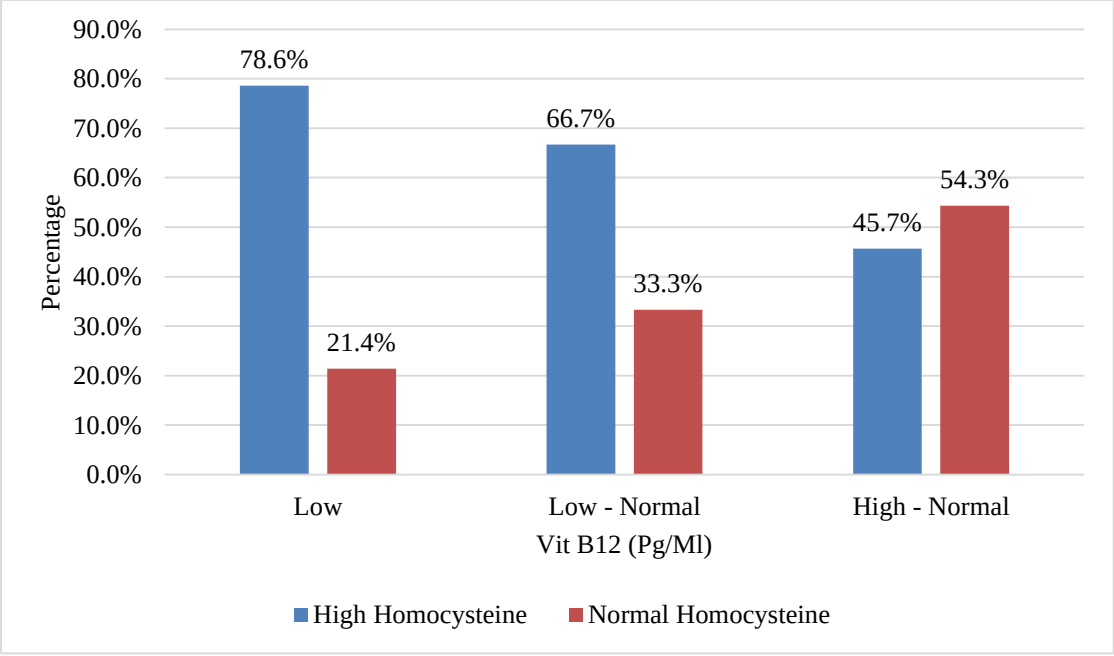


Table 8: Comparison of MMA and Homocysteine across vit B12 (pg./ml) in mothers (N=83)

Mothers	Vit B12 (pg/ml)			Chi square	P value
	Low (N=67)	Low (Normal) (N=11)	High (Normal) (N=5)		
MMA					
High	23 (34.33%)	5 (45.45%)	0 (0%)	3.23	0.199
Normal	44 (65.67%)	6 (54.55%)	5 (100%)		
Homocysteine					
High	44 (65.67%)	4 (36.36%)	1 (20%)	6.71	0.035
Normal	23 (34.33%)	7 (63.64%)	4 (80%)		

Figure 32: Cluster bar chart of comparison of MMA across vit B12 (pg /ml) (N=83)

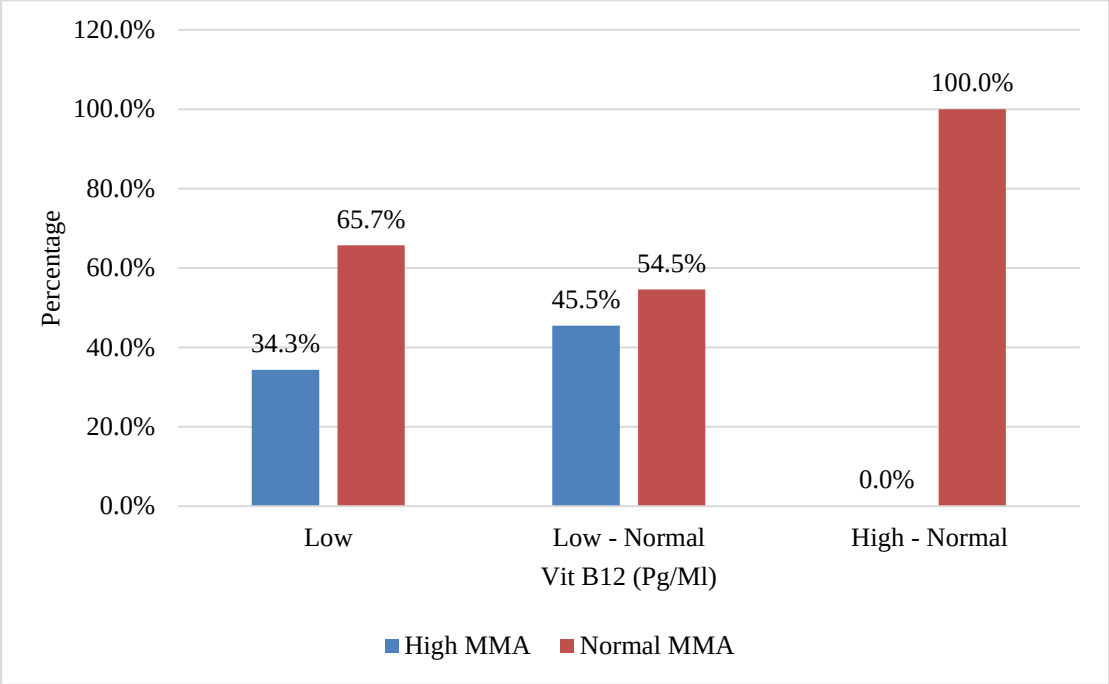
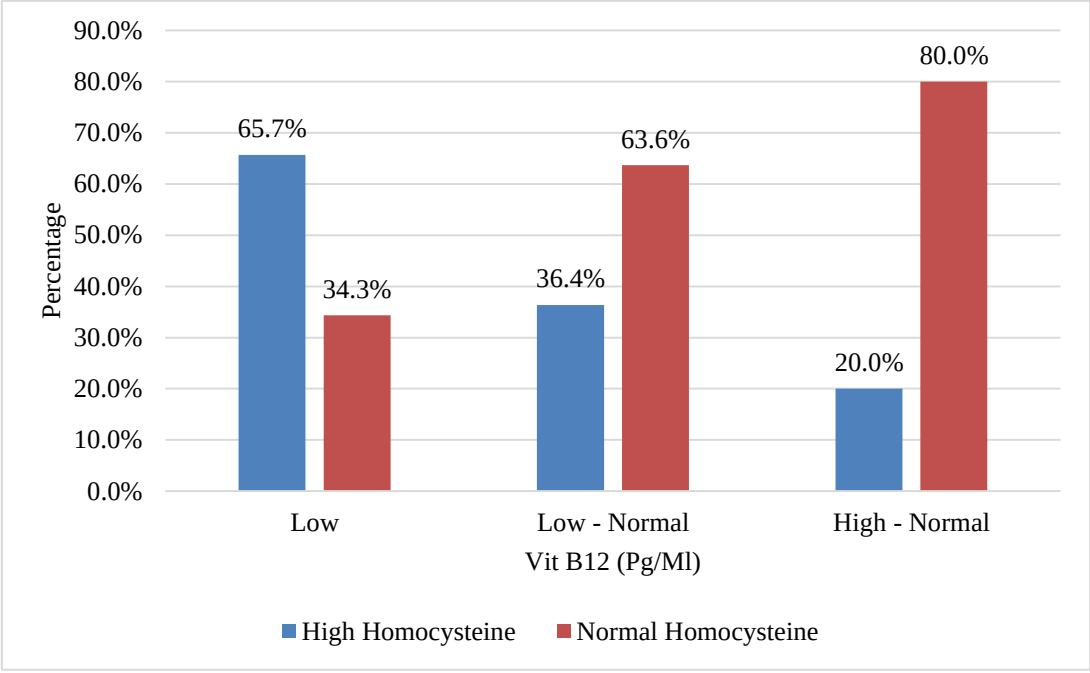


Figure 33: Cluster bar chart of comparison of Homocysteine (mcmol/l) across vit B12 (pg/ml) (N=83)



- **Comparison of MMA across Vitamin B12 Levels in Infants:**

  Among infants with low vitamin B12 levels, 53.57% had elevated MMA, suggesting a high prevalence of functional B12 deficiency in this group. In contrast, 33.33% of infants with low-normal B12 levels and 45.65% of those with high-normal B12 levels also had elevated MMA. However, the chi-square test (χ^2 = 1.19, p = 0.519) indicates that this difference is not statistically significant.

- **Comparison of Homocysteine across Vitamin B12 Levels in Infants:**

  A significant association was observed between vitamin B12 levels and homocysteine levels in infants (χ^2 = 8.043, p = 0.018). High homocysteine levels were most prevalent in infants with low B12 levels (78.57%), followed by those with low-normal B12 (66.67%) and high-normal B12 (45.65%). This suggests that lower vitamin B12 levels may contribute to increased homocysteine, which is a known marker of B12 deficiency and potential metabolic dysfunction.

- **Comparison of MMA across Vitamin B12 Levels in Mothers:**

  Among mothers with low vitamin B12 levels, 34.33% exhibited high MMA, whereas 45.45% of those with low-normal B12 and none of those with high-normal B12 had elevated MMA. Although there was a trend toward lower MMA levels in mothers with higher B12 status, the chi-square test (χ^2 = 3.23, p = 0.199) did not show statistical significance.

- **Comparison of Homocysteine across Vitamin B12 Levels in Mothers:**

  A significant association was found between maternal vitamin B12 levels and homocysteine levels (χ^2 = 6.71, p = 0.035). Elevated homocysteine was observed in 65.67% of mothers with low B12 levels, 36.36% of those with low-normal B12, and only 20% of those with high-normal B12. This indicates that lower maternal vitamin B12 levels are associated with increased homocysteine, which could have implications for both maternal and infant health.

Table 9: Comparison of MCV and RDW between functional B12 deficiency in infants (N=83)

Infants	Functional B12 Deficiency		Chi square	P value
	Present (N=39)	**Absent (N=44)**		
MCV				
Macrocytosis	14 (35.9%)	21 (47.73%)	1.186	0.276
Normal	25 (64.1%)	23 (52.27%)		
RDW				
High	32 (82.05%)	36 (81.82%)	0.001	0.978
Normal	7 (17.95%)	8 (18.18%)		

Figure 34: Cluster bar chart of comparison of MCV between functional B12 deficiency (N=83)

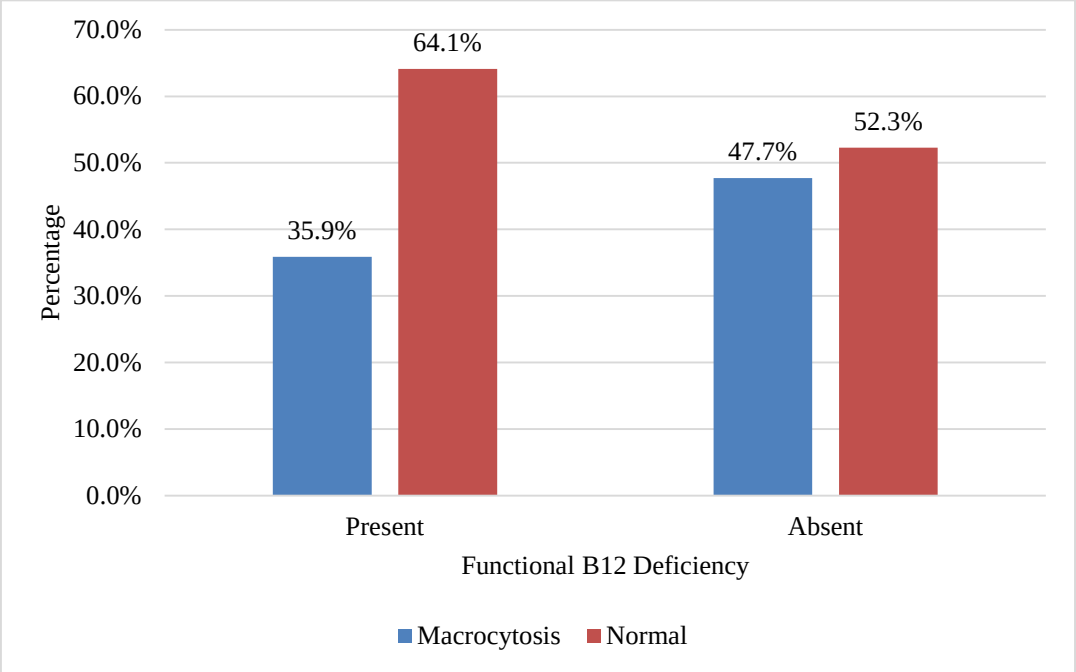


Figure 35: Cluster bar chart of comparison of RDW between functional B12 deficiency (N=83)

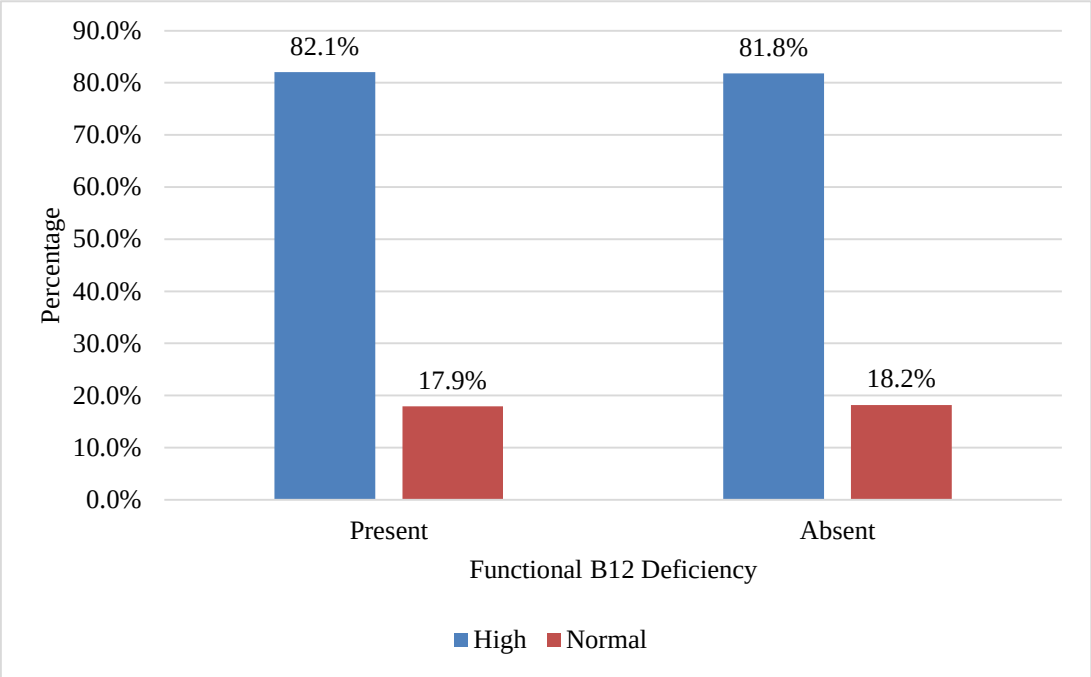


Table 10: Comparison of MCV and RDW between functional B12 deficiency in Mothers (N=83)

Mothers	Functional B12 Deficiency		Chi square	P value
	Present (N=28)	Absent (N=55)		
MCV				
Macrocytosis	22 (78.57%)	24 (43.64%)	9.17	0.002
Normal	6 (21.43%)	31 (56.36%)		
RDW				
High	18 (64.29%)	42 (76.36%)	1.35	0.245
Normal	10 (35.71%)	13 (23.64%)		

Figure 36: Cluster bar chart of comparison of MCV between functional B12 deficiency (N=83)

