B.L.D.E (DEEMED TO BE UNIVERSITY)

SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE

VIJAYAPURA, KARNATAKA

DISSERTATION

On

"PREVALENCE OF VITAMIN B12 DEFICIENCY IN ELDERLY WITH DIABETES MELLITUS ON METFORMIN THERAPY AND ITS ASSOCIATION WITH COGNITION LEVEL"

Submitted in partial fulfillment of

M.D DEGREE EXAMINATION
GERIATRICS

By

DR. KUSHAL BHANGALE

P.G. IN GERIATRICS

UNDER THE GUIDANCE OF
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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation **"PREVALENCE OF VITAMIN B12 DEFICIENCY IN ELDERLY WITH DIABETES MELLITUS ON METFORMIN THERAPY AND ITS ASSOCIATION WITH COGNITION LEVEL"** is a bonafide and genuine research work carried out by me under the guidance of **DR. ANAND P AMBALI,** Professor & Head**,** Department of Geriatrics at BLDE (Deemed to be university) Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.

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ACKNOWLEDGEMENT

On completion of my post-graduation journey and this scientific document, I would like to acknowledge the immense help received from my mentors in the Department of Geriatrics.

I wish to express my sincere gratitude and thanks to my Head of Department and my guide Prof. Dr. Anand P. Ambali, for his guidance, wisdom, and constant motivation during the course of the study.

I would like to express my sincere gratitude to Associate Professor Dr. Aniruddha Umarji, for his guidance and support during the course of this study.

I am forever grateful to Assistant Professors Dr.Mohammed Abrar Ul Huq and Dr.Muddasirahmed Indikar, Senior residents Dr.Vijayalakshmi and Dr. Suma Rathod for their guidance and encouragement provided to me to achieve new heights professionally over my course period.

I am extremely thankful to Professor Dr. Aravind V Patil, Principal, Faculty of Medicine and Professor Dr. R.M.Honnutagi, Medical Superintendent, BLDE (Deemed to be University) Shri. B. M. Patil Medical College Hospital and Research Centre, Vijayapura for permitting me to utilize resources in the completion of my work.

I would like to thank the statistician for their timely support and help.

ACKNOWLEDGEMENT

I would like to extend my sincere thanks to all my colleagues Dr. Sandeep Kumar, Dr.Vigneshwaran, Dr.Harini.T, Dr.Karthik.S, Dr.Kokila and Dr Tabussum for their support.

I would be failing in my duty if I would not acknowledge my thanks to all the PATIENTS who were kind enough to be a part of this study.

I would also like to thank my Parents Dr.Ravindra Bhangale, Dr Chandrika Bhangale, my sisters Dr Komal Sarode and Dr Tejal Rane without their constant encouragement & moral support, my studies would have been a distant dream.

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ABBREVIATIONS

Abbreviation	Full Form
ADA	American Diabetes Association
A1c	Hemoglobin A1c
AMPK	Adenosine Monophosphate-Activated Protein Kinase
B12	Vitamin B12 (Cobalamin)
BCSH	British Committee for Standards in Haematology
BMI	Body Mass Index
CVD	Cardiovascular Disease
DALYs	Disability-Adjusted Life Years
DM	Diabetes Mellitus
ER	Endoplasmic Reticulum
FFA	Free Fatty Acid
FPG	Fasting Plasma Glucose
GA	Glycated Albumin
GA/HbA1c	Glycated Albumin to Hemoglobin A1c Ratio
GDM	Gestational Diabetes Mellitus
GI	Gastrointestinal

Abbreviation	Full Form
HbA1c	Hemoglobin A1c
H2RA	Histamine H2-Receptor Antagonist
IF	Intrinsic Factor
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
IL-6	Interleukin-6
IR	Immediate Release
MCI	Mild Cognitive Impairment
MMA	Methylmalonic Acid
MoCA	Montreal Cognitive Assessment
MMSE	Mini-Mental State Examination
MUI	Metformin Usage Index
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NCD	Noncommunicable Disease
OGTT	Oral Glucose Tolerance Test
PCOS	Polycystic Ovary Syndrome

Abbreviation	Full Form
PG	Plasma Glucose
PPI	Proton Pump Inhibitor
ROS	Reactive Oxygen Species
RPG	Random Plasma Glucose
SOD	Superoxide Dismutase
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
THF	Tetrahydrofolate
UPR	Unfolded Protein Response
XR	Extended Release

ABSTRACT

Introduction: Vitamin B12 is indispensable for hematopoiesis and neurologic function. Metformin, the drug of choice for T2DM, has been noted to cause abnormal vitamin B12 absorption with prolonged therapy. Older patients are especially vulnerable because of age-related gastrointestinal changes and concomitant nutritional deficits.

Aim: To assess the frequency of vitamin B12 deficiency among older patients with Type 2 diabetes who have been on metformin for a minimum of three years and assess its association with cognitive performance.

Methods: In an 18-month, observational, cross-sectional study at our tertiary care hospital, 75 patients aged ≥60 years on metformin therapy (≥3 years) were enrolled. Patients with confounding conditions (e.g. pernicious anemia, gastrointestinal surgeries/disorders, chronic alcohol use, altered renal/hepatic function, or on proton pump inhibitors) were excluded. A structured questionnaire collected demographic data, metformin dose/duration, comorbidities, dietary patterns, and smoking status. A thorough clinical evaluation focused on neuropathic symptoms. Venous blood was drawn for serum vitamin B12 (categorized as deficient: <200 pg/mL, borderline: 200–400 pg/mL, or adequate: >400 pg/mL), blood sugar parameters, HbA1c, and other routine tests. Patients with vitamin B12 deficiency underwent cognitive evaluation using the Montreal Cognitive Assessment (MoCA). Statistical comparisons and multivariate logistic regression were performed using IBM SPSS version 30.0 with significance set at $p < 0.05$.

Results: Overall, 21.3% (16/75) were vitamin B12 deficient. Only 2 patients on metformin for <5 years were deficient compared with 11 of 13 (84.6%) on therapy for >10 years. Deficient patients had a significantly lower mean MoCA scores (22 ± 3). Our result indicates

that there is no statistically significant association between dietary habits (vegetarian vs. non-vegetarian) and Vitamin B12 levels in the cases with vitamin b12 deficient states.

Conclusion: Elderly with type 2 diabetes mellitus who have been on long-term metformin treatment face a considerable risk of vitamin B12 deficiency, a condition that is closely linked to peripheral neuropathy and could also play a role in cognitive decline. Routine screening and early intervention in this high-risk population are recommended.

INTRODUCTION

Cobalamin, also known as Vitamin B12, is a crucial nutrient necessary for the synthesis of DNA, the production of blood cells, and the maintenance of healthy neurological function. It is soluble in water Deficiency primarily leads to hematological and neurocognitive impairments [1].

In individuals with type 2 diabetes mellitus (T2DM), vitamin B12 deficiency caused by metformin is linked to various mechanisms, one of which is the change in small bowel motility that encourages bacterial overgrowth, resulting in decreased absorption of B12. Alternative suggested mechanisms involve the competitive inhibition or inactivation of vitamin B12 uptake, alterations in intrinsic factor (IF) levels, and its interaction with the cubulin endocytic receptor. [2]

Furthermore, metformin disrupts the calcium-dependent uptake of the B12-IF complex in the terminal ileum, a mechanism that can be reversed by supplementing with calcium. [3] Vitamin B12 levels can begin to decrease around the fourth month of using metformin; however, because the liver has substantial reserves, clinical symptoms may not appear for 5 to 10 years. [2,4]

For individuals with type 2 diabetes, we should check for vitamin B12 deficiency prior to starting metformin therapy, also we need to perform annual screenings in older patients who have been on metformin long-term for three to four years or more, those taking high doses of metformin (2 g/day or more), or those experiencing worsening diabetic distal polyneuropathy, even if there are no hematological issues present. [5]

The method for screening vitamin B12 deficiency in individuals with diabetes mirrors that of the general population. Serum vitamin B12 concentration measurement serves as the initial screening method for patients with Type 2 DM. Serum vitamin B12 levels below 200 pg/ml are generally indicative of a deficiency, whereas levels exceeding 400 pg/ml confirm that there is

no deficiency present. For patients who have borderline serum vitamin B12 levels ranging from 200 to 400 pg/ml and exhibit minor hematological changes, assessing serum 'Methylmalonic acid' (MMA) or 'homocysteine' levels offers a sensitive screening method, particularly in individuals with type 2 diabetes mellitus.

As per the consensus from 'American Diabetes mellitus Association – European Association' for the Study of Diabetes mellitus (ADA-EASD) consensus, 'The American Association of Clinical Endocrinology' (AACE), 'International Diabetes mellitus Federation' (IDF), and 'The National Institute for Health and Care Excellence' (NICE) guidelines, metformin is still considered the primary treatment for type 2 diabetes mellitus (T2DM) unless there are contraindications or patient intolerance. Various studies have indicated a heightened incidence of vitamin B12 deficiency among individuals with T2DM. [7–11]

Metformin's origins trace back to *Galega officinalis* (goat's rue), a European herbal remedy rich in guanidine, known to lower blood glucose since 1918. While early guanidine compounds were employed in the treatment of diabetes during the 1920s and 1930s, they were eventually discarded because of their toxic properties and the emergence of insulin. Metformin was rediscovered in the 1940s during antimalarial research and noted for lowering blood sugar during influenza treatment. Its potential was realized by French physician Jean Sterne, who introduced it as a diabetes treatment in 1957. [12] The use of metformin has repeatedly been recognized as the main cause of vitamin B12 deficiency in individuals with diabetes. [11] Research assessing patients with T2DM who are on metformin has reported vitamin B12 deficiency prevalence rates that vary between 5.8% and 33%.[11,13]

The discovery of vitamin B12 and its role in metabolism unfolded over a century and earned two Nobel Prizes. Clinical observations of pernicious anemia in the 19th century laid the groundwork for Minot and Murphy's landmark treatment study, which won them the Nobel Prize. Later, Castle identified the missing gastric component in these patients—intrinsic factor—marking another major breakthrough in understanding and treating B12 deficiency.[14]

The wide variation in reported prevalence is likely due to differences in study definitions of vitamin B12 deficiency, which may include varying diagnostic thresholds and assessment methods.

In India, a large portion of the population chooses a vegetarian lifestyle driven by cultural and religious beliefs, resulting in a considerable incidence of vitamin B12 deficiency among the general public. While vitamin B12 deficiency typically shows symptoms like anemia, peripheral neuropathy, depression, and cognitive decline, these are often absent in those who have only a biochemical deficiency of vitamin B12. [15]

A noticeable link between insufficient vitamin B12 and cognitive decline highlights the harmful impact of this widespread nutritional deficiency on mental health across individuals of all ages. Although vitamin B12 deficiency is primarily common in older individuals, it is also found in a relatively younger demographic. Potential factors that could contribute include malabsorption due to gastritis, overuse of drugs like proton pump inhibitors, pernicious anemia, or nutritional deficiencies linked to inadequate socioeconomic status. The signs of a vitamin B12 deficiency are often unclear and can be easily overlooked [16] . Various research studies indicates that individuals with Alzheimer's disease generally exhibit reduced levels of vitamin B12 and elevated levels of homocysteine when compared to individuals without dementia. [17]

Tackling risk factors related to ‘Vitamin B12 deficiency’, including the excessive use of antibiotics and proton pump inhibitors (PPIs) for H. pylori infections, along with fostering awareness of nutritious and hygienic eating practices, is essential for its prevention and management. [18]

Vitamin B12 deficiency is associated with cognitive impairment, and supplementation may help improve cognitive outcomes in affected patients. [16]

OBJECTIVE OF THE STUDY

1. To study the prevalence of Vitamin B12 deficiency in elderly patients on Metformin therapy for Diabetes Mellitus Type 2.
2. To record the demographic details including age, sex, BMI of the patient.
3. To check the cognition status in Vitamin B12 deficient patients.

REVIEW OF LITERATURE

DIABETES MELLITUS- HISTORICAL BACKGROUND [19]

This detailed historical overview of diabetes mellitus underscores its evolution from being considered a kidney disease in antiquity, attributed to the kidneys' supposed inability to retain substances, to its recognition in later centuries as a metabolic disorder linked to elevated blood sugar levels. The sweet taste of diabetic urine, noted in ancient texts and later by notable figures like Avicenna and Morgagni, marked a significant diagnostic clue. Thomas Willis's differentiation of diabetes based on the sweetness of urine in 1674 was pivotal, eventually leading to Matthew Dobson's identification of sugar in both blood and urine, solidifying diabetes as a condition of elevated blood sugar rather than a kidney issue. The experimental research conducted in the late 19th and early 20th centuries, reaching its peak with the discovery of insulin in 1922, firmly identified diabetes as an endocrine disorder and revolutionized its treatment. Subsequent research has linked diabetes to complications such as kidney disease, marking another chapter in its medical history.

EPIDEMIOLOGY IN INDIA

Diabetes has indeed emerged as a critical global health issue, aligning with other major non-communicable diseases (NCDs) like cardiovascular disease, respiratory disease, and cancer in terms of its impact on mortality. As reported by the World Health Organization (WHO), diabetes contributed to numerous fatalities in 2019, positioning it as the ninth most common cause of death globally. The prevalence of T2DM, previously associated with affluent Western nations, has now become widespread globally, affecting people across all demographics and significantly impacting younger age groups as well.

Looking ahead, the projections indicate a concerning trend, with the number of diabetes-related deaths expected to increase substantially by 2035. This underscores the urgent need for

continued global efforts in prevention, management, and research to address the increasing burden of diabetes on public health worldwide. [20]

Over the past three decades, diabetes has witnessed a significant rise in India, contributing substantially to the global burden of the disease. Our nation has experienced an epidemiological shift, characterized by a significant reduction in deaths caused by 'communicable, maternal, neonatal, and nutritional diseases' (CMNNDs), while non-communicable diseases (NCDs) and injuries have emerged as primary factors contributing to the considerable impact of illness on the society and mortality. In 1990, the contribution of CMNNDs represented 61% of the overall disability-adjusted life years (DALYs) in India, whereas NCDs accounted for 30% and injuries comprised 9%. However, due to notable changes in epidemiology over the years, by 2016, the proportion of CMNNDs had reduced to 33%, while NCDs and injuries had risen to 55% and 12%, respectively. [20]

Diabetes has shown a dramatic rise in its disease burden ranking in India, climbing from the 35th leading cause of DALYs in 1990 to the 13th in 2016. The prevalence of diabetes has been steadily increasing since 1990, with an accelerated rise after 2000. As per the International Diabetes Federation (IDF), the rate of T2DM in India rose from 7.1% in 2009 to 8.9% in 2019. At present, India holds the second position worldwide, following China, in terms of the T2DM crisis, with 7.7 crores individuals impacted. Among this population, there are 1.21 crores individuals aged 65 and older, a figure projected to grow to 2.75 crores by the year 2045. Furthermore, it is approximated that 2.52 crores people have impaired glucose tolerance (IGT), with this number anticipated to rise to 3.57 crores by 2045. Worryingly, nearly 57% of adults living with diabetes in India are undiagnosed, representing around 4.39 crore people.[20]

According to the Diabetes study conducted by the "India State-Level Disease Burden Initiative", the incidence and total cases of T2DM in India increased from 5.5% and 2.6 crores in 1990 to 7.7% and 6.50 crores by 2016. Among the various states in India, 'Tamil Nadu' had the highest prevalence in 2016, followed by 'Kerala', 'Delhi', 'Punjab', 'Goa', and

'Karnataka'. The 'ICMR–India Diabetes' study, which stands as the largest nationwide epidemiological survey concerning diabetes and prediabetes, reviewed data from 15 states and union territories. This research revealed that diabetes prevalence in rural areas varied between 3.5% and 8.7%, while urban regions saw a range from 5.8% to 15.5%, with Chandigarh showing the highest prevalence at 13.6% and Bihar the lowest at 4.3%. Overall, urban areas exhibited a notably higher rate of diabetes prevalence (11.2%) in comparison to the countryside (5.2%). Furthermore, the prevalence of prediabetes was also significant, ranging from 5.8% to 14.7% in rural settings and from 7.2% to 16.2% in urban settings. In many states, the rate of prediabetes surpassed that of diabetes, underscoring the substantial number individuals who may be susceptible to developing T2DM in the upcoming future. [20]

RISK FACTORS OF DIABETES MELLITUS [21]

Studies on twins have provided compelling evidence of a strong genetic component in T2DM, with higher concordance rates observed in monozygotic twins (96%) compared to dizygotic twins in some studies. Furthermore, first-degree relatives of individuals with T2DM have a considerably higher chance of developing diabetes, with a 40% risk compared to only 6% in the general population. Various candidate genes have been recognized as contributing to genetic susceptibility to T2DM, such as 'KCNJ11' ('potassium inwardly rectifying channel, subfamily J, member 11'), 'TCF7L2' ('transcription factor 7-like 2, noted as the most strongly associated genetic locus for T2DM'), 'IRS1' ('insulin receptor substrate 1'), 'MTNR1B' ('gene associated with melatonin receptors'), 'PPARG2' ('peroxisome proliferator-activated receptor gamma 2'), 'IGF2BP2' ('insulin-like growth factor 2 binding protein 2'), 'CDKN2A' ('cyclin-dependent kinase inhibitor 2A'), 'HHEX' ('haematopoietically expressed homeobox'), and 'FTO' ('gene associated with fat mass and obesity').

In addition to genetic predispositions, various lifestyle factors play a significant role in the onset of T2DM. Factors such as sedentary behavior, lack of physical activity, smoking, and alcohol use have all been associated with a heightened risk of the disease. Epidemiological

research suggests that obesity is the most significant risk factor, as it substantially contributes to insulin resistance and the advancement of diabetes. The World Health Organization (WHO, 2011) reports that almost 90% of those diagnosed with T2DM are overweight or obese.

Dietary habits also play a crucial role in T2DM risk. A diet that is low in fiber and has a high glycemic index is significantly linked to an increased risk of developing the disease, whereas different types of dietary fatty acids affect insulin resistance to different extents.

High total and saturated fat intake increase the risk of T2DM, independent of body mass index (BMI), whereas higher linoleic acid intake has a protective effect, particularly among younger and leaner men. Consuming processed meats consistently, in comparison to other types of meat, has been linked to an increased likelihood of developing T2DM, even when factors like body mass index (BMI), previous weight fluctuations, alcohol consumption, and total energy intake are taken into account. Moreover, habitual consumption of sugary drinks, such as sodas, has been linked to both an increased risk of T2DM and metabolic syndrome because of their direct effect on BMI.

New research suggests that vitamin D may play an important role in the management of T2DM, as there are seasonal fluctuations in glycemic control, with a decline in glycemic levels during winter commonly associated with vitamin D deficiency. Moreover, vitamin K deficiency has been associated with poor glycemic control and reduced bone quality in T2DM patients, making it essential to assess and address vitamin K status in individuals with the disease.

