

Original article:

Status of vitamin b12 in type 2 diabetes mellitus patients taking metformin based oral hypoglycemic agent- a cross sectional study

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ABSTRACT

INTRODUCTION: Diabetes Mellitus is a group of disorders that develops a consequence of absolute or relative deficiency of the hormone insulin. Metformin is an oral agent in Biguanide class that produces its most prominent effect by decreasing gluconeogenesis and thus reduces hepatic glucose production. HbA1c reflects the average blood glucose levels for preceding 8 to 12 weeks and HbA1c of more than 6.5% is recommended for the diagnosis of diabetes. Vitamin B12 is water soluble vitamin that plays a crucial role in cell reproduction, erythropoiesis, one carbon metabolism, nucleoprotein and myelin synthesis. The objective of this study was to see the status of Vitamin B12 in T2DM patients on metformin therapy.

METHOD: Total 100 T2DM patients attending the Medicine OPD of KLE'S Dr. Prabhakar Kore Charitable Hospital. Serum Vitamin B12 was estimated by ECLIA on cobas e immunoassay analyzer and HbA1c is estimated by HPLC on Biorad D 10.

RESULT: Vitamin B12 levels in patients taking metformin was 161.99pg/ml, whereas patients without metformin therapy was 344.34pg/ml of Vitamin B12 levels. A significant positive correlation exist between metformin therapy and Vitamin B12 deficiency with a p value of <0.05. No correlation exists between HbA1c and Metformin dose, metformin duration and diabetic duration.

CONCLUSION: The present study concludes that metformin induced vitamin B12 deficiency leads to neurologic damage with symptomatology of peripheral neuropathy which may be alternatively diagnosed as diabetic neuropathy. The diabetic patient on metformin therapy should be screened 6 monthly or annually for the subclinically diagnosis of Vitamin B12 deficiency.

KEYWORDS: Diabetes Mellitus, Vitamin B12, Metformin, Glycated hemoglobin, Neuropathy

INTRODUCTION

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.^{1, 2,3}

T2DM earlier referred as non-insulin dependent diabetes or adult onset diabetes, which accounts for 90-95% of all diabetes⁴, T2DM is heterogenous, initially, insulin resistance develops. Elevated insulin secretion to maintain

normal blood glucose levels and pancreatic beta cells are unable to maintain increased demand of insulin and slowly develops insulin deficiency⁵ and have impaired insulin action.¹ Chronic complication are divided into microvascular (neuropathy, retinopathy, nephropathy) and macrovascular complication (coronary heart disease, peripheral arterial disease, cerebrovascular disease). 5% T2DM occurs due to genetic defect of pancreatic cell function (glucokinase gene on chromosome 7p) at younger age known as MODY⁶.

Metformin is widely used as first line therapy for insulin resistance. It is considered as insulin sensitizer because it helps in lowering insulin levels, reduces hepatic glucose production, improves peripheral glucose utilization and helps in uptake and utilization of gut glucose. It activates AMP-dependent protein kinase and enters cell through organic cation transporters.^{7,8} The main site of action for metformin is liver where it suppresses glucose production and promotes glucose uptake by tissues via glucose transporters and inhibit hepatic gluconeogenesis by mediating activation of liver kinase B 1(LKB1), thus promotes synthesis of gluconeogenesis enzymes. It leads to hyperglycaemia by suppressing glucose production by liver.⁹

Metformin was approved by FDA in 1994 and in 1996 it was approved in America, when it was found to reduce mortality and decreased diabetes related complication in Type 2 obese diabetes patients.^{10,11} It is estimated that routinely 120 million diabetic patients around the world are prescribed with metformin.¹²