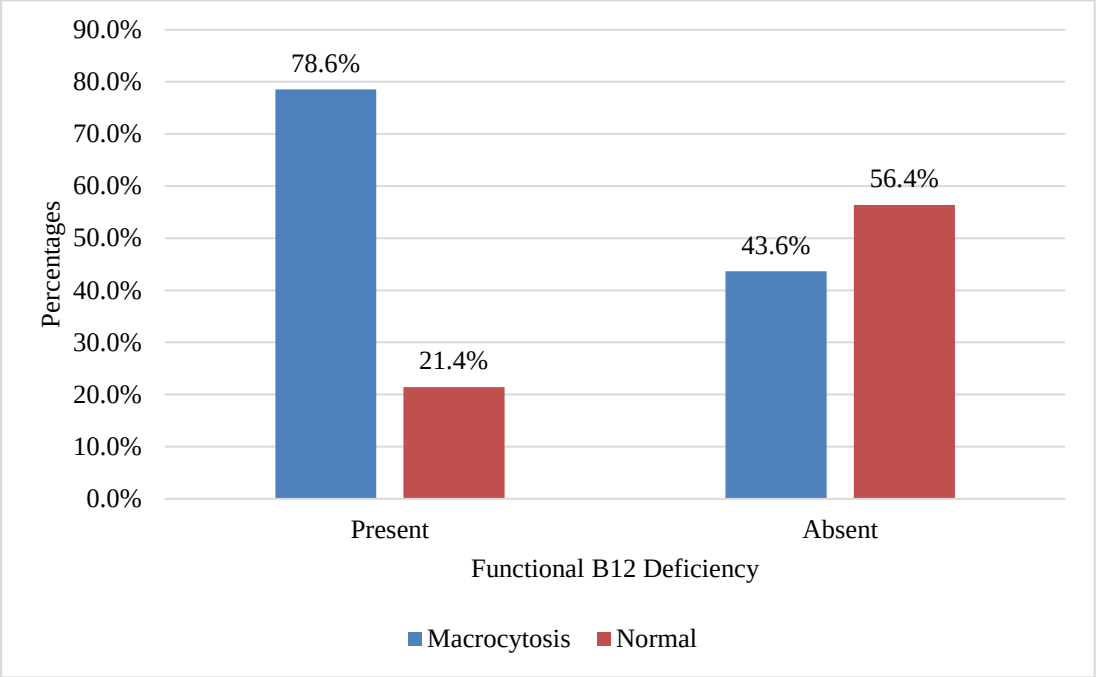
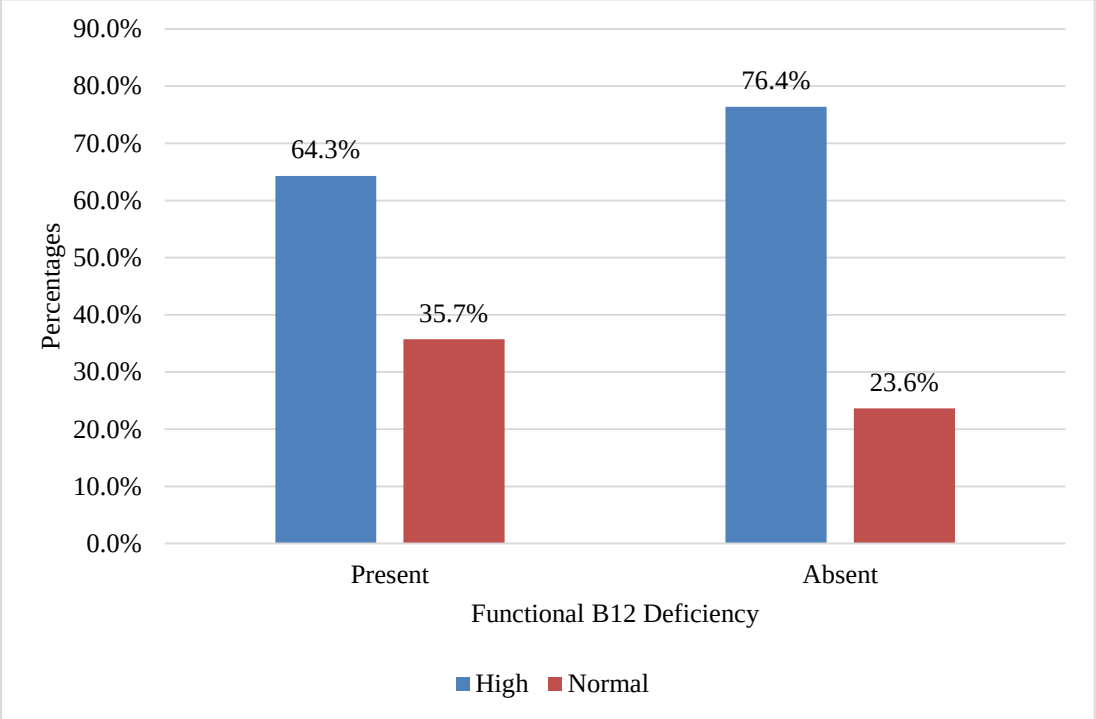


Figure 37: Cluster bar chart of comparison of RDW between functional B12 deficiency (N=83)



- **Comparison of MCV across Functional B12 Deficiency in Infants:**

  Among infants with functional B12 deficiency, 35.9% had macrocytosis, compared to 47.73% of those without deficiency. However, this difference was not statistically significant ($\chi^2 = 1.186$, $p = 0.276$), suggesting that macrocytosis may not be a reliable indicator of functional B12 deficiency in infants.

- **Comparison of RDW across Functional B12 Deficiency in Infants:**

  A high RDW, which indicates red blood cell size variability, was observed in 82.05% of infants with functional B12 deficiency and 81.82% of those without it. The chi-square test ($\chi^2 = 0.001$, $p = 0.978$) showed no significant difference, suggesting that RDW is not strongly associated with functional B12 deficiency in this population.

- **Comparison of MCV across Functional B12 Deficiency in Mothers:**

  In mothers with functional B12 deficiency, 78.57% had macrocytosis, compared to 43.64% of those without deficiency. This difference was statistically significant ($\chi^2 = 9.17$, $p = 0.002$), indicating a strong association between functional B12 deficiency and macrocytosis in mothers. This suggests that in adults, macrocytosis may serve as a useful haematological marker of functional B12 deficiency.

- **Comparison of RDW across Functional B12 Deficiency in Mothers:**

  A high RDW was found in 64.29% of mothers with functional B12 deficiency and 76.36% of those without it. The difference was not statistically significant ($\chi^2 = 1.35$, $p = 0.245$), implying that RDW does not consistently differentiate between those with and without functional B12 deficiency in mothers.

Table 11: Comparison of MCV and RDW across vit B12 in Infants (pg/ml) (N=83)

Infants	Vit B12 (Pg/Ml)			Chi square	P value
	Low (N=28)	Low - Normal (N=9)	High - Normal (N=46)		
MCV					
Macrocytosis	21 (75%)	2 (22.22%)	12 (26.09%)	18.723	<0.001
Normal	7 (25%)	7 (77.78%)	34 (73.91%)		
RDW					
High	27 (96.43%)	6 (66.67%)	35 (76.09%)	6.452	0.040
Normal	1 (3.57%)	3 (33.33%)	11 (23.91%)		

Figure 38: Cluster bar chart of comparison of MCV across vit B12 (pg/ml) (N=83)

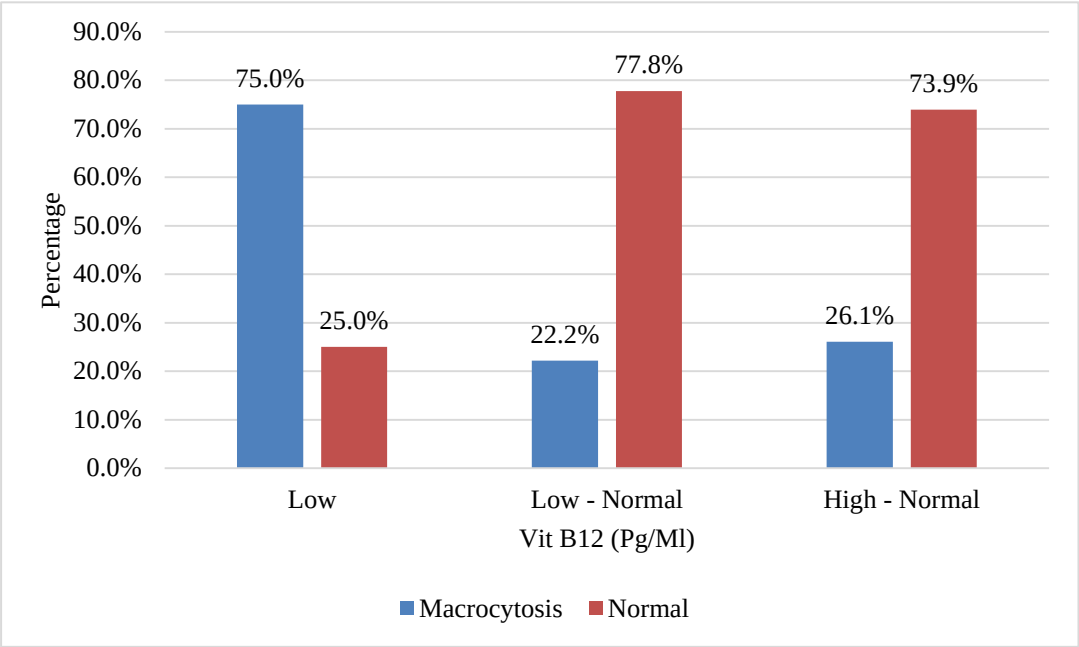


Figure 39: Cluster bar chart of comparison of RDW across vit B12 (pg/ml) (N=83)

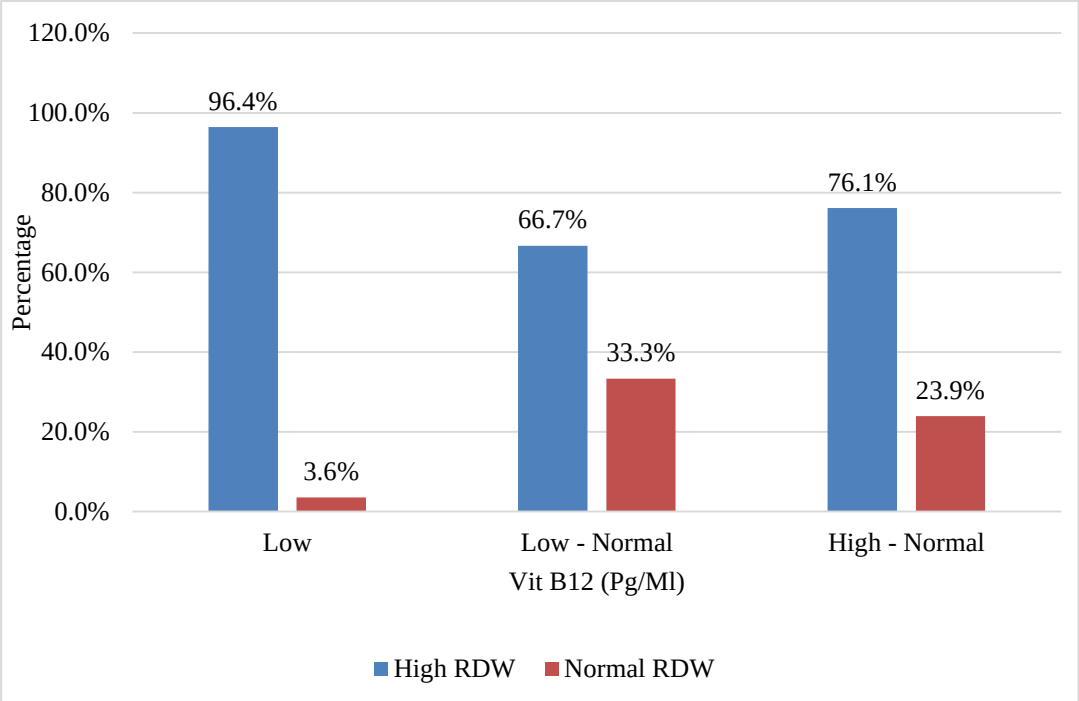


Table 12: Comparison of MCV and RDW across vit B12 in mothers (pg/ml) (N=83)

Mothers	Vit B12 (Pg/Ml)			Chi square	P value
	Low (N=67)	Low -Normal (N=11)	High – Normal (N=5)		
MCV					
Macrocytosis	42 (62.69%)	4 (36.36%)	0 (0%)	9.265	0.010
Normal	25 (37.31%)	7 (63.64%)	5 (100%)		
RDW					
High	52 (77.61%)	6 (54.55%)	2 (40%)	5.279	0.071
Normal	15 (22.39%)	5 (45.45%)	3 (60%)		

Figure 40: Cluster bar chart of comparison of MCV across vit B12 (pg/ml) (N=83)

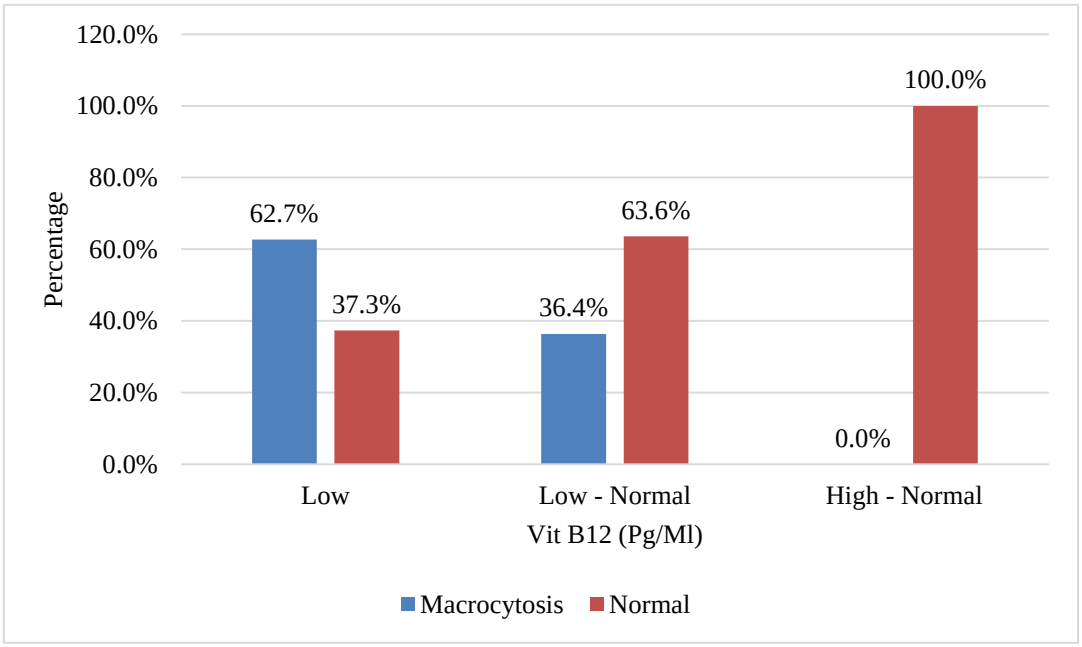
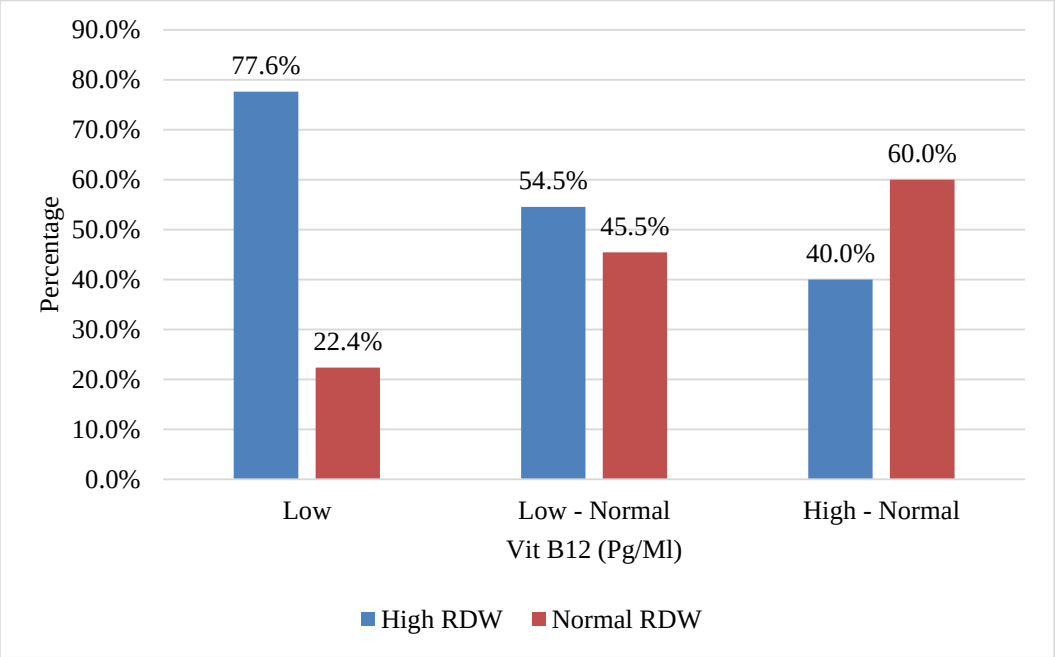


Figure 41: Cluster bar chart of comparison of RDW across vit B12 (pg/ml) (N=83)



- **Comparison of MCV Across Infant Vitamin B12 Levels:**

  Macrocytosis was significantly more common in infants with low vitamin B12 levels (75%) compared to those with low-normal (22.22%) and high-normal (26.09%) B12 levels. This association was statistically significant (χ^2 = 18.723, $p < 0.001$), indicating that low vitamin B12 levels are strongly linked to macrocytosis in infants. This suggests that vitamin B12 deficiency has a noticeable impact on red blood cell size, making macrocytosis a potential haematological marker for low B12 status in infants.

- **Comparison of RDW Across Infant Vitamin B12 Levels:**

  A high RDW was observed in 96.43% of infants with low B12 levels, 66.67% of those with low-normal B12, and 76.09% of those with high-normal B12. The chi-square test (χ^2 = 6.452, $p = 0.040$) showed a statistically significant association, suggesting that increased RDW is more prevalent in infants with

low vitamin B12. This indicates that red blood cell size variability may be influenced by B12 status, potentially serving as an additional marker of B12 deficiency.

- **Comparison of MCV Across Maternal Vitamin B12 Levels:**

  Macrocytosis was significantly more common in mothers with low vitamin B12 levels (62.69%) compared to those with low-normal (36.36%) and high-normal (0%) B12 levels. This association was statistically significant (χ^2 = 9.265, p = 0.010), indicating that lower maternal B12 levels are strongly linked to macrocytosis. The absence of macrocytosis in mothers with high-normal B12 levels further supports the role of vitamin B12 in maintaining normal red blood cell size.

- **Comparison of RDW Across Maternal Vitamin B12 Levels:**

  A high RDW was observed in 77.61% of mothers with low B12 levels, 54.55% of those with low-normal B12, and 40% of those with high-normal B12. Although there was a trend suggesting that lower B12 levels are associated with a higher RDW, the chi-square test (χ^2 = 5.279, p = 0.071) did not reach statistical significance. This suggests that while RDW may be influenced by B12 status, it is not a consistently reliable marker of B12 deficiency in mothers.