Major risk factors for T2DM are [22]

1. Family history of T2DM
2. Age more than 35 years
3. Overweight (Body Mass Index ≥25 kg/m²) and obesity (Body Mass Index ≥30 kg/m²)
4. Enlarged waist or upper body adiposity (≥90 cm for men & ≥80 cm for women)

5. Systemic hypertension

6. Recent increase in body weight

7. ‘Sedentary lifestyle’

8. ‘Gestational diabetes’

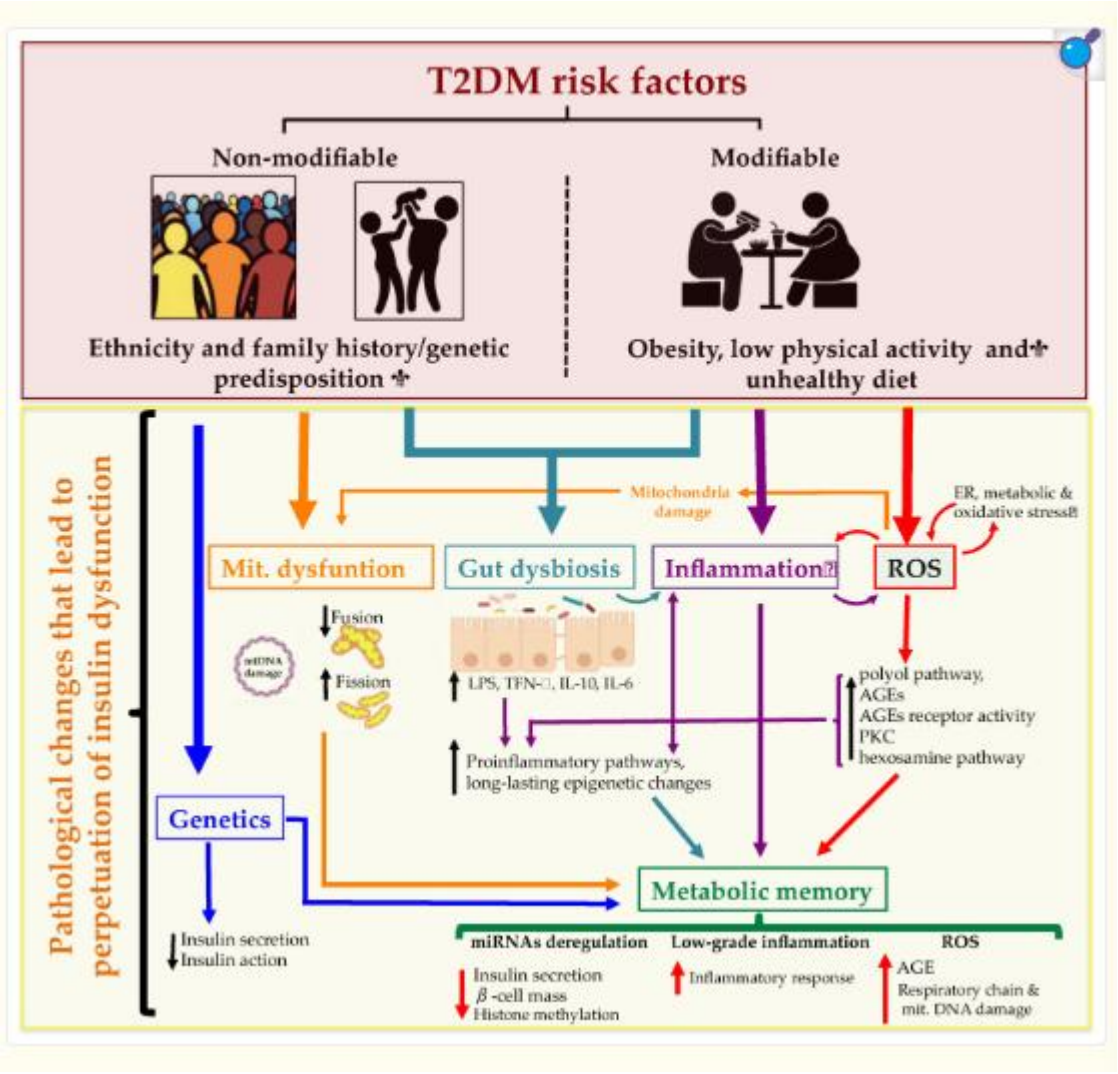


FIGURE 1. Type 2 Diabetes Mellitus (T2DM) risk factors and the pathological changes leading to the perpetuation of insulin dysfunction. [23]

PATHOPHYSIOLOGY OF DIABETES [23]

B-CELL DYSFUNCTION

Dysfunction of β-cells has historically been associated with β-cell death; nevertheless, new research suggests that its influence in T2DM is determined by a interaction between environmental influences and different molecular pathways that regulate cellular functions. In conditions of excessive nutrition, such as obesity, the presence of hyperglycemia and hyperlipidemia[23] promotes insulin resistance and chronic inflammation. Amid these metabolic challenges, β-cells may experience harmful stressors based on their genetic predisposition, all of which jeopardize islet integrity.

Increased concentrations of free fatty acids (FFAs) and high blood sugar levels lead to β-cell impairment by inducing endoplasmic reticulum (ER) stress via the activation of 'apoptotic unfolded protein response' (UPR) pathways. Lipotoxicity, glucotoxicity, and glucolipotoxicity, which are frequently linked to obesity, trigger metabolic and oxidative stress that results in harm to β-cells. Saturated free fatty acids (FFAs) increase stress responses through various mechanisms, such as inhibiting the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), which is essential for mobilizing Ca^{2+} in the endoplasmic reticulum, activating inositol trisphosphate (IP3) receptors, or directly disturbing endoplasmic reticulum (ER) homeostasis. Furthermore, prolonged high blood sugar levels boost proinsulin production and raise the synthesis of 'islet amyloid polypeptide' (IAAP) in β-cells. This causes an accumulation of improperly folded insulin and IAAP, which leads to a rise in reactive oxygen species (ROS) due to oxidative protein folding. These molecular disruptions hinder normal ER Ca^{2+} dynamics, amplify pro-apoptotic signalling, degrade proinsulin mRNA, and trigger the release of interleukin (IL)-1β, which subsequently attracts macrophages and worsens inflammation in the local islets cells.

Ensuring accurate insulin release is vital for fulfilling metabolic requirements, which makes it essential to maintain islet structure so that β-cells can adequately respond to physiological demands. In pathological contexts, the processes outlined previously can lead to the deterioration of islet organization, hindering communication between cells within pancreatic islets. This disturbance results in irregular control of insulin and glucagon secretion, worsening hyperglycemia.

Issues related to the synthesis of insulin precursors or the production of insulin itself, in addition to issues with the secretion process, cause dysfunction in insulin secretion— a key factor in β-cell failure in T2DM. For example, a decrease in the expression of the GLUT2 glucose transporter may interfere with downstream signalling pathways, while mis-folding of proinsulin is often associated with inadequate insulin production and the development of diabetes.

TYPES OF DIABETES [24]

Diabetes mellitus is a long-term disease with a complicated pathophysiology. It is characterized by high levels of blood sugars, known as hyperglycemia, which arises from issues with either insulin secretion, insulin action, or both.

Diabetes mellitus (DM) is a condition with various clinical manifestations, rendering any classification somewhat arbitrary. Nevertheless, classification is still useful as it assists in clinical evaluations and treatment choices, often shaped by the physiological circumstances present during diagnosis. The existing classification framework is founded on both the causes and mechanisms of the illness, offering an organized way to comprehend its different types. In accordance with this framework, DM is divided into four major categories: ‘type 1 diabetes mellitus’ (T1DM), ‘type 2 diabetes mellitus’ (T2DM), ‘gestational diabetes mellitus’ (GDM), and diabetes linked to specific conditions, diseases, or disorders.

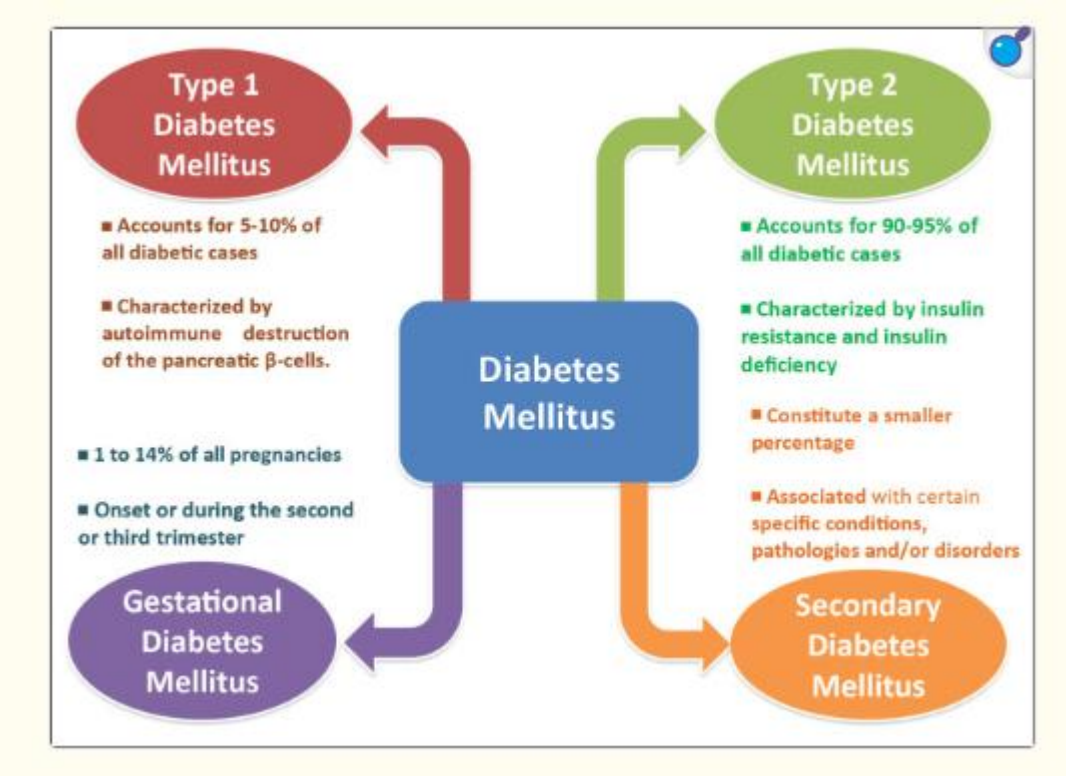


FIGURE 2 - Four types of diabetes mellitus[24]

SIGNS AND SYMPTOMS OF DIABETES [22]

Many individuals overlook the signs and symptoms of diabetes due to its chronic and gradual progression. Unlike acute illnesses, the effects of hyperglycemia do not manifest immediately, leading people to underestimate its seriousness. What remains largely unrecognized is that diabetes-related damage can begin years before noticeable symptoms appear. This lack of awareness is concerning, as early recognition of symptoms allows for timely intervention, helping to control the disease effectively and prevent the onset of vascular complications.

Classical symptoms of diabetes includes polyuria, polydipsia, and polyphagia, which are frequently observed in T1DM due to its rapid onset and severe hyperglycemia. These symptoms can also appear in T2DM when blood glucose levels become significantly elevated. Severe weight loss is primarily associated with type 1 diabetes or long-undetected cases of type 2 diabetes. Moreover, unaccounted weight loss, lethargy, restlessness, and

bodily discomfort are frequent signs of undiagnosed diabetes. Less severe symptoms or those that progress slowly might be overlooked, which can further postpone diagnosis and treatment.

Warning signs of diabetes [22]

1. Sudden reduction in weight
2. Persistent lethargy
3. Mood swings
4. Frequent infections, especially in:
    - Genital regions
    - Urinary tract
    - Skin
    - Mouth
    - Slow wound healing
5. Dryness in oral cavity
6. Tingling sensation in legs
7. Skin Itching
8. Reactive hypoglycemia
9. 'Acanthosis nigricans' – the appearance of smooth, dark patches on the neck, underarms, or groin, signifying insulin resistance
10. Diminished vision
11. Erectile dysfunction or impotence

DIAGNOSIS OF DIABETES [22]

Individuals of Asian ethnicity aged 35 and over with two or more of the previously mentioned risk factors should be screened for diabetes. The 'oral glucose tolerance test' (OGTT) is a frequently used method for screening. Fasting and 2-hour post-glucose tests assist in identifying various glycemic conditions: 'impaired fasting glucose' (IFG) is diagnosed when fasting glucose levels are between 110–125 mg/dL, while 'impaired glucose tolerance' (IGT) is recognized when 2-hour glucose levels fall between 140–200 mg/dL. A diabetes diagnosis is confirmed if 'fasting glucose levels' exceed 126 mg/dL or if 2-hour glucose levels go over 200 mg/dL. If a random blood glucose test shows a level higher than 150 mg/dL, an OGTT is recommended for confirmation.

In the past few years, glycosylated hemoglobin (HbA1c) has been established as a suggested diagnostic test, with readings of 6.5% or higher indicating diabetes. HbA1c levels ranging from 5.7% to 6.4% indicate the existence of prediabetes.

1. **Hemoglobin A1c (A1c)**:
    1. **Normal**: <5.7%
    2. **Pre-diabetes**: 5.7–6.4%
    3. **Diabetes**: ≥6.5%

       (Diagnosis must be confirmed with a second test.)
2. **Fasting Blood Sugars (FBS)**:
    1. **Normal**: <100 mg/dL
    2. **Pre-diabetes**: 100–125 mg/dL
    3. **Diabetes**: ≥126 mg/dL

       (Requires confirmation with a second test.)

3. **Random Blood Sugar (RBS)**:
    1. **Normal**: <130 mg/dL
    2. **Pre-diabetes**: 130–199 mg/dL
    3. **Diabetes**: ≥200 mg/dL

    (A random glucose level of 130–199 mg/dL is considered abnormal and warrants further testing via FPG, OGTT, or A1c.)
4. **Oral Glucose Tolerance Test (OGTT) – 2 hours post 75g glucose load**:
    1. **Normal**: <140 mg/dL
    2. **Pre-diabetes**: 140–199 mg/dL
    3. **Diabetes**: ≥200 mg/dL

COMPLICATIONS OF DIABETES [25]

The complications associated with diabetes mellitus are categorized into acute and chronic types.

Acute complications are-

1. Diabetic Ketoacidosis (DKA) and Diabetic Coma - DKA is a serious and possibly dangerous complication associated with diabetes, that requires urgent medical attention. It necessitates the immediate hospitalization of the patient due to its critical nature. Key clinical signs include dehydration, Kussmaul breathing (characterized by deep, prolonged, and sighing respirations), and the presence of acetone breath. Frequently, patients also experience diffuse abdominal pain.

   At first, the patient's sense of awareness is not impacted; however, as the situation advances, there may be a slow decrease in mental attentiveness, resulting in lethargy, and, in extreme cases, coma and death. In extreme instances, DKA can result in

hypotension and circulatory shock. Despite its severity, diabetic ketoacidosis is a fully reversible condition with timely and appropriate medical intervention.

2. Hypoglycemia happens when blood sugar levels fall to critically low levels, making it a significant complication in the treatment of diabetes. It is frequently caused by an overdose of insulin, vigorous exercise, or insufficient carbohydrate consumption. The condition manifests with symptoms such as irritability and excessive sweating. In severe cases, it can lead to altered consciousness, loss of consciousness, or even coma.

   Prompt action is essential, necessitating the quick consumption of foods or beverages high in glucose (like candies or sugary sodas). If the individual is unresponsive, administering glucose intravenously is essential.

Hyperglycemia

Hyperglycemia is a condition characterized by elevated levels of blood sugar. If not addressed, it can result in severe and potentially fatal complications related to diabetes. This condition arises when there is a lack of insulin, inadequate insulin production, or insulin that is not working effectively.

Common causes of hyperglycemia include:

- Missing diabetes medications or insulin doses
- Consuming excessive sweets without proper treatment adjustments
- Underlying infections or illnesses

Management of hyperglycemia involves:

- Adjusting the current therapeutic regimen
- Following a balanced diet
- Engaging in regular physical activity

CHRONIC COMPLICATIONS[25]

Long-Term Complications of Diabetes

The primary difficulty faced by individuals with diabetes is the management of the long-term complications associated with the condition. The most prevalent complications include:

1. Macroangiopathy

- Severe damage to the heart and blood vessels
- Hypertension, narrowing of blood vessel, coronary artery disease
- Elevated risk for strokes
- Male erectile dysfunction

2. Diabetic Retinopathy

- Damage to the blood vessels in the eye
- Progressive vision deterioration
- Leading cause of blindness in the Western world

3. Diabetic Nephropathy

- Progressive kidney damage
- Potential for renal insufficiency and failure

4. Diabetic Neuropathy

- Disturbances in sensation and muscle wasting
- Difficulty with walking and the formation of wounds due to injuries
- Intense pain in the lower legs
- May result in autonomic dysfunction that can lead to –

1. Tachycardia
2. Orthostatic hypotension
3. Urinary incontinence
4. Digestive issues (indigestion, nausea, diarrhea, or constipation)

5. Diabetic Foot

- Discomfort, loss of sensation, and dry skin
- Formation of calluses, sores, and ulcers
- Increased likelihood of serious infections
- May advance to gangrene, which could necessitate amputation

6. Other Complications

- Increased vulnerability to infections
- Myopathy and muscle weakness
- Osteoporosis and joint disorders
- Liver damage

METFORMIN

Metformin is a commonly prescribed biguanide medication recognized for its safety, effectiveness, and affordability. For more than six decades, it has been fundamental in managing type 2 diabetes mellitus (T2DM), especially in the initial phases of the condition.

Metformin Dosage and Administration [26,27]

Metformin is a oral medication that is frequently recommended for those with type 2 diabetes mellitus (T2DM). It comes in two different forms:

1. Extended-release (XR) metformin – requires once-daily dosing
2. Immediate-release (IR) metformin – requires twice-daily dosing

Recommended Dosage

- The typical daily dose ranges from 500 mg to 2550 mg.
- To minimize gastrointestinal (GI) side effects, it should be taken with meals.
- Dosage is titrated weekly in 500 mg or 850 mg increments to enhance tolerance.
- Physicians recommend consistent daily intake at the same time each day for optimal effectiveness.

Extended-Release Formulation

• To be taken once per day (ideally during the evening meal)

• Taken whole with a complete glass of water.

Additional Benefits

Metformin is not only an effective, safe, and affordable medication for diabetes management, but it also has cardiovascular benefits, potentially reducing the risk of heart disease and mortality (ADA, 2023).

The recommended starting dose for immediate-release oral metformin is typically 500 mg once or twice daily, or 850 mg once a day. To minimize gastrointestinal side effects, the dose is typically increased weekly in 500 mg or 850 mg increments. The usual maintenance dose is 500 to 1000 mg twice daily.

For the oral formulation designed for extended release, the starting dose is generally 500 mg or 1000 mg taken once a day. The dosage is slowly raised by 500 mg each week for a maximum of six weeks. After the initial titration period, the maximum recommended dose is 2000 mg, taken once or twice daily.

To help prevent type 2 diabetes, treatment with immediate-release metformin usually begins at 850 mg once daily for the first month. If necessary, the dosage can be increased to 850 mg twice daily to reach the intended therapeutic effect.

INDICATIONS

FDA-Approved Indications

1. T2DM
2. GDM (Off-Label Use in Some Regions)

Off-Label Uses

1. Prevention of Type 2 Diabetes
2. Polycystic Ovary Syndrome (PCOS)
3. Weight Management in Insulin-Resistant Individuals
4. Metabolic Syndrome
5. 'Non-Alcoholic Fatty Liver Disease' (NAFLD) and 'Non-Alcoholic Steatohepatitis' (NASH)
6. Antipsychotic-Induced Weight Gain and Hyperglycemia

7. Cancer Prevention and Treatment (Investigational Use)

8. Longevity and Aging (Experimental Use)

ADVERSE EFFECTS OF METFORMIN [28]

Common Side Effects

- Gastrointestinal disturbances (diarrhoea, nausea, vomiting)

Less Common Side Effects May Include:

- Chest pain or discomfort
- Headaches
- Excessive sweating
- Low blood sugar
- General weakness or fatigue
- Nasal congestion or inflammation

Long-Term Effects

- Vitamin B12 deficiency (risk of anemia and peripheral neuropathy)

Severe Adverse Effects (Black Box Warning)

- **Lactic Acidosis** (Rare but serious, ~1 in 30,000 cases)
    - Symptoms: Malaise, respiratory distress, metabolic acidosis
    - Risk factors: Renal/hepatic impairment, hypoxia, advanced age, surgery, alcoholism
    - Potential outcomes: Hypotension, hypothermia, death

CONTRAINDICATIONS OF METFORMIN [29]

Renal Impairment

- **Severe renal dysfunction** (GFR <30 mL/min/1.73m²)
- **Serum creatinine Cutoff levels:**
    - 1.5 mg/dL or higher in men
    - 1.4 mg/dL or higher in women
- **Abnormal creatinine clearance (CrCl)**

Other Contraindications

- Hypersensitivity to metformin
- Metabolic acidosis
- Hepatic impairment
- Unstable heart failure

Situational Discontinuation

- **Before surgery** (hold on the day of surgery)
- **Before contrast procedures** (if GFR <60 mL/min/1.73m² or risk factors for lactic acidosis are present)
- **In cases of:**
    - Nausea, vomiting, dehydration (risk of lactic acidosis)
    - Concurrent use of nephrotoxic drugs

OVERVIEW OF METFORMIN AND ITS ROLE IN DIABETES MANAGEMENT

Metformin, a biguanide derivative, is one of the most frequently prescribed oral medications for managing diabetes, particularly type 2 diabetes. It is especially beneficial for patients who are overweight or obese Compared to treatments like insulin, glibenclamide, and chlorpropamide, metformin has been shown to reduce diabetes-related complications and mortality by approximately 30%. [31–33]

Metformin reduces blood sugar levels via various mechanisms without raising insulin secretion. It enhances cellular insulin sensitivity, suppresses hepatic glucose production by reducing gluconeogenesis and glycogenolysis, and stimulates glucose uptake in muscle cells. These effects are largely mediated by the activation of adenosine monophosphate kinase (AMPK), which regulates glucose metabolism. [31,33,34]

Recent research shows that metformin lowers the risk of microvascular and macrovascular complications by preventing cellular damage in blood vessels. This protective effect is primarily attributed to AMPK activation and a reduction in intracellular 'reactive oxygen species' (ROS). [35,36] Metformin also plays a protective role against diabetic nephropathy by easing oxidative stress and restoring normal biochemical processes within the kidney's tubular structures. This action contributes to minimizing the risk of tubular injury and preserving renal function. [37]

Metformin remains the most widely used oral drug for diabetes management, thanks to its proven efficacy, low side effect profile, and compatibility with other antidiabetic therapies. It is estimated that around 150 million people with diabetes globally take metformin on a regular basis. [33]

