Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i4.2884

PROLONGED DURATION AND DOSE OF METFORMIN THERAPY AND ITS ASSOCIATED EFFECT OF VITAMIN B12 DEFICIENCY AMONG TYPE II DM PATIENTS: A CROSS SECTIONAL STUDY.

Andrew Gnanaprakasam J[1], Veeramani G[2]*, Manjanna K[3]

[1,2*,3]Department of Pharmacy, Annamalai University, Annamalai nagar, Chidambaram, TamilNadu, 608002, India.

***Corresponding Author:** Dr. Veeramani G.
*M.Pharm, Ph.D., Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, 608002. E.mail: veer9282@gmail.com

Abstract:
Objective: The aim of the study is to observe the long-term treatment of metformin and its associated effect on Vitamin B12 among Type II Diabetes mellitus south Indian patients.

Methods: An observational study conducted over a period of 54 months on Type II Diabetes mellitus patients who are in chronic therapy with metformin. The study described the Vitamin B12 levels among the Type II DM patients, which could alert the patients who are in the low and borderline range of serum Vitamin B12, which may prevent the risk of peripheral neuropathic complications and anaemic conditions, etc.

Findings: Totally, 387 patients were enrolled as per the inclusion and exclusion criteria, including 232 males (59.94%) and 155 females (40.05%). A total of 39 patients had been observed to have low levels of Vitamin B12, among them 23 females (Mean 184.17 ± S.D. 10.69) and 16 males (Mean 185.31± S.D 7.56), followed by 16 patients found to be above the normal range of Vitamin B12, among them 02 females (Mean 991 ± S.D. 15.55) and 14 males (Mean 1578.14 ± S.D. 406.93), followed by 76 patients had been found to be borderline of Vitamin B12, among them 43 females (Mean 220.76 ± S.D 6.50).

Conclusion: High doses of chronic Metformin therapy may alter Vitamin B12 levels. Some of the patients had an abnormally high level of serum Vitamin B12, whose medical condition should be ruled out, and such Patients' diet histories were also cross-examined, which was a mixed normal diet, and they had various medical conditions. However, the high chances of metformin-associated effects on Vitamin B12 with higher doses and long-term therapy of metformin, whose duration was 10 to 20 years, Moreover, age is also an important factor, and the study reveals that, among genders, female populations had a higher rate of low-level Vitamin B12 than male populations. Patients in the age group between 50 and 65 years had a higher rate of low-level Vitamin B12 than other age groups, totaling 20 patients, among them 12 females (Mean 183.08 ± S.D. 13.17) and 8 males (Mean 185.5 ± S.D. 4.3). Patients with other comorbid conditions also had low levels and borderline levels of Vitamin B12 and had been on long-term metformin therapy. Therefore, our study concluded that

chronic therapy and higher doses of metformin may lead to a deficit in serum Vitamin B12, which requires regular checks on Vitamin B12 among Type 2 DM patients to avoid the risk of Peripheral neuropathy and other complications.

Keywords: Type II Diabetes Mellitus, Metformin, Vitamin B12, Peripheral neuropathy.

INRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is increasingly becoming a pandemic in developed and developing world [1] In 2010, 285 million people, representing about 6% of the world's adult population, were T2DM patients [2]. This number is expected to reach 439 million by 2030 [3]. The disease is associated with various systemic macro vascular and micro vascular complications. T2DM can lower the quality of life and result in heavy social and economic burdens, making the disease a public health concern. T2DM absorbs 5-10% of healthcare budget in many countries [4]. Both the European and American guidelines recommend the use of metformin as a first-line pharmacological therapy in T2DM [5]. Findings from clinical studies confirmed that the medication improves cardiovascular outcomes in T2DM patients [6]. Due to its proven effectiveness, relative safety and potential for use with other anti-diabetic medications, metformin is currently the most widely prescribed oral anti-diabetic agent [7]. It is estimated that the medication is routinely prescribed to 120 million patients with diabetes around the world [8]. Diabetes is a serious and growing global health concern, according to the latest statistics.

