Original article:

Effect of glycemic control on vitamin b12 status in type 2 diabetes mellitus

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ABSTRACT

BACKGROUND AND OBJECTIVES: Diabetes mellitus is an endocrine disorder and the complications arising from the disease is the leading cause of death worldwide. Vitamin B12, a water soluble vitamin and its deficiency results in disruption of methylation process which leads to accumulation of serum and intracellular homocysteine and methylmalonic acid which gets accumulated in the myelin sheath and leads to formation of abnormal fatty acids, resulting in neurological manifestations.

METHODS: A total 100 Type 2 Diabetes mellitus patients attending in the Medicine OPD of KLE'S Dr. Prabhakar Kore Charitable Hospital. Serum Vitamin B12 was estimated and HbA1c was estimated by HPLC. The data was collected by examining the inclusion and exclusion criteria. The analysis was done by Karl Pearson's correlation coefficient were recruited for the study by using SPSS software

RESULTS: Mean serum Vitamin B12 in the study group was 218.17±118.3 and HbA1c was 7.94±1.99. Positive correlation existed between HbA1c and Vitamin B12 with p value 0.0013 and r value 0.3161 which is statistically significant (p value <0.05)

CONCLUSION: In our study an attempt was made to find the affect of Glycemic control on Vitamin B12 status. The present study concludes that poor glycemic status increases the risk of developing Vitamin B12 deficiency leading to neuropathy. Physicians should recommend measuring the serum Vitamin B12 level for screening and prevention of diabetic neuropathy.

KEYWORDS: Type 2 Diabetes mellitus, Vitamin B12, Glycated hemoglobin

INTRODUCTION

Vitamin B12 is a group of physiologically active substance known as Cobalamins or Corrinoids; composed of tetrapyrrole rings surrounding the central cobalt atom and nucleotide side chains attached to the cobalt atom. The cobalamin tetrapyrrole ring of cobalt and other side chain is called corrin, and the cobalt corrin compex is termed cobamide.[1]

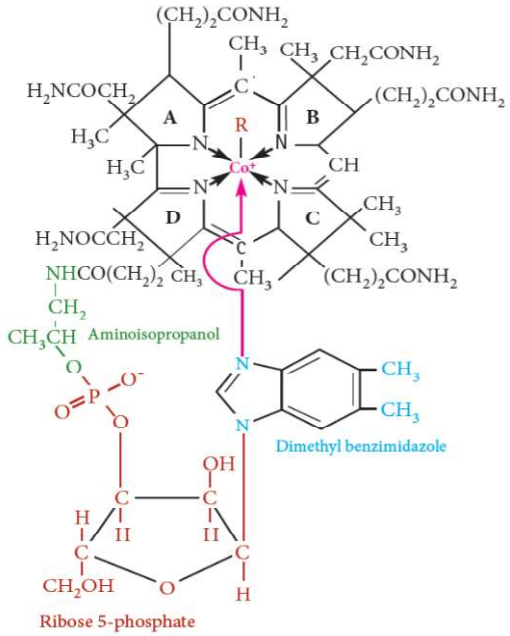

Figure 1: Structure of Vitamin B12[2]

Vitamin B12 plays a crucial role in cell reproduction, normal erythropoiesis, nucleoprotein and myelin synthesis, normal growth, DNA synthesis, and one carbon metabolism.[3,4,5] Vitamin B12 deficiency was first reported in 1849, and it was considered to have a deadly outcome until 1926, when a diet of Liver rich in Vitamin B12 slows down the disease process.[6]

Vitamin B12 exist in number of forms- Cyano, Methyl, Deoxyadenosyl, Aquo, Nitro and Hydroxycobalamin. Vitamin B12 is synthesized in gastrointestinal tract by bacteria *Lactobacillus reuteri*, in animals and found in foods of animal origin; therefore vegetarians are at increased risk for B12 deficiency.[1,6,7,8]