INTRODUCTION TO VITAMIN B12 DEFICIENCY

Vitamin deficiencies pose a worldwide health issue, with the recognition of vitamin B12 deficiency occurring almost a century ago. The criteria for defining vitamin B12 (cobalamin) deficiency differs based on the test employed. [38] Diagnostic criteria consist of a serum cobalamin level lower than 148 pmol/l (200 ng/l) when accompanied by clinical symptoms and/or hematological irregularities, or a serum cobalamin level lower than 148 pmol/L, when accompanied by elevated homocysteine or methylmalonic acid (MMA) levels, may indicate deficiency. Typically, homocysteine concentrations exceeding 15 µmol/L and MMA levels above 0.27 µmol/L are considered the upper limits of normal. However, "The British Society for Standards in Haematology" (BCSH) recommends that individual laboratories establish their own reference ranges for these biomarkers. [39]

The causes of vitamin B12 deficiency are [40]

Cause	Examples
Low vitamin B12 intake	Vegetarian diet, long-term alcohol abuse, and senior citizens
Autoimmune	Pernicious anemia, Sjögren's syndrome
Food-bound cobalamin malabsorption	Atrophic gastritis, chronic gastritis, *Helicobacter pylori*-associated gastritis
Surgery	Post-gastrectomy and ileal resection
Malabsorption	Small intestinal bacterial overgrowth, chronic pancreatic exocrine insufficiency, Crohn's disease, celiac disease, achlorhydria
Obstetric/gynaecological	Oral contraceptive use, hormone replacement therapy, pregnancy
Genetic	Transcobalamin II deficiency
Drugs	Metformin, proton pump inhibitors, histamine H_2-receptor antagonists

TABLE 1 – COMMON CAUSES OF VITAMIN B12 DEFICIENCY

VITAMIN B12 METABOLISM AND PHYSIOLOGICAL ROLE

Vitamin B12 comes from food sources like meat, eggs, and dairy. Typically, people take in about **2.4 µg** of vitamin B12 each day, with an absorption rate of around **50-60%.** [40]

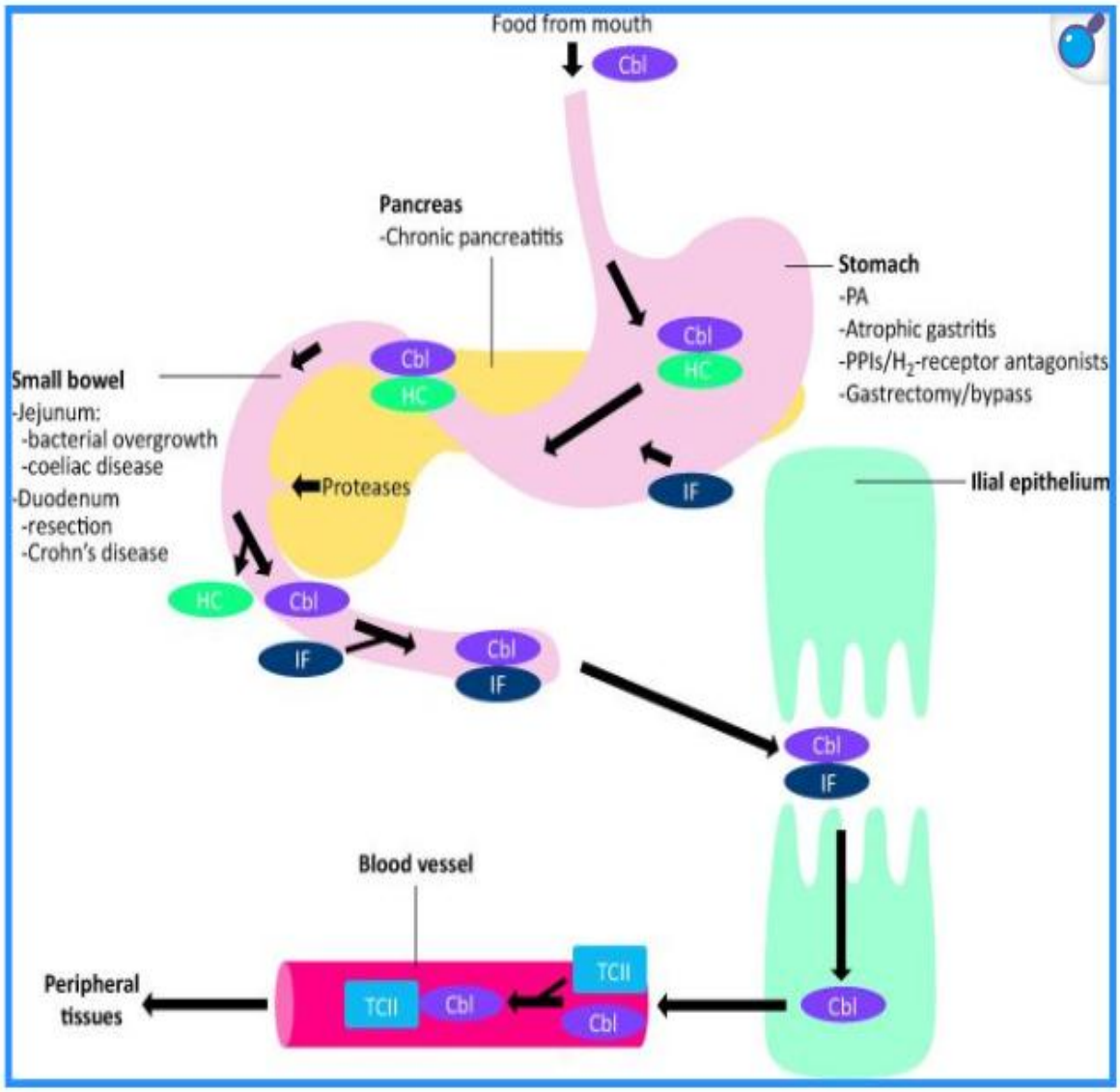


FIGURE-3. Sequence of events which results in the absorption of vitamin B_{12} [40]

A pictorial representation of vitamin B12 (cobalamin) absorption illustrates its complex process. Dietary vitamin B12 is initially associated with food proteins and must be released in the stomach's acidic environment to ensure proper absorption in the small intestine. Once liberated, it quickly attaches to ‘haptocorrin’ (transcobalamin I) and stays bound until it is cleaved by proteolytic enzymes in the duodenum. At this stage, vitamin B12 is released and attaches to intrinsic factor (IF), a transport protein secreted by the stomach's parietal cells, which is essential for its absorption in the terminal ileum. [40]

After passing through the brush border, vitamin B12 separates from intrinsic factor and enters the bloodstream, where it attaches to either ‘transcobalamin II’ or ‘haptocorrin’. This ‘transcobalamin II’ facilitates the delivery of cobalamin to peripheral tissues, while ‘haptocorrin’ primarily transports it to the liver. Abnormalities in this process, such as those caused by pernicious anemia, proton pump inhibitors, or intrinsic factor deficiencies, can lead to impaired vitamin B12 absorption and deficiency. [40]

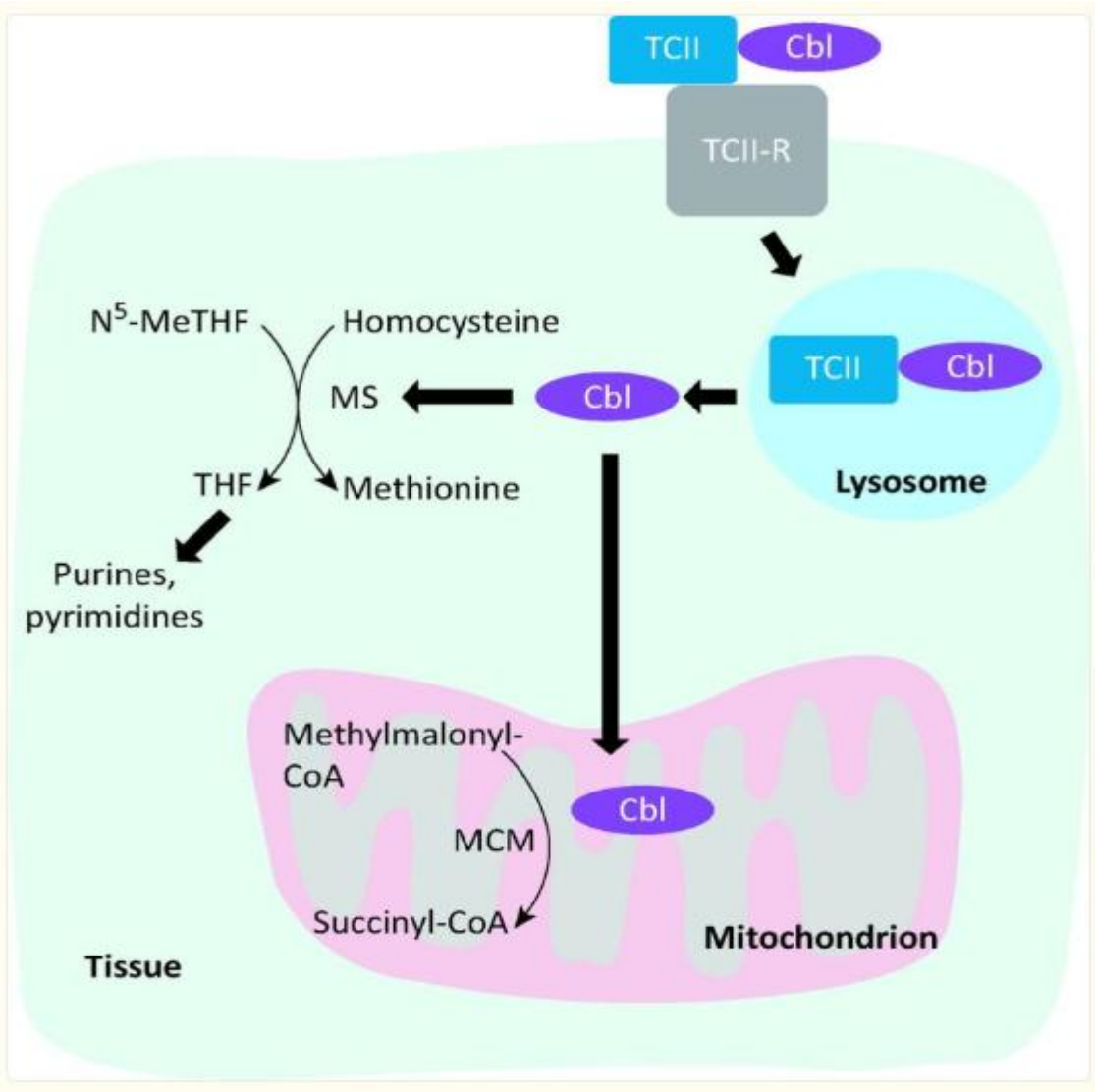


FIGURE-4. Intracellular metabolism of vitamin B 12 [40]

After reaching peripheral tissues, free vitamin B12 takes part in key metabolic reactions. In the 'cytosol, it acts as a cofactor for 'methionine synthase' (MS), an enzyme that facilitates the conversion of 'homocysteine' and 'N5-methyltetrahydrofolate' (N5-MeTHF) into 'methionine' and 'tetrahydrofolate' (THF). THF is crucial for the synthesis of purines and pyrimidines, which are fundamental building blocks for DNA and RNA production, thus accounting for the clinical symptoms associated with vitamin B12 deficiency. The sole other reaction that relies on vitamin B12 happens in the mitochondria, where methylmalonyl-CoA is transformed into

succinyl-CoA through the action of methylmalonyl-CoA mutase (MCM). This process is essential for energy metabolism and proper cellular function. Deficiency in vitamin B12 can disrupt these pathways, leading to hematological and neurological complications. [40]

Methylcobalamin and adenosylcobalamin are the active forms of vitamin B12, making up approximately 75-90% and 10-25% of the body's vitamin B12 reserves, respectively. Cyanocobalamin, a synthetic variant frequently found in supplements, is relatively inactive and is only converted partially into its active forms. Vitamin B12 plays an essential role in the metabolism of carbohydrates, lipids, and proteins, supports red blood cell production, and helps maintain central nervous system function. It serves as a coenzyme for methionine synthase in the cytosol and for methylmalonyl-CoA mutase within the mitochondria. Methionine synthase aids in the transformation of homocysteine into methionine, which helps to avoid the buildup of homocysteine that is linked to vascular diseases and neurological issues like depression and brain atrophy.

Furthermore, methionine is essential for methylation processes that are vital for the synthesis of DNA, RNA, and proteins, affecting the growth of vascular endothelial cells and the production of norepinephrine, which in turn impacts stress response and cardiovascular health. Methylmalonyl-CoA mutase is involved in the breakdown of odd-chain fatty acids, branched-chain amino acids, and cholesterol, resulting in the formation of succinyl-CoA. Impairment in this process is thought to contribute to the neurological consequences associated with vitamin B12 deficiency.[41]

PREVALENCE OF VITAMIN B12 DEFICIENCY IN DIABETIC PATIENTS

Vitamin B12 is primarily obtained from non-vegetarian food sources, making deficiency more relevant in India, where a large portion of the population prefers a vegetarian diet. However, comprehensive population-based data on vitamin B12 levels in India is still emerging, with different studies reporting deficiency rates ranging from 16% to 77%. [42]

A study conducted at Karnataka Institute of Endocrinology, Bangalore, examined 161 type 2 diabetes subjects over six months. Exclusion criteria included alcohol consumption, vitamin B12 supplementation, pregnancy, and type 1 diabetes. Vitamin B12 deficiency was defined as levels below 200 picogram/ml. Only subjects on metformin for more than six months were included. Among individuals with type 2 diabetes receiving metformin treatment, 27.33% were found to have a deficiency in vitamin B12. Deficiency rates increased with higher metformin doses but showed no correlation with treatment duration.[43]

In a research carried out at a tertiary care facility located in the Salem district of Tamil Nadu, India, findings indicate a high prevalence of vitamin B12 deficiency within the sample group, highlighting gaps in the identification and treatment of this condition, as it often goes undiagnosed. Longer duration and higher daily doses of metformin were identified as key risk factors for vitamin B12 insufficiency. Deficiency of vitamin B12 may contribute to worsening diabetic neuropathy, emphasizing the need for regular monitoring. It is advisable to conduct monthly evaluations of vitamin B12 levels in diabetic patients who are undergoing prolonged treatment with high doses of metformin. Supplementation, whether aimed at prevention or treatment, can assist in managing this concern. [44]

RELATIONSHIP BETWEEN METFORMIN AND VITAMIN B12 DEFICIENCY IN PATIENTS WITH T2DM

In the last twenty years, there has been an increasing interest in exploring the relationship between metformin and vitamin B12 deficiency. The initial account of vitamin B12 malabsorption caused by metformin was recorded by Tomkin and colleagues in 1971. [45] Since then, multiple experimental, observational studies, and systematic reviews have explored this association in patients with type 2 diabetes mellitus. [42,46–48] Accurately defining this association is crucial due to the significant clinical impact of vitamin B12 deficiency and its potential to negatively affect the quality of life in people living with diabetes.

Metformin reduces vitamin B12 absorption through mechanisms that remain unclear. [49] Multiple theories propose mechanisms by which metformin affects absorption, including disrupted enterohepatic circulation, enhanced hepatic storage, diminished production of intrinsic factor (IF), and slowed intestinal motility resulting in bacterial overgrowth. [49,50] The most widely accepted mechanism suggests that metformin disrupts the calcium-dependent binding of the intrinsic factor–vitamin B12 complex to the cubilin receptor in the ileum, thereby reducing its uptake through endocytosis and impairing absorption. [41,51]

It has been proposed that metformin imparts a positive charge to the surface membrane of the cubilin receptor. [52] The positive charge repels divalent calcium ions, which hinders the calcium-dependent attachment of the IF-vitamin B12 complex to the cubilin receptor, ultimately resulting in malabsorption of vitamin B12. [50]

The deficiency of vitamin B12 caused by Metformin arises from the compromised absorption of the intrinsic factor-cobalamin complex through the cubilin receptor in the ileum. This deficiency may result in peripheral neuropathy, cardiac autonomic neuropathy, neuropsychiatric issues, or hematological disorders. [53]

Reduced vitamin B12 absorption due to metformin may impact cognitive function, as several studies have linked low vitamin B12 levels to cognitive decline and depressive symptoms. [54] Porter et al.[55] in their cohort study identified an association between metformin usage and lower levels of vitamins B12 and B6. The findings also suggested an increased risk of cognitive decline among the individuals studied. Furthermore, other studies indicated that patients on metformin who had vitamin B12 deficiency demonstrated reduced cognitive abilities and an increased risk of experiencing depression. [56,57]

RISK FACTORS FOR VITAMIN B12 DEFICIENCY IN T2DM PATIENTS ON METFORMIN

For diabetic patients using metformin, the risk of developing a vitamin B12 deficiency is increased by factors such as higher dosages of metformin, extended duration of treatment, and pre-existing conditions like gastrointestinal issues or autoimmune diseases that hinder B12 absorption. [58]

Elevated doses and extended use of metformin are notably associated with a greater likelihood of vitamin B12 deficiency. [59] Some studies indicate that even moderate doses and durations can lead to a noticeable decline in vitamin B12 levels. [60] Research has shown that each additional gram of metformin taken daily is associated with a heightened risk of vitamin B12 deficiency.[61]

One research identified a distinct correlation between the dosage of metformin, the length of its use, and the incidence of vitamin B12 deficiency in individuals with T2DM. Correlation analysis revealed that vitamin B12 deficiency was associated with both increased doses of metformin and extended periods of treatment. Patients who had been on metformin for ten years or more and were taking daily doses of 2,000 mg or higher had about a fourfold increase in the risk of vitamin B12 deficiency when compared to those who used metformin for less than four years with daily doses of 1,000 mg or lower. However, neither the length of time a person had diabetes nor the presence of microvascular complications appeared to influence the likelihood of developing a vitamin B12 deficiency. [59]

The specific mechanisms that lead to vitamin B12 deficiency in patients treated with metformin are not fully understood. Several contributing factors have been proposed, such as small intestinal bacterial overgrowth linked to diabetes, disruptions in gastrointestinal motility, shifts in gut microbiota composition, competitive absorption mechanisms, impaired vitamin B12 uptake, and calcium's influence on cellular membrane function. [59]

COGNITIVE IMPAIRMENT IN THE ELDERLY: ROLE OF VITAMIN B12

Maintaining cognitive abilities in later life is crucial for overall well-being, postponing the development and advancement of dementia, and minimizing societal expenses associated with chronic care and decreased productivity. Since there is currently no cure for dementia, initiatives have focused on discovering adjustable non-genetic risk factors to mitigate its effects. [62] Increased homocysteine concentrations, along with deficiencies in folate, vitamin B12, and vitamin B6—key nutrients involved in regulating homocysteine—have been associated with declines in cognitive function.[63] The occurrence of elevated total serum homocysteine levels tends to rise as people age, and high levels of homocysteine are frequently observed in older adults[64], exhibiting a negative relationship with cognitive function. Deficiency of B vitamins (folate, vitamin B6, and vitamin B12) may contribute to cognitive impairment in the elderly through hyperhomocysteinemia, while elevated plasma homocysteine concentrations serve as a sensitive marker for vitamin B12 and folate deficiency. [65]

Cognitive impairment can result from a variety of factors, including those that can and cannot be changed. With no definitive cure available, efforts are directed toward delaying or halting the progression of the disease. It is crucial to identify modifiable risk factors such as elevated homocysteine levels, deficiencies in vitamin B12, and folate. These nutritional factors have been associated with diminished cognitive function in older adults, although early dementia might also trigger changes in diet, indicating that malnutrition may be a result rather than a cause of cognitive decline. Given that there is currently no definitive cure for dementia, vitamin B12 and folate may play a role in modifying its clinical course and warrant consideration as part of potential therapeutic strategies.[66]

ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND COGNITIVE DECLINE IN DIABETES

Research has shown that there is a significant occurrence of vitamin B12 deficiency in individuals with T1DM and T2DM. This deficiency commonly exhibits a variety of clinical symptoms, such as memory issues, dementia, delirium, peripheral neuropathy. [67]

A deficiency in vitamin B12 has been associated with reduced cognitive function and a heightened risk of developing dementia. In older adults, low levels of vitamin B12 are associated with decreased cognitive performance. A randomized, placebo-controlled study investigated whether vitamin B12 supplementation could slow cognitive decline in older diabetic individuals with borderline vitamin B12 levels. The findings indicated that supplementation had no significant effect on preventing cognitive deterioration in this population. [68]

Due to the significant occurrence of vitamin B12 deficiency among individuals with diabetes and its possible effects on cognitive abilities, regular testing for vitamin B12 levels in this group may be warranted. Early detection and appropriate supplementation could potentially mitigate the risk of cognitive decline associated with vitamin B12 deficiency. [67]

NEUROCOGNITIVE ASSESSMENT IN TYPE 2 DIABETIC OLDER PATIENT

Patients with T2DM experience a notably increased occurrence of central nervous system disorders, including cognitive impairment, when compared to those without diabetes. [69] As the occurrence of T2DM increases among older adults, the likelihood of experiencing cognitive decline in this demographic is significantly heightened. Cognitive impairment in individuals with diabetes has been associated with oxidative stress[70] and inflammation[71], with research showing that higher levels of plasma interleukin-6 (IL-6) correlate with decreased cognitive function. [72]

Studies involving rat models of type 2 diabetes mellitus (T2DM) have demonstrated that individuals with cognitive deficits exhibit notably decreased levels of superoxide dismutase (SOD) activity in the brain when compared to those with normal cognitive abilities. [73] Moreover, rapid changes in blood glucose levels have been shown to be associated with cognitive abilities in elderly with T2DM. Glycated albumin (GA) acts as a marker for blood glucose variations over a period of 2 to 3 weeks[74] , The GA to glycated hemoglobin (GA/HbA1c) ratio offers an even more immediate indication of variability in glucose levels.[75] Both GA and HbA1c levels can be easily measured during standard hospital tests.