Table 13: Comparison of mothers vit B12 (pg/ml) between functional B12 deficiency (Infants) (N=83)

Vit B12 (Pg/ml) (Mothers)	Functional B12 Deficiency (Infants)		Chi square	P value
	Present (N=39)	Absent (N=44)		
Low	36 (92.31%)	31 (70.45%)	7.371	0.025
Low (Normal)	3 (7.69%)	8 (18.18%)		
High (Normal)	0 (0%)	5 (11.36%)		

Figure 42: Cluster bar chart of comparison of mothers vit B12 (pg/ml) between functional B12 deficiency (infants) (N=83)

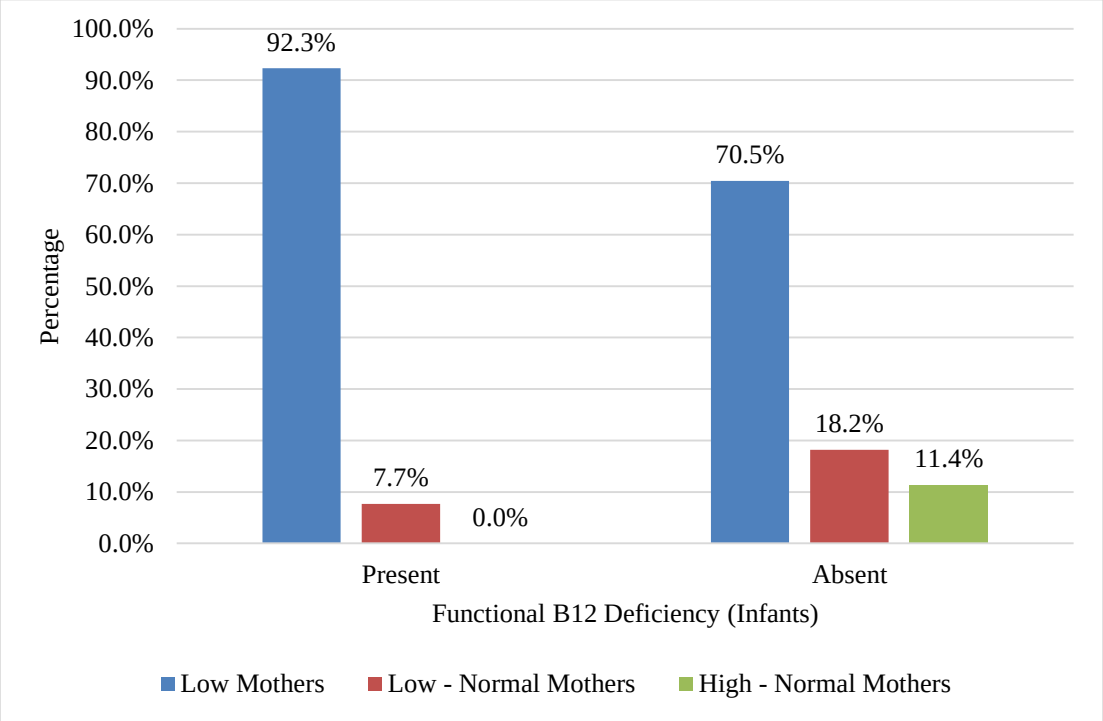


Table 14: Comparison of functional B12 deficiency (mothers) between functional B12 deficiency (infants) (N=83)

Functional B12 Deficiency (Mothers)	Functional B12 Deficiency (Infants)		Chi square	P value
	Present (N=39)	Absent (N=44)		
Present	12 (30.77%)	16 (36.36%)	0.289	0.591
Absent	27 (69.23%)	28 (63.64%)		

Figure 43: Cluster bar chart of comparison of functional B12 deficiency (mothers) between functional B12 deficiency (infants) (N=83)

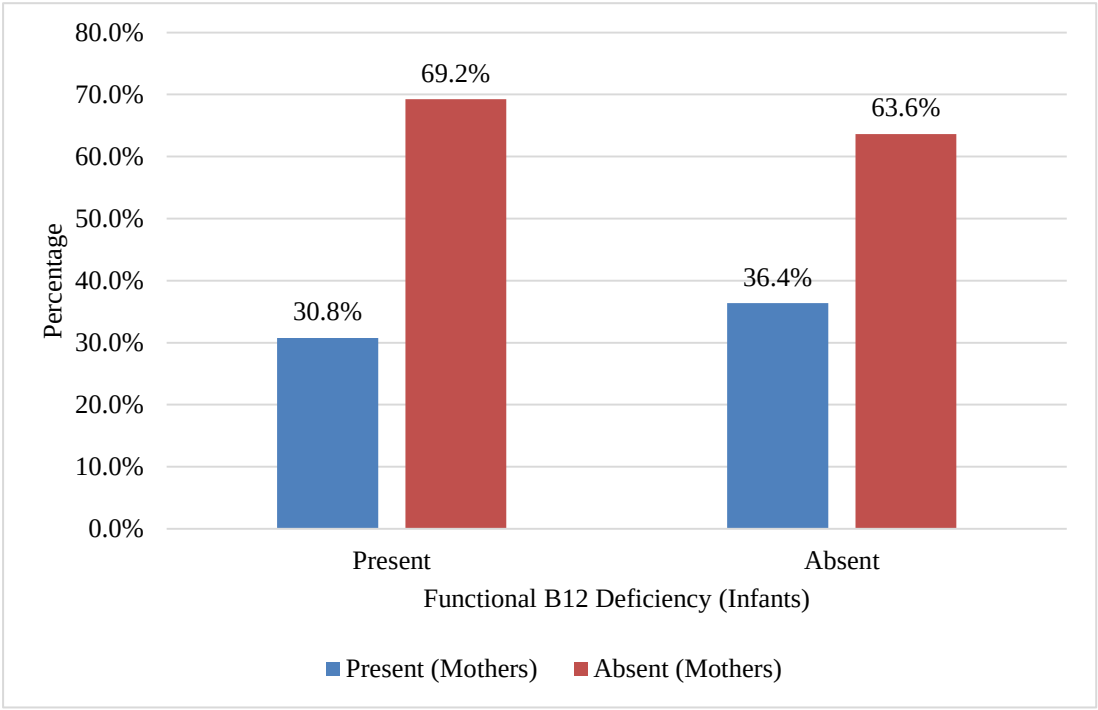
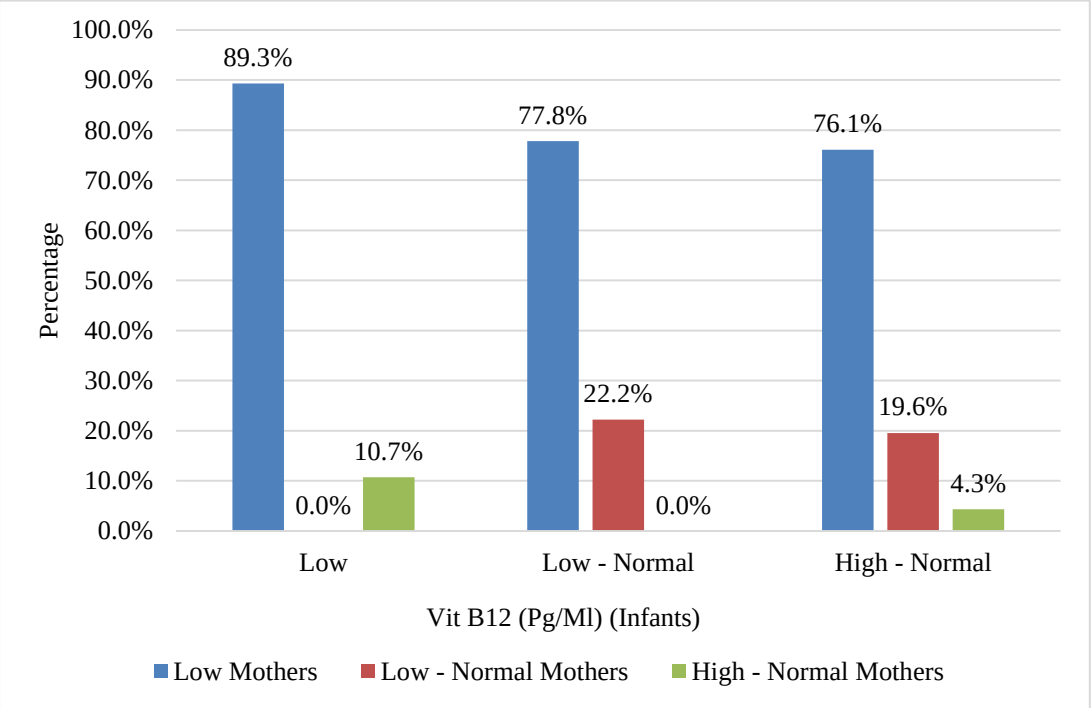


Table 15: Comparison of Vit B12 (mothers) across Vit B12 (infants) (N=83)

Vit B12 (Pg/ml) (Mothers)	Vit B12 (Pg/ml) (Infants)			Chi square	P value
	Low (N=28)	Low (Normal) (N=9)	High (Normal) (N=46)		
Low	25 (89.29%)	7 (77.78%)	35 (76.09%)	7.81	0.099
Low (Normal)	0 (0%)	2 (22.22%)	9 (19.57%)		
High (Normal)	3 (10.71%)	0 (0%)	2 (4.35%)		

Figure 44: Cluster bar chart of comparison of Vit B12 (mothers) across Vit B12 (infants) (N=83)



- **Comparison of Maternal Vitamin B12 Levels Across Infant Functional B12 Deficiency:**

  Among mothers of infants with functional B12 deficiency, 92.31% had low vitamin B12 levels, compared to 70.45% of mothers whose infants did not have the deficiency. This difference was statistically significant ($\chi^2 = 7.371$, $p = 0.025$), suggesting that lower maternal B12 levels are associated with a higher likelihood of functional B12 deficiency in infants. This finding reinforces the importance of adequate maternal B12 levels during pregnancy and lactation for infant health.

- **Comparison of Functional B12 Deficiency in Mothers and Infants:**

  Functional B12 deficiency in mothers was present in 30.77% of infants with the condition and in 36.36% of those without it. The chi-square test ($\chi^2 = 0.289$, $p = 0.591$) showed no statistically significant association, suggesting that maternal functional B12 deficiency does not strongly predict functional B12 deficiency in infants.

- **Comparison of Maternal Vitamin B12 Levels Across Infant Vitamin B12 Status:**

  Mothers with low vitamin B12 levels were more likely to have infants with low B12 (89.29%), low-normal B12 (77.78%), or high-normal B12 (76.09%). However, the chi-square test ($\chi^2 = 7.81$, $p = 0.099$) did not show statistical significance, suggesting that while there is a trend indicating maternal B12 levels influence infant B12 status, the association is not strong enough to be conclusive.

- **Comparison of Developmental Delay Across Infant Functional B12 Deficiency:**

None of the infants had developmental delay based on the screening test performed by Trivandrum Development Screening Test. However, in this study, we performed only screening test and we did not perform detailed developmental assessment in each domain.

Table 16: Depiction of mean Hb (g/dl) in infants with functional B12 deficiency (N=83)

Parameter	Functional B12 deficiency (Mean± SD)		P value
	Present (N=39)	Absent (N=44)	
Hb (g/dL)	10.97 ± 1.54	10.29 ± 2.01	0.091

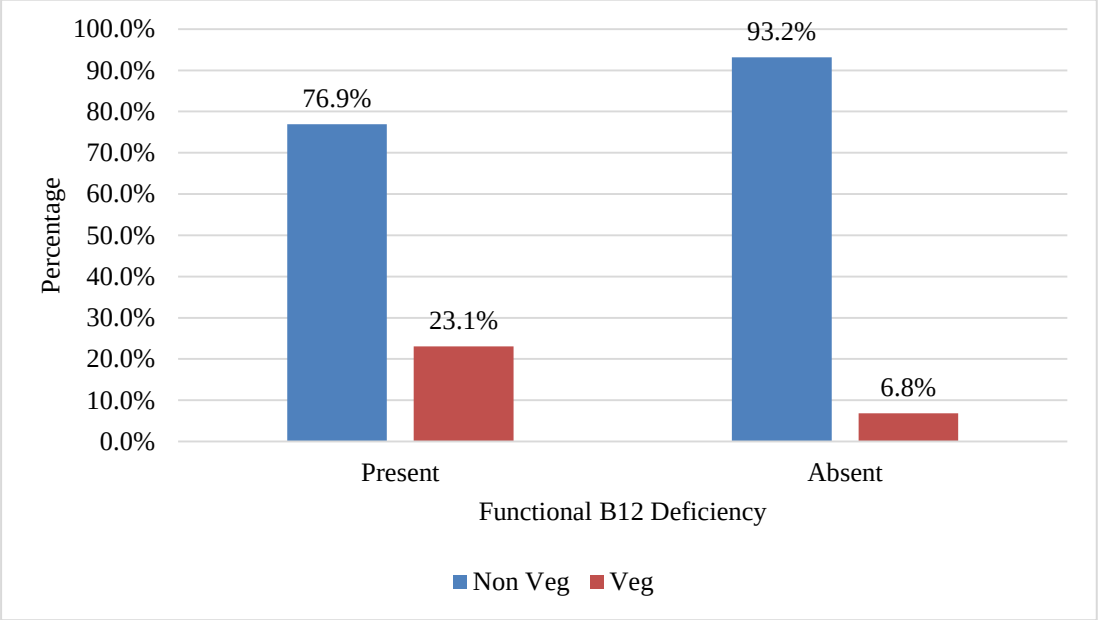
Table 17: Depiction of levels of mean Hb (g/dl) in Low, Low-Normal, High-Normal vit B12 levels (N=83)

Parameter	Vit B12 (Pg/ml) (Mean± SD)			P Value
	Low (N=28)	Low - Normal (N=9)	High - Normal (N=46)	
Hb (g/dL)	10.96 ± 2.16	10.17 ± 1.8	10.48 ± 1.61	0.407

Table 18: Relation between maternal dietary status and functional B12 deficient infants (N=83)

Mother's Dietary Status	Infants with Functional B12 Deficiency		Chi square	P value
	Present (N=39)	Absent (N=44)		
NonVegetarian	30 (76.92%)	41 (93.18%)	4.419	0.036
Vegetarian	9 (23.08%)	3 (6.82%)		

Figure 45: Cluster bar chart of comparison of mother's dietary status between functional B12 deficiency (N=83)



- In our study, the mother's dietary status did not impact the functional B12 status. Majority of the mothers whose infants had functional B12 deficiency were on non-vegetarian diet (n=30, 76.92 %) and the correlation between dietary pattern (vegetarian or nonvegetarian) of mothers was not statistically significant (p=0.036).

DISCUSSION

Our study aimed to find out the prevalence of functional Vitamin B12 deficiency amongst exclusively breastfed infants and to study the vitamin B12 status of the mothers of the infants who had Functional vitamin B12 deficiency. A total of 83 exclusively breastfed infants were recruited in our study, based on the inclusion and exclusion criteria. The prevalence of functional B12 deficiency in exclusively breastfed infants was 47% (n=39). However, the prevalence of low vitamin B12 levels in exclusively breastfed infants was 33.7% (n=28).

Among the infants with functional vitamin B12 deficiency, 38.5% (n=15) had low vitamin B12 levels and 61.5% (n=24) had normal vitamin B12 levels. When we studied the B12 status of the mothers of infants with functional vitamin B12 deficiency, we found that 30.8% (n=12) had functional B12 deficiency, 92.3% (n=36) had low vitamin B12 levels and only 7.7% (n=3) had normal vitamin B12 levels.

Although there is plenty of literature regarding B12 deficiency in exclusively breastfed infants but after a thorough review of literature, we could find very few studies regarding functional vitamin B12 deficiency in exclusively breastfed infants which is primarily diagnosed based on the MMA levels. Schroder TH et al, studied methylmalonic acid levels of dried blood spots of 160 newborns and established the reference range of 16.8 pmol (95% CI: 15.9–17.6 pmol)/8-mm spot. The reference interval (2.5th to 97.5th percentile) for MMA was 9.89 to 29.3 pmol/8-mm spot (0.450 to 1.33 μmol/L whole blood)[70]. Guidelines for functional vitamin B12 deficiency shows that despite normal or elevated serum B12 levels due to impaired cellular uptake, metabolism or utilization. Accurate diagnosis requires combining metabolic markers like methylmalonic acid and homocysteine rather than relying on

serum B12 alone[71]. This is the first study regarding the correlation of functional vitamin B12 deficiency among exclusively breastfed infants and their maternal B12 status. It is of paramount importance to detect functional vitamin B12 deficiency before severe vitamin B12 deficiency sets in, so that the potential complications related to this deficiency can be prevented and addressed[70]. In a study done by Duggan et al., maternal supplementation with oral vitamin B12 during pregnancy and early lactation significantly increased breast milk vitamin B12 concentrations at 6 weeks postpartum (136 vs. 87 pmol/L, $p < 0.001$). This, in turn, improved infant cobalamin status, as evidenced by higher plasma B12 (199 vs. 139 pmol/L, $p < 0.001$) and lower methylmalonic acid and homocysteine levels[72]. In a study done by Greibe E et al, breast milk cobalamin concentrations decline significantly from a median of 760 pmol/L at 2 weeks to 290 pmol/L at 4 months, then rise moderately to 440 pmol/L by 9 months.

This decline at 4 months correlates with biochemical signs of low vitamin B12 status in exclusively breastfed infants, suggesting that breast milk alone may be insufficient to meet their needs beyond this age[73].

In the study conducted by Kadiyala A et al, prevalence of low vitamin B12 levels was reported to be 63.7% in exclusively breastfed infants between age group of 1 to 6 months and the incidence was higher as compared to our study.[4] Cobalamin levels in breast milk declines notably by 4 months postpartum, correlating with decreased cobalamin status in infants. This suggests that exclusive breastfeeding beyond this period may not ensure adequate vitamin B12 supply for infants[73].

In the present study, the prevalence of low vitamin B12 levels was 33.7% (n=28) (Table-6). A higher prevalence of 57% was reported in the study conducted by

Mittal M et al and prevalence of 46% was seen in their mothers[22]. Low Vitamin B12 levels amongst the exclusively breastfed infants between 0-6 months of age was reported to be 16% in the study conducted by Azad C et al., which was lower than the prevalence reported in our study[74]. A similar study by Singhal A et al. reported a positive correlation ($p<0.001$) between serum vitamin B12 levels in exclusively breastfed infants and their mothers[75]. Another study by Inderpal S Grover et al. demonstrated a statistically significant positive correlation between serum vitamin B12 levels in exclusively breastfed infants and their mothers ($p < 0.001$)[76]. Thus, all these Indian studies have shown higher prevalence of low Vitamin B12 levels in exclusively breastfed infants and in the mothers of these infants. When we reviewed the western literature, regarding the correlation between infant and maternal B12 status, we found a study done by Yıldırmak et al., in which they studied the cord blood vitamin B12 and homocysteine levels of the new born babies and their mothers and they found that there was a statistically significant correlation between the vitamin B12 levels ($p=0.001$) and homocysteine levels($p=0.0001$) of mothers and their infant[77]. Similar study done by Reischl-Hajiabadi et al., also found that exclusively breastfed infants of mothers with low vitamin B12 status had vitamin B12 deficiency[65].