Liver, meat, fish, eggs, milk, curd and cheese are good sources of Vitamin B12.[2]

Table 1. Recommended Dietary Allowance in different age group[9]

ABSORPTION OF VITAMIN B12

Dietary cobalamin binds to R protein (glycoprotein) secreted by salivary gland; belongs to the family-Haptocorrins.[10,11] The R protein is hydrolyzed in the duodenum in alkaline medium and pancreatic proteases to release Vitamin B12, which later binds with Intrinsic Factor (IF) a glycoprotein with molecular weight of 50kDa.[1,3] IF (gene at chromosome 11q13), produced in stomach, resistant to proteolytic digestive enzymes. Pancreatic secretions raise the pH and vitamin B12 gets released from diet and switches from haptocorrin to IF in the duodenum. Vitamin B12 binds to IF protecting from being used by intestinal flora.[2] Vitamin B12-IF attaches to specific Cubilin receptor in ileum on the microvillus membrane of the enterocytes. Further cubilin with its ligand Vitamin B12-IF complex, enters the ileal cell where it is destroyed.[10]

Vitamin B12 enters the circulation after 2-3 hours of ingestion and appears in portal blood bound to Transcobalamin 2.[6,10]

TRANSPORT

Transcobalamin 1(TC-1) and Transcobalamin 2 (TC-2) transports Vitamin B12. TC-1 is derived from specific granules in neutrophils, also known as Haptocorrin and encoded by TCN 1 gene. Glycoprotein receptors removes TC-1 from plasma. TC-2, gene is present on chromosome 22q11q13.1 with a molecular weight of

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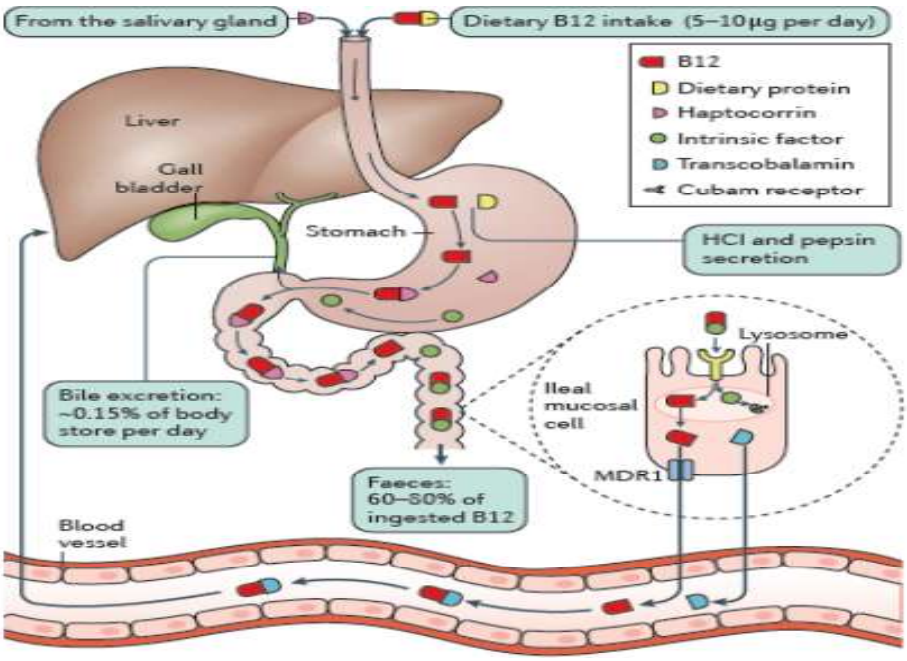
43kDa synthesized by liver, macrophage, ileum and vascular endothelium.[1,10] 80% of vitamin is transported in circulation as inactive TC-1 and 20% vitamin is transported in circulation in active form called Transcobalamin 2 (TC-2).