Earlier research has indicated that reduced hippocampal volume correlates with higher serum GA and GA/HbA1c concentrations in older adults. Moreover, cognitive deterioration in older women has been associated with heightened GA levels, emphasizing the significance of glucose metabolism in neurodegenerative events. [75]

The Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA) are commonly used tools for evaluating cognitive function. However, MMSE outcomes can be influenced by an individual's educational level, limiting its diagnostic utility in certain cases. In contrast, the MoCA was developed for use with elderly who have at least a secondary education and is generally more effective in detecting mild cognitive impairment

(MCI). Despite this, it tends to be less reliable in identifying moderate to advanced stages of Alzheimer's disease. Both assessments have limitations, and their effectiveness is dependent on proper administration—yet consistent training among healthcare providers in using these tools for elderly is not always ensured.[75]

People living with diabetes face a 1.2 to 1.5 times greater likelihood of experiencing cognitive decline and are approximately 1.6 times more prone to developing dementia than individuals without diabetes. Cognitive deterioration tends to progress more rapidly in elderly with diabetes compared to the general population, and such impairments can further complicate the management and progression of T2DM. In one research, 63.6% of elderly with T2DM showed signs of cognitive impairment. These results underscore the importance of early identification and standardized treatment for cognitive dysfunction to enhance clinical outcomes in this demographic. [75]

HbA1c is recognized as the primary method for assessing average blood glucose levels over the past 8 to 12 weeks and has a strong correlation with cognitive function in individuals with diabetes. Nonetheless, people exhibiting similar HbA1c values may experience different levels of complication risks. Another key aspect of glycemic management is glycemic fluctuation, as larger variations can elevate the risk of complications. Research indicates that fluctuations in blood glucose significantly impact microglial activity, with increased variability contributing to cognitive decline. In comparison to HbA1c, GA/HbA1c and GA provide a more accurate representation of blood glucose fluctuations in both T1DM and T2DM. The GA/HbA1c ratio has been associated with the onset of Alzheimer's disease and is also considered an independent risk factor for cognitive deterioration in elderly individuals with type 2 diabetes. [75]

In the research conducted by Biessels GJ and Despa F, individuals in the cognitive dysfunction group exhibited higher GA and GA/HbA1c levels in comparison to those with normal cognitive

abilities, indicating that patients with cognitive impairments faced more significant variations in blood glucose and had worse glycemic control. [69]

Nevertheless, there was no significant difference in HbA1c levels between the two groups. Univariate regression analysis revealed no association between HbA1c and MMSE or MoCA scores, consistent with earlier studies suggesting that GA and GA/HbA1c are indicators of cognitive decline in older T2DM patients, regardless of HbA1c levels. [76] Additional analysis indicated an inverse relationship between GA/HbA1c levels and MMSE/MoCA scores, aligning with previous research that associates GA/HbA1c with cognitive deterioration in older adults. [77,78]

Logistic regression analysis identified age and the GA/HbA1c ratio as independent predictors of cognitive impairment, implying that variability in blood glucose levels may play a direct role in cognitive decline among elderly patients with type 2 diabetes. In contrast, univariate analysis did not demonstrate a significant association between age, GA, and the GA/HbA1c ratio. Furthermore, when participants were divided into tertiles or quartiles according to age, no meaningful differences were observed in GA or GA/HbA1c levels across these subgroups.

The MoCA is particularly good at identifying mild cognitive impairments, while the MMSE is better at recognizing cognitive decline and functional issues. In one research, both assessment tools were utilized for screening; however, the MMSE detected fewer individuals with cognitive dysfunction, probably because it is less sensitive than the MoCA. [79]

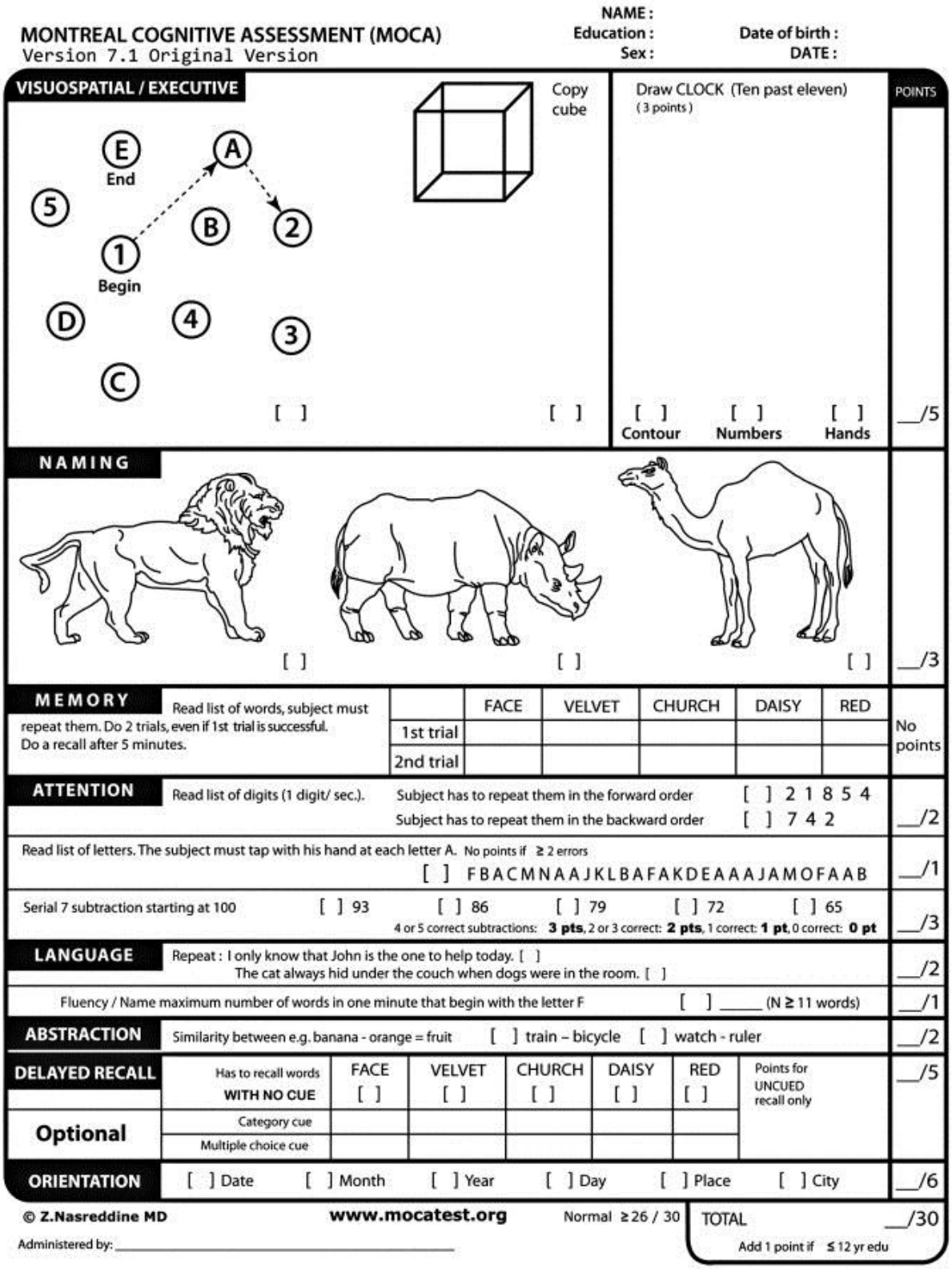


FIGURE-5. MONTREAL COGNITIVE ASSESSMENT[79]

VITAMIN B12 DEFICIENCY IN METFORMIN-TREATED PATIENTS: PROPOSED CRITERIA FOR A COST-EFFECTIVE SCREENING

At present, there are no clear guidelines for screening vitamin B12 deficiency in individuals using metformin, resulting in a common under-diagnosis within this group. The British Society for Haematology's guidelines on diagnosing and managing vitamin B12 deficiency do not specify how often serum vitamin B12 levels should be monitored in individuals with T2DM who are being treated with metformin. Nevertheless, these guidelines do suggest evaluating serum vitamin B12 levels when there is a significant clinical suspicion of deficiency. [49]

According to recent findings, a study by Marco Infante and colleagues has put forth a series of guidelines for cost-effective screening of vitamin B12 deficiency in patients treated with metformin. These guidelines can act as a useful reference for pinpointing individuals at high risk who could gain from vitamin B12 supplementation and regular checks of their cobalamin levels. Importantly, screening for vitamin B12 deficiency should be considered for specific individuals, even if they do not exhibit symptoms of deficiency. Adopting these screening guidelines may assist in preventing or alleviating the health issues associated with vitamin B12 deficiency, such as anemia and peripheral neuropathy, by facilitating early detection and prompt treatment. [49]

Proposed criteria

1. Comprehensive analysis of Vitamin B12 Deficiency:

- Use a circulating biomarker (e.g., methylmalonic acid [MMA] or total homocysteine) with a functional biomarker (e.g., total vitamin B12 or holotranscobalamin [HoloTC]) for efficient detection.
- Add a recent full blood count (CBC) to the workup.

2. Screening Recommendations:

- Screen with presence of risk factors such as:

- High clinical suspicion (e.g., macrocytic anemia without explanation, peripheral neuropathy).
- Diabetic peripheral/autonomic neuropathy, established.
- Metformin treatment ≥ 5 years.
- Age ≥ 65 years.
- Increased cumulative metformin exposure (Metformin Usage Index [MUI] > 5; computed as daily dose × years ÷ 1000).
- Metformin ≥ 1500 mg/day for ≥ 6 months (with increased risk ≥ 2000 mg/day).
- Long-term acid-suppressing therapy (≥ 12 months, eg, proton-pump inhibitors [PPIs] or H2-receptor antagonists [H2RAs]).
- Other comorbidities or risk factors with higher risk of deficiency.

3. Peripheral Neuropathy Definition:

According to Diabetes Prevention Program results, neuropathy is characterized by abnormally positive monofilament testing.

4. Routine Screening in High-Risk Groups:

All metformin-treated diabetic patients with existing neuropathy need to be screened regularly. Early treatment of metformin-associated vitamin B12 deficiency is important to avoid worsening of nerve damage or mixed neuropathy.

5. Metformin Use Index (MUI):

- MUI = (Metformin daily dose in mg × duration in years) ÷ 1000.
- Used to estimate total metformin exposure in patients treated for ≥6 months.

This guideline gives more importance to early detection, risk stratification, and early treatment to avoid vitamin B12 deficiency in susceptible populations.

TREATMENT OF VITAMIN B12 DEFICIENCY IN METFORMIN-INDUCED VITAMIN B12 DEFICIENCY

For individuals at an increased risk of marginal vitamin B12 deficiency, the main objective is to avert the development of a clinically significant deficiency and to ensure proper replenishment when a deficiency is present. The recommended daily intake of vitamin B12 is approximately 2.4 micrograms for adult men and non-pregnant women, while pregnant individuals are advised to consume around 2.6 micrograms per day. Nevertheless, research indicates that a daily intake ranging from 4-7 µg is linked to lower levels of serum MMA. There is no defined upper limit for vitamin B12 intake, and since it is low-cost, safe, and generally well tolerated, a tailored approach to supplementation is usually considered safe.

The British Society for Haematology guidelines state that there are no specific suggestions for preventive oral vitamin B12 supplementation for patients taking metformin.
[62] Although there are no specific guidelines, individuals on metformin who have a vitamin B12 deficiency should be given cobalamin supplements to correct the deficiency and avoid complications such as nerve damage and other related issues. Timely administration of vitamin B12 is especially crucial for patients experiencing neurological or hematological symptoms, including peripheral neuropathy and megaloblastic anemia. Treating vitamin B12 deficiency in those who use metformin is likely a cost-effective approach, although the best method for replenishing it is still uncertain. Since metformin interferes with vitamin B12 absorption in the small intestine, alternative routes such as intramuscular injections or sublingual administration may offer greater efficacy than traditional oral supplements, as these methods circumvent intestinal absorption. Nonetheless, high-dose oral vitamin B12 supplementation might still prove effective in addressing the malabsorption caused by metformin. Additional studies are necessary to establish the most efficient and practical method for administering vitamin B12 to this group of patients

Furthermore, because oral calcium supplements have demonstrated the ability to counteract vitamin B12 malabsorption caused by metformin, upcoming research should investigate if calcium supplementation might be a safe and effective alternative treatment for vitamin B12 deficiency related to metformin use. [49]

In individuals experiencing vitamin B12 deficiency due to metformin, supplementation offers a straightforward, safe, and effective method to prevent or reduce peripheral nerve damage, anemia, and other related symptoms of deficiency. Currently, there are no specific recommendations for addressing metformin-induced vitamin B12 deficiency. As a result, healthcare providers should address and rectify vitamin B12 deficiency in patients on metformin in accordance with the British Society for Haematology guidelines regarding the diagnosis and treatment of vitamin B12 deficiency. [49]

Considering the proven safety and effectiveness of metformin as an insulin-sensitizing medication, it is not advisable to stop its use in patients who have recently been diagnosed with vitamin B12 deficiency. Nevertheless, it is recommended to regularly monitor vitamin B12 levels in patients on metformin, as they could still be at a heightened risk of deficiency even if they receive proper supplementation.[49]

CURRENT GUIDELINES AND RECOMMENDATIONS FOR VITAMIN B12 MONITORING IN METFORMIN USERS [80]

The research conducted by Longo, Stacy L et al. deepens our comprehension of existing vitamin B12 monitoring practices among a diverse urban population and underscores the necessity for better adherence to established screening guidelines. The findings indicate that most individuals with type 2 diabetes who are on metformin therapy are not undergoing routine screening for vitamin B12 deficiency, despite recommendations outlined in national diabetes guidelines and metformin prescribing information. Within a five-year timeframe, only 25% of metformin users had vitamin B12 testing, despite the suggestions for regular screening. While the American Diabetes Association (ADA) suggests regular monitoring, metformin's prescribing information specifically recommends annual blood count evaluations and checking vitamin B12 levels every two to three years.

Earlier research focused on mainly Caucasian male veteran populations has identified monitoring shortcomings, with Kancherla et al. noting that 63% of patients were not monitored and Pierce et al. documenting a 47% gap. In this above mentioned study, the rate of non-compliance with monitoring guidelines was even greater (74.8%) among a more racially and gender-diverse cohort.

Variations in monitoring practices were noted between Federally Qualified Health Centres (FQHCs) and the Program of All-Inclusive Care for the Elderly (PACE). In contrast to FQHCs, PACE patients were more likely to receive vitamin B12 supplementation (41%) and present with conditions such as peripheral neuropathy (28%) and proton pump inhibitor prescriptions (41%). Furthermore, 97% PACE patients underwent testing for vitamin B12, which was significantly higher than the rates observed in FQHCs. However, it remained uncertain whether the monitoring within PACE was driven by the use of metformin or other reasons.

An evaluation of vitamin B12 levels revealed that 87.7% were within the normal range, 11.1% were low, and 1.2% were deficient. Premarketing trials of metformin indicated a 7% reduction in vitamin B12 levels over a 29-week period, which aligns with this study's results, where 88% of patients taking metformin for more than a year maintained normal levels. Interestingly, 28% of patients with normal vitamin B12 levels were using supplementation, In contrast, 80% of individuals with low or deficient vitamin B12 levels were not receiving an active prescription for supplementation. Furthermore, nearly 10% of patients who had not undergone vitamin B12 testing had been diagnosed with peripheral neuropathy, and 5% were receiving supplementation without having been tested. These results highlight the importance of educating providers and enhancing patient care processes to ensure proper monitoring and timely intervention to avoid complications.

The association between metformin-induced vitamin B12 deficiency and the development of peripheral neuropathy remains inconclusive and not yet fully understood. Ahmed et al. found no relationship, while Aroda et al. indicated a higher incidence of neuropathy in those with low vitamin B12 levels. In this study, 7.1% of patients diagnosed with peripheral neuropathy had not received a vitamin B12 assessment within the past five years. Nevertheless, all 15 patients who were tested displayed normal levels, and 53.3% were actively prescribed supplements. Although vitamin B12 deficiency was not identified in this cohort, it is crucial to rule it out as a potential contributing factor. In instances where both neuropathy and vitamin B12 deficiency coexist, supplementation might assist in decreasing the dependence on prescription medications for managing neuropathy.

CONCLUSION

Various studies mentioned thoroughly investigates the link between prolonged metformin use and Vitamin B12 deficiency in individuals with T2DM, focusing on the elderly population. The results indicate a significant occurrence of B12 deficiency among those on metformin, with indications of reductions in serum Vitamin B12 levels that are influenced by both dosage and duration of treatment. Furthermore, the studies highlight the potential impact of vitamin B12 deficiency on cognitive function, especially in elderly patients, underscoring the need for routine monitoring of B12 levels in individuals with diabetes who are treated with metformin.

A considerable part of the research examines the pathophysiological mechanisms through which metformin hinders B12 absorption, notably by affecting the calcium-dependent binding of the B12–intrinsic factor complex in the terminal ileum. Supporting literature provides consistent findings regarding this interaction, highlighting the importance of clinical awareness.

Moreover, some studies frames its results within the framework of the Indian population, where vegetarian eating habits and socio-cultural influences increase the likelihood of B12 deficiency. This localized perspective enhances the importance of the international evidence, calling for focused public health initiatives and affordable screening measures, especially for elderly individuals and those using high doses or prolonged courses of metformin.

In terms of cognition, the research supports prior association between B12 deficiency and elevated homocysteine levels with cognitive decline, such as in Alzheimer's disease. Although certain trials have not demonstrated cognitive enhancement with B12 supplementation, the advantages of early detection and addressing deficiency still outweigh the potential drawbacks.

MATERIALS AND METHODOLOGY

SETTING:

Elderly aged over 60 years, including those visiting the outpatient department and those admitted to the Geriatrics ward at Shri B M Patil Medical College Hospital & Research Centre, were included in the study.

ETHICAL COMMITTEE APPROVAL:

Ethical committee clearance was obtained from the Institutional Ethical committee of Shri B M Patil Medical College & Research Centre as per the meeting held on 01/04/2023.

STUDY DESIGN:

Observational Cross-Sectional Study.

STUDY PERIOD:

18 months

CONSENT:

Informed consent was obtained from all participants involved in the study.

FINANCIAL SUPPORT:

Nil

STUDY POPULATION:

Patient presenting as a known case of diabetes mellitus type 2 taking metformin for at least 3 years.

INCLUSION CRITERIA:

- Age above 60 yrs.
- Individuals diagnosed with type 2 diabetes mellitus who had been on metformin therapy for a minimum duration of three years were included.

EXCLUSION CRITERIA:

- Patients with newly diagnosed type 2 diabetes mellitus patients (< 3 years of Metformin therapy)
- Patients who may develop vitamin b12 deficiency due to other causes like pernicious anaemia, GI surgeries (gastrectomy, colectomy) , GI disorders (inflammatory bowel disease, crohn's disease) and chronic alcoholics .
- Patients with severe medical illnesses (severe infection, sepsis or malignancy).
- Patients with altered renal or hepatic function.
- Patients taking B12 supplements or patients taking proton pump inhibitors (PPIs)
- Patients having severe form of dementia or neurocognitive disorders like parkinsonism or Alzheimer's disease.
- Patients presenting with acute hyperglycemic conditions like diabetic ketoacidosis and hyperosmolar hyperglycemic state as it may affect cognition levels
- Patients who had a poor compliance to oral metformin therapy.

DETAILS OF THE STUDY

A total of 75 patients who satisfied the inclusion and exclusion criteria were taken for the study.

All participants underwent comprehensive history taking and a detailed clinical examination. Neuropathy symptoms were especially asked and evaluated.

The following pertinent examinations were carried out after 10ml of venous blood was obtained from patients with their consent using dry syringe and aseptic measures.

All cases underwent Vitamin B12 evaluation and patients with lab Vitamin B12 values of <200pg/ml were subjected to Montreal Cognitive Assessment (MOCA). All the scores were recorded and classified based on their severity.

Other investigations including FBS, PPBS, HbA1c were also performed.