A study done by Kalay Z et al., depicted that plasma methylmalonic acid (MMA) is highly sensitive and reliable marker for detecting Vitamin B12 deficiency in newborns[78]. A similar study done by Karademir F et al., showed that exclusively breastfed infants had significantly lower serum vitamin B12 and folate levels and higher plasma homocysteine levels at 2 to 6 months of age compared to those fed with formula milk[79].

In our study, the mother's dietary pattern (vegetarian or nonvegetarian) did not impact the functional B12 status. Majority of the mothers whose infants had functional B12 deficiency were on non-vegetarian diet (n=30, 76.92 %) and the correlation between dietary pattern of mothers was not statistically significant(p=0.036) (Table-18). Similarly, a study done by Kadiyala A et al, revealed that majority of infants with low B12 status (63.7%) had mothers who followed non vegetarian diet (86.6%)[4]. In the study conducted by Mittal M et al, regarding the maternal dietary status of the exclusively breastfed infants with Vitamin B12 deficiency, it was observed that, 52.1% of the mothers were on non-vegetarian diet[22]. Similar finding was reported by the study conducted by Azad C et al in which, 49.5% of the mothers of these exclusively breastfed infants with vitamin B12 deficiency, were on non-vegetarian diet[74].

Usually, serum vitamin B12 level is the first assay performed to determine vitamin B12 status. However, one of the major limitations of this biomarker is that it assesses total circulating vitamin B12, of which approximately 80% is bound to haptocorrin and hence, not available for cellular uptake. Another limitation is its unreliability to reflect cellular vitamin B12 levels[71]. Also, the children with inborn errors of vitamin B12 metabolism can have normal values of serum B12, despite being deficient at the cellular level[57]. Interestingly, functional vitamin B12 deficiency due to oxidative stress has been identified in patients exhibiting normal values of serum vitamin B12 levels[80]. MMA and Homocysteine are considered as important biomarkers for B12 deficiency. MMA is more specific as it is raised only in B12 deficiency, whereas Homocysteine can be raised in both Vitamin B12 deficiency and Folate deficiency[57]. One of the limitations of MMA as a biomarker for vitamin B12 deficiency is that it can be falsely elevated in cases of impaired renal functions and in

such cases, holotranscobalamin assay would be a more reliable biomarker than MMA[81].

In our study, 53.57 % of infants who had low vitamin B12 levels had high MMA levels and 33.33 % of infants with low normal vitamin B12 levels had high MMA levels, but the association was not statistically significant (p=0.519) (Table-7). This could be explained by the fact, that MMA may not be elevated, when vitamin B12 deficiency is at a subclinical stage and majority of our subjects had subclinical vitamin B12 deficiency (66.3%)[82]. In our study, 78.57 % of infants who had low vitamin B12 levels had high Homocysteine levels and 66.67 % of infants with low normal vitamin B12 levels had high Homocysteine levels and the association was statistically significant (p=0.018) (Table-7). Our study was in accordance with other studies in which low B12 levels led to increase in homocysteine levels. A study done by Pinto et al., revealed that in infants, a significant inverse correlation was observed between vitamin B12 and homocysteine levels ($p < 0.001$)[8]. Similar finding was reported in study done by Ünsür EK et al., a significant inverse correlation between neonatal homocysteine and vitamin B12 levels ($r = -0.394$, $P < 0.001$), indicating that infants with lower B12 levels had higher homocysteine concentrations[83]. In a study done by Ljungblad et al., on 46% of presumed healthy infants, raised serum homocysteine levels was found to be significantly associated with symptoms like tremor and excessive sleep, indicating underlying functional vitamin B12 deficiency ($p = 0.001$)[84].

In the present study, the mean hemoglobin level among infants with functional vitamin B12 deficiency and those with low serum B12 levels were 10.97 ± 1.54 and 10.96 ± 2.16, respectively (Table-16, 17). Mittal M et al. reported a median hemoglobin level of 10.3 (9.6–11) among exclusively breastfed infants with low

vitamin B12 levels[22] whereas Azad C et al., observed significantly lower mean hemoglobin levels in infants with vitamin B12 deficiency (7.0 ± 3.0) compared to those with normal B12 levels (10.0 ± 2.0) ($p < 0.01$)[74].

In the present study, the mean vitamin B12 levels among infants with functional B12 deficiency and their mothers were 528.01 ± 417.98 pg/mL and 119.61 ± 55.92 pg/mL, respectively. In the study by Azad C et al among infants aged between one month to one year, the mean vitamin B12 level was reported as 357 ± 172 pg/mL[55]. Mittal M et al., reported median vitamin B12 levels of 176.5 (118–251) pg/mL in infants and 216 (159.5–278.5) pg/mL in mothers[22]. The study by Dag H et al. highlighted that exclusively breastfed infants had lower vitamin B12 levels as compared to formula fed infants[67]. These findings emphasize the critical need for adequate maternal vitamin B12 supplementation during pregnancy and lactation to prevent deficiency in infants. Collectively, these studies underline the importance of ensuring optimal maternal nutrition and implementing effective supplementation strategies to reduce the risk of early-life vitamin B12 deficiency and its associated hematological and neurological complications.

In the present study, the mean MCV among infants with functional vitamin B12 deficiency and low serum B12 levels was 90.07 ± 16.53 fL and 89.05 ± 11.21 fL, respectively (Table-9, 11). In the study by Azad C et al., the mean MCV was significantly higher in the vitamin B12 deficient group (87.0 ± 13.0 fL) compared to the normal B12 group (77.0 ± 11.0 fL) ($p = 0.02$)[55]. Mittal M et al. reported median MCV levels of 82.4 (74.8–87.7) fL in infants with B12 deficiency which was slightly lower than those observed in the present study[22]. The relatively low MCV values in these studies may reflect concurrent iron deficiency in the infant population. The higher mean MCV observed in our study compared to Mittal M et al., may be

influenced by differences in the concurrent iron deficiency status. Furthermore, Azad C et al. reported a statistically significant difference in MCV between vitamin B12 deficient and normal vitamin B12 level groups, thus supporting the influence of vitamin B12 status on red blood cell indices[74].

In the present study, the mean MCH among infants with functional vitamin B12 deficiency and low serum B12 levels was 24.77 ± 3.46 and 26.10 ± 3.90 pg, respectively. Azad C et al., reported mean MCH values of 29.0 ± 6.0 pg in vitamin B12 deficient infants and 25.0 ± 5.0 pg in normal B12 level infants, with the difference being statistically significant ($p = 0.02$)[74]. Mittal M et al. observed median MCH levels of 27 (23.8–29) pg among both infants and their mothers, which was comparable to our study[22].

In the present study, the mean MCHC among infants with functional vitamin B12 deficiency and low serum B12 levels was 29.87 ± 1.80 g/dL and 30.24 ± 1.91 g/dL, respectively. Azad M et al. reported mean MCHC values of 31.0 ± 6.0 g/dL in vitamin B12 deficient infants and 31.0 ± 3.0 g/dL in normal B12 level infants, showing no significant difference between groups[74]. Mittal M et al. documented median MCHC levels of 32 (30.3–34) g/dL among both infants and their mothers[22]. Since MCHC reflects the concentration of hemoglobin in red blood cells, lower levels may signify impaired hemoglobin synthesis associated with vitamin B12 deficiency.

Methylmalonic acid (MMA) is a recognized marker of vitamin B12 deficiency and elevated levels typically suggest B12 deficiency, which can result in macrocytosis (increased MCV). In our study, among infants with low vitamin B12 levels, 53.57% had elevated MMA, 33.33% of infants with low-normal B12 levels and 45.65% of those with high-normal B12 levels also had elevated MMA (Table-7). However, this

difference was not statistically significant (χ^2 = 1.19, p = 0.519) in this population, higher MMA levels were not associated with macrocytosis, possibly due to confounding factors such as iron deficiency or the presence of mixed nutritional deficiencies[13]. Iron deficiency could lead to masking of megaloblastic anemia caused by vitamin B12 deficiency. In a study done by Remacha AF et al., Vitamin B12 deficiency was found in 18% of patients with iron deficiency anemia, often without macrocytosis[85].

In our study, no statistical significance was observed between MCV and functional B12 deficiency in infants (χ^2 = 1.186, p = 0.276) (Table-6). However, a statistically significant association was found between MCV and low vitamin B12 levels in infants (χ^2 = 18.723, p <0.001) (Table-11). Among mothers, there was statistical significance observed between MCV and functional B12 deficiency (χ^2 = 9.17, p = 0.002) and between MCV and low vitamin B12 levels (χ^2 = 9.265, p= 0.010)[76]. In a study done by Dağ H et al., vitamin B12 levels were significantly lower in exclusively breastfed infants compared to those receiving formula ($p < 0.001$), while MCV values were higher in the breastfed group but not statistically significant ($p > 0.05$). This suggests that elevated MCV may precede anemia in B12 deficiency, warranting early screening even in the absence of clinical signs[86].

In our study, no significant association was observed between RDW and functional B12 deficiency in infants (χ^2 = 0.001, p = 0.978) (Table-9). However, a statistically significant association was found between RDW and low vitamin B12 levels in infants (χ^2 = 6.452, p = 0.040) (Table-8). Among mothers, no significant association was observed between RDW and functional B12 deficiency (χ^2 = 1.35, p = 0.245) (Table-10) or between RDW and low vitamin B12 levels (χ^2 = 5.279, p = 0.071) (Table-12). Our study differed from studies conducted by Rajashekar RB et al.,

in which they found negative correlation between RDW and serum vitamin B12 levels ($p = 0.029$) in adults[87].

In our study, none of the infants with functional B12 or low vitamin B12 levels had developmental delay. Similar finding was reported in the study conducted by Adhualia et al, reported developmental delay in children was not significantly associated with vitamin B12 deficiency[88].

In our study, among functional B12 deficient infants, 7.7% (n=3) had hyperpigmentation. Among infants with low Vitamin B12 levels, 10.7% (n=3) had hyperpigmentation. In Kaur S and Goraya JS study 95.3% of the study participants with low vitamin B12 levels had hyperpigmentation[89].

Vitamin B12 deficiency is a prevalent health issue, particularly in developing nations. This essential nutrient plays a crucial role in fetal, neonatal and infant development, with deficiencies linked to various health complications, including neurological and developmental disorders. To support optimal growth, mothers are encouraged to consume vitamin B12-rich foods during lactation.

Limitations

This was a single center study and hence results cannot be generalized. The sample size was small and long-term follow-up of developmental status was not done.

CONCLUSION

- The prevalence of functional vitamin B12 deficiency among exclusively breastfed infants was 47%.
- In our study, 53.57 % of infants with low vitamin B12 levels (<200pg/ml) had high MMA value. 33.33 % of infants with low-normal vitamin B12 levels (200-300pg/ml), had high MMA value and 45.65 % of infants with high vitamin B12 levels (>300pg/ml) had high MMA values. However, there was no statistically significant correlation between low serum vitamin B12 levels and elevated serum MMA in infants (p=0.519).
- In our study, 78.57 % of infants with low vitamin B12 levels had high Homocysteine value. 66.67 % of infants with low-normal vitamin B12 levels, had high Homocysteine value and 45.65 % of infants with high vitamin B12 levels had high Homocysteine values. A statistically significant association was observed between low serum vitamin B12 levels and elevated homocysteine levels (p=0.018).
- Among mothers, there was no statically significant correlation between low serum vitamin B12 levels and elevated MMA. A statistically significant association was found between low serum vitamin B12 and elevated homocysteine levels (p=0.035).
- In exclusively breastfed infants, no statistically significant correlation was found between functional B12 deficiency and elevated MCV (p=0.276). However, statistically significant correlation was observed between low serum B12 levels and high MCV (p<0.001).
- In mothers, functional B12 deficiency showed a significant correlation with elevated MCV (p=0.002) and even low vitamin B12 levels also showed

statistically significant correlation with high MCV (p=0.010).

- Among infants with functional B12 deficiency, 82.05 % of infants had high RDW, however there was no statistically significant correlation observed between functional B12 deficiency and high RDW (p=0.978). Infants with low vitamin B12 levels showed a statistically significant association with elevated RDW (p=0.040).
- In mothers, 64.29 % of mothers with functional B12 deficiency had high RDW and 77.61 % of mothers with low Vitamin B12 levels had high RDW. However, neither functional B12 deficiency (p=0.245) nor low serum B12 levels (p=0.071) showed a statistically significant correlation with high RDW.
- Among infants with functional B12 deficiency, 30.8% of their mothers also had functional B12 deficiency and 92.3% mothers had low serum B12 levels. However, there was no statistically significant correlation between infant and maternal functional B12 deficiency (p=0.591) or between their low B12 levels (p=0.099).
- None of the infants with functional B12 deficiency or low vitamin B12 levels, showed developmental delay assessed by Trivandrum Development Screening Test.

SUMMARY

- In our study, the prevalence of functional B12 deficiency in infants was 47% (n=39). Among the functional B12 deficient infants, 38.5% (n=15) had low vitamin B12 levels and 61.5% (n=24) did not have low vitamin B12 levels.
- Among the infants with functional B12 deficiency (n=39), 38.5% (n=15) had low vitamin B12 levels (<200 pg/mL).
- Among the mothers, 85.5% (n=71) of them followed non-vegetarian diet and 14.5% (n=12) were vegetarian diet.
- Among the functional B12 deficient infants (n=39), 76.9% (n=30) of the mother's diet were non-vegetarians. The functional B12 deficiency status and the mother's diet status were statistically significant (p=0.036).
- The correlation between functional B12 deficiency and low vitamin B12 levels in the infants was not statistically significant (p=0.266).
- Among the study participants with low vitamin B12 levels (n=28), macrocytosis was reported in 61.8% (n=21) of the study participants. The association between low vitamin B12 levels and macrocytosis was statistically significant (p<0.001).
- Among the study participants with low vitamin B12 levels (n=28), 34.9% had high RDW levels. It was statistically significant (p=0.011).
- A negative correlation (Pearson's correlation, r=-0.176 and p value of 0.112) was found between MCV and the functional B12 deficiency in infants (methylmalonic acid levels).
- A negative correlation (Pearson correlation, r=-0.360, p=0.001) was found between RDW and Vitamin B12 levels in infants.

- Developmental delay was not found in any infants with low vitamin B12 levels and functional B12 deficient infants.
- Among infants with low Vitamin B12 levels, 10.7% (n=3) had hyperpigmentation.

BIBLIOGRAPHY

1. Venkatesh U, Sharma A, Ananthan VA, Subbiah P, Durga R. Micronutrient's deficiency in India: a systematic review and meta-analysis. Journal of Nutritional Science. 2021;10.
2. Singla R, Garg A, Surana V, Aggarwal S, Gupta G, Singla S. Vitamin B12 deficiency is endemic in Indian population: A perspective from North India. Indian Journal of Endocrinology and Metabolism. 2019;23(2):211.
3. Singh J, Dinkar A, Gupta P, Atam V. Vitamin B12 deficiency in northern India tertiary care: Prevalence, risk factors and clinical characteristics. Journal of Family Medicine and Primary Care. 2022;11(6):2381.
4. Kadiyala A, Palani A, Rajendraprasath S, Venkatramanan P. Prevalence of Vitamin B12 Deficiency among Exclusively Breast Fed Term Infants in South India. Journal of Tropical Pediatrics. 2020 Dec 21;67(1).
5. Jatoi S, Hafeez A, Riaz SU, Ali A, Ghauri MI, Zehra M, et al. Low Vitamin B12 Levels: An Underestimated Cause Of Minimal Cognitive Impairment And Dementia. Cureus Journal of Medical Science [Internet]. 2020 Feb 13;12(2). Available from: https://www.cureus.com/articles/23152-low-vitamin-b12-levels-an-underestimated-cause-of-minimal-cognitive-impairment-and-dementia
6. Bahadir A, Reis PG, Erduran E. Oral vitamin B12 treatment is effective for children with nutritional vitamin B12 deficiency. Journal of Paediatrics and Child Health. 2014 Jun 18;50(9):721–5.
7. Guez S, Chiarelli G, Menni F, Salera S, Principi N, Esposito S. Severe vitamin B12 deficiency in an exclusively breastfed 5-month-old Italian infant born to a mother receiving multivitamin supplementation during pregnancy. BMC

pediatrics [Internet]. 2012 [cited 2019 Dec 3];12:85. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22726312

8. Lipari Pinto P, Florindo C, Janeiro P, Santos RL, Mexia S, Rocha H, et al. Acquired Vitamin B12 Deficiency in Newborns: Positive Impact on Newborn Health through Early Detection. Nutrients. 2022 Oct 20;14(20):4397.
9. Langan RC, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. American Family Physician [Internet]. 2017 Sep 15;96(6):384–9. Available from: https://www.aafp.org/pubs/afp/issues/2017/0915/p384.html
10. Vashi P, Edwin P, Popiel B, Lammersfeld C, Gupta D. Methylmalonic Acid and Homocysteine as Indicators of Vitamin B-12 Deficiency in Cancer. Sengupta S, editor. PLOS ONE [Internet]. 2016 Jan 25;11(1):e0147843. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4725715/
11. Lee SM, Oh J, Chun MR, Lee SY. Methylmalonic Acid and Homocysteine as Indicators of Vitamin B12 Deficiency in Patients with Gastric Cancer after Gastrectomy. Nutrients [Internet]. 2019 Feb 21;11(2):450. Available from: https://www.mdpi.com/2072-6643/11/2/450/htm
12. Solomon LR. Functional vitamin B12 deficiency in advanced malignancy: implications for the management of neuropathy and neuropathic pain. Supportive Care in Cancer. 2016 Mar 22;24(8):3489–94.
13. Ankar A, Kumar A. Vitamin b12 deficiency [Internet]. National Library of Medicine. StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441923/
14. Maner BS, Moosavi L. Mean Corpuscular Volume (MCV) [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545275/