The TC 2 complex also referred as Holo TC 2 is actively taken by the liver, bone marrow and other vital cells.[3,6] Liver can store about 4-5mg, an amount sufficient to meet the body requirements of Vitamin B12 for 4-5 years.[12] So the clinical features of vitamin B12 deficiency become apparent by 5 years.[13]

Figure 2: Absorption and enterohepatic circulation of Vitamin B12[14]



BIOCHEMICAL ROLE OF VITAMIN B12

In the cytoplasm Vitamin B12 helps in methylation of homocysteine to methionine; is activated by S-Adenosyl Methionine, acts as methyl donor to membrane phospholipids, neurotransmitters, amines, DNA and RNA, and myelin basic proteins.[2,15] It also helps in the conversion of methyl tetrahydrofolate, to tetrahydrofolate which helps in nucleotide synthesis.[10] In Vitamin B12 deficiency, conversion of N^5 methyl THF to THF is blocked, by which body folate gets trapped as N^5 methyl THF which results in unavailability of free THF for one carbon Metabolism, synthesis of Purine, pyrimidine and nucleic acids.[2]

In the mitochondria Vitamin B12 (Adenosyl Cobalamine) helps in conversion of methylmalonyl coenzyme A(CoA) to succinyl CoA, for protein and lipid metabolism.[10]

Methylmalonyl CoA mutase
Methylmalonyl CoA ⟹ Succinyl CoA
Isomerase

Reductase
5-Methyl-tetrahydrofolate ⟹ Tetrahydrofolate

Homocysteine ⟹ Methionine
Homocysteine methyl transferase [16]

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DIABETES MELLITUS

Diabetes mellitus is a group of metabolic disorder characterized by insulin resistance initially, impaired insulin secretion, insulin deficiency, increase glucose production and decreased glucose utilization and the complications arising from this disease is the major cause of death worldwide. The cells of the body cannot metabolize carbohydrate due to relative or complete lack of insulin and body breaks its own protein, fat, glycogen resulting in hyperglycemia .[10,17,18]

The term Diabetes was given by the Greek physician Aretaeus (130-200 CE) with symptoms of constant thirst, loss of weight and excessive urination and named the condition diabetes meaning flowing through. First case of diabetes was reported by Thowas Cawley and Paul Langerhan's (1847-1888) first discovered the tissues in the pancreas called Islets of Langerhans. In 1923 Banting and John Macleod were awarded the Nobel Prize for discovery of Insulin.[19] India is the home of 69.1 million diabetic people with a greater degree of insulin resistance and a stronger genetic liability to diabetes.[20]

Diabetes is a common disorder of systemic complication including diabetic peripheral neuropathy which is seen in upto 50% of diabetic patients.[21]

Neuropathy is seen in 50% of diabetic patients. The most common complication of Type 2 diabetes mellitus is neuropathy which is the greatest source of morbidity and mortality.[19] Vitamin B12 deficiencies sometimes co-exist with diabetes that is often overlooked. Metformin used in diabetic Patients shows low Vitamin B12 levels that is associate with vitamin B12 deficiency.[22]

ADA CLASSIFICATION OF DIABETES[23]

Diabetes can be classified into the following categories:

1. Type 1 diabetes (due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency)
2. Type 2 diabetes (due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance)
3. Gestational diabetes mellitus (diabetes diagnosed in the second and third trimester of pregnancy that was not clearly overt diabetes prior to gestation)
4. Specific type of diabetes due to other causes, e.g, monogenic diabetic syndrome (such as neonatal diabetes and maturity-0nset diabetes of the young[MODY]), diseases of the exocrine pancreas (such as with glucocorticoid use, in the treatment of HIV/AIDS, or other organ transplantation).