Routine investigations including Complete Blood Count (CBC), Serum Creatinine, Serum Urea and Urine routine were also carried out.

All the above relevant data was compiled and correlated.

STATISTICAL ANALYSIS

The data obtained was entered in Microsoft Excel Sheet and statistical analysis was done in IBM SPSS (Statistical package for Social Science software) version 30.0. A p- value of <0.05 was considered significant. The following tests were used -

1. **Descriptive Statistics** –
    - Frequencies, percentages for categorical variables
    - Mean ± SD / Median (IQR) for continuous variables
2. **Normality Test** –
    - **Shapiro-Wilk test** (to assess if data is normally distributed)
3. **Comparisons between Groups**:
    - **Independent Samples t-test** → for normally distributed continuous variables (e.g., comparing B12 levels between two groups)
    - **Mann–Whitney U test** → for non-normally distributed continuous variables
4. **Categorical Data Comparison**:
    - **Chi-square test**
    - **Fisher's Exact test** (when expected frequencies are small)
5. **Correlation Analysis**:
    - **Pearson correlation** → for normally distributed continuous variables
    - **Spearman's rank correlation** → for non-normal data or ordinal variables *(used for B12 levels vs MoCA scores, metformin duration vs B12 levels, etc.)*
6. **Regression Analysis (if applicable)**:
    - **Logistic regression** → to assess predictors of B12 deficiency (if done)
7. **Paired Sample Tests** (if pre- and post-comparisons were done):
    - **Paired t-test** or
    - **Wilcoxon signed-rank test**

RESULTS

Table No. 1: Age wise distribution

Sr No	Age group (years)	Number of subjects	%
1	Old (75-85)	14	18.7%
2	Very old (>85)	03	4%
3	Young old (60-74)	58	77.3%

In the present study cohort of 75 subjects, the majority belonged to the **young-old age group (60–74 years)**, comprising **58 individuals (77.3%)**. The **old group (75–85 years)** included **14 participants (18.7%)**, while **3 subjects (4%)** were categorized as **very old (>85 years)**. This age distribution suggests that most participants were in the early elderly stage, reflecting the age range where chronic conditions such as Type 2 diabetes and associated complications are commonly observed.

Graph No.1: Age group distribution

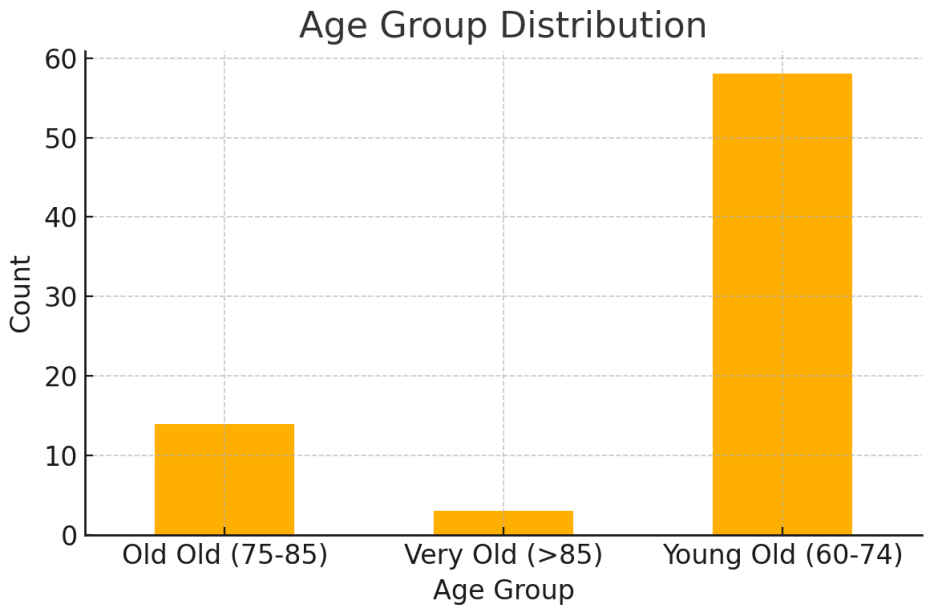


Table No.2: Gender distribution

Sr No	Gender	Number of subjects	%
1	Males	40	53.3%
2	Females	35	46.7%

In the present study involving 75 subjects, there was a nearly equal distribution of gender, with **40 males (53.3%)** and **35 females (46.7%)**. This balanced representation ensures that gender-based comparisons, such as those related to vitamin B12 levels, metformin usage, or cognitive outcomes, can be meaningfully interpreted within the study population.

Graph No.2: Gender distribution

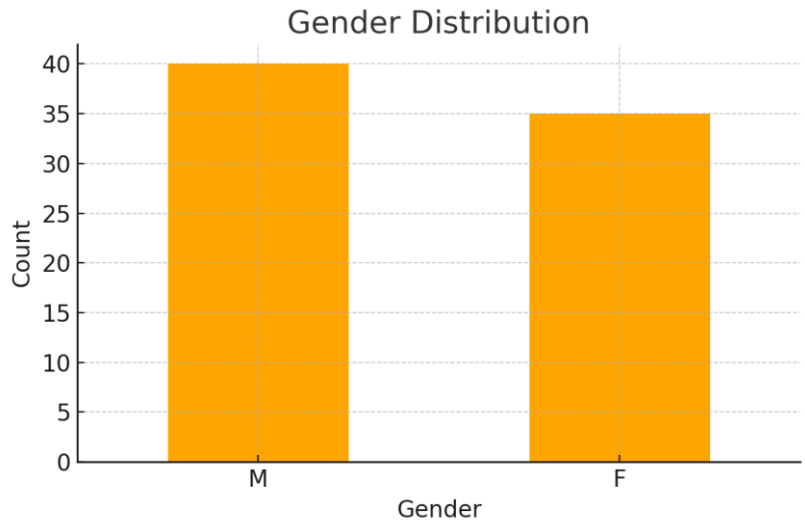


Table No.3: Age Group Distribution by Gender

Age Group	Female (n)	Male (n)	%
Young Old (60–74)	27	31	77.3%
Old (75–85)	7	7	18.7%
Very Old (>85)	1	2	4%
Total	**35**	**40**	**100%**

Among the 75 study participants, age-wise and gender-wise distribution revealed that the **young-old group (60–74 years)** comprised the largest segment, with **27 females** and **31 males**. The **old age group (75–85 years)** included an equal number of **7 males and 7 females**, while the **very old group (>85 years)** had **2 males** and **1 female**. This distribution indicates a relatively higher proportion of younger elderly participants in both genders, with minimal representation in the very old category.

Graph No.3: Age Group Distribution by Gender

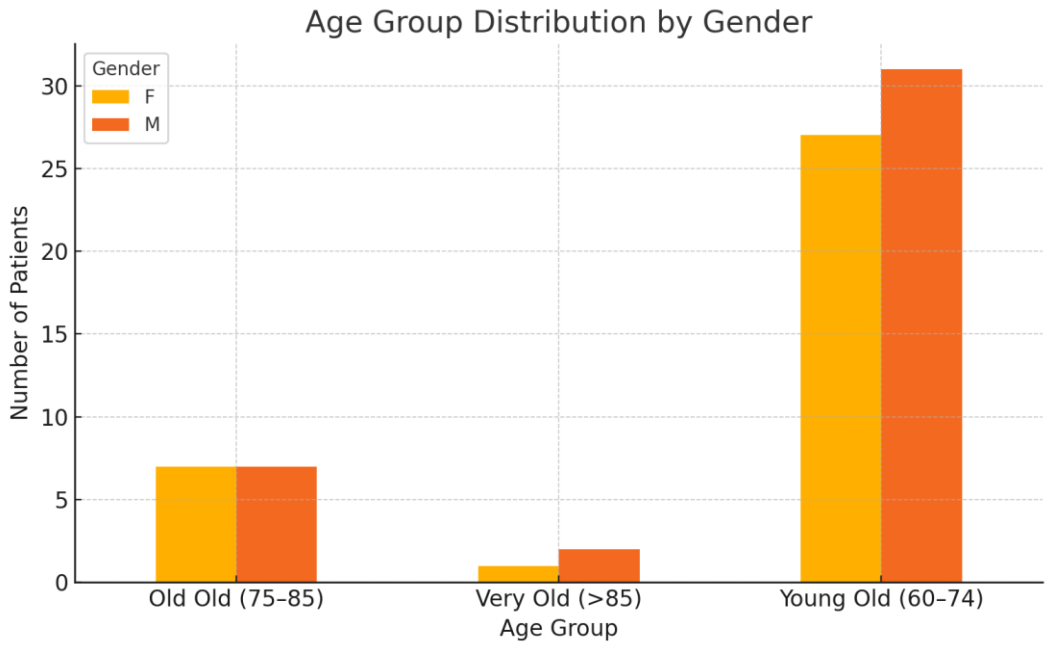


Table No.4 BMI distribution

Sr No	BMI category (kg/m2)	Number of subjects	%
1	< 18.5	7	9.3%
2	18.5- 24.9	31	41.3%
3	25- 29.9	30	40%
4	30 or more	7	9.3%.

In this study cohort of 75 subjects, the **majority had a BMI within the 18.5–29.9 kg/m² range**, with **31 participants (41.3%)** classified within the normal weight range (18.5–24.9 kg/m²) and **30 participants (40%)** classified as **overweight (25–29.9 kg/m²)**. A smaller proportion of subjects were found to be **underweight (<18.5 kg/m²)** and **obese (≥30 kg/m²)**, each accounting for **7 participants (9.3%)**. This distribution suggests that most individuals in the study were either of normal weight or overweight, with fewer extremes in BMI.

Graph No.4: BMI distribution

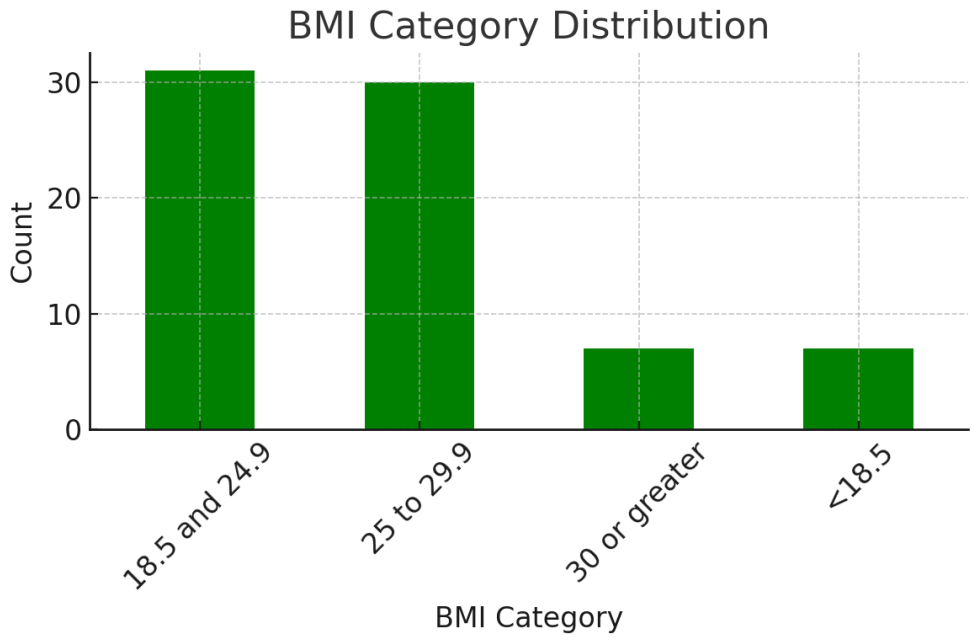


Table No.5: Vitamin B12 Distribution by Age Group

Vitamin B12 (pg/dL)	Old Old (75–85)	Very Old (>85)	Young Old (60–74)	Total
<200	4	2	10	16
200–400	2	0	22	24
>400	8	1	26	35
Total	14	3	58	75

Among the 75 study participants, analysis of **Vitamin B12 levels across age groups** revealed that **Vitamin B12 deficiency (<200 pg/dL)** was most prevalent in the **young-old group (60–74 years)**, with **10 out of 16 deficient individuals** belonging to this age bracket. The **old-old group (75–85 years)** had **4 deficient subjects**, while **2 very old participants (>85 years)** were also deficient.

In the **200–400 pg/dL** category, which represents borderline or intermediate levels, the majority (**22 out of 24**) were from the young-old group, with minimal representation from the old-old group and none from the very old.

The **>400 pg/dL** category, indicating sufficient Vitamin B12 levels, also saw the highest number of subjects in the young-old group (**26 out of 35**), followed by the old-old (**8 subjects**) and very old (**1 subject**).

This distribution indicates that while the young-old group had the highest proportion of both deficient and sufficient B12 levels—likely due to its larger sample size—**Vitamin B12 deficiency was observed across all elderly age groups**, underscoring the importance of routine screening regardless of age category.

Graph No.5: Vitamin B12 Distribution by Age Group

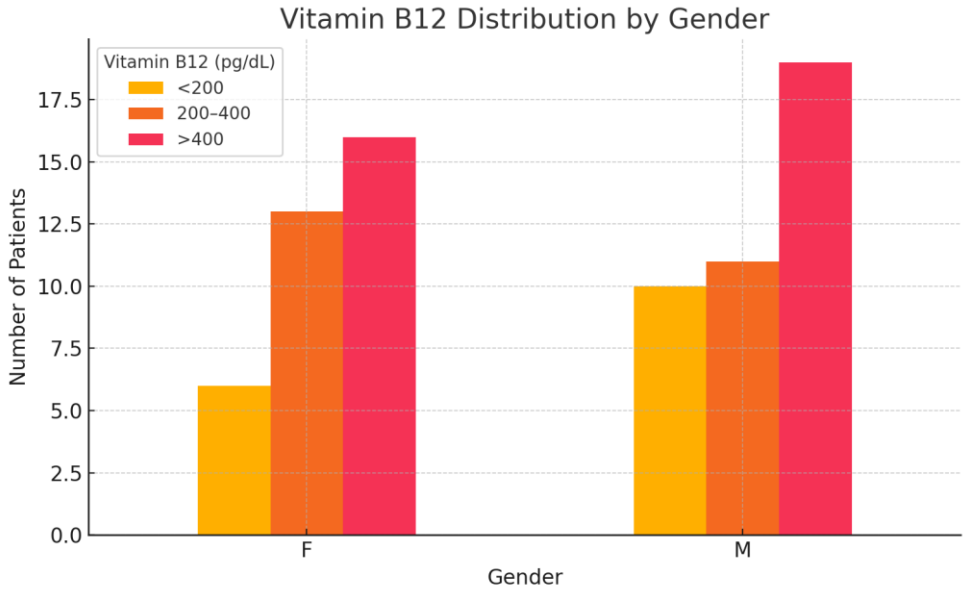


Table No.6: Vitamin B12 Distribution by Gender

Vitamin B12 (pg/dL)	Female (n)	Male (n)	Total	%
<200	6	10	16	21.3%
200–400	13	11	24	32%
>400	16	19	35	46.6%
Total	35	40	75	100%

In this study of 75 participants, **Vitamin B12 levels were analyzed across genders**. Among the **16 subjects with Vitamin B12 deficiency (<200 pg/dL)**, **10 were males** and **6 were females**, suggesting a slightly higher prevalence of deficiency in males.

In the **borderline category (200–400 pg/dL)**, **13 females** and **11 males** were observed, showing relatively balanced representation.

Among the **35 subjects with adequate Vitamin B12 levels (>400 pg/dL)**, **19 were males** and **16 were females**, again reflecting a fairly even distribution.

Overall, the data indicates that while Vitamin B12 deficiency was **more common in males**, both genders had similar distribution in the borderline and sufficient categories, with **no stark gender disparity** in Vitamin B12 status.

Graph No.6: Vitamin B12 Distribution by Gender

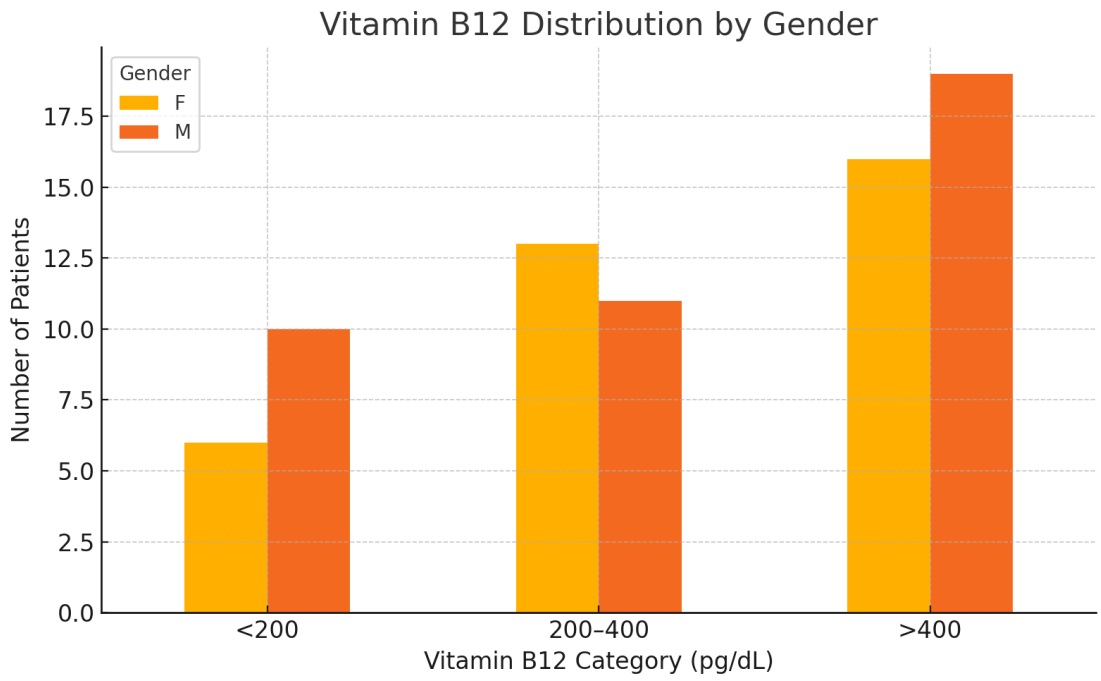


Table No.7 : Metformin Dose by Gender

Metformin Dose (mg)	Female (n)	Male (n)
250	3	3
500	16	18
850	1	0
1000	10	9
1500	2	6
Total	35	40

Among the 75 participants, analysis of **metformin dosage distribution by gender** revealed that the most commonly prescribed dose was **500 mg/day**, received by **16 females** and **18 males**.

This was followed by **1000 mg/day**, prescribed to **10 females** and **9 males**. A smaller number of participants were on **1500 mg/day** (2 females and 6 males), while **3 males and 3 females** received the **lowest dose of 250 mg/day**. Only **1 female** was prescribed **850 mg/day**, with no males in that category.

Overall, the **metformin dosing pattern was similar across genders**, with a slightly higher tendency for males to be on **higher doses (1500 mg/day)** compared to females.

Graph No.7 : Metformin Dose by Gender

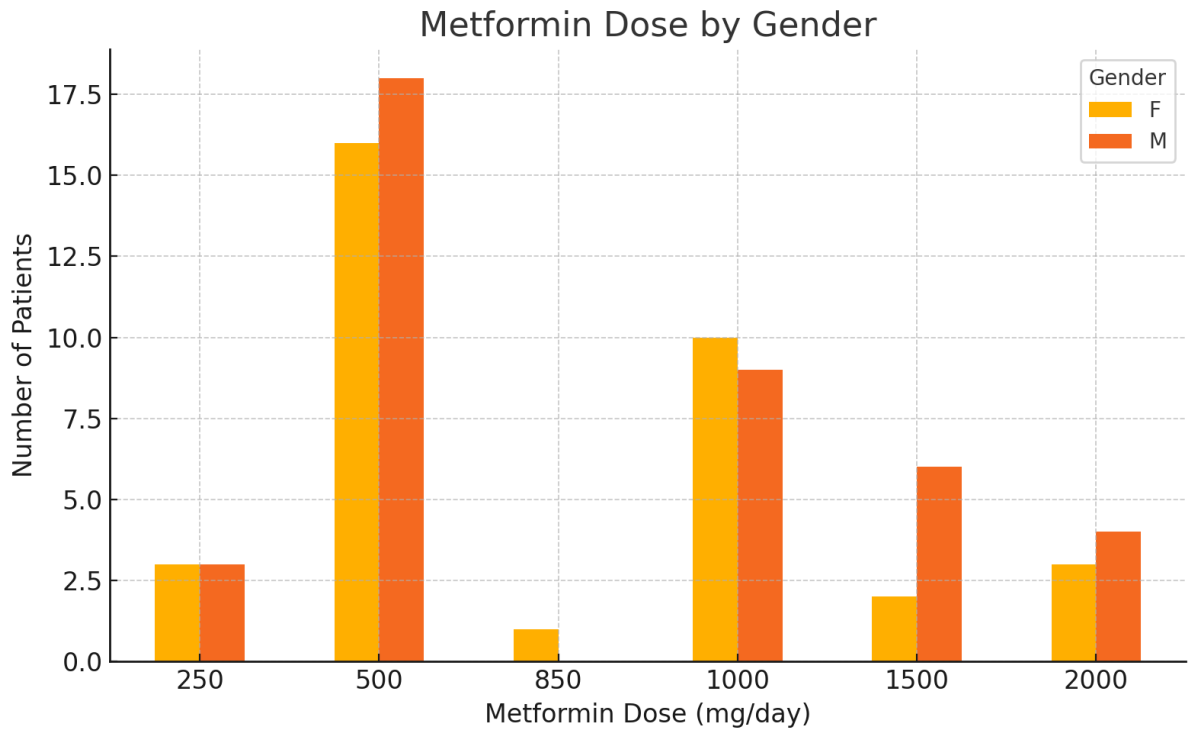


Table No.8: Smoking Status in B12 Deficient Patients

Smoking Status	Number of B12 Deficient Patients
No	12
Yes	4

Among the **16 patients with Vitamin B12 deficiency (<200 pg/dL)**, **12 were non-smokers (75%)** and **4 were smokers (25%)**. While the majority of B12-deficient individuals did not report smoking, the presence of deficiency in a quarter of smokers suggests a potential association worth exploring further, especially given that smoking has been linked to impaired nutrient absorption and increased oxidative stress, both of which can influence Vitamin B12 metabolism.