In 1985 WHO report mentioned the utility of HbA1c in diabetes. By 2010 ADA and major expert committee and association across the globe recommended HbA1c for the diagnosis of type 2 Diabetes mellitus¹³ In 2010 ADA-Organized international expert committee and recommended adoption of HbA1c for the diagnosis of diabetes at a cut off 6.5%¹⁴ clinical significance of HbA1c in T1DM was given by Diabetes Control and Complications Trial and significance on T2DM was given by United Kingdom Prospective Diabetes Study. Measurement of HbA1C and Blood Glucose levels are used in routine management of patients with T1DM and T2DM¹⁵

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.¹⁶

HbA1c measurement is recommended for monitoring glycemic control in pre-diabetic and diabetic patients with elevated level. HbA1c of more than 6.5% is recommended for the diagnosis of diabetes.^{15,17}

Vitamin B12 is also known as cobalamin¹⁸, extrinsic factor of castle and anti-pernicious anaemia factor. It is water soluble, heat stable and red colour and contains 4.35% cobalt by weight.¹⁹

Vitamin B12 is not present in vegetarian diet except curd (3.0µg) is the good source because lactobacillus synthesizes Vitamin B12. Egg yolk, meat, cheese and liver is the richest source.^{19,20}

Vitamin B12 helps in synthesis of methionine from homocysteine and conversion of methylmalonylcoA to succinylcoA. Methionine is converted to SAM which donates its methyl group to myelin, membrane phospholipids and various neurotransmitters and free THF is liberated from N⁵ methyl THF which is used in synthesis of purine, pyrimidine and nucleic acid.^{20,21}

Vitamin B12 is mainly stored in liver (2-3mg) which is sufficient to cater the needs for 3 to 4 years, storage in small amount is observed in leukocytes and gastric mucosa. It is stored as a complex with Transcobalamin 1 and Transcobalamin 3.^{6,22}

In India vitamin B12 deficiency ranges from 16 to more than 60%. About 65 million people in India have diabetes. People receiving metformin therapy are at greater risk of developing vitamin B12 deficiency which is influenced by increasing age, metformin dose and duration of therapy.^{23,24}

Study Design- 100 Patients attending Medicine OPD of KLE's Dr.PrabhakarKore Charitable Hospital, Belagavi were selected according to the inclusion criteria and reports of HbA1c and vitamin B12 were collected and details of metformin based therapy was enquired. Serum Vitamin B12 was estimated by Cobae e immunoanalyzer and HbA1c is estimated by Biorad D 10.

CASE SELECTION:

After obtaining Ethical Clearance from Ethics Committee for research on human subjects of KAHER's Jawaharlal Nehru Medical College, Nehru Nagar, Belagavi dated 03/05/2018 with reference number MDC/DOME/103. A total 100 subjects were included in the study after following the inclusion and exclusion criteria.

Inclusion Criteria: Type 2 diabetic mellitus patients on metformin therapy between age group of 30 to 70 years, patients with symptoms of peripheral neuropathy.

Exclusion Criteria: 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.

RESULTS

Figure 1: Gender wise distribution

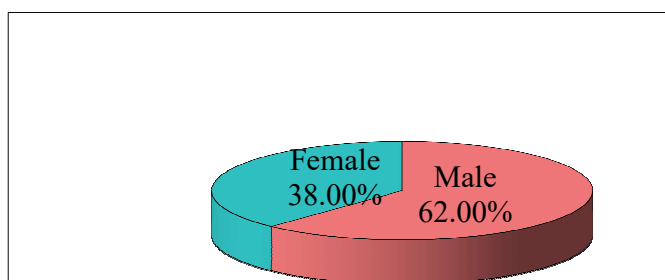


Figure 2: Age wise distribution

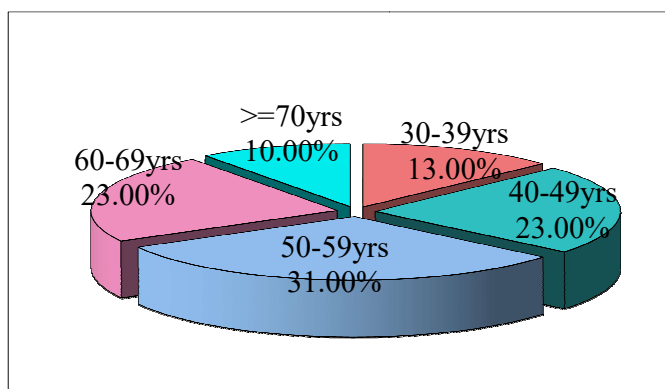


Figure 3: Duration of diabetes wise distribution.