15. Snow CF. Laboratory Diagnosis of Vitamin B12 and Folate Deficiency. Archives of Internal Medicine. 1999 Jun 28;159(12):1289.
16. Oosterhuis WP, Niessen RW, Bossuyt PM, Sanders GT, Sturk A. Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency [Internet]. www.ncbi.nlm.nih.gov. Centre for Reviews and Dissemination (UK); 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK68152/
17. Kumar S, Mahto M. Cerebral White Matter Demyelination in Vitamin B12 Deficiency: A Case Report. Indian Journal of Clinical Medicine. 2023 Mar 5;13(1):39–42.
18. Oh S, Cave G, Lu C. Vitamin B12 (Cobalamin) and Micronutrient Fortification in Food Crops Using Nanoparticle Technology. Frontiers in Plant Science. 2021 Aug 23;12.
19. Vargas-Uricoechea H, Nogueira JP, Pinzón-Fernández MV, Agredo-Delgado V, Vargas-Sierra HD. Population Status of Vitamin B12 Values in the General Population and in Individuals with Type 2 Diabetes, in Southwestern Colombia. Nutrients [Internet]. 2023 May 18 [cited 2023 Oct 18];15(10):2357. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10221874/
20. Shalini T, Pullakhandam R, Ghosh S, Kulkarni B, Rajkumar H, Sachdev HS, et al. Prevalence of Vitamin B12 and Folate Deficiencies in Indian Children and Adolescents. Nutrients [Internet]. 2023 Jul 3 [cited 2024 Mar 12];15(13):3026. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10346745/#:~:text=The%20prevalence%20of%20B12%20and%20FA%20deficiency%20was%20high

21. Sujatha Jali, Gajjar R, Mahesh Kamate. Study of serum vitamin B12 levels in pregnant mothers and their newborns and its relation to growth of the infants. Indian Journal of Child Health. 2020 Jun 15;4(2):203–6.
22. Mittal M, Bansal V, Jain R, Dabla PK. Perturbing Status of Vitamin B12 in Indian Infants and Their Mothers. Food and Nutrition Bulletin. 2017 Mar 7;38(2):209–15.
23. Kumawat U, Amolsing, Thakur R. To study the status of vitamin b12 deficiency in pregnancy and its impact on the maternal and fetal outcome. International Journal of Clinical Obstetrics and Gynaecology. 2021 May 1;5(3):243–55.
24. Wolffenbuttel BHR, Heiner-Fokkema MR, Green R, Gans ROB. Relationship between serum B12 concentrations and mortality: experience in NHANES. BMC Medicine. 2020 Oct 9;18(1).
25. Periyasamy Kuppusamy, Ranjan Kumar Prusty, Khan S. Assessing the prevalence and predictors of anemia among pregnant women in India: findings from the India National Family Health Survey 2019–2021. Current Medical Research and Opinion. 2023 Nov 11;40(1):51–8.
26. Marol R, Marol R. Prevalence of anemia in exclusively breastfed full term babies between 3-6 months of age. International Journal of Contemporary Pediatrics. 2021 Jan 22;8(2):300.
27. Al Amin ASM, Gupta V. Vitamin B12 (Cobalamin) [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559132/
28. Schleicher E, Triantafyllos Didangelos, Evangelia Kotzakioulafi, Čegan A, Peter A, Konstantinos Kantartzis. Clinical Pathobiochemistry of Vitamin B12 Deficiency: Improving Our Understanding by Exploring Novel Mechanisms

with a Focus on Diabetic Neuropathy. Nutrients [Internet]. 2023 Jun 1;15(11):2597–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10255445/

29. Watanabe F. Vitamin B12 sources and bioavailability. Experimental biology and medicine (Maywood, NJ) [Internet]. 2007;232(10):1266–74. Available from: https://www.ncbi.nlm.nih.gov/pubmed/17959839
30. Fang H, Li D, Kang J, Jiang P, Sun J, Zhang D. Metabolic engineering of Escherichia coli for de novo biosynthesis of vitamin B12. Nature Communications. 2018 Nov 21;9(1).
31. Bjørke-Monsen AL, Ueland PM. Cobalamin status in children. Journal of Inherited Metabolic Disease. 2010 May 27;34(1):111–9.
32. Obeid R, Morkbak AL, Munz W, Nexo E, Herrmann W. The Cobalamin-Binding Proteins Transcobalamin and Haptocorrin in Maternal and Cord Blood Sera at Birth. Clinical Chemistry. 2006 Feb 1;52(2):263–9.
33. Blom H, Schiff M. Treatment of Inherited Homocystinurias. Neuropediatrics. 2012 Nov 2;43(06):295–304.
34. Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, et al. Vitamin B12 deficiency. Nature Reviews Disease Primers. 2017 Jun 29;3(1).
35. Nawaz A, Khattak NN, Khan MS, Nangyal H, Sabri S, Shakir M. Deficiency of vitamin B12 and its relation with neurological disorders: a critical review. The Journal of Basic and Applied Zoology. 2020 Mar 18;81(1).
36. Guéant JL, Guéant-Rodriguez RM, Alpers DH. Vitamin B12 absorption and malabsorption. Vitamins and Hormones [Internet]. 2022;119:241–74. Available from: https://pubmed.ncbi.nlm.nih.gov/35337622/

37. Vaqar S, Shackelford K. Pernicious anemia [Internet]. Nih.gov. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK540989/
38. Htut TW, Thein KZ, Oo TH. Pernicious anemia: Pathophysiology and diagnostic difficulties. Journal of Evidence-Based Medicine [Internet]. 2021 May;14(2):161–9. Available from: https://pubmed.ncbi.nlm.nih.gov/34015185/
39. Shah SC, Piazuelo MB, Kuipers EJ, Li D. AGA Clinical Practice Update on the Diagnosis and Management of Atrophic Gastritis: Expert Review. Gastroenterology. 2021 Oct;161(4):1325-1332.e7.
40. Mohamed M, Thio J, Thomas RS, Phillips J. Pernicious anaemia. BMJ (Clinical research ed) [Internet]. 2020 Apr 24;369(8244):m1319. Available from: https://pubmed.ncbi.nlm.nih.gov/32332011/
41. Esposito G, Dottori L, Pivetta G, Ligato I, Dilaghi E, Lahner E. Pernicious Anemia: The Hematological Presentation of a Multifaceted Disorder Caused by Cobalamin Deficiency. Nutrients. 2022 Apr 17;14(8):1672.
42. Lahner E, Annibale B. Pernicious anemia: New insights from a gastroenterological point of view. World Journal of Gastroenterology [Internet]. 2009;15(41):5121. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2773890/
43. Castellana C, Eusebi LH, Dajti E, Iascone V, Vestito A, Fusaroli P, et al. Autoimmune Atrophic Gastritis: A Clinical Review. Cancers [Internet]. 2024 Jan 1;16(7):1310. Available from: https://www.mdpi.com/2072-6694/16/7/1310
44. Iwamuro M, Tanaka T, Otsuka M. Update in Molecular Aspects and Diagnosis of Autoimmune Gastritis. Current Issues in Molecular Biology

[Internet]. 2023 Jun 21 [cited 2023 Nov 1];45(7):5263–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10378041/

45. Singh S, Chakole S, Agrawal S, Shetty N, Prasad R, Lohakare T, et al. A Comprehensive Review of Upper Gastrointestinal Symptom Management in Autoimmune Gastritis: Current Insights and Future Directions. Cureus [Internet]. 2023 Aug 13;15(8). Available from: https://assets.cureus.com/uploads/review_article/pdf/169618/20230813-25724-1y6y2qz.pdf
46. Hall SN, Appelman HD. Autoimmune Gastritis. Archives of Pathology & Laboratory Medicine. 2019 Nov;143(11):1327–31.
47. Kingma SDK, Neven J, Bael A, Meuwissen MEC, van den Akker M. Imerslund-Gräsbeck syndrome: a comprehensive review of reported cases. Orphanet Journal of Rare Diseases [Internet]. 2023 Sep 14;18:291. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10500774/#CR1
48. NIH. Transcobalamin II deficiency (Concept Id: C0342701) - MedGen - NCBI [Internet]. Nih.gov. 2024 [cited 2025 Mar 29]. Available from: https://www.ncbi.nlm.nih.gov/medgen/137976
49. Lanzkowsky P. Megaloblastic Anemia. Lanzkowsky's Manual of Pediatric Hematology and Oncology. 2016;84–101.
50. Labpedia. Anemia:- Part 3 - Megaloblastic Anemias, Macrocytic, Vitamin B12 and Folic Acid Deficiency, and Lab findings - Labpedia.net [Internet]. Labpedia. 2020. Available from: https://labpedia.net/anemia-part-3-megaloblastic-anemia-macrocytic-vitamin-b12-and-folic-acid-deficiency-and-lab-findings/

51. Hariz A, Bhattacharya PT. Megaloblastic Anemia [Internet]. National Library of Medicine. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537254/
52. Halczuk K, Kaźmierczak-Barańska J, Karwowski BT, Karmańska A, Cieślak M. Vitamin B12—Multifaceted In Vivo Functions and In Vitro Applications. Nutrients [Internet]. 2023 Jun 13;15(12):2734–4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10305463/
53. Olders H. B12 and cognition: What the guidelines don't say. Parkhurst Exchange [Internet]. 2011 May 1;19(4):38–44. Available from: https://www.researchgate.net/publication/259786487_B12_and_cognition_What_the_guidelines_don%27t_say
54. Srivastava A, Choudhary S. Knuckle Pigmentation as an Early Cutaneous Sign of Vitamin B12 Deficiency: A Case Report. Journal of Nepal Medical Association. 2020 Oct 15;58(230).
55. Mohankar A, Swain JP, Verma S. Knuckle Hyperpigmentation Is A Marker Of Vitamin B12 Deficiency In Vegetarian Population. Journal of Evidence Based Medicine and Healthcare. 2018 May 9;5(20):1559–61.
56. Behere RV, Deshmukh AS, Otiv S, Gupte MD, Yajnik CS. Maternal Vitamin B12 Status During Pregnancy and Its Association With Outcomes of Pregnancy and Health of the Offspring: A Systematic Review and Implications for Policy in India. Frontiers in Endocrinology. 2021 Apr 12;12.
57. Hannibal L, Lysne V, Bjørke-Monsen AL, Behringer S, Grünert SC, Spiekerkoetter U, et al. Biomarkers and Algorithms for the Diagnosis of Vitamin B12 Deficiency. Frontiers in Molecular Biosciences [Internet]. 2016;3(27):27. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27446930

58. Sobczyńska-Malefora A, Delvin E, McCaddon A, Ahmadi KR, Harrington DJ. Vitamin B12 status in health and disease: a critical review. Diagnosis of deficiency and insufficiency – clinical and laboratory pitfalls. Critical Reviews in Clinical Laboratory Sciences. 2021 Apr 21;58(6):399–429.
59. Al-Dirbashi OY, Sharma C, Al Dahouri N, Al Aidaros A, Al-Muhairi S, Beiram R, et al. Role of Functional Biomarkers to Identify Early Vitamin B12 Deficiency in Patients with Sleeve Gastrectomy: A Cross-Sectional Study. Medicina. 2020 Mar 20;56(3):142.
60. Chan CQH, Low LL, Lee KH. Oral Vitamin B12 Replacement for the Treatment of Pernicious Anemia. Frontiers in Medicine [Internet]. 2016 Aug 23;3(38). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4993789/
61. Nair MKC, Nair GSH, George B, Suma N, Neethu C, Leena ML, et al. Development and Validation of Trivandrum Development Screening Chart for Children Aged 0-6 years [TDSC (0-6)]. The Indian Journal of Pediatrics. 2013 Sep 8;80(S2):248–55.
62. Tangeraas T, Ljungblad UW, Lutvica E, Kristensen E, Rowe AD, Bjørke-Monsen AL, et al. Vitamin B12 Deficiency (Un-)Detected Using Newborn Screening in Norway. International Journal of Neonatal Screening [Internet]. 2023 Mar 1 [cited 2023 Jan 10];9(1):3. Available from: https://www.mdpi.com/2409-515X/9/1/3
63. Reischl-Hajiabadi AT, Garbade SF, Feyh P, Weiss KH, Mütze U, Kölker S, et al. Maternal Vitamin B12 Deficiency Detected by Newborn Screening—Evaluation of Causes and Characteristics. ProQuest [Internet]. 2022;3767. Available from:

https://www.proquest.com/docview/2716558603?accountid=8018&pq-origsite=primo

64. National Institutes of Health. Office of Dietary Supplements - Vitamin B12 [Internet]. Nih.gov. National Institutes of Health; 2024. Available from: https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/
65. Lopez MJ, Tadi P. Drug Labeling [Internet]. Nih.gov. StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557743/
66. Finkelstein JL, Fothergill A, Krisher JT, Thomas T, Kurpad AV, Dwarkanath P. Maternal vitamin B12 deficiency and perinatal outcomes in southern India. Szecsi PB, editor. PLOS ONE. 2021 Apr 6;16(4):e0248145.
67. Brihi JE, Pathak S. Normal and Abnormal Complete Blood Count with Differential [Internet]. www.ncbi.nlm.nih.gov. StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK604207/
68. Bain BJ, Bates I, Laffan MA. Dacie and Lewis Practical Haematology | ScienceDirect [Internet]. www.sciencedirect.com. 2017. Available from: https://www.sciencedirect.com/book/9780702066962/dacie-and-lewis-practical-haematology
69. IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp [Internet]. Ibm.com. 2018. Available from: https://www.ibm.com/support/pages/how-cite-ibm-spss-statistics-or-earlier-versions-spss
70. Schroder TH, Mattman A, Sinclair G, Vallance HD, Lamers Y. Reference interval of methylmalonic acid concentrations in dried blood spots of healthy, term newborns to facilitate neonatal screening of vitamin B12 deficiency. Clinical Biochemistry [Internet]. 2016 Sep 1;49(13-14):973–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27040901/

71. Lysne V, Bjørndal B, Grinna ML, Midttun Ø, Ueland PM, Berge RK, et al. Short-term treatment with a peroxisome proliferator-activated receptor α agonist influences plasma one-carbon metabolites and B-vitamin status in rats. PloS one [Internet]. 2019 May;14(12):e0226069. Available from: https://pubmed.ncbi.nlm.nih.gov/31805132/
72. Duggan C, Srinivasan K, Thomas T, Samuel T, Rajendran R, Muthayya S, et al. Vitamin B-12 Supplementation during Pregnancy and Early Lactation Increases Maternal, Breast Milk, and Infant Measures of Vitamin B-12 Status. The Journal of Nutrition. 2014 Mar 5;144(5):758–64.
73. Greibe E, Lildballe DL, Streym S, Vestergaard P, Rejnmark L, Mosekilde L, et al. Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: a 9-mo longitudinal study. The American journal of clinical nutrition [Internet]. 2013 Aug;98(2):389–95. Available from: https://pubmed.ncbi.nlm.nih.gov/23783295/
74. Azad C, Jat KR, Kaur J, Guglani V, Palta A, Tiwari A, et al. Vitamin B_{12} status and neurodevelopmental delay in Indian infants: a hospital-based cross-sectional study. Paediatrics and International Child Health. 2019 Jul 3;40(2):78–84.
75. Singhal A, Singh A, Sharma D, Kritanjali Sahu Bose. Correlation between maternal and infant vitamin B12 levels in severe malnutrition in infants under 6 months. Irish journal of medical science. 2024 Mar 11;
76. Grover IS, Bharti R, Kapahi R, Sodhi MK, Kapahi R. Correlations between serum vitamin B12 and folate levels among mother–infant dyads in Punjab state, North-West India. Journal of Paediatrics and Child Health. 2022 Sep 13;58(12):2243–7.

77. Yıldırmak ZY, Genç DB, Kural A, Mıhmanlı V, Salman S, Doğan K, et al. Assessment of vitamin B12 and homocysteine levels in pregnant women admitted for delivery and cord blood samples of their newborn babies: a multicenter study. The Turkish journal of pediatrics [Internet]. 2024 Jul;66(4):429–38. Available from: https://pubmed.ncbi.nlm.nih.gov/39387428/
78. Kalay Z, Islek A, Parlak M, Kirecci A, Guney O, Koklu E, et al. Reliable and powerful laboratory markers of cobalamin deficiency in the newborn: plasma and urinary methylmalonic acid. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians [Internet]. 2016;29(1):60–3. Available from: https://pubmed.ncbi.nlm.nih.gov/25385266/
79. Karademir F, Suleymanoglu S, Ersen A, Aydinoz S, Gultepe M, Meral C, et al. Vitamin B_{12}, Folate, Homocysteine and Urinary Methylmalonic Acid Levels in Infants. Journal of International Medical Research. 2007 May 1;35(3):384–8.
80. Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. Blood. 2005 Feb 1;105(3):978–85.
81. Iqbal N, Azar D, Yun YM, Ghausi O, Ix J, Fitzgerald RL. Serum methylmalonic acid and holotranscobalamin-II as markers for vitamin B12 deficiency in end-stage renal disease patients. Annals of clinical and laboratory science [Internet]. 2013;43(3):243–9. Available from: https://pubmed.ncbi.nlm.nih.gov/23884217/

82. Carmel R. Subclinical cobalamin deficiency. Current Opinion in Gastroenterology. 2012 Mar;28(2):151–8.
83. ÜNSÜR E, KINAŞ B. The relationship between maternal and neonatal vitamin B12 and folate levels, anthropometric measurements, and metabolic indicators. Journal of Surgery and Medicine. 2020 Jan 24;
84. Ljungblad UW, Paulsen H, Mørkrid L, Pettersen RD, Hager HB, Lindberg M, et al. The prevalence and clinical relevance of hyperhomocysteinemia suggesting vitamin B12 deficiency in presumed healthy infants. European Journal of Paediatric Neurology. 2021 Nov;35:137–46.
85. Remacha AF, Sardà MP, Canals C, Queraltò JM, Zapico E, Remacha J, et al. Combined cobalamin and iron deficiency anemia: a diagnostic approach using a model based on age and homocysteine assessment. Annals of Hematology [Internet]. 2013 Apr 1 [cited 2021 May 11];92(4):527–31. Available from: https://pubmed.ncbi.nlm.nih.gov/23183879/
86. Dağ H, Özberk Koç M, Dikker O, Dursun H. Vitamin B12 Serum Levels of Six to Nine-monthold Infants According to Feeding Practices. The Journal of Pediatric Research [Internet]. 2020 Mar 1;7(1):1–6. Available from: https://jpedres.org/articles/vitamin-b12-serum-levels-of-six-to-nine-monthold-infants-according-to-feeding-practices/doi/jpr.galenos.2019.00377
87. Rajashekar RB, Patel S, Kulkarni P. Discriminant Functions in the Diagnosis of Vitamin B12 Deficiency Anemia, the Value of RDW-SD: An Analytical Study. DOAJ (DOAJ: Directory of Open Access Journals). 2017 Jan 1;
88. Adhualia A, Maurya M, Tewari AD. Development delay in children with severe acute malnutrition and its association with Vitamin B12 deficiency. International Journal of Contemporary Pediatrics. 2019 Oct 21;6(6):2484.