The global prevalence of impaired glucose tolerance was estimated at 7.5% (374million) in 2019 and is projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045.

The number of people with diabetes is anticipated to increase to 693 million by 2045 [9]. Most people with diabetes live in low- and middle-income countries, at approximately 79%, where a rapid increase in the number of diabetic cases is expected over the next 22 years by 13.3% and 13.9% in the Middle East and North Africa (MENA) Region [10, 11]. Diabetes can be classified into two main categories: (i) insulin-dependent diabetes mellitus (T1DM), or type 1 diabetes mellitus, characterized by the lack of insulin secretion (without daily administration of insulin, T1DM rapidly becomes fatal); and (ii) non-insulin-dependent diabetes mellitus, or type 1 diabetes mellitus (T2DM) [12,13]. T2DM results from the body's ineffective use of insulin. About 90% of people with diabetes worldwide have T2DM, which is mainly associated with high body weight and physical inactivity (WHO, 2019). All guidelines, including the European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA), consider metformin (1,1-dimethylpiguanide hydrochloride) a cornerstone and first-line treatment, along with lifestyle intervention, for managing hyperglycemia in patients with T2DM (14). Furthermore, metformin has beneficial effects on carbohydrate metabolism, weight loss, and prevention of vascular disease [15].

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that is primarily obtained from animal-sourced foods such as red meat, poultry, shellfish, milk, eggs and other dairy products, or vitamin B12-fortified foods [16]. Once ingested, vitamin B12 is released from its food carrier proteins by proteolysis in the acidic environment of the stomach, where it binds to a glycoprotein called haptocorrin (also referred to as Rfactor or R-protein). Haptocorrin is produced and secreted by the salivary glands. The haptocorrin-vitamin B12 complex protects vitamin B12 from degradation in the acidic environment of the stomach. Once the haptocorrin-vitamin B12 complex reaches the duodenum, pH change and degradation of haptocorrin by pancreatic proteases favor vitamin B12 cleavage from haptocorrin, resulting in the release of vitamin B12 in its free form. In the duodenum, the free vitamin B12 binds to intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells, resulting in the formation of an IFvitamin B12 complex. The newly formed IF-vitamin B12 complex subsequently binds, in a calcium-dependent manner, to the cubilin receptor (a protein encoded by the CUBN gene) on the enterocytes of the distal ileum, resulting in the absorption of vitamin B12 by receptor-mediated endocytosis. Upon internalization, the IF-vitamin B12 complex is released from its receptor, IF is degraded in lysosomes, and vitamin B12 enters the circulation via the multidrug

resistance protein 1 (MDR1) transporter [17]. In the circulation, approximately 20%-25% of vitamin B12 is bound to its binding protein transcobalamin. The transcobalamin-vitamin B12 complex is also known as holotranscobalamin (HoloTC), which represents the biologically active form of cobalamin and allows for cellular uptake of vitamin B12 through specific cell surface transcobalamin receptors [18]. The remaining 75%-80% of vitamin B12 is bound to haptocorrin and is stored in the liver. Some vitamin B12 is excreted in bile and undergoes enterohepatic circulation [19]. Vitamin B12 also acts as a cofactor for the enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, a reaction that takes place in the mitochondria [20]. Thus, vitamin B12 deficiency results in the accumulation of methylmalonyl-CoA that is subsequently converted to methylmalonic acid (MMA), whose plasma levels are often, elevated in patients with vitamin B12 deficiency. In subjects with vitamin B12 deficiency, increased levels of MMA and homocysteine have been suggested to contribute to myelin damage (myelopathy) and, as a consequence, to peripheral and autonomic neuropathy [21, 22]. Neurologic manifestations of vitamin B12 deficiency include progressive axonal demyelination, impaired sensory and peripheral nerve function, sub-acute combined degeneration of the spinal cord, areflexia and loss of proprioception and vibration sensitivity [23, 24].