GLYCATED HEMOGLOBIN

Glycated Hb also known as HbA1c , is used as a guideline to check the status of patients glycemic status for preceding 3 months or it represents value for glucose preceding 8 to 12 weeks and provides criteria for assessing glycemic control.[1,24]

HbA1c of 6.5% is recommended for the diagnosis of diabetes.[25]

Human Hb consists of HbA (97% of the total, it is made up of 4 polypeptide chains, 2α and 2β), HbA_2 (2.5% , made up of 2α and 2δ) and HbF (0.5%, and made up of 2α and 2γ). Several minor hemoglobin's are identified in chromatographic analysis of HbA_1, namely, HbA_{1a}, HbA_{1b}.[1,26] Aldehyde group of glucose and hexoses non-enzymatically combines and binds with amino terminal of the β-chain of Hb and forms an Schiff base (aldimine, pre-HbA1c) and undergoes an Amadori rearrangement to form a more stable ketoamine, HbA1c and

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this process is known as glycation of Hb which occurs in life span of 120 days. So this characteristic of Hb biomarker is used to monitoring the average blood glucose levels. [26,27]

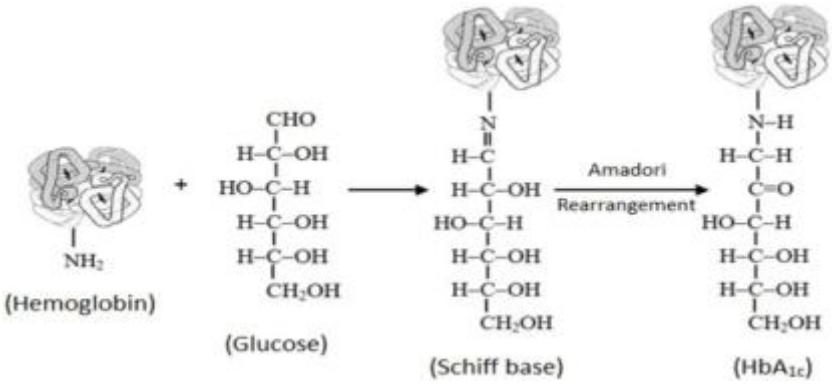

Figure 3. Formation of HbA1c[26]

So the measurement of HbA1c is used for checking blood sugar in pre-diabetic patients and monitoring sugar levels in patients with elevated HbA1c.[28]

Vitamin B12 deficiency in Type 2 diabetes mellitus is yet camouflaged in North Karnataka. Type 2 diabetes mellitus is a disease with multiple comorbidities. Various reasons lie behind Vitamin B12 deficiency in Type 2 diabetic patient's viz dietary insufficiency, adverse effect of oral hypoglycemic agents, dyslipidemia and non-alcoholic steatohepatitis deranging Vitamin B12 storage. Neuropathy is one of the major health problem from vitamin B12 deficiency overlapping with paraesthesia, nerve damage, foot ulcers and its complications. Thus evaluating Vitamin B12 levels in serum would provide early diagnosis of the deficiency status. This would provide an opportunity for the reversal of damage caused by routine screening and early diagnosis.

INCLUSION CRITERIA:

- Cases of Diabetes Mellitus aged between 30-75 years.
- Patients with symptoms of peripheral neuropathy.

EXCLUSION CRITERIA:

- Patients on Metformin therapy
- K/C/O Intrinsic factor deficiency
- Gastrectomy
- Terminal ileal resection
- K/C/O pernicious anaemia
- Pregnancy
- Chronic Giardiasis
- Alcoholism
- End stage renal and hepatic disease

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RESULT:

A total 100 subjects were included in the study. Serum Vitamin B12 was estimated by "ECLIA" on Cobas e analyzer and HbA1c (whole blood) was estimated by HPLC in Biorad D10.

Table 1. Recommended Dietary Allowance in different age group[9]

Age group	RDA(mcg)
Adults	2.0
Children	1.4
Infants	0.5
Pregnant and lactating women	2.6

The subjects were bracketed in to 5 age groups. Only 8% of the patients (n=8) were in the age group 30-39 years. The maximum number of patients 31% (n=31) were in the age group 50-59 years. Mean age of the patients was 54.47 years. The prevalence of Type 2 diabetes mellitus is maximum in the age group 50-59 years.