89. Kaur S, Goraya JS. Dermatologic findings of vitamin B12 deficiency in infants. Pediatric Dermatology. 2018 Oct 11;35(6):796–9.

ANNEXURES

ANNEXURE – I - INFORMED CONSENT FORM

KAHERS JNMC BELAGAVI

"PREVALENCE OF FUNCTIONAL B12 DEFICIENCY AMONGST EXCLUSIVELY BREASTFED INFANTS BETWEEN 1 TO 6 MONTHS OF AGE.A CROSS SECTIONAL STUDY AT KLE'S DR PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI"

Name of Student/Principal Investigator: _____

Name of Guide/Co Investigators: _____

Introduction: You are being invited to participate in this study to find out Prevalence of functional B12 deficiency amongst exclusively breastfed infants between 1 to 6 months of age. Participation of your baby will help us to know the Serum B12 level, Methylmalonic acid level and serum Homocysteine level. If an infant is found to be B12 deficient by the above analysis then subject the mother to the same tests and do the developmental assessment of infants with B12 deficiency by Trivandrum Development Screening Test. Vitamin B12 deficiency or megaloblastic anemia is not only an important cause of hematological complications but it also leads to significant neurological issues as it is essential for myelination. Its deficiency can result in axonal degeneration and cerebral atrophy, which can manifest as hypotonia, cognitive deficits and developmental delay. This study would also help in the early diagnosis of B12 deficiency before the neurologic complications set in as vit B12 deficiency is the 2nd commonest preventable cause of mental retardation.

Explanation of procedure: 2ml of Infant's blood and the blood of functional vitamin B12 deficient infant's mother will be collected in Plain bulbs and in EDTA bulbs each. The complete blood counts and liver function tests will be processed. Few basic demographic and birth history will be asked to you as a part of the proforma.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: The early detection of vitamin B12 level in your baby can be life saving since the deficiency is the 2nd commonest preventable cause of mental retardation. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the principal investigator/Participant.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Questions: If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"PREVALENCE OF FUNCTIONAL B12 DEFICIENCY AMONGST EXCLUSIVELY BREASTFED INFANTS BETWEEN 1 TO 6 MONTHS OF AGE.A CROSS SECTIONAL STUDY AT KLE'S DR PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI".** My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE II: PROFORMA

PROFORMA

Name-

Age- Sex-

Address-

Phone no- Email ID-

Birth weight- Period of Gestation-

Current weight- Length/Height- Head circumference-

Regression of Milestones- YES NO

Interpretation as per the standard growth chart-

History:

Developmental delay (as per scale)-

Gross Motor	Fine Motor	Language	Social	Global

Developmental Quotient in each domain-

Any antenatal complication-

Any problem in Neonatal period-

H/O exclusively breastfeeding-

Recurrent Infections- YES NO

Site and Type of infections-

Family History-

Physical Examination-

Pallor	Yes	No
Knuckle pigmentation	Yes	No
Generalised Hyper pigmentation	Yes	No
Tremors	Yes	No

Any Other—

Systemic Examination-

CNS- Tremors

CVS- Features of Hyperdynamic circulation

Hemic murmur

Venous murmur

P/A- Hepatomegaly-

Splenomegaly-

Surgical scar-

Investigations-

1.) Complete Hemogram—

HB	
RBC	
MCV	
MCH	
MCHC	
RDW	
Retic Count	
White Blood Cell Count	
Neutrophils	
Eosinophils	

Monocytes	
Basophils	
Platelets	
Peripheral Smear	

2.) Vitamin B12 metabolism profile—

	BABY	MOTHER
Serum B12		
Serum Homocysteine		
Serum Methylmalonic acid		

3.) LDH—

4.) LFT—

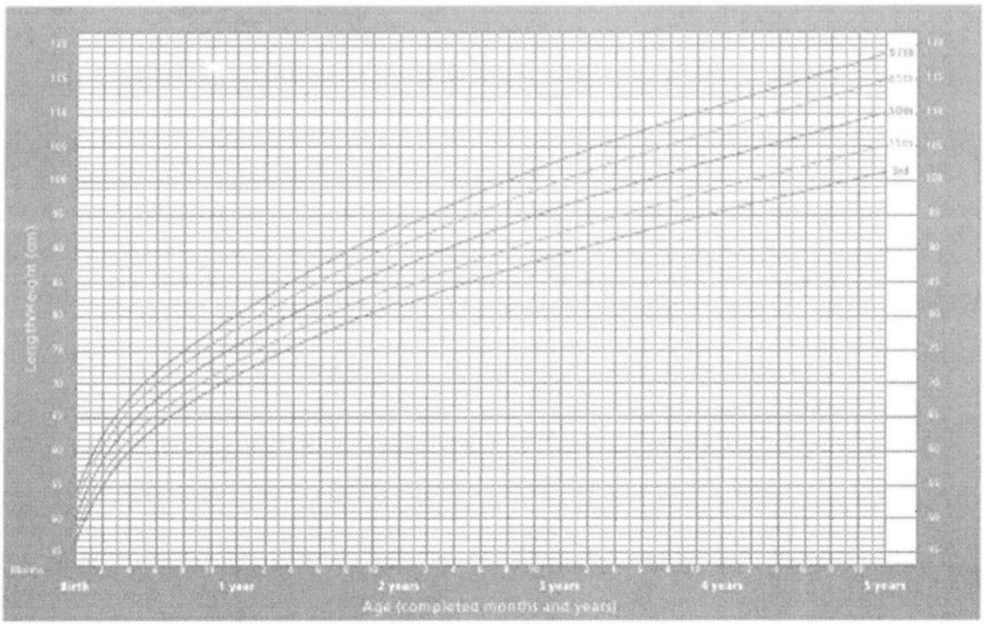
Total Bilirubin		
Direct Bilirubin		
Indirect Bilirubin		
Total Protein		
Albumin		
Globulin		
A/G Ratio		
ALT (SGPT)		
AST (SGOT)		
Alk. Phosphatase		

5.) Any Other Positive Investigation—

Length/height-for-age BOYS

Birth to 5 years (percentiles)

World Health Organization

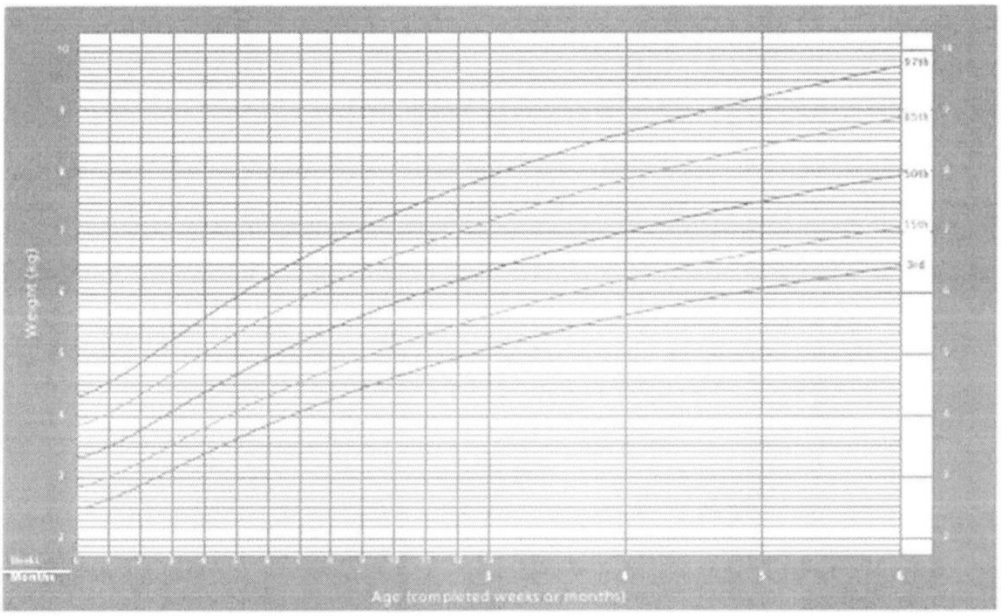


WHO Child Growth Standards

Weight-for-age BOYS

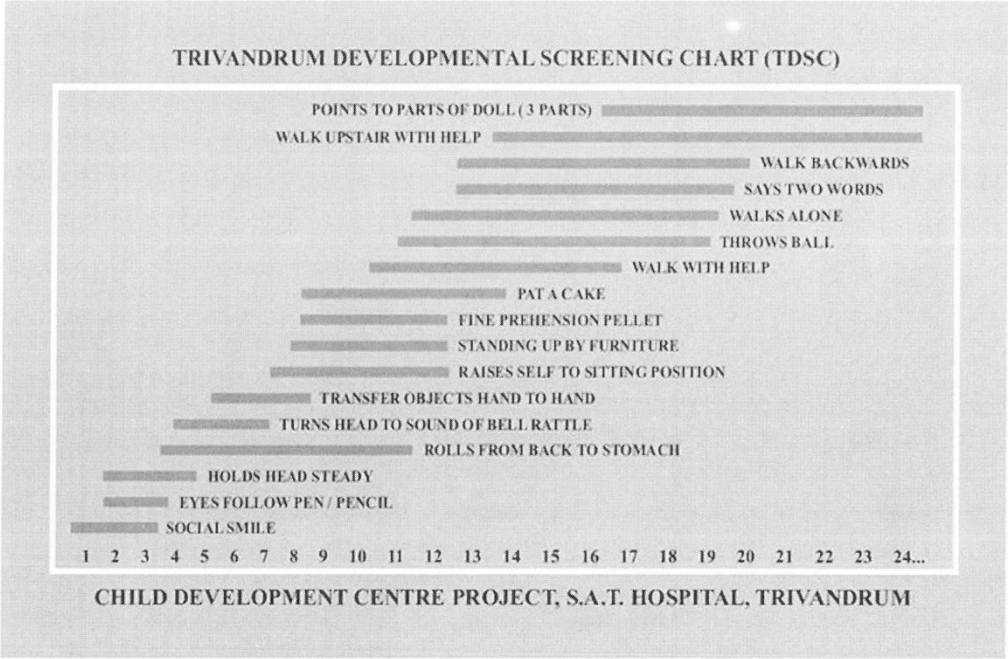
Birth to 6 months (percentiles)

World Health Organization



WHO Child Growth Standards

Initial developmental assessment by Trivandrum Developmental Screening Test-



ANNEXURE III: MASTER CHART

S no	Name	Sex	Age (months)	B.Wt (kg)	Current Wt (kg)	Length (cm)	HC (cm)	POG	Regression of Milestones	Developmental Delay	Hyperpigmentation	Mother's Dietary Status	Vit B12 (pg/ml)	MMA (pmol/L)	Homocysteine (mcmol/L)	Hb (g/dL)	MCV	MCH	MCHC	RDW	RBC	WBC	Platelets	PS	TB/DB	ALT	AST	ALP	LDH
1	B/O Ambika	F	3m 20d	2.9	4.8	56	40	Term	No	No	No	Non Veg	349.693	153.99	21.67	10	75.8	23	32	16	4.17	7.2	403K	Microcytic Hypochromic An emia	0.13/0.09	112	180	217	424
2	Shravan Patil	M	5m 19d	2.5	6.7	68	42	Term	No	No	No	Non Veg	1138.54	426.79	5.434	12	77.8	21	28	16	5.41	12	568K	Microcytic Hypochromic, mild thrombocytosis	0.4/0.1	12	27	901	331
3	Yallanagouda	M	4m 12d	1.9	4.9	56	40	Late PT	No	No	No	Non Veg	660.08	302.19	24.08	9.8	72.4	22	31	17	4.41	15	303K	Microcytic Hypochromic Anemia, Neutrophilia	1.16/0.6	28	36	203	473
4	Sadananda gouda	M	4m 12d	1.8	4.4	55	39	Late PT	No	No	No	Non Veg	1138.54	422.11	15.9	12	79.4	23	29	16	5.24	20	826K	Neutrophilic leucocytosis, Thrombocytosis	0.36/0.22	51	46	378	437
5	Mutturaj Sarav	M	2m 6d	2.5	3.8	52	37	Term	No	No	No	Non Veg	177.557	110.93	6.248	13	112	30	31	19	4.12	16	366K	Macrocytic Normochromic	1.6/0.82	10	20	285	310
6	Driti Badiger	F	5m 7d	2.1	5.4	58	39	Term	No	No	No	Non Veg	1138.54	426.79	10.98	9.6	72.2	23	31	17	4.45	14	301.5K	Microcytic Hypochromic Anemia, Neutrophilia	0.4/0.2	28	33	200	470
7	B/O Geetanjali	M	1m 8d	3.3	3.9	57	40	Term	No	No	No	Non Veg	1138.54	426.79	18.71	11	78	22	28	17	5.6	13	560K	Microcytic Hypochromic with mild thrombocytosis	0.28/0.14	12	28	748	289
8	Renuka	F	3m	2.4	4.8	54	37	Term	No	No	No	Non Veg	250.749	291.52	18.82	12	85	24	29	16	5.24	19	393K	Normocytic Hypochromic Anaemia	0.48/0.32	19	40	682	289
9	Abhinav	M	2m 13d	2	3	54	39	Late PT	No	No	No	Non Veg	234.484	100.37	30.66	11	85.2	23	35	16	4.5	15	572K	Normocytic Hypochromic Anaemia	5.0/4.01	26	26	758	295
10	Tahira Tumbagi	F	3m 8d	2.3	3.6	54	36	Term	No	No	No	Non Veg	114.909	38.776	37.17	9.9	91.1	28	30	24	3.6	13	220K	Macrocytic Normochromic anaemia with Eosinophilia	8.23/6.45	136	258	362	302
11	Lalitesh	M	1m 15d	3.4	4.2	56	38	Term	No	No	No	Non Veg	189.756	99.492	22.52	12	88.9	28	32	21	4.07	3.7	366K	Leucopenia	0.81/0.44	56	66	360	412
12	Rahul Gadade	M	6m	2.6	6.3	62	40	Term	No	No	No	Non Veg	192.467	95.41	35.43	18	74.2	21	29	16	8.26	12	220K	Erythrocytosis	0.67/0.19	5	37	303	487

13	Trupti Kurbeta	F	1m 16d	3	3.8	54	36	Term	No	No	No	Non Veg	109.142	96.435	24.34	7	118	30	29	26	2.21	12	418K	Macrocytic Normochromic Anemia	3.66/2.69	134	148	551	469
14	Pranathi Harijan	F	6m	1.9	4.7	60	39	Late PT	No	No	No	Non Veg	1138.54	411.42	16.2	11	79.7	23	29	19	4.62	17	467K	Normocytic Hypochromic anaemia with Thrombocytosis	0.38/0.27	17	31	122	291
15	Arpita Dandin	F	1m 1d	3.3	4.1	54	35	Term	No	No	Yes	Non Veg	105.721	271.84	34.21	14	128	32	31	34	4.32	16	365K	Macrocytic, Leucocytosis	1.6/0.82	10	20	285	382
16	Vaishnavi Ganti	F	6m	2.6	6.5	60	40	Term	No	No	No	Non Veg	1138.54	426.79	1.624	12	77.8	21	28	16	5.41	12	568K	Normocytic Hypochromic mild thrombocytosis	0.28/0.14	12	27	901	331
17	B/O Snehal Patil	F	4m 22d	3.4	7	63	41	Term	No	No	No	Non Veg	1138.54	310.87	6.285	9.8	72.4	22	31	17	4.41	15	303K	Normocytic Hypochromic Anemia, Neutrophilia	1.16/0.6	28	36	203	473
18	Samanvi Kalakutagi	F	1m 6d	2.8	2.9	51	36	Term	No	No	No	Veg	70.251	275.96	23	12	130	31	32	36	3.74	12	448K	Macrocytic Normochromic	4.94/1.34	18	35	334	311
19	Nagaraj Walikar	M	5m 18d	2.5	3.9	57	38	Term	No	No	No	Non Veg	1138.54	89.008	34.77	11	87.3	26	29	16	4.32	12	517K	Thrombocytosis	0.08/0.04	26	45	271	288
20	Rahman Badekhan	M	6m	2.5	4.7	58	40	Term	No	No	No	Non Veg	1138.54	65.247	9.725	9.9	83.9	23	31	15	4.02	14	512K	Normocytic Hypochromic Anaemia	0.24/0.13	11	25	243	324
21	Rayanna Sheregar	M	1m 3d	2.6	2.9	50	36	Term	No	No	No	Non Veg	119.128	49.115	25.82	9.3	133	29	29	21	3.21	11	259K	Macrocytic Hypochromic anaemia with Leucopenia with Thrombocytosis	0.49/0.29	6	17	288	298
22	Shoury Khanapuri	M	6m	1.7	5.6	64	42	Late PT	No	No	No	Non Veg	1138.54	51.52	22.08	6.9	64.4	15	24	24	4.49	13	418K	Microcytic Hypochromic Anaemia	0.3/0.18	9	33	204	211
23	Nayana Pujeri	F	6m	2.5	5.6	60	41	Term	No	No	No	Non Veg	1138.54	68.185	5.841	8.7	65.7	19	28	19	4.69	8.6	544K	Microcytic Hypochromic Anaemia	0.3/0.21	7	39	182	351
24	Sanjithgouda	M	6m	2.3	6.4	64	41	Term	No	No	No	Non Veg	881.01	139.58	7.505	10	75.8	24	32	16	4.17	7.2	403K	Normocytic Normochromic Anemia	0.13/0.09	112	180	217	424
25	Kartik Totagi	M	6m	2.7	6.5	63	41	Term	No	Yes	No	Non Veg	1138.54	143.92	12.46	9.8	66.5	20	31	17	4.83	15	385K	Microcytic Hypochromic anaemia with Eosinophilia	0.31/0.17	22	38	254	309
26	Agathsya Shinge	M	6m	2.6	5.2	62	40	Term	No	No	No	Non Veg	1138.54	139.1	12.5	6.2	103	32	31	18	1.91	7.8	97K	Macrocytic Hyperchromic Anemia, Thrombocytopenia	0.33/0.24	206	429	362	841
27	Vaibhav Ramaganatti	M	6m	2.8	5.5	62	42	Term	No	No	Yes	Veg	138.536	275.98	22.78	9.5	102	24	29	19	4.02	5.3	585K	Macrocytic Hypochromic anaemia with Leucopenia with Thrombocytosis	0.12/0.07	10	36	164	473
28	Shanur Mujawar	M	4m 1d	2.3	4	58	38	Term	No	No	No	Non Veg	1122.27	86.553	22.23	12	83.1	24	29	15	4.84	18	383K	Normocytic Normochromic Blood Picture	0.44/0.26	17	40	723	287
29	Samarth Gadadi	M	4m 1d	2.5	5	56	40	Term	No	No	No	Veg	191.111	345.3	18.23	12	92.4	27	30	20	4.56	15	639K	Lymphocytosis with Thrombocytosis	0.28/0.14	27	30	351	310

