

Serum Vitamin B12 Levels and Lipid Profile in Patients with Type 2 Diabetes Mellitus

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Abstract: *Diabetes Mellitus stands as a significant global health concern and is recognized as a leading cause of mortality worldwide. Its associated complications, including peripheral neuropathy, nephropathy, retinopathy, coronary artery disease, and cerebrovascular disease, contribute significantly to the morbidity and mortality rates associated with the condition. Vitamin B12, a water-soluble vitamin, plays a crucial role in the physiological processes related to the methylation of the myelin sheath. Deficiency in Vitamin B12 can disrupt this methylation process, leading to the accumulation of intracellular and serum homocysteine. Elevated homocysteine levels are recognized as potentially toxic to both neurones and vascular endothelium. The present study aims to assess and compare the levels of serum Vitamin B12 in individuals diagnosed with type 2 diabetes mellitus against a control group. This comparative analysis seeks to shed light on potential variations in Vitamin B12 levels in individuals with diabetes, offering insights into its role in the context of diabetes-related complications. Furthermore, the study seeks to establish correlations between serum Vitamin B12 levels and the lipid profile of individuals with type 2 diabetes mellitus. Understanding the interplay between Vitamin B12 and lipid metabolism may provide valuable information for the management and prevention of complications associated with diabetes. By exploring these relationships, the study aspires to contribute to the existing body of knowledge regarding the complex interconnections between Vitamin B12 status, diabetes mellitus, and associated complications. The findings may have implications for the development of targeted interventions and treatment strategies aimed at mitigating the impact of diabetes-related complications. All the parameters, FBS, HbA1c, cholesterol, triglyceride levels were found to be increased in the patients of Type 2 Diabetes Mellitus as compared to controls except Vitamin B12 and HDL. The correlation of mean serum Vitamin B12 levels with other parameters was significant. From the present study, it is concluded that there is decrease in serum vitamin B12 Level with increase HbA1c, cholesterol and triglycerides levels.*

Keywords: Diabetes Mellitus, Glycated Hemoglobin, Fasting Blood Sugar, Impaired Glucose Tolerance, Impaired Fasting Glycemia, Gestational Diabetes Mellitus, High Density Lipoprotein Post- Prandial Blood Sugar Very Low Density Lipoprotein Diphosphopyridine Nucleotide

1. Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which there is hyperglycemia due to insulin resistance or defective insulin action or both. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Diabetes is rapidly gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. Vitamin B12, a crucial co-factor in various metabolic pathways, plays a pivotal role in the methylation process, influencing the conversion of homocysteine to methionine. This process is essential for the synthesis of S-adenosyl-methionine, a compound that donates methyl groups critical for the formation of myelin, neurotransmitters, and membrane phospholipids. Consequently, Vitamin B12 deficiency disrupts this methylation process, leading to the accumulation of intracellular and serum homocysteine. Elevated homocysteine levels, termed hyperhomocysteinemia, have been associated with potentially toxic effects on both neurones and the vascular endothelium. This underscores the importance of Vitamin B12 in mitigating risks associated with diabetes-related complications. Furthermore, Vitamin B12 deficiency adversely affects the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA, a process essential for fatty acid synthesis in neuronal membranes. The impairment of this pathway, along with the synthesis of neurotransmitters like serotonin and dopamine, contributes to the increased morbidity observed in diabetics with Vitamin B12 deficiency. Early detection and treatment of Vitamin B12 deficiency are crucial, as it can lead to irreversible and clinically significant complications. The

synthesis of monoamines, critical for neurological function, is impaired in the absence of sufficient Vitamin B12. Therefore, integrating measures for the timely identification and management of Vitamin B12 deficiency is essential in preventing complications and improving the overall health outcomes of individuals with diabetes mellitus^{1,2,3}. In 2000, India topped the world with the highest number of people with diabetes mellitus (31.7 million) followed by China physiological changes. Some of these infections may result in serious consequences to both maternal and fetal health, if not treated properly. Diabetes mellitus is a disorder of carbohydrate metabolism in which there is hyperglycemia due to insulin resistance or defective insulin action or both. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Diabetes is rapidly gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India topped the world with the highest number of people with diabetes mellitus (31.7 million) followed by China process of homocysteine to methionine and the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. Vitamin B12 as a co-factor facilitates the methylation of homocysteine to methionine which is later activated into S-adenosyl-methionine that donates its methyl group to methyl acceptors such as myelin, neurotransmitters and membrane phospholipids. Metabolically significant vitamin B12 deficiency hence will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurones and the vascular endothelium. This reaction is also essential in the conversion of dietary folate (methyl-tetrahydrofolate) to its active metabolic form, tetrahydrofolate. In another essential

enzymatic pathway, vitamin B12 as a co-factor mediates the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. In the presence of vitamin B12 deficiency, this conversion pathway is diminished and an increase in the serum methylmalonic acid (MMA) ensues. This is followed by defective fatty acid synthesis of the neuronal membranes². Vitamin B12 is also essential in the synthesis of monoamines or neurotransmitters like serotonin and dopamine³.