Table 2: Distribution of patients by gender

Gender	**No. of subjects**	**%**
Males	59	59.00
Females	41	41.00
Total	100	100.00

Table 3: Distribution of patients by age groups

Age groups	Number of patients	% of patients
30-39yrs	8	8.00
40-49yrs	26	26.00
50-59yrs	31	31.00
60-69yrs	25	25.00

Table 4: HbA1c and Vitamin B12 levels in different age group of patients

Age Group	No of Patients	HbA1c	Vitamin B12
30-39	8	6.75±0.62	233.82±128.93
40-49	26	8.07±1.39	326.73±146.35
50-59	31	8.52±1.95	222.51±165.08
60-69	25	8.66±2.10	256.80±233.23
≥70	10	9.11±1.84	184.55±81.16
Mean Vitamin B12	218.17±118.3		
Mean HbA1c	7.94±1.99		

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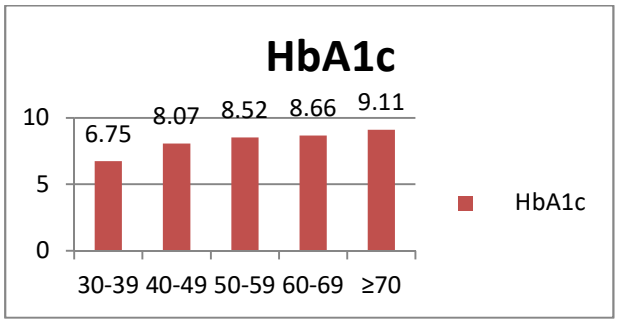
Table 5: Correlation (r) between HbA1c and Vitamin B12 levels by Karl Pearson's correlation coefficient method:

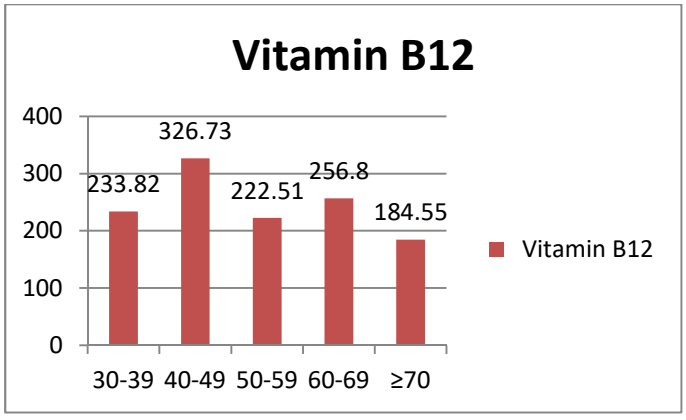
Variables	Correlation between HbA1c with			
	r-value	r^2	t-value	p-value
Vitamin B12	0.3161	0.0999	3.298	0.0013*

Graph 1: HbA1c levels in different Age group of patients



Blood HbA1c was found high in the age group of ≥70 years with a mean of 9.11. In our study results depicts that only 8% of patients in the age group 30-39 years had good glycemic control[33]. Remaining 92% of the patients had poor glycemic control with HbA1c ranging between 8.07% to 10.95%. As the duration of disease progresses HbA1c levels increased and serum Vitamin B12 levels decreased.(Table 7 and Graph 1)