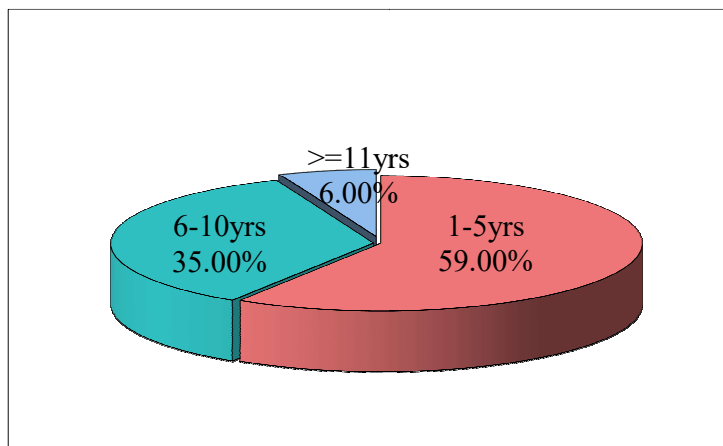
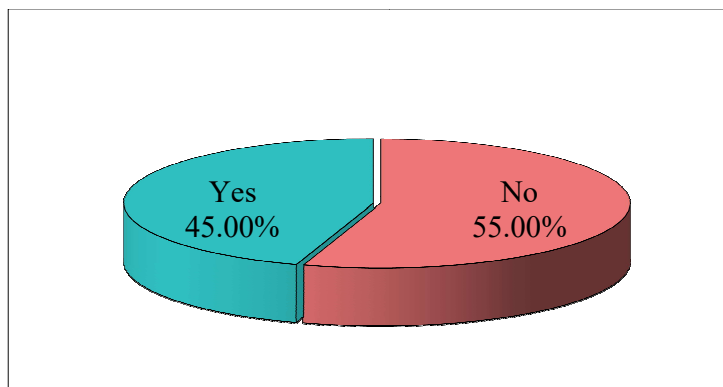


Figure 4: Metformin therapy wise distribution



A Total 100 type 2 Diabetes Mellitus subjects were included in the present study.

In the present study, 38 patients were female and 62 patients were male (Figure 1). Mean age was 53.17 ± 11.67 years with a minimum age of 30 and maximum age of 76 years (Figure 2) and the duration of diabetes was 4.86 ± 3.57 years (Figure 3). 45% of patients were on Metformin Therapy (Figure 4).

Table 1: Summary of Metformin dose (mg) and duration of Metformin therapy (years)

Summary	Metformin dose(mg)	Metformin therapy duration (years)
Min	500.00	1.00
Max	1000.00	6.00
Mean	606.82	3.23
Median	500.00	3.00
SD	201.33	1.17
SE	30.35	0.18