2. Methodology

METHOD USED: ELISA Method

Name of KIT

Vitamin B12 ELISA KIT

Steps

- 1) Summary and explanation of the test
- 2) Biological principle of the procedure
- 3) Specimen collection and storage
- 4) Sample Extraction
- 5) Storage and Stability
- 6) Test procedure
- 7) Results

3. Intended Use

Vitamin B12 ELISA kit is used for the quantitative detection of vitamin B12 in human serum. For in vitro research use only (RUO)

1) Summary and Explanation of the Test

Vitamin B12, vitamin B12 or vitamin B-12, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism. Neither fungi, plants, nor animals are capable of producing vitamin B12. Only bacteria and archaea have the enzymes required for its synthesis, although many foods are a natural source of B12 because of bacterial symbiosis. The vitamin is the largest and most structurally.

2) Biological Principle of the Procedure

Vitamin B12 ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with VB16. During the reaction, VB12 in the sample or standard competes with a fixed amount of VB12 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to VB12. Excess conjugate and unbound sample or standard are washed from the plate, and biotinylated conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of the V12 in the sample is then determined by comparing the OD of the samples to the Standard curve.

3) Specimen Collection and Storage

The specimens shall be blood, serum in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, at fasting morning serum sample should be obtained.. The blood should be collected in a red top venipuncture tube(s) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

4) Sample Extraction

Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µl) of the prepared extraction agent to each test tube, shaking after each addition. Let the reaction proceed for 15 min. At end of the 15min, dispense 0.050 ml (50µl) of the neutralizing buffer, vortex. After the neutralization buffer is added and mixed, let the reaction go to completion by waiting an additional 5 min before dispensing into the microwells.

Wash Buffer (50X): Dilute the wash buffer with distilled water (dissolve content of 1 bottle (20 ml) into 980 ml water. Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8°C.

5) Storage And Stability

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C. until the expiration date printed on the label. The whole kit stability is usually 6 months from date of shipping under appropriate storage conditions. Do not freeze and thaw.

4. Test Procedure

Prepare working solutions of biotin-antibody, HRP conjugate and wash buffer. Bring all reagents and solutions to room temp. (25-28°C).

- 1) Organize the microplates' wells for each serum reference standard, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
- 2) Pipette 50 µL of Vitamin B12 standard, control or specimen into the assigned well.
- 3) Add 50 µl of the Vitamin B12 Biotin Reagent to all wells.
- 4) Mix the microplate gently for 20-30 seconds by gentle tapping against the palm. Cover and incubate for 45 minutes at room temp.
- 5) Add 50 µl of Vitamin B12 Enzyme Reagent to all wells. Add directly on top the reagents dispensed in the wells. Mix the microplate gently for 20-30 seconds by tapping against the palm of your hand.
- 6) Cover and incubate for 30 minutes at room temp.

- 7) Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- 8) Add 350 μ l of 1X wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
- 9) Add 100 μ l of TMB substrate reagent to all wells. Note: Always add reagents in the same order to minimize reaction time difference between wells. Do not shake the plate after substrate addition.
- 10) Incubate at room temperature for twenty (20) minutes.
- 11) Add 50 μ l of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
- 12) Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within fifteen (15) minutes of adding the stop solution

5. Results

The age distributions in the two groups were 52.15 ± 8.66 and 54.14 ± 7.14 respectively in controls and cases which were statistically matched. Among the cases 40% were female and 60% were male and in the control group 30% were female and 70% were male (Table 1). The means of vitamin B12 in controls and cases were 597.47 and 404.36 respectively and it was significantly low in cases ($p < 0.0001$) (It was found that, among the cases, prevalence of vitamin B12 deficiency was 10%, borderline deficiency was 17.5%. Vitamin B12 deficiency was significantly associated with increasing age