30	Basavaraj Vakkund	M	6m	2.5	5.5	60	40	Term	No	No	No	Non Veg	1138.54	91.083	2.956	11	95.2	29	31	17	3.8	20	606K	Macrocytosis, Leucocytosis, Thrombocytosis	0.37/0.18	24	37	215	223
31	Ayan Onti	M	3m 1d	2.6	4.1	56	37	Term	No	No	No	Non Veg	588.244	121.71	21.82	11	84.2	27	31	16	4.12	14	560K	Normocytic Normochromic Anemia	5.01/4.25	24	24	706	442
32	B/O Seema Gadadi	M	3m 10d	2.3	4.2	59	38	Term	No	No	No	Non Veg	193.822	309.11	16.94	12	99.7	27	30	16	4.56	15	639K	Lymphocytosis with Thrombocytosis	0.28/0.14	27	30	351	310
33	Ruthvi Khot	F	6m	2.6	5.2	63	41	Term	No	No	No	Non Veg	1138.54	75.84	20.71	12	79.4	23	29	16	5.24	20	826K	Neutrophilic leucocytosis, Thrombocytosis	0.36/0.22	51	46	378	437
34	Shourya Nandihalli	M	6m	2.9	6	62	42	Term	No	No	No	Veg	914.895	86.222	10.13	6.8	91.1	24	27	24	2.8	3.1	48K	Pancytopenia	0.1/0.16	35	27	80	356
35	Rohit Bachagoudra	M	6m	2.5	5.4	61	40	Late PT	No	No	No	Non Veg	967.756	90.668	21.08	9.4	79.1	22	28	16	4.21	34	909K	Normocytic Hypochromic Anemia, Neutrophilic leucocytosis, Thrombocytosis	0.17/0.13	36	63	278	682
36	Abhay Done	M	6m	3	6.5	62	42	Term	No	No	No	Non Veg	973.177	124.58	10.54	11	72.2	23	31	15	4.73	16	452K	Normocytic Hypochromic Anemia	0.38/0.18	23	43	398	373
37	Advit Matawadi	M	5m 2d	2.4	5.4	63	41	Term	No	No	No	Non Veg	1138.54	72.089	10.17	9.8	87.3	27	31	12	3.63	14	356K	Macrocytic Normochromic Anaemia	0.28/0.16	18	21	186	442
38	Shrinivas Shivapure	M	6m	2.6	6.5	63	42	Term	No	No	No	Non Veg	185.69	70.167	7.838	8.5	96.2	25	33	23	2.56	10	301K	Macrocytic Normochromic Anemia, Lymphocytosis with Atypical cells	0.45/0.3	10	26	155	286
39	Samayara Dharennavar	F	6m	2.2	3.8	52	37	Early PT	No	No	No	Non Veg	108.432	63.655	17.09	9.5	126	37	30	36	1.48	6.3	149K	Macrocytic anaemia with Lymphocytosis and Thrombocytopenia	1.05/0.62	9	32	122	600
40	Rekha Badakali	F	6m	2.8	4.2	60	42	Term	No	No	No	Non Veg	689.899	82.835	4.102	9.4	99.5	29	29	30	3.24	18	492K	Macrocytic Normochromic Anemia, Leucocytosis	0.48/0.19	10	36	392	344
41	Bhagyashree Marapur	F	1m 18d	2.5	3.3	48	36	Late PT	No	No	No	Non Veg	231.773	82.801	22.63	7.7	114	33	29	18	2.32	21	389K	Macrocytic Hyperchromic Anemia, Leucocytosis, Lymphocytosis	0.45/0.24	382	560	240	216
42	B/O Basavakumari L	F	2m 7d	2.8	4	54	36	Term	No	No	No	Non Veg	295.477	80.337	38.65	8.3	68.3	20	27	19	4.65	21	450K	Microcytic Hypochromic Anemia with Leucocytosis	4.5/3.16	49	159	757	1182
43	Laxmi Pudabangi	F	5m	2.6	4.8	50	36	Term	No	No	No	Non Veg	78.871	426.79	9.762	12	105	25	30	21	4.4	13	591K	Lymphocytosis with Thrombocytosis	0.25/0.09	16	36	303	309
44	B/O Prathiba Burud	F	1m 16d	3	3.8	52	36	Term	No	No	No	Veg	795.62	129.31	2.512	12	94.4	29	30	14	4.33	19	546K	Normocytic Hypochromic, Thrombocytosis	0.24/0.18	17	18	279	255

45	Niranjan Kumar	M	6m	3.5	6.8	62	41	Term	No	No	No	Non Veg	262.948	426.79	6.248	9.3	77.8	22	29	14	4.15	6.6	394K	Normocytic Hypochromic Anemia	0.5/0.27	15	41	235	353
46	B/O Shireen Bepari	F	3m	3	3.8	56	40	Term	No	No	No	Non Veg	749.536	298.42	13.5	14	87.3	26	30	16	5.28	21	611K	Leucocytosis with Thrombocytosis	0.87/0.32	38	55	369	367
47	Lohit Yarazarvi	M	6m	2.4	6.8	62	42	Term	No	No	Yes	Non Veg	158.584	426.79	9.799	8.6	65.7	19	28	25	4.69	8.6	544K	Microcytic Hypochromic Anaemia, Thrombocytosis	0.2/0.17	25	43	267	396
48	Ahmed Shaikh	M	2m 15d	3.5	5	59	38	Term	No	No	No	Non Veg	531.317	160.94	21.45	11	97.4	28	29	26	3.79	14	452K	Normocytic Hypochromic Blood Picture	0.31/012	14	78	225	456
49	Minaxi Kaleri	F	6m	2.9	5.2	62	41	Term	No	No	No	Non Veg	409.331	426.79	34.1	9.5	80.8	25	30	19	3.86	8.5	319K	Normocytic Normochromic Anemia	0.22/0.13	13	32	123	270
50	Abhirvey Hadapad	M	3m	3	5.2	52	36	Term	No	No	No	Veg	99.246	347.44	26.93	8.5	111	28	25	33	1.77	41	132K	Macrocytic Hypochromic Anaemia, Leucocytosis, Thrombopenia	1.88/0.98	40	81	359	1657
51	B/O Rajashri Soudatti	M	1m 7d	3.4	4.7	56	37	Term	No	No	No	Non Veg	103.01	76.713	32.07	11	101	30	31	17	3.59	17	436K	Normocytic Hypochromic blood picture	0.82/0.43	4	21	456	296
52	B/O Shruti Patil	F	2m 15d	2.5	5.2	52	36	Late PT	No	No	No	Non Veg	112.498	330.26	24.71	13	95.9	30	33	19	3.93	12	437K	Normocytic Normochromic Anemia with Lymphocytosis	1.85/0.89	38	74	689	392
53	B/O Pooja Bhavi	F	2m 20d	2.4	4	54	36	Term	No	No	No	Non Veg	508.275	87.264	11.35	9.5	84.5	32	37	16	3.01	7.1	439K	Normocytic Normochromic Anemia with Lymphocytosis	0.81/0.5	25	43	417	300
54	Tanvith Hosamani	M	3m	3	5.2	52	36	Term	No	No	No	Veg	135.54	401.15	18.09	9.3	91.8	19	31	19	4.89	16	387K	Normocytic Hypochromic Anemia with Lymphocytosis	0.39/0.25	12	48	370	308
55	B/O Rajashree Soudatti	F	2m	3	4.8	54	36	Term	No	No	No	Non Veg	100.3	27.521	30.62	11	116	30	31	20	3.84	9.2	420K	Macrocytic Normochromic Blood Picture	0.68/0.36	38	59	153	308
56	Spandana Huli	F	3m 15d	2.8	5.2	61	45	Term	No	No	No	Non Veg	243.972	73.148	22.86	13	86.7	25	29	12	5.05	21	585K	Leucocytosis with Thrombocytosis	0.32/0.18	21	42	409	444
57	Amshika Anjanayya	F	1m 15d	2.9	3.7	48	36	Term	No	No	No	Non Veg	584.177	74.892	25.34	12	104	31	30	19	4.05	8.3	384K	Macrocytic Normochromic Anemia with Neutrophilia	0.25/0.08	24	28	287	305
58	B/O Yasmeen Sayhad	M	5m 28d	2.6	5.6	62	42	Term	No	No	No	Non Veg	887.787	426.79	13.76	10	78.2	22	28	19	4.64	19	741K	Normocytic Hypochromic Anemia with Leucocytosis with Lymphocytosis with Thrombocytosis	0.12/0.09	11	40	314	333

59	B/O Nagavva Iragar	M	4m	3.8	6	60	39	Term	No	No	No	Non Veg	336.139	426.79	14.61	9.7	90.4	28	31	14	3.42	7.6	340K	Macrocytic Normochromic Anemia	0.17/0.09	12	29	242	225
60	B/O Fathima Hawaldar	M	3m	2.4	4	52	36	Term	No	No	No	Non Veg	100.285	303.59	23.26	11	118	29	29	20	3.74	19	770K	Macrocytic Hypochromic Anemia with Thrombocytosis	0.28/0.15	15	34	203	415
61	B/O Savita Kadlaskar	M	3m 15d	2.8	5.2	61	45	Term	No	No	No	Veg	77.142	426.79	19.45	14	122	24	30	23	5.66	6.7	348K	Macrocytic Normochromic Blood Picture	0.34/0.13	80	26	163	250
62	B/O Laxmi Nadugeri	M	2m	2.4	3.8	54	36	Term	No	No	No	Non Veg	174.683	86.663	30.81	13	84.9	28	33	19	4.74	4.7	339K	Normocytic Normochromic, Leucopenia	6.68/0.62	25	30	578	330
63	Britlyn Rachel	M	2m	3.2	4.1	52	34	Term	No	No	No	Non Veg	483.878	126.62	14.98	12	106	30	29	30	4.04	14	405K	Macrocytic blood picture	0.63/0.30	381	560	240	289
64	Mahalaxmi Pujeri	F	4m	2.8	4	57	37	Term	No	No	No	Non Veg	100.382	155.97	14.58	9.5	87.4	23	26	20	4.14	9.4	364K	Macrocytic Hypochromic Anemia	0.31/0.10	14	72	219	429
65	Araham Kadakol	M	2m 16d	2.7	3	56	38	Term	No	No	No	Non Veg	837.637	71.42	29.37	11	91.4	25	27	28	4.44	8.7	406K	Normocytic Normochromic Blood Picture	0.5/0.23	29	40	256	376
66	Vedant Marakatti	M	5m	2.5	6	67	42	Term	No	No	No	Non Veg	288.7	146.33	11.59	8.9	75.6	23	31	19	3.85	18	256K	Dimorphic Anemia	21.1/15.4	248	662	888	359
67	Zafirah Rafiyi	F	3m 15d	3.5	5.3	57	39	Term	No	No	No	Veg	765.801	84.227	24.3	9.4	96.9	32	33	14	2.91	6.5	394K	Macrocytic Hyperchromic Anemia	0.63/0.24	34	75	159	520
68	Manikant Makalageri	M	4m	3	6.3	60	40	Term	No	No	No	Non Veg	206.021	112.75	19.97	9.7	78.7	25	31	16	3.97	11	512K	Normocytic Normochromic Anemia with mild Thrombocytosis	0.2/0.09	10	21	176	313
69	Kalmesh Jadagoppogol	M	4m	2.5	5.2	56	37	Term	No	No	No	Non Veg	520.474	275.93	19.79	14	84.1	21	26	14	6.67	12	146K	Dimorphic blood picture with Thrombocytpenia	0.57/0.28	38	57	148	420
70	Chinmay Kamble	M	6m	3.5	6.5	61	42	Term	No	No	No	Non Veg	914.895	426.79	21.82	8.9	68.3	19	28	19	4.64	21	449K	Microcytic Hypochromic Anemia with Leucocytosis	0.3/0.16	12	30	116	412
71	Shreshta Kurthkoti	F	6m	2.8	6.2	60	40	Term	No	No	No	Veg	1069.41	426.79	19.64	12	79.3	22	28	19	5.26	17	436K	Normocytic Hypochromic Blood Picture	0.46/0.24	18	60	261	387
72	B/O Ashwini Jattennavar	F	2m	2.7	4.8	54	36	Term	No	No	No	Non Veg	471.679	426.79	11.46	9.3	90.9	29	32	14	3.18	9.5	548K	Normocytic Normochromic Anemia with Thrombocytosis	0.43/0.25	37	46	291	263
73	Ayukta Kambar	F	5m	2.5	5.4	59	40	Late PT	No	No	No	Non Veg	696.676	426.79	10.87	10	72.6	23	31	17	4.56	15	696K	Dimorphic anemia with Neutrophilia with Thrombocytosis	0.28/0.14	11	30	229	333
74	Samantha More	F	5m	2.9	5.8	67	42	Term	No	No	No	Non Veg	878.299	277.44	9.503	9.8	83.1	24	29	19	4.08	15	462K	Normocytic Normochromic Anemia	0.21/0.13	10	20	184	302
75	B/O Rajeshwari Meti	F	6m	3.5	6.5	61	42	Term	No	No	No	Non Veg	198.849	426.79	22.81	11	95.7	24	32	15	4.61	12	477K	Macrocytic Normochromic Blood Picture	0.28/0.15	25	51	291	318

76	B/O Chaya Shahpurkar	M	1m 15d	2.9	3.1	54	36	Term	No	No	No	Non Veg	195.178	83.352	2.556	11	109	29	31	14	3.78	10	542K	Thrombocytosis	1.0/0.49	24	32	279	300
77	B/O Aaianamarziya	F	1m 25d	3.5	4	57	37	Term	No	No	No	Veg	1012.48	295.48	17.94	12	93.5	31	33	16	4	13	539K	Normal blood picture	0.74/0.31	15	34	331	314
78	B/O Srushti Kajagar	M	3m	2.2	4	58	36	Late PT	No	No	No	Non Veg	47.439	426.79	19.65	8.9	105	27	31	40	3.33	6.8	334K	Macrocytic Normochromic Anemia	0.22/0.14	23	32	243	264
79	B/O Poornima Patil	M	3m 10d	2.3	4.7	59	37	Term	No	No	No	Non Veg	51.505	301.49	22.6	11	112	29	32	26	3.73	9.4	373K	Macrocytic Anaemia	0.32/0.19	23	37	472	268
80	B/O Mohini Maruche	F	3m	3.3	5	56	38	Term	No	No	No	Non Veg	1026.04	29.334	9.984	11	87.2	26	30	13	4.05	9.9	481K	Normocytic Normochromic Anemia	0.66/0.33	38	47	190	408
81	B/O Jyoti Kumbhar	M	3m 20d	2.8	5.8	60	40	Term	No	No	No	Veg	672.278	426.79	2.584	11	78.7	25	32	13	4.47	12	362K	Normocytic Normochromic Anemia with Lymphocytosis	0.57/0.45	32	53	428	306
82	B/O Sheetal Chinchangi	M	5m	2.7	5.5	58	40	Term	No	No	No	Non Veg	864.745	276.83	20.95	12	80.9	25	31	12	4.76	13	351K	Normal blood picture	0.11/0.08	42	51	237	238
83	Prem Rajagolkar	M	3m	2.7	5.8	59	41	Term	No	No	No	Non Veg	206.021	426.79	14.28	12	97.8	30	30	13	4.1	17	437K	Leucocytosis with Thrombocytosis	0.37/0.25	32	56	330	342