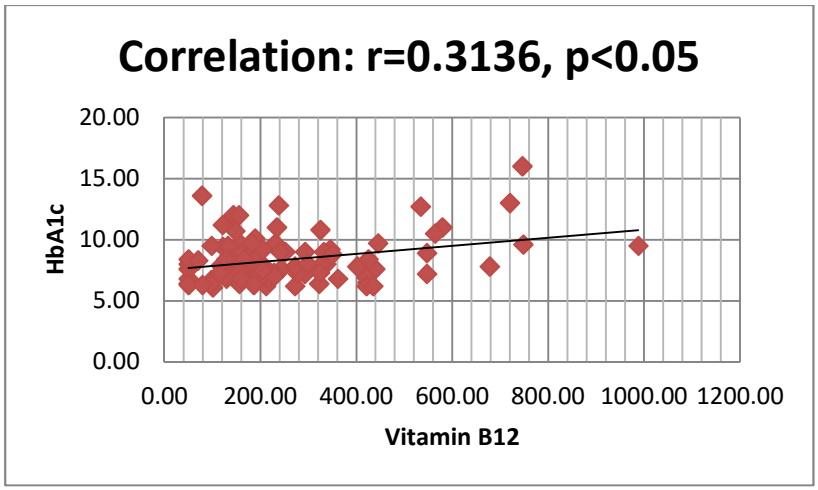
Graph 2: Vitamin B12 levels in Different age Groups of Patients



Serum Vitamin B12 levels were found lowest in the age group of ≥70 years with a mean of 184.55 pg/ml. As the duration of disease progresses Vitamin B12 levels were found to decline. (Table 7 and Graph 2)

Present study was statistically significant with a p value less than 0.05 concluding that a significant correlation exists between the parameters studied.(Table 8 and Graph 3)

Graph 3: Scatter diagram of correlation between HbA1c (%) and Vitamin B12 (pg/ml)



The above scatter diagram shows the association between HbA1c and Vitamin B12. Coefficient of correlation is calculated for the given data (r=0.3136) which means a positive linear relationship exists.

DISCUSSION

Type 2 diabetes mellitus is a multifactorial disease linked to energy metabolism mainly carbohydrates and fats and has macrovascular and microvascular complications.[14,24]

Glycated hemoglobin is used for assessing the glycemic status of diabetic patients of previous 3 months. Chronic hyperglycemia is linked with long term damage and failure of organs mainly eyes, kidney, nerves, heart and blood vessel.

The correlation between Vitamin B12 and HbA1c is explained by role of Vitamin B12 in the carbohydrate metabolism.[15,29] Type 2 Diabetes Mellitus is a disease associated with oxidative stress.[14] Vitamin B12 is a potent antioxidant it stimulates methionine synthase activity through glutathione sparing effect and modifying signaling molecules to decrease the oxidative stress.[30] RBC of Vitamin B12 deficient patients contains dwindled glutathione or enzymes, which were essential for degradation of glucose to ribose and further leads to elevation of coenzyme A in the liver.[15] The increased fatty acid metabolism leads to increased intra-mitochondrial acetyl Coenzyme A; which inhibits pyruvate dehydrogenase resulting in inhibition of phosphofructokinase the key enzyme in glycolysis.[31] Changing dietary pattern and sedentary lifestyle leads to insulin resistance worldwide and accumulation of fat in liver and develops NAFLD which progress to cirrhosis and carcinoma. Type 2 diabetes mellitus is also associated with NAFLD which leads to insulin resistance and increased free fatty acid flux that develops steatosis and disrupting storage of Vitamin B12. In liver 4-5mg of Vitamin B12 is stored which fulfill the body requirement for 4-5years.[32,15] Vitamin B12 acts as a co-factor in the form of methyl cobalamin for methionine synthase, as adenosyl cobalamin for methylmalonyl CoA mutase influencing its effect in mitochondria and cytoplasm.[11] Vitamin B12 is required for the production of methionine a precursor of S-Adenosyl methionine (SAM) for methylation reactions; where SAM acts as a methyl donor that is required in myelin formation, has important immunomodulator and neurotrophic effects. In mitochondria, conversion of methylmalonyl CoA to succinyl CoA exists; if there is any disruption in the pathway it produces elevated levels of homocysteine. Methylmalonic acid causes abnormal fatty acids formation, when these fatty acids are incorporated into sphingolipids (the component of myelin sheath) nerve impulse transmission may be