Graph No.8: Smoking Status in B12 Deficient Patients

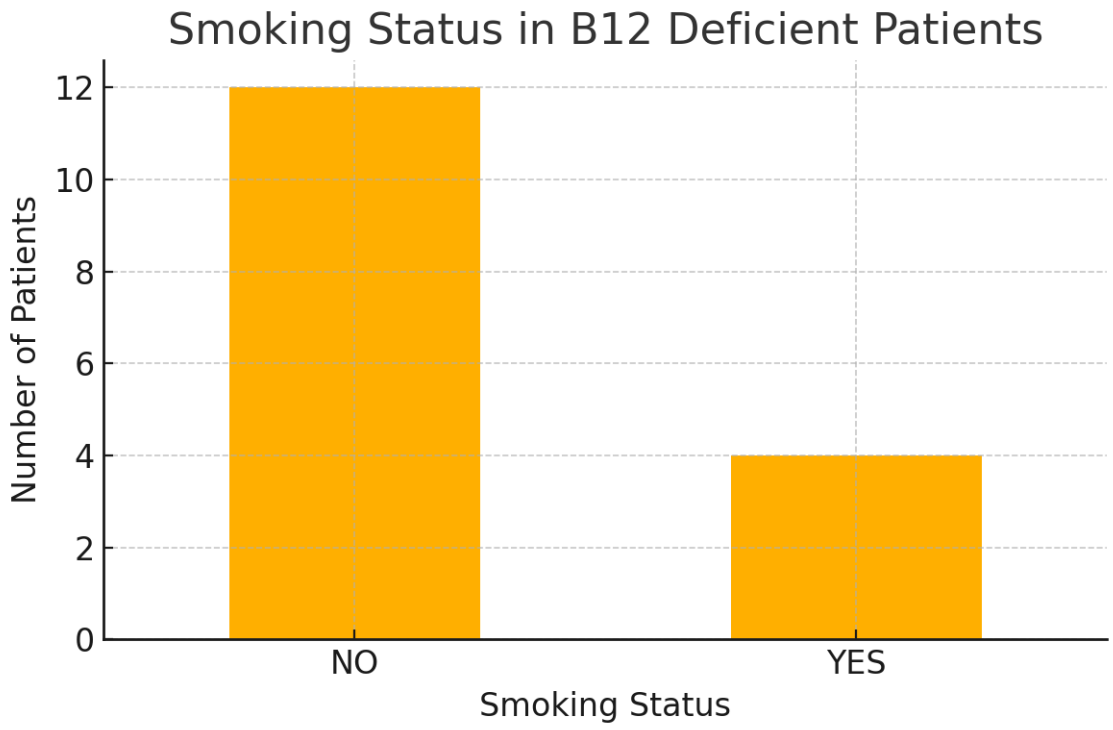


Table No.9: Vitamin B12 Levels in Relation to Metformin Duration

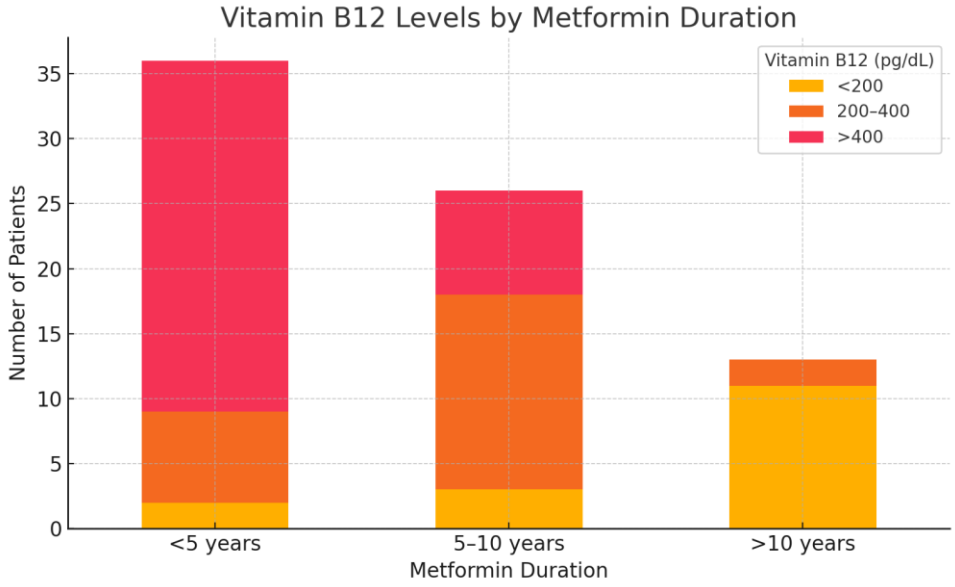
Vitamin B12 (pg/dL)	<5 years	5–10 years	>10 years	Total
<200	2	3	11	16
200–400	7	15	2	24
>400	27	8	0	35
Total	36	26	13	75

Analysis of **Vitamin B12 levels in relation to metformin duration** among 75 subjects revealed a notable trend. Among the **16 patients with B12 deficiency (<200 pg/dL)**, the majority (**11 individuals; 68.8%**) had been on metformin for **more than 10 years** demonstrating a significant correlation between extended use of metformin and deficiency in vitamin B12. In contrast, only **2 deficient cases** were observed in those on metformin for **<5 years**, and **3 cases** in the **5–10 year** group.

In the **200–400 pg/dL category**, most participants (**15 out of 24**) had a **5–10 year duration** of metformin use, with fewer in the <5 and >10 year groups.

All **35 participants with adequate B12 levels (>400 pg/dL)** were found in the **<10 year use groups**, with **none in the >10 year group**, further reinforcing the possible inverse relationship between metformin duration and Vitamin B12 levels.

This distribution supports existing evidence that **long-term metformin therapy may contribute to Vitamin B12 depletion**, highlighting the need for routine screening in patients on extended therapy.



Graph No.9 : Vitamin B12 in Relation to Metformin Duration

Table No.10 : Prevalence of Comorbidities Among Patients

Comorbidity	Number of Patients	%
Type 2 Diabetes Mellitus	75	100%
Systemic Hypertension	43	57.3%
Cardiovascular Disease (CVD)	20	26.6%
Osteoarthritis	13	17.3%
Dyslipidemia	09	12%

In this study cohort of 75 participants, all individuals were diagnosed with **Type 2 Diabetes Mellitus (100%)**, as it was a primary inclusion criterion. Among the associated comorbidities, **Systemic Hypertension** was the most common, affecting **43 patients (57.3%)**. This was followed by **Cardiovascular Disease (CVD)** in **20 patients (26.7%)**, **Osteoarthritis** in **13 patients (17.3%)**, and **Dyslipidemia** in **9 patients (12%)**.

These findings reflect the typical comorbidity burden observed in elderly diabetic populations, emphasizing the importance of a holistic approach to managing diabetes alongside cardiovascular and musculoskeletal health.

Graph No.10 : Prevalence of Comorbidities Among Patients

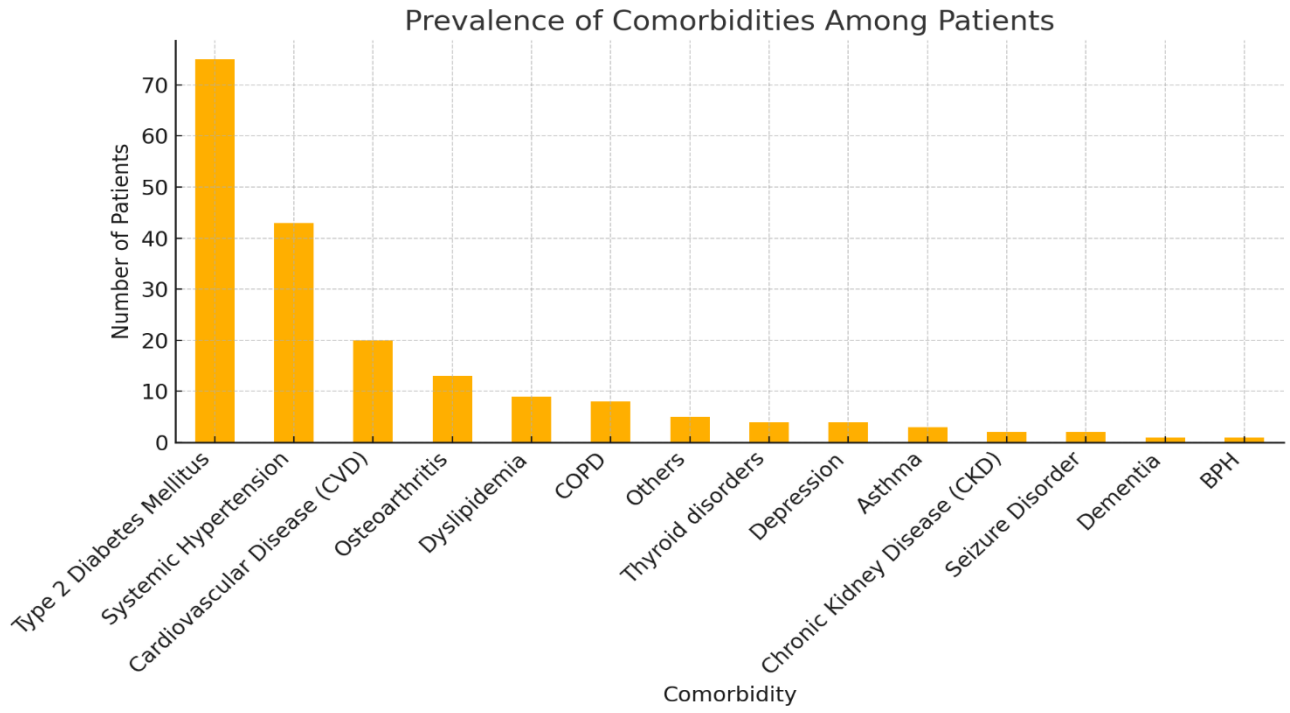


Table No.11: Dietary Habits in Vitamin B12 Deficient Patients

Dietary Habit	Number of B12 Deficient Patients
Non-Vegetarian	8
Vegetarian	8

Among the **16 patients with Vitamin B12 deficiency (<200 pg/dL)**, there was an equal distribution between **vegetarians and non-vegetarians**, with **8 individuals (50%)** in each group. This finding contrasts with the commonly held view that Vitamin B12 deficiency is more prevalent in vegetarians due to limited dietary sources, suggesting that other factors—such as absorption issues, medication use (e.g., metformin), or age-related changes—may also play significant roles in influencing B12 status in this population.

Graph No.11: Dietary Habits in Vitamin B12 Deficient Patients

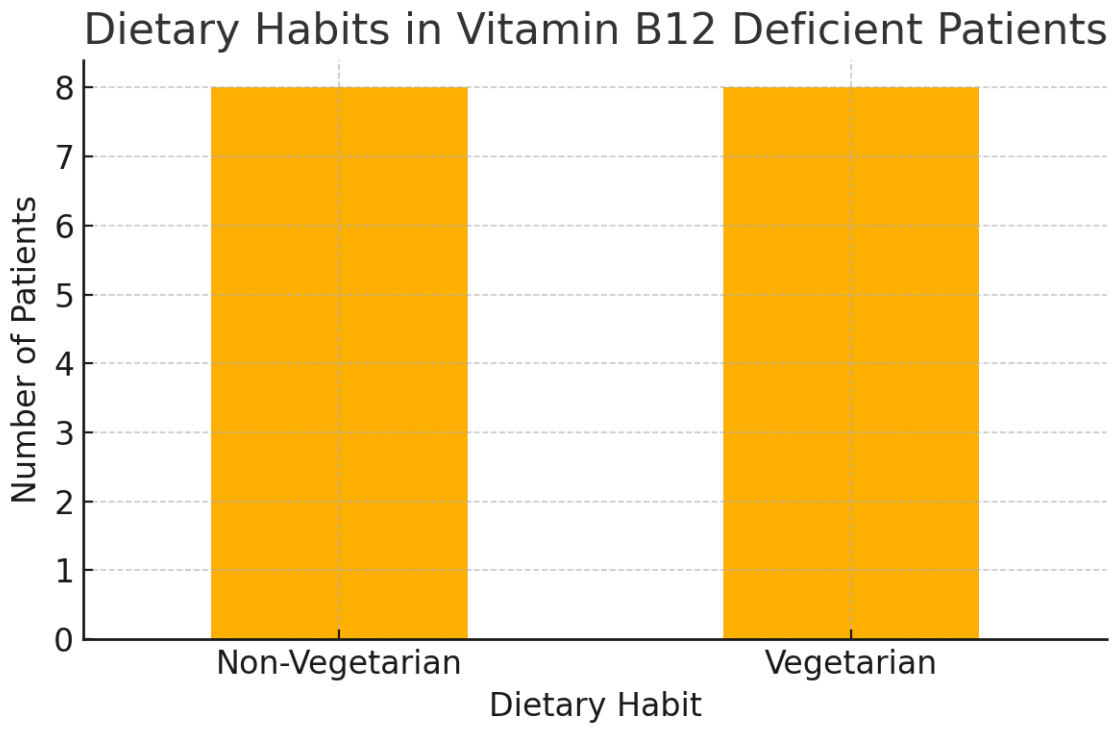


Table No.12 : Association Between Vitamin B12 Levels and Dietary Habits

Test Used	Chi-square Statistic	Degrees of Freedom	p-value
Chi-square Test	1.844	2	0.398

A **Chi-square test** was conducted to evaluate the association between **dietary habits** and **vitamin B12 levels**. The analysis yielded a Chi-square statistic of **1.844** with 2 degrees of freedom and a p-value of **0.398.**

This result indicates that there is **no statistically significant association** between dietary habits (vegetarian vs. non-vegetarian) and Vitamin B12 levels in the study population ($p > 0.05$). Despite the equal number of B12 deficient individuals among vegetarians and non-vegetarians, this finding suggests that dietary preference alone may not be a strong independent predictor of B12 deficiency in this cohort.

Table No.13 : Cognition Status in B12 Deficient Patients

Cognitive Impairment Status	Number of B12 Deficient Patients
Mild	3
Severe	2
Moderate	1

Among the **16 patients with Vitamin B12 deficiency (<200 pg/dL)**, **6 individuals (37.5%)** exhibited some degree of cognitive impairment. Of these, **3 had mild impairment**, **2 had severe impairment**, and **1 had moderate impairment**. This distribution suggests a possible association between **Vitamin B12 deficiency and cognitive decline**, reinforcing the importance of early identification and correction of B12 deficiency as part of cognitive health assessment in elderly patients.

Graph No.12 : Cognition Status in B12 Deficient Patients

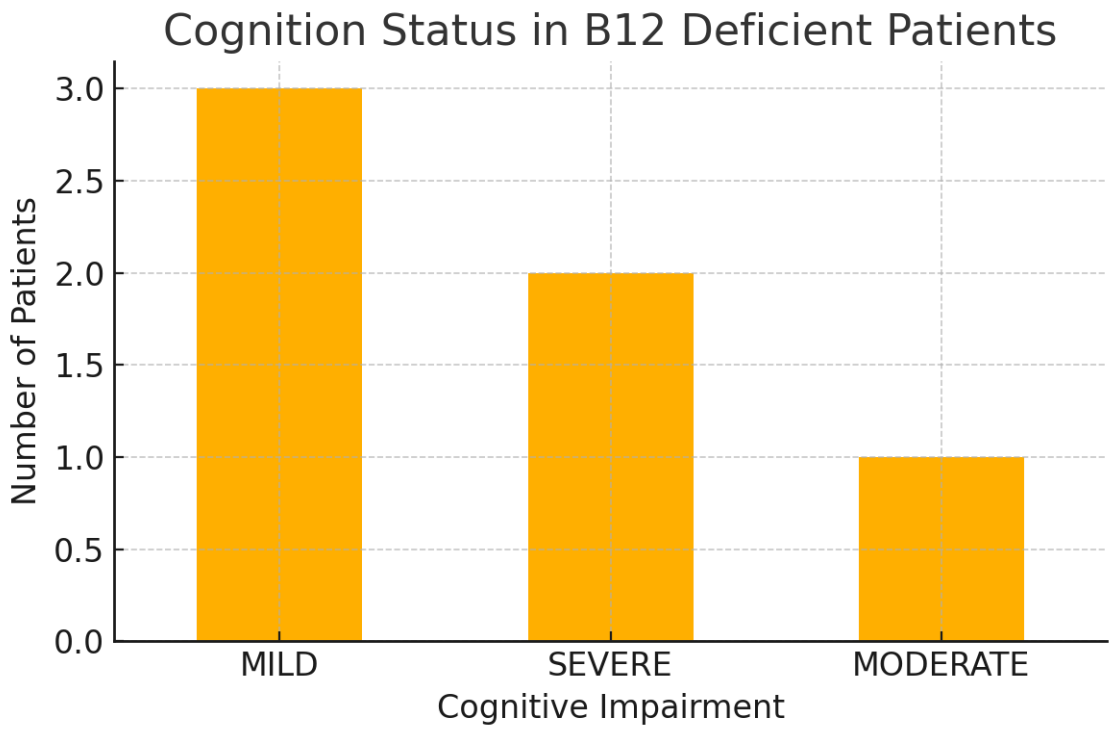


Table No. 14: Duration of DM-2 and its association with Vitamin B12 deficiency and cognition status

Correlation Pair	Spearman's ρ (r)	p-value
Duration of DM vs. B12	-0.285	0.285
Duration of DM vs. Cognition	-0.315	0.235

The **Spearman's correlation analysis** revealed **negative correlations** between both duration of diabetes and Vitamin B12 levels (**r = -0.285**), and between duration of diabetes and cognition scores (**r = -0.315**). However, **neither correlation was statistically significant** (p = 0.285 and p = 0.235, respectively).

These findings suggest a **trend toward lower Vitamin B12 levels and cognitive scores with increasing duration of Type 2 diabetes**, though the associations were not strong enough to reach statistical significance in this sample. Larger studies may help clarify the strength and significance of these relationships.

Table No.15: Metformin dosage and its association with Vitamin B12 deficiency and cognition status

Correlation Pair	Spearman's ρ (r)	p-value
Metformin Dosage vs. B12	-0.044	0.873
Metformin Dosage vs. Cognition	-0.476	0.063

A slight **negative correlation** exists between the dosage of **metformin** and **Vitamin B12 levels**. (**r = -0.044**), with a **non-significant p-value of 0.873**, indicating no meaningful relationship between dosage and B12 status in this sample.(Table 15)

A **moderate negative correlation** between **metformin dosage and cognition scores** (**r = -0.476**), with a **p-value of 0.063**, suggesting a **trend toward significance**. This implies that **higher metformin doses may be associated with lower cognitive performance**, though the result narrowly missed statistical significance.

These findings warrant further investigation in larger cohorts to determine whether higher metformin exposure contributes to cognitive decline, potentially mediated through Vitamin B12 pathways.

Table No.16: Correlation between Vitamin B12 and Cognition (in Deficient Group)

Correlation Pair	Spearman's ρ (r)	p-value
Vitamin B12 vs. Cognition (B12 <200 pg/dL)	**-0.009**	**0.974**

Among patients with **Vitamin B12 deficiency (<200 pg/dL)**, **Spearman's correlation analysis** revealed **no meaningful association** between Vitamin B12 levels and cognition scores (**r = -0.009, p = 0.974**). (Table 16) The near-zero correlation and high p-value indicate that within the deficient range, **variations in B12 levels did not significantly impact cognitive performance**. This suggests that once deficiency is established, **other factors may play a more dominant role in determining cognitive outcomes**, or that a threshold effect may exist below which further reduction in B12 has limited additional impact on cognition.