Therefore, mentioned neurologic manifestations can be erroneously interpreted as features of diabetic neuropathy in diabetic patients who are chronically treated with metformin. Failure to identify the cause of neuropathy can lead to progression of central and/or peripheral nerve damage, which may be arrested, but not completely reversed, by vitamin B12 replacement in some instances [25]. Neurocognitive manifestations such as poor memory performance, cognitive impairment, dementia, delirium, depression and episodes of psychosis are also possible in the presence of severe and chronic vitamin B12 deficiency [26]. Other symptoms that have been reported in adult patients with vitamin B12 deficiency include glossitis, skin hyperpigmentation and infertility, hearing loss, bone disease and macular degeneration [27].

METFORMIN-INDUCED VITAMIN B12 DEFICIENCY: CLINICAL EVIDENCE

More than 60 years after its first clinical use, metformin is still recommended as the first-line oral glucose-lowering drug in most clinical guidelines on the management of type 2 diabetes (T2D) thanks to its well-established long-term safety and efficacy profile. Possible side effects of metformin include gastrointestinal intolerance [28] and the rare occurrence of lactic acidosis, which is most likely in the presence of moderate to severe chronic kidney disease. However, moderate to severe renal impairment is a major contraindication to the clinical use of metformin [29]. Metformin is usually well tolerated and effective in maintaining glucose control in the long-term [30]. Indeed, metformin is still the most widely used oral anti-hyperglycemic (insulin sensitizing) agent, being prescribed to more than 100 million people worldwide, including patients with pre-diabetes, insulin resistance and polycystic ovary syndrome (PCOS) [31]. Yet, in recent decades, several observational studies, systematic reviews and meta-analyses have reported an association between long-term metformin therapy and biochemical vitamin B12 deficiency, including frank deficiency or borderline vitamin B12 status [32-36].

MECHANISM OF METFORMIN-INDUCED MALABSORPTION OF VITAMIN B12

Many mechanisms were proposed to explain how Metformin interferes with the absorption of vitamin B12. Intestinal bacteria overgrowth resulting in the binding of IF-vitamin B12 complex to bacteria instead of being absorbed was an early suggested mechanism (37). It was also proposed that metformin reduces the vitamin absorption by altering the intestinal motility (38). The most currently accepted mechanism suggests that metformin antagonizes the calcium cation and interferes with the calcium-dependent IF-vitamin B12 complex binding to the ileal cubilin receptor (39). The reversal of metformin-associated vitamin B12 malabsorption by calcium supplementation greatly supported the latter mechanism. The study of Bauman et al. proposed the mechanism that describes the malabsorption of vitamin B12 by metformin (39). Type-2 diabetic participants were divided into two

groups: the first group was given metformin, and the second (control) group was given a sulfonylurea. The metformin group, but not the control group, showed a statistically significant gradual decrease in serum vitamin B12 and holo-TC-II levels over the first three months. Oral calcium supplementation was then introduced to the metformin group for one month. At the end of that month, holo-TC-II levels increased in the metformin group by 53%. The absence of bacterial overgrowth was confirmed by hydrogen breath tests and by similar baseline and study end concentrations of serum vitamin B12 analogues. The authors built on the previously reported ability of biguanides to give a positive charge to the membrane's surface and on the essential role the calcium plays in the binding of IF vitamin B12 complex to ileal receptors to introduce the theory of the mechanism by which metformin inhibits vitamin B12 absorption (40). They proposed that the protonated metformin molecule directs itself towards the hydrocarbon core of the ileal cell membrane and positively charges the membrane surface, displacing the divalent calcium cations by repulsion forces. Such displacement impairs the calcium-dependent binding of IF-vitamin B12 complex to the ileal cubilin receptor and malabsorption of the vitamin ensues. Since bile is secreted into the duodenum, the above theory connotes that metformin may inhibit the absorption of bile vitamin B12 later in the distal ileum. Therefore, the medication has the theoretical potential to inhibit both dietary and enterohepatic vitamin B12 absorption.