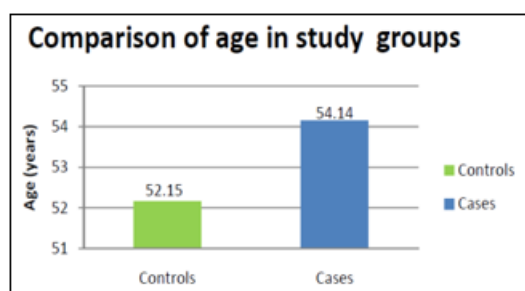


Figure 1: Showing comparison of mean age in controls and cases

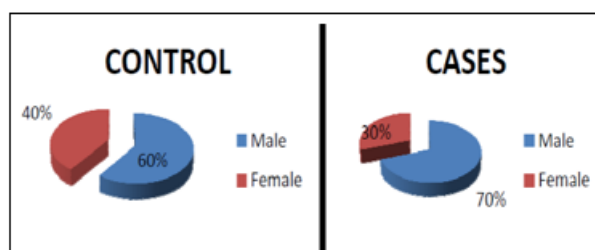


Figure 2: sex distribution in the controls and cases

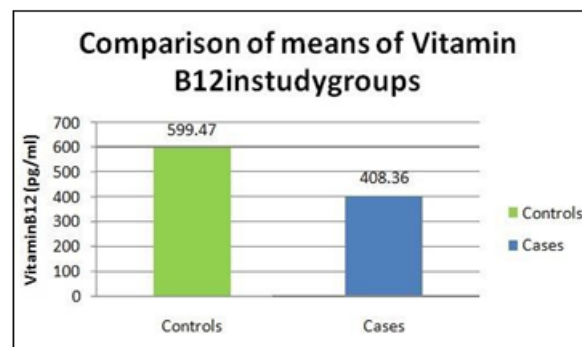


Figure 3: Comparison of mean vitamin B12 in controls and cases

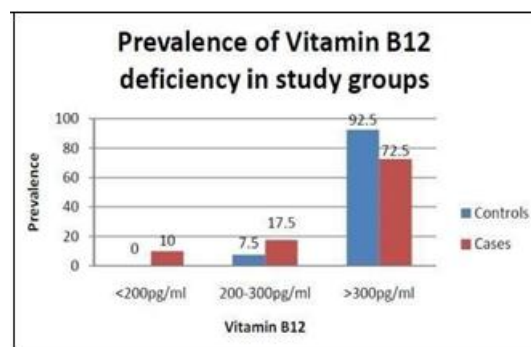


Figure 4: Prevalence of vitamin B12 deficiency in controls and cases

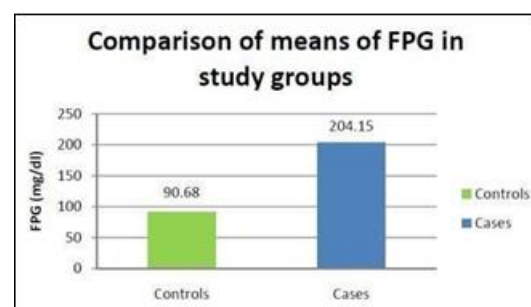


Figure 5: Showing mean FPG in the controls and cases

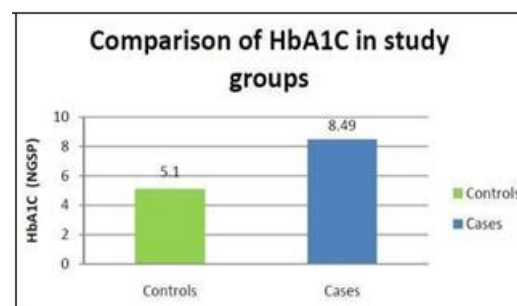


Figure 6: comparison of mean HbA1c in controls and cases

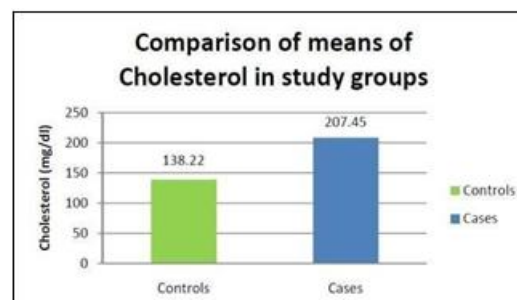


Figure 7: Comparison of mean cholesterol in controls and cases

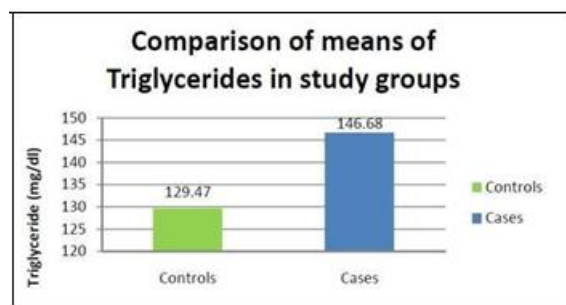


Figure 8: comparison of mean triglyceride in controls and cases

6. Conclusion

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. There is either insulin resistance or defective insulin action or both. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Prevention of Diabetes and its associated burden, primarily Neuropathy and cardiovascular morbidity and mortality has become a major health issue worldwide. Vitamin B12 is a water soluble vitamin that exerts its physiological effects through mediating two principal enzymatic pathways i.e. as cofactor it facilitates the methylation of homocysteine to methionine which is later activated into S-adenosyl-methionine that donates its methyl group to methyl acceptors such as myelin, neurotransmitters and membrane phospholipids and also converts methylmalonyl coenzyme A (CoA) to succinyl-CoA. Hence vitamin B12 deficiency will lead to accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurones and the vascular endothelium resulting in neuropathy and cardiovascular diseases. A considerable number of patients with Type 2 diabetes Mellitus have subnormal B12 levels. Periodic screening for serum vitamin B12 level may be of clinical Benefit in such patients. There is an decrease in serum Vitamin B12 Level with increase in HbA1C, cholesterol and triglyceride levels, which is potentially toxic to the neurons and the vascular endothelium. The relationship between serum vitamin B12 levels and lipid profile is an area of growing research, with some studies suggesting potential association, although findings are not entirely consistent. The relationship between serum vitamin B12 levels and diabetes mellitus (particularly type 2 diabetes, T2DM) is complex and multifaceted.

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