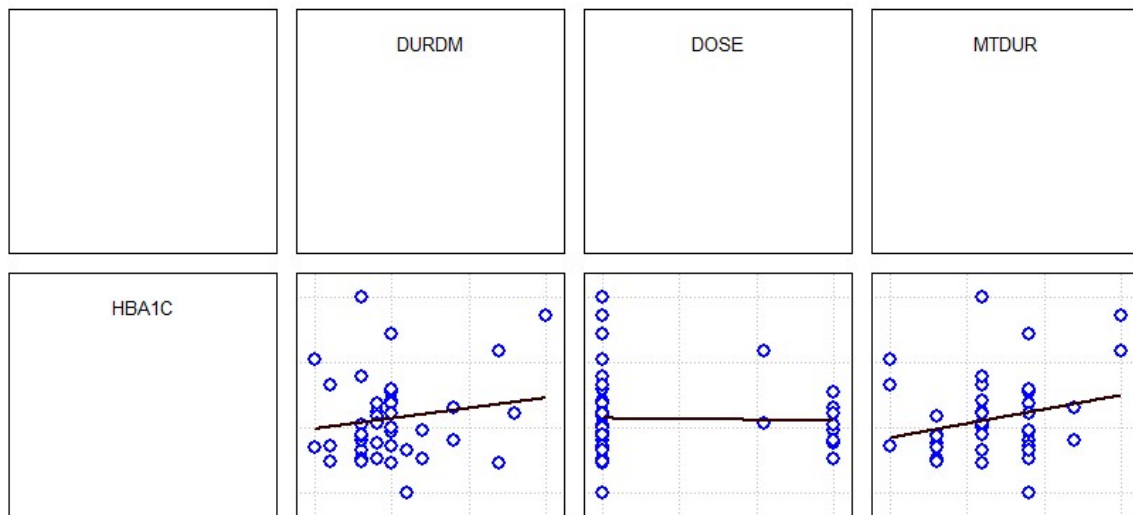
The metformin therapy ranged between 500 mg once daily to 1000mg twice daily for 1 to 6 years.

Table 2: Correlation between HbA1c with duration of diabetes, Metformin dose, Metformin duration by Karl Pearson’s correlation coefficient method

Variables	Correlation between HbA1c with		
	r-value	t-value	p-value
Duration of diabetes	0.1634	1.0605	0.2951
Metformin dose	-0.0245	-0.1571	0.8759
Metformin duration	0.2389	1.5755	0.1228

Glycated Hb is correlated with the duration of diabetes with a p value of 0.29, with metformin dose (p value of 0.87) and with metformin duration (p value of 0.12) which is not statistically significant.

Diagram 1: Scatter diagram of correlation between HbA1c with duration of diabetes, Metformin dose, Metformin duration.



The above scatter diagram shows that there is a positive correlation between duration of T2DM and HbA1c as the duration of diabetes increases HbA1c also increases showing poor glycaemic control. There exists inverse correlation between metformin Dose and HbA1c levels with a (r value of -0.024) showing that as the dose of metformin levels increases HbA1c levels decreases showing improvement in glycaemic control exists. There is a positive correlation between metformin duration and HbA1c levels with a (r value of 0.238).

Table 3: Comparison of Metformin dose (No and Yes) with HbA1c scores by t test

Metformin dose	Mean	SD	SE	t-value	P-value
No	7.75	2.12	0.29	-1.1074	0.2709
Yes	8.19	1.83	0.27		

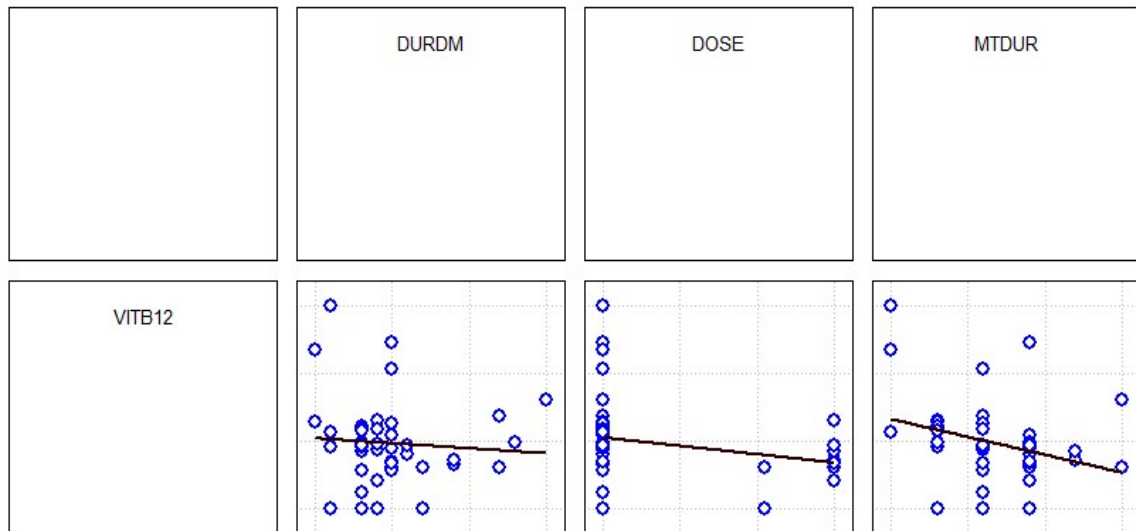
t value when correlated with duration of diabetes and HbA1c the t value is (1.0605) and correlation between metformin therapy duration has a greater t value (-1.1074) thus showing strong evidence against null hypothesis but with insignificant p value.