MATERIALS AND METHOD:

An observational study conducted in the tertiary care teaching hospital. The protocol was reviewed and approved by the Institutional ethics committee. This study was conducted over a period of 54 months. Based on inclusion and exclusion criteria, patients were recruited for the study. A total of 387 patients had enrolled with their informed consent. Data had been collected from the patient's demographic details, clinical data, treatment chart, lab investigations, etc. Based on study criteria, both inpatients and outpatients of either gender were investigated. All the patients were on chronic metformin therapy between the ages of 18 and 65. Blood samples were collected for the investigations. The following patients were excluded from the study: emergency visits, those who were not willing to participate in the study, and those who were on a vitamin B12 supplement. This study also cross-examined the past medical history and diet history of the patients and carefully recruited them. This study assessed the range of serum Vitamin B12 for those who have been on chronic metformin therapy for at least 2 years. We observed that 4.13% of the patients had an abnormally high level of Vitamin B12, followed by 19.6% of the patients who were found to be borderline in Vitamin B12, and 10.06% of patients who were found to be in the low range of Vitamin B12. Patients in the age group between 50 and 65 years had a higher rate of low-level Vitamin B12 than other age groups, totalling 20 patients, among them 12 females (Mean 183.08 ± S.D. 13.17) and 8 males (Mean 185.5 ± S.D. 4.3).

RESULTS

An observational study conducted in the tertiary care teaching hospital. The protocol was reviewed and approved by the Institutional ethics committee. This study was conducted over a period of 48 months. Based on inclusion and exclusion criteria, patients were recruited for the study. A total of 387 patients had enrolled with their informed consent. Data had been collected from the patient's demographic details, clinical data, treatment chart, lab investigations, etc. Based on study criteria, both inpatients and outpatients of either gender were investigated. All the patients were on chronic metformin therapy between the ages of 18 and 65. Blood samples were collected for the investigations. The following patients were excluded from the study: emergency visits, those who were not willing to participate in the study, and those who were on a vitamin B12 supplement. This study also cross-examined the past medical history and diet history of the patients and carefully recruited them. This study assessed the range of serum Vitamin B12 for those who have been on chronic metformin therapy for at least 2 years. We observed that 4.13% of the patients had an abnormally high level of Vitamin B12, followed by 19.6% of the patients who were found to be

borderline in Vitamin B12, and 10.06% of patients who were found to be in the low range of Vitamin B12. Patients in the age group between 50 and 65 years had a higher rate of low-level Vitamin B12 than other age groups, totalling 20 patients, among them 12 females (Mean 183.08 ± S.D. 13.17) and 8 males (Mean 185.5 ± S.D. 4.3).

Patient distribution according to the age and sex group: Patients age group between 18 and 30 years (16 males, 14 females), followed by 31 and 40 years (41 males, 28 females), followed by 41 and 50 years (74 males, 42 females), and patient age group between 51 and 65 years (101 males and 71 females). Among genders, the female population had a higher rate of low-level vitamin B12 than the male population. Moreover, patients in the age group between 51 and 65 had a higher rate of low-level vitamin B12 than others.

Table. 1 Patients Age & Gender wise Distribution

Age Group (Years)	Sex / Gender	
	Male (n=232)	Female (n=155)
18 - 30	16 (6.8%)	14 (9%)
31 - 40	41 (17.6%)	28 (18%)
41- 50	74 (31.8%)	42 (27%)
51 - 65	101 (43.5%)	71 (45.8%)

The above table represents the age group distributions among either gender. Age between 51 and 65 and above shows the highest patient population.