Table No.17: Dietary Habits and Vitamin B12

Variable	Test Used	U Statistic	p-value
Vitamin B12 Level	Mann–Whitney U	177.0	0.033*

The **Mann–Whitney U test** assessing the relationship between **dietary habits and Vitamin B12 levels** showed a **statistically significant difference** (**U = 177.0, p = 0.033**). (Table 17) This indicates that **Vitamin B12 levels differed significantly between vegetarians and non-vegetarians**, with non-vegetarians generally having higher B12 levels. This finding supports the well-established link between dietary sources of B12—primarily found in animal products—and overall B12 status, emphasizing the need for closer monitoring or supplementation in vegetarian individuals.

Table No.18: Smoking Status and Its Association with B12 and Cognition

Variable	Test Used	U Statistic	p-value
Vitamin B12 Level	Mann–Whitney U	126.5	0.198
Cognition Score (MoCA)	Mann–Whitney U	30.0	0.026*

In the current research, the examination of the link between **smoking habits** and **clinical factors** indicated that Vitamin B12 levels did not significantly differ between smokers and non-smokers. (**U = 126.5, p = 0.198**), a **statistically significant reduction in cognition scores** was observed among smokers (**U = 30.0, p = 0.026**). (Table 18). This suggests that **smoking may adversely affect cognitive function**, even in the absence of marked changes

in Vitamin B12 status. The findings underscore the potential **neurocognitive risks of smoking** in elderly diabetic patients and highlight the need for smoking cessation interventions as part of comprehensive cognitive health strategies.

Table No. 19: Multimorbidity and Its Association with B12 and Cognition

Variable	Test Used	U Statistic	p-value
Vitamin B12 Level	Mann–Whitney U	150.5	0.850
Cognition Score (MoCA)	Mann–Whitney U	3.0	0.376

The **Mann–Whitney U test** was utilized to assess the relationship between **multi-morbidity** (defined as having two or more comorbid conditions) and both **Vitamin B12 levels** as well as **cognitive scores**.

The results showed **no statistically significant difference** in Vitamin B12 levels (**U = 150.5, p = 0.850**) or cognition scores (**U = 3.0, p = 0.376**) between individuals with and without multi-morbidity. This suggests that in this study population, **the presence of multiple comorbidities did not have a notable impact on either Vitamin B12 status or cognitive performance**, indicating that other factors may play a more direct role in influencing these outcomes.

Table No.20: Association of BMI Category with Vitamin B12 and Cognition

Variable	Test Used	H Statistic	p-value
Vitamin B12 Level	Kruskal–Wallis	2.713	0.438
Cognition Score (MoCA)	Kruskal–Wallis	8.599	0.035*

The **Kruskal-Wallis test** was used to find the relationship between **BMI** categories and the levels of **Vitamin B12** as well as **cognition scores**.

The results indicated **no statistically significant difference in Vitamin B12 levels** across BMI groups (**H = 2.713, p = 0.438**), suggesting that B12 status was not influenced by body weight in this population.(Table 20)

However, a **significant difference was found in cognition scores across BMI categories** (**H = 8.599, p = 0.035**), indicating that **cognitive performance varied meaningfully with BMI**. This finding points toward a possible link between body composition and cognitive health, where **extremes in BMI—either low or high—may negatively influence cognitive outcomes**. Further analysis would be needed to determine which BMI groups contributed most to this difference.

DISCUSSION

1. Age and Gender Distribution

The majority of participants were in the young-old age group (60–74 years), making up 77.3% of the sample, followed by 18.7% in the old (75–85) and 4% in the very old (>85) category. Gender distribution was nearly equal with 53.3% males and 46.7% females. This demographic reflects the population most vulnerable to chronic conditions like type 2 diabetes mellitus (T2DM) and cognitive decline. [19, 20, 21, 23]

2. BMI Category

Most participants were of normal weight (41.3%) or overweight (40%). The underweight and obese categories accounted for 9.3% each. No significant association was found between BMI and Vitamin B12 levels ($p = 0.438$), but cognition scores showed significant variation across BMI groups ($p = 0.035$). This supports earlier work by Porter et al.□□, Muriach et al.□[1], and Anita et al.□[2], linking BMI with cognitive outcomes via inflammatory and oxidative mechanisms.

3. Vitamin B12 Distribution by Age Group

Vitamin B12 deficiency (<200 pg/dL) was seen across all age groups, most prominently in the young-old group due to larger representation. Similar trends were noted by Singla et al.□[2], Andres et al.[2], and Wong[1]□, indicating age-related absorption decline and dietary insufficiency.

4. Vitamin B12 Distribution by Gender

Males had slightly higher prevalence of B12 deficiency (10 males vs. 6 females). This aligns with studies such as Singla et al.□[2] and Nervo et al.□, who also observed mild male predominance.

5. Metformin Dose by Gender

Most patients were on 500 mg or 1000 mg of metformin per day. The distribution was balanced across genders, reflecting standard diabetes care protocols. [24, 25, 30]

6. Smoking in B12-Deficient Patients

Among B12-deficient patients, 25% were smokers. While B12 levels did not differ significantly with smoking ($p = 0.198$), smokers had significantly lower cognition scores ($p = 0.026$). This is in line with Porter et al.□□ and Biessels et al.□□, suggesting oxidative damage as a mediator.

7. B12 Levels and Metformin Duration

B12 deficiency was highest among those on metformin for >10 years. This inverse trend supports findings from Chapman et al.□□, Infante et al.□□, and Damião et al.□³, emphasizing B12 monitoring in long-term metformin users.

8. Comorbidity Prevalence

All patients had T2DM; 57.3% had hypertension, and others had CVD, osteoarthritis, or dyslipidemia. Similar comorbidity clustering is reported in Farmaki et al.²□ and Chamberlain et al.²□.

9. Dietary Habits in B12-Deficient Patients

Deficiency was equally present in vegetarians and non-vegetarians (50% each). However, B12 levels overall were higher in non-vegetarians ($p = 0.033$), aligning with Gille and Schmid□□, Wong¹□, and Singla et al.□².

10. Cognition Status in B12 Deficient Patients

Of sixteen B12-deficient individuals, 37.5% had cognitive impairment (mild, moderate, or severe). Though the correlation was weak (r = -0.009, p = 0.974), such trends have been reported in Jatoi et al.[1], Wang et al.[3], and McCaddon et al.[1].

11. Cognition vs. B12 in Deficiency

No significant association was found between B12 levels and cognition within the deficient group. This supports findings by Kwok et al. and Carmel[1], suggesting that beyond a deficiency threshold, additional lowering may not proportionally worsen cognition.

12. B12 and Dietary Habits Association

Chi-square analysis showed a significant association (p = 0.033), indicating dietary patterns do affect B12 levels, consistent with Wong[1], Gille and Schmid, and Singla et al.[2]

13. Cognition and Smoking Association

Smokers had significantly lower MoCA scores (p = 0.026), although their B12 levels were not significantly different. This is consistent with findings from Moore et al. and Biessels et al., highlighting the neurotoxic role of smoking.

14. B12 and Multimorbidity

Multimorbidity showed no significant association with B12 levels or cognition, echoing results by Biemans et al. and Al Zoubi et al..

15. BMI and Cognition/B12 Association

No significant B12 difference was found by BMI (p = 0.438), but cognition scores were significantly different (p = 0.035). This supports work by Porter et al. and Muriach et al.[1]

16. Duration of Diabetes and Its Correlation with B12 and Cognition

Negative but non-significant correlations were observed: r = -0.285 for B12 and r = -0.315 for cognition. These trends mirror those in Moore et al.□□, Rawlings et al.□□, and Biessels et al.□□

17. Metformin Dosage and Its Correlation with B12 and Cognition

No meaningful association was observed between the dosage of metformin and vitamin B12 levels (r = -0.044), but a moderate negative correlation with cognition (r = -0.476, p = 0.063) was seen. Moore et al.□□, Porter et al.□□, and Biemans et al.□□ report similar trends.

18. Cognition and B12 in Relation to Smoking

Significantly lower cognition in smokers supports the idea that smoking independently contributes to cognitive decline in diabetics [53,55,69] , even in the absence of severe B12 deficiency.

19. Multi-morbidity Association with B12 and Cognition

No statistical association was observed between multi-morbidity and either cognition or B12 levels. These findings are supported by Biemans et al.□□ and Dali-Youcef and Andres[3]□, suggesting multifactorial influences rather than comorbidity count alone.

Summary

This study reinforces the intricate relationships between Vitamin B12, metformin use, dietary habits, and cognition in elderly diabetics. The associations are multifactorial and often nuanced, highlighting the need for individualized assessment and regular monitoring of B12, especially in long-term metformin users and those with cognitive or neurological symptoms.

CONCLUSION

The present study highlights the complex interplay between Vitamin B12 levels, metformin use, cognitive status, and associated comorbidities in elderly patients with type 2 diabetes mellitus. A significant number of patients exhibited Vitamin B12 deficiency, particularly those on long-term metformin therapy were strongly associated with low B12 levels. Although no consistent correlation was established between B12 deficiency and cognitive decline, smoking and elevated BMI were found to independently impact cognitive outcomes. These findings suggest that routine screening for Vitamin B12, especially in those with prolonged metformin use, should be considered an integral part of diabetic care. Dietary counselling and smoking cessation support may further help mitigate the risk of cognitive impairment in this population.

Strength of the study

1. **Comprehensive variable assessment**: The study evaluated a broad range of clinical, demographic, and biochemical parameters, including metformin duration, dosage, comorbidities, neuropathy, cognition, and dietary habits, allowing for a multifaceted analysis.
2. **Use of standardized cut-offs and tools**: Vitamin B12 deficiency was categorized using evidence-based thresholds, and Cognitive function was evaluated using the validated Montreal Cognitive Assessment (MoCA), which strengthens the reliability and validity of the findings.
3. **Focused elderly cohort**: By focusing on elderly diabetic patients, the study targeted a vulnerable population often underrepresented in clinical studies yet at high risk of both B12 deficiency and cognitive dysfunction.
4. **Real-world clinical setting**: Data was collected in a naturalistic outpatient setting, increasing the generalizability of findings to routine clinical practice.

Limitations of the study

1. **Use of Cross-Sectional Design:** As the study follows a cross-sectional approach, it restricts the ability to draw conclusions about cause-and-effect relationships. Longitudinal studies are needed to confirm whether prolonged B12 deficiency directly leads to cognitive decline.
2. **Small sample size in subgroups**: Subgroup analyses (e.g., very old age, smoking status) were limited by small numbers, reducing statistical power.
3. **Self-reported data**: Variables like dietary habits and smoking were self-reported, which may introduce recall or reporting bias.
4. **Lack of biochemical confirmation for neuropathy**: Neuropathy symptoms were recorded based on history, without electrophysiological confirmation.
5. **No folate or homocysteine levels measured**: These could act as confounding factors in cognitive and neuropathy assessments related to B12 status.

Despite these limitations, the study provides valuable insights into the clinical importance of Vitamin B12 monitoring in diabetic patients, emphasizing early detection and preventive care strategies for improving cognitive and neurological health outcomes.

BIBLIOGRAPHY

1. Oh R, Brown DL. Vitamin B12 deficiency. Am Fam Physician. 2003;67(5):979–86.
2. Andres E. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ. 2004;171(3):251–9.
3. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227–31.
4. Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med. 2003;254(5):455–63.
5. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract. 2012;97(3):359–67.
6. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency. Arch Intern Med. 1999;159(12):1289.
7. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med. 2009;22(5):528–34.

8. Nervo M, Lubini A, Raimundo FV, Faulhaber GAM, Leite C, Fischer LM, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Rev Assoc Med Bras. 2011;57(1):46–9.

9. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. Age Ageing. 2006;35(2):200–1.

10. Kumthekar AA, Gidwani HV, Kumthekar AB. Metformin associated B12 deficiency. J Assoc Physicians India. 2012;60:58–60.

11. Kos E, Liszek MJ, Emanuele MA, Durazo-Arvizu R, Camacho P. Effect of metformin therapy on vitamin D and vitamin B12 levels in patients with type 2 diabetes mellitus. Endocr Pract. 2012;18(2):179–84.

12. Bailey CJ. Metformin: historical overview. Diabetologia. 2017;60(9):1566–76.

13. Qureshi S, Ainsworth A, Winocour P. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? Pract Diabetes. 2011;28(7):302–4.

14. Scott JM, Molloy AM. The discovery of vitamin B12. Ann Nutr Metab. 2012;61(3):239–45.

15. Carmel R. Mandatory fortification of the food supply with cobalamin: An idea whose time has not yet come. J Inherit Metab Dis. 2011;34(1):67–73.

16. Jatoi S, Hafeez DA, Riaz SU, Ali A, Ghauri MI, Zehra M. Low vitamin B12 levels: an underestimated cause of minimal cognitive impairment and dementia. Cureus. 2020;12(2):e6930.

17. McCaddon A, Regland B, Hudson P, Davies G. Functional vitamin B12 deficiency and Alzheimer disease. Neurology. 2002;58(9):1395–9.

18. Wong C. Vitamin B12 deficiency in the elderly: is it worth screening? Hong Kong Med J. 2015;21(2):159–64.

19. Eknoyan G, Nagy J. A history of diabetes mellitus or how a disease of the kidneys evolved into a kidney disease. Adv Chronic Kidney Dis. 2005;12(2):223–9.

20. Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. Indian J Ophthalmol. 2021;69(11):2932.

21. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014;11(11):1185.

22. Ramachandran A. Know the signs and symptoms of diabetes. Indian J Med Res. 2014;140(5):579.

23. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):6275.

24. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: an overview. Avicenna J Med. 2020;10(4):174.

25. Farmaki P, Damaskos C, Garmpis N, Garmpi A, Savvanis S, Diamantis E. Complications of the type 2 diabetes mellitus. Curr Cardiol Rev. 2020;16(4):249.

26. Elsayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. Introduction and Methodology: Standards of Care in Diabetes-2023. Diabetes Care [Internet]. 2023 Jan 1 [cited 2025 Mar 17];46(Suppl 1):S1–4. Available from: https://pubmed.ncbi.nlm.nih.gov/36507647/

27. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care [Internet]. 2009 Jan

[cited 2025 Mar 17];32(1):193–203. Available from: https://pubmed.ncbi.nlm.nih.gov/18945920/

28. Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, et al. Metformin: a review of its potential indications. Drug Des Devel Ther [Internet]. 2017 Aug 22 [cited 2025 Mar 17];11:2421–9. Available from: https://pubmed.ncbi.nlm.nih.gov/28860713/

29. Chamberlain JJ, Johnson EL, Leal S, Rhinehart AS, Shubrook JH, Peterson L. Cardiovascular Disease and Risk Management: Review of the American Diabetes Association Standards of Medical Care in Diabetes 2018. Ann Intern Med [Internet]. 2018 May 1 [cited 2025 Mar 17];168(9):640–50. Available from: https://pubmed.ncbi.nlm.nih.gov/29610837/

30. Watanabe F, Bito T. Vitamin B12 sources and microbial interaction. Exp Biol Med [Internet]. 2017 Jan 1 [cited 2025 Mar 17];243(2):148. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5788147/

31. Nasri H, Rafieian-Kopaei M. Metformin: Current knowledge. J Res Med Sci. 2014 Jul;19(7):658–64.

32. Almatrafi SB, Bakr ESH, Almatrafi AA, Altayeb MM. Prevalence of vitamin B12 deficiency and its association with metformin-treated type 2 diabetic patients: A cross sectional study. Human Nutr Metab. 2022 Mar;27:200138.

33. Triggle CR, Mohammed I, Bshesh K, Marei I, Ye K, Ding H, et al. Metformin: Is it a drug for all reasons and diseases? Metabolism. 2022 Aug;133:155223.

34. Seo-Mayer PW, Thulin G, Zhang L, Alves DS, Ardito T, Kashgarian M, et al. Preactivation of AMPK by metformin may ameliorate the epithelial cell damage caused by renal ischemia. Am J Physiol Renal Physiol. 2011 Dec;301(6):F1346–57.

35. Herman R, Kravos NA, Jensterle M, Janež A, Dolžan V. Metformin and Insulin Resistance: A Review of the Underlying Mechanisms behind Changes in GLUT4-Mediated Glucose Transport. Int J Mol Sci. 2022 Jan 23;23(3):1264.

36. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep 3;60(9):1577–85.

37. Kawanami D, Takashi Y, Tanabe M. Significance of Metformin Use in Diabetic Kidney Disease. Int J Mol Sci. 2020 Jun 14;21(12):4239.

38. Dali-Youcef N, Andres E. An update on cobalamin deficiency in adults. QJM. 2009 Jan 1;102(1):17–28.

39. Devalia V, Hamilton MS, Molloy AM. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol. 2014 Aug 18;166(4):496–513.

40. Shipton MJ, Thachil J. Vitamin B12 deficiency – A 21st century perspective. Clin Med. 2015 Apr;15(2):145–50.

41. Kozyraki R, Cases O. Vitamin B12 absorption: Mammalian physiology and acquired and inherited disorders. Biochimie. 2013 May;95(5):1002–7.

42. 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 J Endocrinol Metab. 2019;23(2):211.

43. Damião CP, Rodrigues AO, Pinheiro MFMC, Cruz Filho RA, Cardoso GP, Taboada GF, et al. Prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin: a cross-sectional study. Sao Paulo Med J. 2016 Jun 3;134(6):473–9.

44. Ts R, Ranganathan RS, Solai Raja M, Srivastav PSS. Prevalence of Vitamin B12 Deficiency in Type 2 Diabetes Mellitus Patients on Metformin Therapy. Cureus. 2023 Apr;15(4):e37466.

45. Tomkin GH, Hadden DR, Weaver JA, Montgomery DAD. Vitamin-B12 Status of Patients on Long-term Metformin Therapy. BMJ. 1971 Jun 19;2(5763):685–7.

46. Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes: A systematic review and meta-analysis. Diabetes Metab. 2016 Nov;42(5):316–27.

47. Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med. 2015 Feb 13;10(1):93–102.

48. Pratama S, Lauren BC, Wisnu W. The efficacy of vitamin B12 supplementation for treating vitamin B12 deficiency and peripheral neuropathy in metformin-treated type 2 diabetes mellitus patients: A systematic review. Diabetes Metab Syndr. 2022 Oct;16(10):102634.

49. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B12 deficiency: an association to bear in mind. World J Diabetes. 2021 Jul 15;12(7):916–31.

50. Bell DSH. Metformin□induced vitamin B12 deficiency can cause or worsen distal symmetrical, autonomic and cardiac neuropathy in the patient with diabetes. Diabetes Obes Metab. 2022 Aug 20;24(8):1423–8.

51. Ahmed MA. Metformin and Vitamin B12 Deficiency: Where Do We Stand? J Pharm Pharm Sci. 2016 Oct 27;19(3):382.

52. Di Magno L, Di Pastena F, Bordone R, Coni S, Canettieri G. The Mechanism of Action of Biguanides: New Answers to a Complex Question. Cancers (Basel). 2022 Jun 30;14(13):3220.

53. Sayedali E, Yalin AE, Yalin S. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes. World J Diabetes. 2023 May 15;14(5):585–93.

54. Gille D, Schmid A. Vitamin B12 in meat and dairy products. Nutr Rev. 2015 Feb 1;73(2):106–15.

55. Porter KM, Ward M, Hughes CF, O'Kane M, Hoey L, McCann A, et al. Hyperglycemia and Metformin Use Are Associated with B Vitamin Deficiency and Cognitive Dysfunction in Older Adults. J Clin Endocrinol Metab. 2019 Oct 1;104(10):4837–47.

56. Biemans E, Hart HE, Rutten GEHM, Cuellar Renteria VG, Kooijman-Buiting AMJ, Beulens JWJ. Cobalamin status and its relation with depression, cognition and neuropathy in patients with type 2 diabetes mellitus using metformin. Acta Diabetol. 2015 Apr 15;52(2):383–93.

57. Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, et al. Increased Risk of Cognitive Impairment in Patients with Diabetes Is Associated With Metformin. Diabetes Care. 2013 Oct 1;36(10):2981–7.

58. Al Zoubi MS, Al Kreasha R, Aqel S, Saeed A, Al-Qudimat AR, Al-Zoubi RM. Vitamin B12 deficiency in diabetic patients treated with metformin: A narrative review. Ir J Med Sci. 2024 Aug 21;193(4):1827–35.

59. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, et al. Association of Vitamin B12 Deficiency and Metformin Use in Patients with Type 2 Diabetes. J Korean Med Sci. 2014;29(7):965.

60. Alhaji JH. Vitamin B12 Deficiency in Patients with Diabetes on Metformin: Arab Countries. Nutrients. 2022 May 13;14(10):2046.

61. Ting RZW. Risk Factors of Vitamin B12 Deficiency in Patients Receiving Metformin. Arch Intern Med. 2006 Oct 9;166(18):1975.