S no	Name	Vit B12 (pg/ml)	MMA (pmol/L)	Homocysteine (mcmol/L)	Hb (g/dL)	MCV	MCH	MCHC	RDW	RBC	WBC	Platelets	PS	TB/DB	ALT	AST	ALP	LDH
1	M/B/O/Ambika	212.798	105.564	5.243	9.8	83.1	24	28.9	19.3	4.08	14.8	462K	Normocytic Hypochromic Anaemia, Thrombocytosis	1.09/0.56	28	26	81	225
2	M/O Shravan Patil	157.226	88.873	34.343	11.1	75.7	24.1	31.8	22.3	4.61	11.5	477K	Microcytic Hypochromic Anemia, Thrombocytosis	0.52/0.24	27	18	124	245
3	M/O Yallanagouda	28.742	66.974	12.499	10.9	118.7	28.8	30.8	21.5	3.78	10.1	542K	Macrocytic Normochromic Anemia, Thrombocytosis	2.21/0.84	16	14	82	251
4	M/O Sadananda gouda	121.384	83.039	14.241	12.3	112.4	30.8	32.9	15.9	4	13.2	539K	Macrocytic Normochromic picture	1.92/0.72	14	23	96	173
5	M/O Mutturaj Sarav	375.446	70.59	7.505	8.9	85.3	26.7	31.3	12.1	3.33	6.8	334K	Normocytic Normochromic Anemia	0.71/0.42	18	22	114	219
6	M/O Driti Badiger	199.244	88.238	32.414	12.7	89.8	28.7	31.9	18.5	3.73	9.4	373K	Normal blood picture	0.48/0.26	12	16	108	179
7	M/B/O Geetanjali	47.439	318.113	17.566	10.5	115.2	25.9	29.7	20.4	4.05	9.9	481K	Macrocytic Normochromic Anemia	0.23/0.14	26	27	124	204
8	M/O Renuka	176.202	66.686	22.228	11.1	78.7	24.8	31.5	19.5	4.47	12.3	362K	Microcytic Normochromic Anaemia, Leucocytosis	1.05/0.52	23	17	75	412
9	M/O Abhinav	100.3	71.378	17.462	11.9	80.9	25	30.9	12.4	4.76	13.1	351K	Normocytic Normochromic Anaemia, Leucocytosis	1.38/0.51	18	19	115	228
10	M/O Tahira Tumbagi	92.167	65.789	24.004	12.2	107.8	29.8	30.4	13.2	4.1	16.5	437K	Macrocytic Normochromic, Leucocytosis, Thrombocytosis	0.3/0.08	46	36	143	178
11	M/O Lalitesh	318.519	94.436	22.08	9.4	96.9	32.2	33.2	13.7	2.91	6.5	394K	Normocytic Hyperchromic Anemia	0.31/0.14	13	17	101	156
12	M/O Rahul Gadade	14.957	81.472	25.816	9.7	78.7	23.5	31.2	15.8	3.97	10.6	512K	Microcytic Hypochromic Anaemia, Thrombocytosis	0.54/0.2	28	41	113	383
13	M/O Trupti Kurbeta	79.257	81.726	12.499	14.3	84.1	21.4	25.5	14.1	6.67	11.7	176K	Leucocytosis	1.22/0.51	25	22	72	234
14	M/O Pranathi Harijan	115.209	109.534	6.284	8.9	109.2	19.2	28.1	32	3.64	20.7	449K	Macrocytic Hypochromic Anaemia, Leucocytosis	0.26/0.1	23	40	114	391
15	M/O Arpita Dandin	81.324	63.037	9.725	11.8	112.1	22.4	28.3	19.1	5.26	17	436K	Macrocytic Hypochromic Anemia	0.11/0.09	25	20	139	181
16	M/O Vaishnavi Ganti	269.725	66.974	7.505	9.3	90.9	29.2	32.2	14.1	3.18	9.5	548K	Normocytic Normochromic Anaemia, Thrombocytosis	0.18/0.09	13	18	150	226
17	M/B/O Snehal Patil	124.697	88.068	24.201	10.3	110.6	22.5	31	22.4	4.56	15.2	696K	Macrocytic Hypochromic Anaemia, Thrombocytosis	0.51/0.17	13	21	128	200
18	M/O Samanvi K	80.215	355.085	33.528	9.8	108.4	22.3	30.8	32.3	4.41	14.5	303K	Macrocytic Hypochromic Anaemia,Leucocytosis	0.33/0.17	29	22	118	185
19	M/O Nagaraj Walikar	56.248	394.038	18.824	12.2	116.9	26.3	30.3	13.8	4.64	6.2	336K	Macrocytic Hypochromic Anaemia	0.3/0.17	14	18	98	179
20	M/O Rahman B	97.116	102.473	16.198	11.3	107.3	25.7	29.4	13.9	4.4	10.6	331K	Macrocytic Normochromic Anemia	0.17/0.08	14	14	128	186
21	M/O Rayanna Sheregar	60.993	426.793	18.653	12.2	109.9	22.4	31.3	13.2	4.29	6.8	167K	Macrocytic Hypochromic	0.23/0.00	12	19	107	246
22	M/O Shoury Khanapuri	206.021	426.793	15.206	9.4	96.9	32.2	33.2	13.7	2.91	6.5	394K	Normocytic Hyperchromic Anemia	0.54/0.3	14	16	67	147
23	M/O Nayana Pujeri	276.502	194.428	21.767	9.7	108.7	23.5	31.2	15.8	3.97	10.6	512K	Macrocytic Hypochromic Anemia	0.27/0.14	12	14	119	121
24	M/O Sanjithgouda	130.118	426.793	21.542	9.3	111.2	19.1	30.9	12.1	4.88	15.7	387K	Macrocytic Hypochromic Anaemia, Leucocytosis	0.95/0.41	17	16	127	190
25	M/O Kartik Totagi	150.449	304.268	19.89	11	109.5	29.5	31.2	15.2	3.84	9.2	420K	Macrocytic Normochromic Anemia	0.7/0.36	14	15	129	168
26	M/O Agathsya S	231.474	196.096	24.561	12.7	106.7	25.1	29	12.4	5.05	20.8	585K	Leucocytosis	0.6/0.36	14	10	117	176
27	M/O Vaibhav R	79.969	317.148	9.738	12.4	103.5	30.6	29.6	19.4	4.05	8.3	384K	Macrocytic Hyperchromic	0.25/0.14	122	61	128	169
28	M/O Shanur Mujawar	145.028	104.065	23.481	10.3	78.2	22.2	28.4	19.2	4.64	18.8	741K	Microcytic Hypochromic Anaemia, Thrombocytosis	0.47/0.25	8	12	96	213
29	M/O Samarth Gadadi	71.822	401.482	20.148	9.7	102.5	28.4	31.4	27.8	3.42	7.6	340K	Macrocytic Normochromic Anemia	1.34/0.45	23	23	75	374
30	M/O Basavaraj V	94.878	164.281	5.624	10.7	97.9	21.6	29.2	15.9	3.74	19.1	770K	Normocytic Hypochromic Anaemia, Leucocytosis, Thrombocytosis	0.39/0.17	12	12	127	144
31	M/O Ayan Onti	306.32	87.992	12.578	13.9	81.6	24.6	30.1	13.8	5.66	6.7	348K	Normal Blood Picture	2.28/0.97	14	16	84	215
32	M/B/O Seema G	98.599	426.793	19.891	8.3	111.9	28	33	28.7	4.74	4.7	339K	Macrocytic Anemia	1.05/0.47	22	15	85	214
33	M/O Ruthvi Khot	207.376	248.666	28.844	10.2	105.7	30.2	28.6	29.6	4.04	14.1	405K	Macrocytic Hyperchromic Anemia	1.09/0.56	28	26	81	225
34	M/O Shourya N	138.251	246.49	24.884	9.5	109.6	22.9	26.2	12.2	4.14	9.4	364K	Macrocytic Hypochromic Anemia	1.02/0.33	9	20	115	290
35	M/O Rohit Bachagoudra	124.697	318.359	18.672	9.3	107.8	22.4	28.8	13.9	4.15	6.6	394K	Macrocytic Hypochromic Anemia	0.52/0.2	27	19	120	238
36	M/O Abhay Done	242.617	426.793	10.982	13.8	87.3	26.1	29.9	15.5	5.28	21.3	611K	Thrombocytosis	0.42/0.11	31	26	146	246
37	M/O Advit M	97.589	120.815	26.363	8.6	105.7	18.6	28.2	19.1	4.69	8.6	544K	Macrocytic Hypochromic Anemia	0.31/0.12	17	21	152	382
38	M/O Shrinivas S	43.373	91.295	30.661	10.7	97.4	28.2	29	26.3	3.79	13.5	452K	Normocytic Normochromic Anemia	0.24/0.13	13	16	96	259
39	M/O Samayara D	98.944	259.565	16.924	9.5	112.8	23.6	30.4	18.7	3.86	8.5	319K	Macrocytic Hypochromic Anemia	0.3/0.14	24	26	115	182

40	M/O Rekha B	177.557	100.432	16.336	8.5	111.3	28.2	25.4	27.8	1.77	11	132K	Macrocytic Normochromic Anaemia, Thrombocytopenia	0.4/0.2	11	20	110	176
41	M/O Bhagyashree M	180.268	231.417	20.142	11.3	101.1	21.5	31.1	14.1	3.59	16.5	436K	Macrocytic Hypochromic Anaemia, Leucocytosis	0.67/0.43	12	16	110	167
42	M/B/O Basavakumari L	164.003	33.102	17.285	12.5	95.9	31.8	33.2	12.8	3.93	12.2	437K	Normal blood picture	0.4/0.13	16	23	152	280
43	M/O Laxmi Pudabangi	131.474	150.327	18.248	9.5	108.6	29.5	37.3	23.2	3.01	7.1	439K	Macrocytic Normochromic Anemia	0.25/0.09	16	36	303	309
44	M/B/O Prathiba Burud	42.851	82.488	9.725	9.4	109.1	22.3	28.2	16.1	4.21	14.3	909K	Macrocytic Hypochromic Anaemia, Thrombocytosis	0.35/0.16	28	23	106	229
45	M/O Niranjan Kumar	117.92	115.427	22.041	10.7	107.2	22.7	31.4	19.6	4.73	16.3	452K	Macrocytic Hypochromic Anemia	0.77/0.32	88	46	132	245
46	M/B/O Shireen Bepari	90.812	82.352	5.845	13.8	111.9	26.1	29.9	18.7	5.28	21.3	611K	Macrocytic Normochromic, Thrombocytosis	0.28/0.16	19	17	89	145
47	M/O Lohit Yarazarvi	73.416	75.756	1.612	13.9	81.6	24.6	24.6	30.1	2.56	10	301K	Normal Blood Picture	0.64/0.18	25	34	128	411
48	M/O Ahmed Shaikh	141.385	119.348	7.505	13.3	84.9	28	33	35.5	1.48	6.3	159K	Normocytic Normochromic	0.16/0.08	11	21	125	218
49	M/O Minaxi Kaleri	180.168	78.415	16.195	12.2	105.7	30.2	28.6	29.6	3.24	17.6	492K	Macrocytic Hyperchromic	0.3/0.11	28	31	161	241
50	M/O Abhirvey Hadapad	68.246	284.359	22.065	9.5	107.4	22.9	26.2	25.9	2.32	21.1	389K	Macrocytic Hypochromic Anaemia, Leucocytosis	0.34/0.2	33	26	88	221
51	M/B/O Rajashri S	131.474	63.731	20.417	11.1	91.4	25	27.3	19.1	4.65	23.5	450K	Normocytic Normochromic Anaemia, Leucocytosis	0.5/0.21	12	13	102	201
52	M/B/O Shruti Patil	78.613	278.669	24.884	8.9	105.6	23.1	30.6	28.6	3.85	17.7	256K	Macrocytic Hypochromic Anaemia, Leucocytosis	0.68/0.31	27	25	120	318
53	M/B/O Pooja Bhavi	147.739	61.241	14.214	12.8	85	26	29	15.6	5.24	18.6	393K	Normal blood picture	0.41/0.28	25	28	118	183
54	M/O Tanvith Hosamani	174.847	285.376	16.924	10.9	85.2	27	35	15.9	3.5	15.2	572K	Normocytic Normochromic Anaemia, Thrombocytosis	0.42/0.27	11	14	160	220
55	M/B/O Rajashree S	314.453	71.844	13.754	9.9	91.1	27.5	30.2	15.6	3.6	12.8	220K	Normocytic Normochromic Anemia	1.14/042	16	17	142	189
56	M/O Spandana Huli	200.599	62.359	9.851	11.5	88.9	28.4	32	15.6	4.07	5.7	366K	Normocytic Normochromic Anemia	0.68/0.15	25	27	128	335
57	M/O Amshika Anjanayya	93.523	79.389	14.514	13.6	74.2	21.3	28.8	21.5	8.26	12	220K	Microcytic Hypochromic	0.85/0.3	34	27	173	207
58	M/B/O Yasmeen S	103.01	71.962	7.249	7	109.5	31.7	28.9	29.2	2.21	12.4	418K	Macrocytic Hyperchromic Anemia	0.24/0.12	68	32	177	219
59	M/B/O Nagavva Iragar	86.746	68.693	22.142	10.7	116.4	23.2	29.1	19.2	4.62	16.5	467K	Macrocytic Hypochromic Anemia	0.24/0.07	24	24	113	207
60	M/B/O Fathima H	29.352	279.215	18.254	13.6	112	31.5	30.8	17.7	4.32	16.3	365K	Macrocytic Hyperchromic	0.69/0.37	24	39	456	304
61	M/B/O Savita Kadlaskar	142.563	295.122	19.563	11.6	107.8	21.4	27.6	16.4	5.41	12.1	568K	Macrocytic Hypochromic Anemia	0.55/0.26	100	64	159	193
62	M/B/O Laxmi Nadugeri	112.341	65.831	5.872	9.8	72.4	22.3	30.8	17.2	3.41	14.5	303K	Microcytic Hypochromic Anemia	0.68/0.26	25	20	101	219
63	M/O Britlyn Rachel	134.245	387.713	32.684	11.7	96.5	31.3	32.4	13.5	3.74	11.6	448K	Normocytic Hyperchromic Anemia	0.35/0.16	49	44	126	276
64	M/O Mahalaxmi Pujeri	93.523	45.956	19.142	12	87.3	25.5	29.2	16.4	4.32	12.1	417K	Normal blood picture	0.2/0.03	35	71	91	238
65	M/O Araham Kadakol	178.368	426.793	16.438	9.9	83.9	25.2	30.8	14.9	4.02	14.1	512K	Normocytic Normochromic Anemia	0.2/0.06	22	22	89	277
66	M/O Vedant Marakatti	203.01	69.548	13.934	9.3	99.7	29	29.1	14.9	3.21	10.9	259K	Normocytic Normochromic Anemia	1.21/0.48	10	21	132	220
67	M/O Zafirah Rafiyi	142.317	426.793	29.143	6.9	104.4	15.4	23.9	23.9	4.49	13.3	418K	Macrocytic Hypochromic Anemia	0.31/0.14	18	20	127	245
68	M/O Manikant Makalag	145.028	74.638	8.528	8.7	65.7	18.6	28.2	19.1	4.69	8.6	544K	Microcytic Hypochromic Anemia	0.36/0.16	13	15	74	174
69	M/O Kalmesh J	93.523	70.091	21.349	10	106.8	23.1	31.7	32.2	4.17	7.2	403K	Macrocytic Hypochromic Anemia	0.63/0.33	11	16	134	181
70	M/O Chinmay Kamble	97.589	33.601	34.954	9.8	110.5	20.3	30.5	16.7	4.83	14.8	385K	Macrocytic Hypochromic Anemia	0.2/0.09	16	25	111	278
71	M/O Shreshta Kurthkoti	111.143	94.885	10.724	6.2	102.6	32.2	31.4	32.5	1.91	7.8	7K	Macrocytic Hyperchromic Anemia	0.28/0.07	17	33	78	426
72	M/B/O Ashwini J	119.275	94.047	11.982	9.5	109.8	23.6	29.1	19.8	4.02	5.3	585K	Macrocytic Hypochromic Anemia	0.44/0.21	44	29	129	242
73	M/O Ayukta Kambar	227.707	86.849	8.326	11.6	83.1	24	28.9	14.9	4.84	17.8	383K	Microcytic Normochromic Anemia	0.18/0.01	27	36	117	382
74	M/O Samantha More	98.944	104.073	17.482	12.1	119.7	26.5	29.6	13.6	4.56	14.6	639K	Macrocytic Normochromic, Thrombocytosis	0.2/0.09	15	16	93	159
75	M/B/O Rajeshwari Meti	128.763	62.266	14.968	11	95.2	29	30.5	17	3.8	20	606K	Normocytic Normochromic Anaemia, Leucocytosis	0.21/0.1	23	28	94	228
76	M/B/O Chaya S	112.498	98.73	8.671	10.9	84.2	26.5	31.4	15.5	4.12	14.3	560K	Normocytic Normochromic Anemia	0.44/0.18	22	17	115	186
77	M/B/O Aaianamarziya	131.474	152.96	14.585	12.1	89.7	26.5	29.6	13.6	4.56	14.6	439K	Normal blood picture	0.3/0.11	27	22	84	225
78	M/B/O Srushti Kajagar	146.383	295.694	34.162	12.1	79.4	23.1	29.1	15.8	5.24	20.2	826K	Thrombocytosis	0.47/0.23	10	15	86	187
79	M/B/O Poornima Patil	107.077	304.475	22.415	6.8	113.7	24.3	26.7	35.1	2.8	3.1	118K	Macrocytic Normochromic Anemia	0.16/0.07	23	24	124	270
80	M/B/O Mohini Maruche	1137.181	100.644	7.412	12.1	79.4	23.1	29.1	15.8	5.24	20.2	826K	Microcytic Hypochromic, Thrombocytosis	0.26/0.14	39	27	63	217
81	M/B/O Jyoti Kumbhar	264.003	126.793	14.826	12.8	101.8	31.2	30.6	17.6	4.12	16.2	366K	Macrocytic Hyperchromic	0.55/0.26	15	22	148	239
82	M/B/O Sheetal Chincha	153.16	62.266	11.235	9.6	105.6	22.5	31	17.4	4.45	14.3	301.5K	Macrocytic Hypochromic Anemia	0.94/0.36	19	25	85	200
83	M/O Prem Rajagolkar	56.927	83.911	14.531	11.4	118	22	28	24.7	5.6	12.6	560K	Macrocytic Hypochromic Anaemia, Thrombocytosis	0.66/0.32	25	32	91	215