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interrupted affecting the neuronal membrane resulting in neurological manifestations.[2,11,15] Myelin basic protein has methyl arginine at position 107 in humans. Methylation of this protein is responsible for its integrity and stability. A defect in methylation due to Vitamin B12 deficiency produces an unstable MBP which leads to neurological disorder.[33]

Accumulation of methylmalonyl CoA also inhibits the enzyme of fatty acid oxidation, carnitine palmitoyl transferase 1 causing lipogenesis.[34] Hyperglycemia develops advanced glycation end products (AGE). AGE contributes to pathogenesis of demyelination by macrophages. Schwann cell abnormalities include both reactive changes (accumulation of lipid droplets, glycogen and pi granules of reich) and degenerative changes (effacement of cristae, mitochondrial enlargement, degeneration of abaxonal and adaxonal cytosol and organelles) leading initially to demyelination with development of neuropathy, axonal degeneration and inducing loss of nerve fibre.[35] Thus serum vitamin B12 deficiency leads to peripheral neuropathy.

So investigation of Vitamin B12 is important to monitor in patients with Type 2 Diabetes Mellitus as the declining Vitamin B12 levels hampers the maintenance of enzyme system essential for utilization of carbohydrates and fats.[36]

In the present study, the age distribution of patients was between 30 and 75 years with the mean age of 54.47±10.54 and the findings of the present study was in support with the study done by Shamin Ahmed et al. where they found mean age of the study group as 54.14±7.14years.[15]

In our study most of the subjects were in age group of 50-59 years and the findings of the present study was in corroboration with studies done by Anand et al and Shamin et al where the age group was 45-59 years and 51-60years. Showing the higher prevalence of Type 2 diabetes mellitus in this age group.

In the present study it was found that poor glycemic control depicted by higher HbA1c levels leads to Vitamin B12 deficiency. In our study it was found that diabetic patients had mean value of Vitamin B12 i.e 218±118.3 pg/ml and higher level of HbA1c 7.94±1.99 which is similar to the findings of Sujatha Rani Akuri where B12 levels in cases were 214.63 pg/ml[8] and Anand et al where HbA1c in Vitamin B12 deficient patients was 7.9%.

CONCLUSION

Chronic hyperglycemia represented by HbA1c values more than 7% affecting Vitamin B12 metabolism and storage. Vitamin B12 has a role in the form of co-enzymes (Methyl-cobalamin, Adenosyl-cobalamin) in carbohydrate and lipid metabolism. Vitamin B12 has a role in formation of myelin sheath by conversion of methylmalonyl CoA to succinyl CoA. Hyperglycemia forms of AGE products on nerve myelin contributing to demyelination with development of neuropathy and accumulation of methylmalonyl CoA leads to incorporation of faulty fatty acids into spingolipids affecting nerve impulse transmission. Hyperglycemia in T2DM caused by insulin deficiency or insulin resistance affects glucose uptake and metabolism; further production of NADPH and formation of glutathione is deranged leading to oxidative stress. Increased fatty acid metabolism leads to increased acetyl CoA, inhibiting pyruvate dehydrogenase; which in turn increases intracellular levels of pyruvate in liver, muscle and adipose tissue. Pyruvate inhibits phosphofructokinase one of the rate limiting enzyme of glycolytic pathway. Increased flux of fatty acids due to insulin resistance causes NAFLD deranging Vitamin B12 metabolism and storage.

In our study an attempt was made to find the affect of Glycemic control on Vitamin B12 status. The present study concludes that poor glycemic control (with mean HbA1c of 7.94±1.99%) increases the risk of

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developing Vitamin B12 deficiency (with mean 218.17±118.3) leading to neuropathy. Physicians should recommend measuring the serum Vitamin B12 level for screening and prevention of diabetic neuropathy.

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