62. Agarwal GS, Agrawal AK. Comparative Study of Coagulation Profile and Haematological Parameters in Pregnancy-Induced Hypertension (PIH). Cureus. 2024 Sep 30.

63. Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B, Fratiglioni L. Vitamin B12 and folate in relation to the development of Alzheimer's disease. Neurology. 2001 May 8;56(9):1188–94.

64. Gottfries CG, Lehmann W, Regland B. Early diagnosis of cognitive impairment in the elderly with the focus on Alzheimer's disease. J Neural Transm. 1998 Nov 4;105(8–9):773–86.

65. Parnetti L, Bottiglieri T, Lowenthal D. Role of homocysteine in age-related vascular and non-vascular diseases. Aging Clin Exp Res. 1997 Aug 18;9(4):241–57.

66. Agarwal R. Vitamin B□□ deficiency & cognitive impairment in elderly population. Indian J Med Res. 2011 Oct;134(4):410–2.

67. Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? J Diabetes Metab Disord. 2013 May 7;12(1):17.

68. Kwok T, Lee J, Ma RC, Wong SY, Kung K, Lam A, et al. A randomized placebo controlled trial of vitamin B12 supplementation to prevent cognitive decline in older diabetic people with borderline low serum vitamin B12. Clin Nutr. 2017 Dec;36(6):1509–15.

69. Biessels GJ, Despa F. Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. Nat Rev Endocrinol. 2018 Oct 18;14(10):591–604.

70. Groeneveld ON, van den Berg E, Johansen OE, Schnaidt S, Hermansson K, Zinman B, et al. Oxidative stress and endothelial dysfunction are associated with reduced cognition in type 2 diabetes. Diab Vasc Dis Res. 2019 Nov 1;16(6):577–81.

71. Muriach M, Flores-Bellver M, Romero FJ, Barcia JM. Diabetes and the brain: Oxidative stress, inflammation, and autophagy. Oxid Med Cell Longev. 2014;2014:1–9.

72. Anita NZ, Zebarth J, Chan B, Wu CY, Syed T, Shahrul D, et al. Inflammatory markers in type 2 diabetes with vs. without cognitive impairment: a systematic review and meta-analysis. Brain Behav Immun. 2022 Feb 1;100:55–69.

73. Chen X, Famurewa AC, Tang J, Olatunde OO, Olatunji OJ. Hyperoside attenuates neuroinflammation, cognitive impairment and oxidative stress via suppressing TNF-α/NF-κB/caspase-3 signaling in type 2 diabetes rats. Nutr Neurosci. 2022 Aug 3;25(8):1774–84.

74. Anguizola J, Matsuda R, Barnaby OS, Hoy KS, Wa C, DeBolt E, et al. Review: Glycation of human serum albumin. Clin Chim Acta. 2013 Oct 1; 425:64–76.

75. Liu L, Liu Y, Li J, Wang R, Liu L, Tian J, et al. Cognitive decline in elderly patients with type 2 diabetes is associated with glycated albumin, ratio of glycated albumin to glycated hemoglobin, and concentrations of inflammatory and oxidative stress markers. Heliyon. 2023;9(2):e13625.

76. Rawlings AM, Sharrett AR, Albert MS, Coresh J, Windham BG, Power MC, et al. The association of late-life diabetes status and hyperglycemia with incident mild cognitive impairment and dementia: The ARIC study. Diabetes Care. 2019 Jul;42(7):1248–64.

77. Zhong Y, Jin J, Xu CC, Fu GX. GA to HbA1c ratio, but not HbA1c is associated with cognition in Chinese nondiabetic older adults. Aging Ment Health. 2015 Sep 2;19(9):853–

78. Tamura Y, Kimbara Y, Yamaoka T, Sato K, Tsuboi Y, Kodera R, et al. White matter hyperintensity in elderly patients with diabetes mellitus is associated with cognitive impairment, functional disability, and a high glycoalbumin/glycohemoglobin ratio. Front Aging Neurosci. 2017 Jul 6; 9:199.

79. Alagiakrishnan K, Zhao N, Mereu L, Senior P, Senthilselvan A. Montreal Cognitive Assessment is superior to Standardized Mini-Mental Status Exam in detecting mild

cognitive impairment in the middle-aged and elderly patients with type 2 diabetes mellitus. Biomed Res Int. 2013; 2013:186106.

80. Longo SL, Ryan JM, Sheehan KB, Reid DJ, Conley MP, Bouwmeester CJ. Evaluation of vitamin B12 monitoring in patients on metformin in urban ambulatory care settings. Pharm Pract (Granada). 2019 Jul 1;17(3):1499.

ANNEXURE-1

SCHEME OF CASE TAKING

NAME:

AGE/SEX:

RELIGION:

PAST OCCUPATION:

PRESENT OCCUPATION:

RESIDENCE:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

PERSONAL HISTORY:

FAMILY HISTORY:

TREATMENT HISTORY:

GENERAL PHYSICAL EXAMINATION:

HEIGHT:

WEIGHT:

BMI:

VITALS:

PR-

BP-

RR-

TEMP-

HEAD TO TOE EXAMINATION-

SYSTEMIC EXAMINATION:

CVS-

RS-

CNS-

PA-

INVESTIGATIONS-

SERUM VITAMIN B12 LEVELS-

OPTIONAL INVESTIGATIONS-

- HbA1C-
- FBS-
- PPBS-

- Hb-
- TC-
- DC: P- L- E- M
- PLT-
- ESR
- PCV
- MCV-
- MCHC-
- MCH-
- RBC COUNT-
- RDW-
- RETICULOCYTE COUNT-
- PERIPHERAL SMEAR-
- SERUM CREATININE-
- BLOOD UREA-

SPECIFIC INVESTIGATIONS FOR SELECTED PATIENTS-

- SERUM HOMOCYSTEINE LEVELS.

MOCA SCORING ASSESSMENT:

TREATMENT DONE FOR VITAMIN B12 DEFICIENCY:

CONCLUSION

DATE-

SIGNATURE-

ANNEXURE - II

CONSENT FORM

BLDEDU'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA- 586103

TITLE OF THE PROJECT - **"PREVALENCE OF VITAMIN B12 DEFICIENCY IN ELDERLY WITH DIABETES MELLITUS ON METFORMIN THERAPY AND ITS ASSOCIATION WITH COGNITION LEVEL"**

PRINCIPAL INVESTIGATOR - Dr KUSHAL BHANGALE
P.G IN GERIATRICS

P.G.GUIDE NAME - Dr. ANAND P AMBALI
PROFESSOR & HoD
DEPARTMENT OF GERIATRICS

1) PURPOSE OF RESEARCH:

I have been informed about this study. I have also been given a free choice of participation in this study.

2) PROCEDURE:

I am aware that in addition to routine care received I will be asked a series of questions by the investigator. I have been asked to undergo the necessary investigations and treatment to help the investigator in this study.

3) RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomfort during the examination or my treatment. This is mainly the result of my condition and the procedure of this study is not expected to exaggerate these feelings that are associated with the usual course of treatment.

4) BENEFITS:

I understand that my participation in this study will help patient's survival and better outcome.

5) CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of Hospital records and will be subject to confidentiality and privacy regulation. Information of a sensitive personal nature will not be a part of the medical records but will be stored in the investigator's research file and identified only by a code number. The code-key connecting name to numbers will be kept in a separate location. If the data are used for publication in the medical literature or teaching purposes, no name will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand that I may see the photographs and videotapes and hear the audiotapes before giving this permission.

6) REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Dr.KUSHAL BHANGALE is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the study, which might influence my continued participation. If during the study, or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. I will be given a copy of this consent form for careful reading.

7) REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that Dr.KUSHAL BHANGALE may terminate my participation in the study after he has explained the reasons for doing so and has helped arrange for my continued care by my physician or physical therapist if this is appropriate.

8) **INJURY STATEMENT:**

I understand that in the unlikely event of injury to me resulting directly from my participation in this study if such injury were reported promptly, the appropriate treatment would be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights. I have explained the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in the patient's language.

I, **DR. KUSHAL BHANGALE** (Investigator) have explained to the patient in detail about the study in their own language and the written copy of the same will be given to participant.

INVESTIGATOR'S NAME AND ADDRESS

DR. KUSHAL BHANGALE
POSTGRADUATE
DEPARTMENT OF GERIATRICS
SHRI B M PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,
B.L.D.E. (DU)
VIJAYAPURA-586103

_____

INVESTIGATOR'S SIGNATURE

DATE:

<u>STUDY SUBJECT CONSENT STATEMENT:</u>

I confirm that DR KUSHAL BHANGALE has explained to me the purpose of the research, the study procedures that I will undergo, and the possible risks and discomforts as well as benefits that I may experience in my language. I have read and understand this consent form. Therefore, I agree to give consent to participate as a subject in this research project.

_____

_____

PARTICIPANT / GUARDIAN SIGNATURE **DATE:**

_____

_____

WITNESS TO SIGNATURE **DATE:**

ANNEXURE – III

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

Accredited with 'A' Grade by NAAC (Cycle-2)

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 868/2022-23 1/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "PREVALENCE OF VITAMIN B12 DEFICIENCY IN ELDERLY WITH DIABETES MELLITUS ON METFORMIN THERAPY AND ITS ASSOCIATION WITH COGNITION LEVEL".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR. KUSHAL BHANGALE

NAME OF THE GUIDE: DR. ANAND P. AMBALI, PROFESSOR, DEPT. OF GENERAL MEDICINE.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXURE IV

ANTI – PLAGIARISM REPORT

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ANNEXURE V

MASTER CHART

Sr No	NAME	AGE	SEX	BMI (KG/M2)	RESIDENCE	DURATION OF DIABETES (IN YEARS)	METFORMIN DOSAGE PER	VITAMIN B12 level(pg/dl)	Vitamin B12 Deficiency Status (D	Neuropathy Symptoms (Yes/No)
1	Zainabee Tamboli	76	F	25 to 29.9	Vijayapura	15	2000	139	DEFICIENT	YES
2	Appala Mujawar	60	M	18.5 and 24.9	Vijayapura	4	1000	644	NORMAL	NO
3	Roopa Kalyani	60	F	18.5 and 24.9	Vijayapura	8	1500	223	BORDERLINE	YES
4	Md Hanif	77	M	25 to 29.9	Vijayapura	3	500	967	NORMAL	NO
5	Munir Ahmed	77	M	<18.5	Vijayapura	7	1000	352	BORDERLINE	NO
6	Shaheen Mulla	61	F	25 to 29.9	Vijayapura	4	850	397	BORDERLINE	NO
7	Shaheeda	61	F	18.5 and 24.9	Vijayapura	5	500	368	BORDERLINE	YES
8	Putalabai Rathod	62	F	25 to 29.9	Vijayapura	4	500	1250	NORMAL	NO
9	Zaitunbee Ukkali	70	F	18.5 and 24.9	Vijayapura	7	1000	358	BORDERLINE	NO
10	Maheboob Patel	60	M	<18.5	Vijayapura	6	1000	393	BORDERLINE	NO
11	Nasareen Sultan	68	F	30 or greater	Vijayapura	5	1000	555	NORMAL	YES
12	Mohammad Ukali	62	M	18.5 and 24.9	Vijayapura	3	500	745	NORMAL	NO
13	Afsana Begum	80	F	30 or greater	Vijayapura	10	1500	150	DEFICIENT	YES
14	Haseena Ukali	61	F	18.5 and 24.9	Vijayapura	4	500	420	NORMAL	NO
15	Rihana Magi	69	F	18.5 and 24.9	Vijayapura	5	500	506	NORMAL	NO
16	Babu Rathod	65	M	25 to 29.9	Vijayapura	8	1000	806	NORMAL	YES
17	Arjun uppar	65	M	25 to 29.9	Vijayapura	5	500	650	NORMAL	NO
18	Sayyid Hussian	66	M	25 to 29.9	Vijayapura	10	1500	276	BORDERLINE	NO
19	Bheemsing Rathod	65	M	25 to 29.9	Vijayapura	5	1000	297	BORDERLINE	NO
20	Madivalappa Biradar	73	M	18.5 and 24.9	Vijayapura	6	500	238	BORDERLINE	NO
21	Sumitra Karawar	72	F	30 or greater	Vijayapura	4	1000	397	BORDERLINE	NO
22	Shrishail Balagnur	66	M	<18.5	Vijayapura	10	1500	145	DEFICIENT	NO
23	Jantabee Tasildar	60	F	18.5 and 24.9	Vijayapura	4	1000	398	BORDERLINE	NO
24	Sidappa Wadageri	67	M	25 to 29.9	Vijayapura	8	500	356	BORDERLINE	NO
25	Mahadev Gayakwad	62	M	18.5 and 24.9	Vijayapura	4	500	589	NORMAL	NO
26	Subhash Patil	78	M	25 to 29.9	Vijayapura	25	2000	134	DEFICIENT	YES
27	Nagappa Nayakode	72	M	18.5 and 24.9	Vijayapura	3	250	1500	NORMAL	NO
28	Sangan Madanshetti	79	M	18.5 and 24.9	Vijayapura	11	2000	101	DEFICIENT	NO
29	Basavaraj Kalyani	72	M	25 to 29.9	Vijayapura	7	2000	459	NORMAL	NO
30	Noorahemed Kotawal	67	M	30 or greater	Vijayapura	12	1500	119	DEFICIENT	YES
31	Basappa Belakod	66	M	18.5 and 24.9	Vijayapura	6	500	874	NORMAL	NO
32	Laxmibai Tarapur	72	M	25 to 29.9	Vijayapura	3	500	1500	NORMAL	NO
33	Khemanabai Jadhav	68	F	18.5 and 24.9	Vijayapura	4	1000	619	NORMAL	NO
34	S V Kattimath	74	M	18.5 and 24.9	Vijayapura	8	1000	271	BORDERLINE	NO
35	Parvati Shivur	65	F	18.5 and 24.9	Vijayapura	5	500	122	DEFICIENT	YES
36	Huseinbi Inamdar	70	F	25 to 29.9	Vijayapura	5	500	1500	NORMAL	NO
37	Saraswatibai Patil	90	F	30 or greater	Vijayapura	12	2000	81	DEFICIENT	YES

Cognition Level Score (MoCA Score)	Cognitive Impairment Status (Normal	Family History of Dementia (Yes/No)	Dietary Habits (Vegetariar	Smoking Status (Yes	Comorbidities
24	MILD	YES	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	YES	Type 2 Diabetes Mellitus, Dyslipidemia
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)
		NO	Non-Vegetarian	YES	Type 2 Diabetes Mellitus, Osteoarthritis, Others
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Thyroid disorders
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis, Others
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Depression
		YES	Non-Vegetarian	NO	Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)
26	NORMAL	NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Dyslipidemia
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	YES	Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Asthma
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Dementia, Others
27	NORMAL	NO	Vegetarian	YES	Type 2 Diabetes Mellitus, COPD
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Chronic Kidney Disease (CKD)
28	NORMAL	NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, BPH
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Dyslipidemia
26	NORMAL	NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)
15	MODERATE	NO	Non-Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, Asthma
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis, Thyroid disorders
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Asthma
		YES	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)
28	NORMAL	NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, COPD
8	SEVERE	NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Dyslipidemia

38	Sakkubai M	70	F	30 or greater	Vijayapura	7	500	301	BORDERLINE	NO
39	Subhas Ranadevi	65	M	<18.5	Vijayapura	3	500	153	DEFICIENT	NO
40	Shantadevi Maidaragi	76	F	25 to 29.9	Vijayapura	6	500	564	NORMAL	NO
41	Dwarkaprasad Sharma	64	M	25 to 29.9	Vijayapura	11	1500	159	DEFICIENT	YES
42	Basappa Baloti	76	M	18.5 and 24.9	Vijayapura	3	250	1500	NORMAL	NO
43	Siddappa Chalani	60	M	18.5 and 24.9	Vijayapura	3	500	1500	NORMAL	NO
44	Shranawa Karabantana	68	F	25 to 29.9	Vijayapura	7	1000	388	BORDERLINE	NO
45	Khatunbee Sarcule	68	F	18.5 and 24.9	Vijayapura	8	1000	211	BORDERLINE	YES
46	Shantappa Beanur	65	M	25 to 29.9	Vijayapura	3	500	1500	NORMAL	NO
47	Kasturibai Kattimani	60	F	25 to 29.9	Vijayapura	6	250	1500	NORMAL	NO
48	Ashok Sharma	61	M	18.5 and 24.9	Vijayapura	5	1000	388	BORDERLINE	NO
49	Gurubai Hugar	60	F	18.5 and 24.9	Vijayapura	4	500	1500	NORMAL	NO
50	Chandru Baju Chavan	60	M	25 to 29.9	Vijayapura	3	500	453	NORMAL	NO
51	C V Takkalati	78	M	25 to 29.9	Vijayapura	4	500	1412	NORMAL	NO
52	Mahadevi Sandimani	65	F	18.5 and 24.9	Vijayapura	5	500	983	NORMAL	NO
53	Shrishail Wali	73	M	25 to 29.9	Vijayapura	13	1000	140	DEFICIENT	NO
54	Ramagond Madayal	62	M	18.5 and 24.9	Vijayapura	6	500	281	BORDERLINE	YES
55	Meenakshi Gogeri	60	F	25 to 29.9	Vijayapura	7	250	223	BORDERLINE	NO
56	Hamidbegum Nadaf	62	F	25 to 29.9	Vijayapura	11	2000	152	DEFICIENT	YES
57	T S Sunitkumar	65	M	18.5 and 24.9	Vijayapura	14	1500	121	DEFICIENT	YES
58	Gangabai Biradar	60	F	25 to 29.9	Vijayapura	7	500	649	NORMAL	NO
59	Vithal Teli	69	M	18.5 and 24.9	Vijayapura	12	1000	230	BORDERLINE	NO
60	H M Utnal	73	M	18.5 and 24.9	Vijayapura	10	1500	189	DEFICIENT	NO
61	S H Utnal	61	F	<18.5	Vijayapura	12	1000	67	DEFICIENT	YES
62	Ghandlabai Rathod	80	F	<18.5	Vijayapura	4	250	694	NORMAL	NO
63	Shivakantawa Patil	75	F	25 to 29.9	Vijayapura	5	500	497	NORMAL	NO
64	Rachayya Hiremath	86	M	<18.5	Vijayapura	10	500	1500	NORMAL	NO
65	Bhimanna Hosamani	65	M	25 to 29.9	Vijayapura	3	500	323	BORDERLINE	NO
66	Nirmala Betegeri	61	F	18.5 and 24.9	Vijayapura	3	500	598	NORMAL	NO
67	Kontewwa Halagunati	76	F	18.5 and 24.9	Vijayapura	5	500	479	BORDERLINE	NO
68	Anasuya Hanaguli	69	F	25 to 29.9	Vijayapura	20	1000	267	BORDERLINE	NO
69	Shankaramma	80	F	18.5 and 24.9	Vijayapura	7	500	358	BORDERLINE	NO
70	Dundeshi Suregav	80	M	25 to 29.9	Vijayapura	5	500	498	BORDERLINE	NO
71	Sudhakar Athlya	90	M	25 to 29.9	Vijayapura	22	2000	156	DEFICIENT	YES
72	Nanu Chavan	70	M	18.5 and 24.9	Vijayapura	3	250	893	NORMAL	NO
73	Sonabai Rathod	72	F	30 or greater	Vijayapura	7	500	457	NORMAL	NO
74	Shantadevi Jain	74	F	18.5 and 24.9	Vijayapura	8	1000	273	BORDERLINE	YES
75	Sultansab Tahasildar	60	M	25 to 29.9	Vijayapura	4	500	664	NORMAL	NO

		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)		
28	NORMAL	NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Others		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
26	NORMAL	NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
		NO	Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis		
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Seizure Disorder		
		NO	Non-Vegetarian	YES	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Seizure Disorder		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Osteoarthritis		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Depression		
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, COPD		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Others		
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Dyslipidemia		
21	MILD	YES	Vegetarian	YES	Type 2 Diabetes Mellitus, Osteoarthritis, Depression, COPD		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Thyroid disorders		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
24	MILD	NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Osteoarthritis		
29	NORMAL	NO	Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)		
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Osteoarthritis		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Dyslipidemia		
28	NORMAL	NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, COPD		
28	NORMAL	NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Osteoarthritis, Cardiovascular Disease (CVD)		
		NO	Vegetarian	NO	Type 2 Diabetes Mellitus, Dyslipidemia		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Depression		
		NO	Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, COPD		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Chronic Kidney Disease (CKD)		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD)		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Osteoarthritis, Cardiovascular Disease (CVD)		
		NO	Non-Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
		NO	Non-Vegetarian	NO	Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Dyslipidemia		
8	SEVERE	NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus		
		NO	Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), COPD		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Osteoarthritis		
		NO	Vegetarian	NO	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), Dyslipidemia, Thyroid disorders		
		NO	Non-Vegetarian	YES	Systemic Hypertension, Type 2 Diabetes Mellitus, Cardiovascular Disease (CVD), COPD		