Table 4: Correlation between Vitamin B12 levels with duration of diabetes, Metformin dose, Metformin duration by Karl Pearson’s correlation coefficient method.

Variables	Correlation between Vitamin B12 levels with		
	r-value	t-value	p-value
Duration of diabetes	-0.0798	-0.5128	0.6108
Metformin dose	-0.2411	-1.5907	0.1194
Metformin duration	-0.2961	-1.9853	0.0538

Karl Pearson’s correlation between Vitamin B12 levels with duration of diabetes, metformin dose, metformin duration (r value is -0.0798, -0.2411 and -0.2961) depicting that chronicity of T2DM decreases Vitamin B12 levels, higher metformin dose and longer duration of metformin depicts vitamin B12 levels with a significant p value of 0.0538.

Diagram 2: Scatter diagram of correlation between Vitamin B12 levels with duration of diabetes, Metformin dose, Metformin duration



The above scatter diagram shows that there exists a negative correlation between Vitamin B12 levels with duration of diabetes, Metformin dose, Metformin therapy duration as depicts in the scatter diagram above.

Table 5: Comparison of Metformin dose with Vitamin B12 levels by t test

Metformin dose	Mean	SD	SE	t-value	P-value
No	344.34	203.05	27.38	5.7309	0.0001*
Yes	161.99	72.16	10.76		

*p<0.05

Vitamin B 12 levels depicts with higher dose of Metformin therapy shows statistically significant with a p value of <0.0001.

DISCUSSION

India is at the top of the world with highest number of people with diabetes mellitus (31.7 millions). WHO predicted that by 2030 DM may affects upto 79.4 million individuals in India.²³

Vitamin B12 plays a crucial role in carbohydrate utilization and a lower level of Vitamin B12 status causes hyperglycemia.²⁴

Metformin is used for the treatment of diabetes, it affects the calcium dependent ileal cell membrane receptors needed for uptake of B12 Intrinsic factor and it reduces serum Vitamin B12 absorption upto 30%.^{25,26} Metformin induced vitamin B12 deficiency in T2DM patient which include alteration in small bowel motility, bacterial flora, competitive inhibition and inactivation of vitamin B12 absorption, alteration in Intrinsic Factor levels, interaction with cubilin endocytic receptors or the effect of calcium on cell membrane have been suggested to the play role as biguanide group being protonated and gives positive charge to membrane which displace divalent cation such as calcium. So, metformin inhibits calcium dependent absorption of Vitamin B12 IF complex at terminal ileum which is reversed with calcium supplementation. Metformin also reduces the fasting plasma glucose and insulin levels, improves the lipid profile and promotes modest weight loss and the major toxicity of metformin is lactic acidosis is very rare and can be prevented by careful patient selection. In the present study it was found that patients taking metformin (dose,duration) having a lower vitamin B12 levels with a (p value 0.0001). Metformin dose and treatment duration were most compatible risk factor for Vitamin B12 deficiency.^{1,22, 26, 27}

Vitamin B12 deficiency is important because it causes bone marrow failure and demyelinating nerve diseases and even shows neurological symptoms such as paresthesias, impaired vibration sensation and proprioception which are potential result of neurological damage.^{10, 26}

According to Allan S Brett, Glycosylated hemoglobin levels on patients prescribed with metformin reduced mean HbA1c levels by 1.1% and comparison between low dose and high dose metformin, HbA1c level was 0.3% lower with high dose therapy. In our study the mean HbA1c levels is 8.9%. Our study shows a clear relationship between the increase dose of metformin leads to vitamin B12 deficiency.

CONCLUSION

Metformin induced vitamin B12 deficiency is caused by impaired vitamin B12 metabolism as discussed earlier. Deficiency of vitamin B12 leads to neurological damage and 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 interrupted affecting the neuronal membrane resulting in neurological manifestations.^{6,12,28} 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 Myelin Basic Protein which leads to neurological disorder²⁹ and presented as peripheral neuropathy which may be alternative diagnosed with diabetic neuropathy. Low vitamin B12 concentration is associated with impaired nerve conduction velocities and poorer response to light touch by monofilament detection²⁷

- The diabetic patient on metformin should take adequate amount of vitamin B12 to prevent the deficiency.
- Serum vitamin B12 levels should be maintained in normal range to reduce the risk of developing neuropsychiatric disorders.

- Annual screening should be done to reduce the vitamin B12 deficiency status.
- Maximizing metformin dose can improve glycemic control in persons with T2DM which supports that improved and optimized use of metformin can reduced the HbA1c levels by 1.1%

CONFLICTS OF INTEREST:

There exists no conflict of interest amongst the authors regarding the process of publication and position of authors.

REFERENCE

1. Kasper, Fauci, Hauser, Longo, Jameson, Loscalzo. Harrison's Principles of Internal Medicine. 19th edition, Diabetes Mellitus, Cobalamin.year: 2422, 2399
2. Agarwal P, Mital P, Meena VK, Mital P, Nawal CL, Goyal LK. A comparative study of levels of vitamin B12 in patients of type 2 diabetes mellitus on metformin and not on metformin at tertiary care center. International Journal of Advances in Medicine. 2016 Dec 29;3(3):759-63.
3. Badyal A, Kumar S. Role of Vitamin B12 Deficiency in Patients with Type 2 Diabetes Mellitus. Journal of Medical Science and Clinical Research 2018 Feb;2(6):235-238.
4. American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes care. 2017 Jan 1;40(Supplement 1):S11-24.
5. Brian R Walker, Nicki R Colledge, Stuart H Raiston, Ian D Penman. Davidson's Principles and Practice of Medicine. 22nd Edition. Diabetes Mellitus. 805
6. Rafi MD. Textbook of Biochemistry for Medical Students. 2nd ed. Vitamins. 2014.170.
7. Stuart H Ralston, Ian D Penman, Mark WJ Strachan, Richard P Hobson. Davidson's Principles and Practice of Medicine. 23rd Edition. Hepatology. 2018,882-883.
8. Kasper, Fauci, Hauser, Longo, Jameson, Loscalzo. Harrison's Principles of Internal Medicine. 19th edition, Diabetes Mellitus, Cobalamin.year: 2422, 2399
9. Koppad AK, Patil G, Baligar BD, Renukappa VB. Evaluation of Serum Vitamin B12 levels in Type 2 Diabetes Patients on Metformin Therapy attending a Tertiary care Hospital. Journal of Evidence Based Medicine and Healthcare. 2017 Jan 1;4(90):5399-404.
10. Kang D, Yun JS, Ko SH, Lim TS, Ahn YB, Park YM, Ko SH. Higher prevalence of metformin-induced vitamin B12 deficiency in sulfonylurea combination compared with insulin combination in patients with type 2 diabetes: a cross-sectional study. PLoS One. 2014 Oct 9;9(10):e109878.
11. Verma VK, Nim RK, Singh PS, Kumar M, Singh G, Singh AK. Vitamin B12 deficiency among vegetarian and non-vegetarian diabetic population receiving prolonged Metformin based oral hypoglycemic agents therapy. International Journal of Advances in Medicine. 2017 Jul;4(4):1150-4.
12. Ahmed MA, Muntingh G, Rheeder P. Vitamin B12 deficiency in metformin-treated type-2 diabetes patients, prevalence and association with peripheral neuropathy. BMC Pharmacology and Toxicology. 2016 Dec;17(1):44.
13. Sarmah D, Sharma B. Importance and Status of HBA1C in T2DM and its Indian Perspective. Asian Journal of Biomedical and Pharmaceutical Sciences. 2012 Sep 12;2(12).
14. Pathmanathan S, Somasundaram NP. HbA1C and diabetes—an overview. Sri Lanka Journal of Diabetes Endocrinology and Metabolism. 2014 Jan 20;3(2).

15. Sultanpur CM, Deepa K, Kumar SV. Comprehensive review on HbA1c in diagnosis of diabetes mellitus. *Int J Pharm Sci Rev Res*. 2010 Aug;3(2):119-22.
16. Burtis CA, Ashwood ER, Bruns DE. *Tietz Fundamentals of Clinical Chemistry*. 5th edition. Diabetes Mellitus, HbA1c. 2014: 1415,1441-1442.
17. World Health Organization. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. Geneva: World Health Organization; 2011.
18. Vasudevn DM. *Textbook of Biochemistry for medical students*. 8th ed. Vitamins. Jaypee Publication: 2016
19. Jeetendra S, Tushar B. Metformin use and vitamin B 12 deficiency in patients with type-2 diabetes mellitus. *MVP Journal of Medical Science*. 2016 Feb 29;3(1):67-70.
20. A R Aroor. *Medical Biochemistry*. 1st ed. Water soluble vitamins. Jaypee Publications: 2011
21. D Shailendra, Kalani R, Narayan KM, Prabhakaran D, Tandon N, P.S Reddy. Prevalence of Vitamin B12 deficiency among individuals with type 2 diabetes mellitus in a south indian community. *IJBCP*. 2018 Feb;7(2):309-314.
22. Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified?. *Journal of Diabetes & Metabolic Disorders*. 2013 Dec;12(1):17.
23. Badyal A, Kumar S. Role of Vitamin B12 Deficiency in Patients with Type 2 Diabetes Mellitus. *Journal of Medical Science and Clinical Research* 2018 Feb;2(6):235-238.
24. Lotake, D. V., Barsode, D. S. Relation of Vitamins in Diabetes Mellitus. *International Journal of Innovative Research in Medical Science*, 2018; 3(04), 1925 - 1928.
25. Rusher DR, Pawlak R. A review of 89 published case studies of vitamin B12 deficiency. *J Hum Nutr Food Sci*. 2013 Jan 1;1(2):1008.
26. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, Ko SH, Kim HS. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *Journal of Korean medical science*. 2014 Jul 1;29(7):965-72.
27. Raizada N, Jyotsna VP, Sreenivas V, Tandon N. Serum vitamin B12 levels in type 2 diabetes patients on metformin compared to those never on metformin: a cross-sectional study. *Indian journal of endocrinology and metabolism*. 2017 May;21(3):424.
28. Lachner C, Steinle NI, Regenold WT. The neuropsychiatry of vitamin B12 deficiency in elderly patients. *The Journal of neuropsychiatry and clinical neurosciences*. 2012 Jan;24(1):5-15
29. Sponne IE, Gaire D, Stabler SP, Drosch S, Barbé FM, Allen RH, Lambert DA, Nicolas JP. Inhibition of vitamin B12 metabolism by OH-cobalamin c-lactam in rat oligodendrocytes in culture: a model for studying neuropathy due to vitamin B12 deficiency. *Neuroscience letters*. 2000 Jul 21;288(3):191-4.