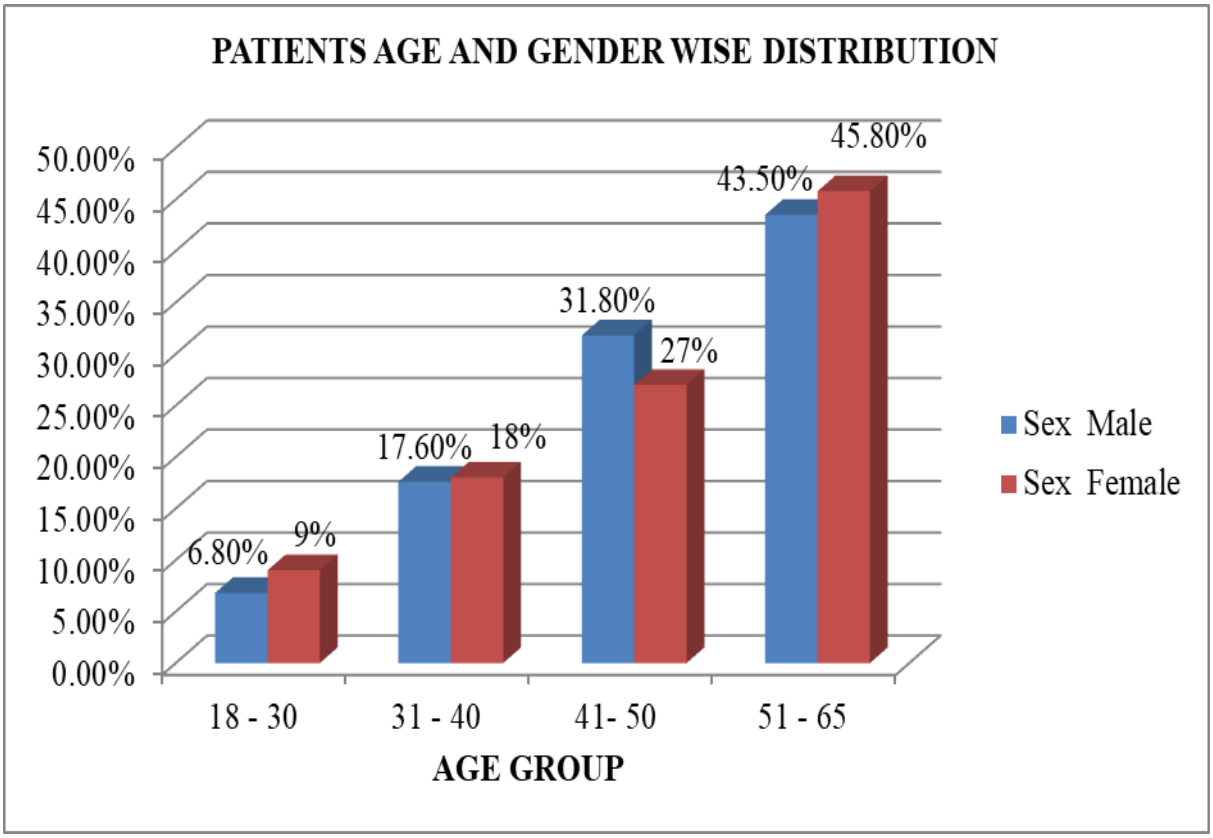

Fig. 1 Represents the age and sex-wise distribution of patients.

Patient's distribution only with Type II Diabetes Mellitus.

A total of 387 patients were diagnosed, among them patients diagnosed only with Type 2 DM (109 males, 72 females). Among them 20 males and 13 females comes under the age group between 18 and 30 followed by 22 males and 14 females comes under the age group between 31 and 40 followed

by 32 males and 22 females comes under the age group between 41 and 50 followed by 35 males and 23 females comes under the age group from 51 and 65 above.

Table.2 Patients only with Type II DM

Patients with Type II DM / Age Group wise	Males (n = 109)	Females (n = 72)
18 – 30	20 (18.3%)	13 (18%)
31 – 40	22 (20.1%)	14 (19.4%)
41 – 50	32 (29.3%)	22 (30.5%)
51 – 65	35 (32.1%)	23 (31.9%)

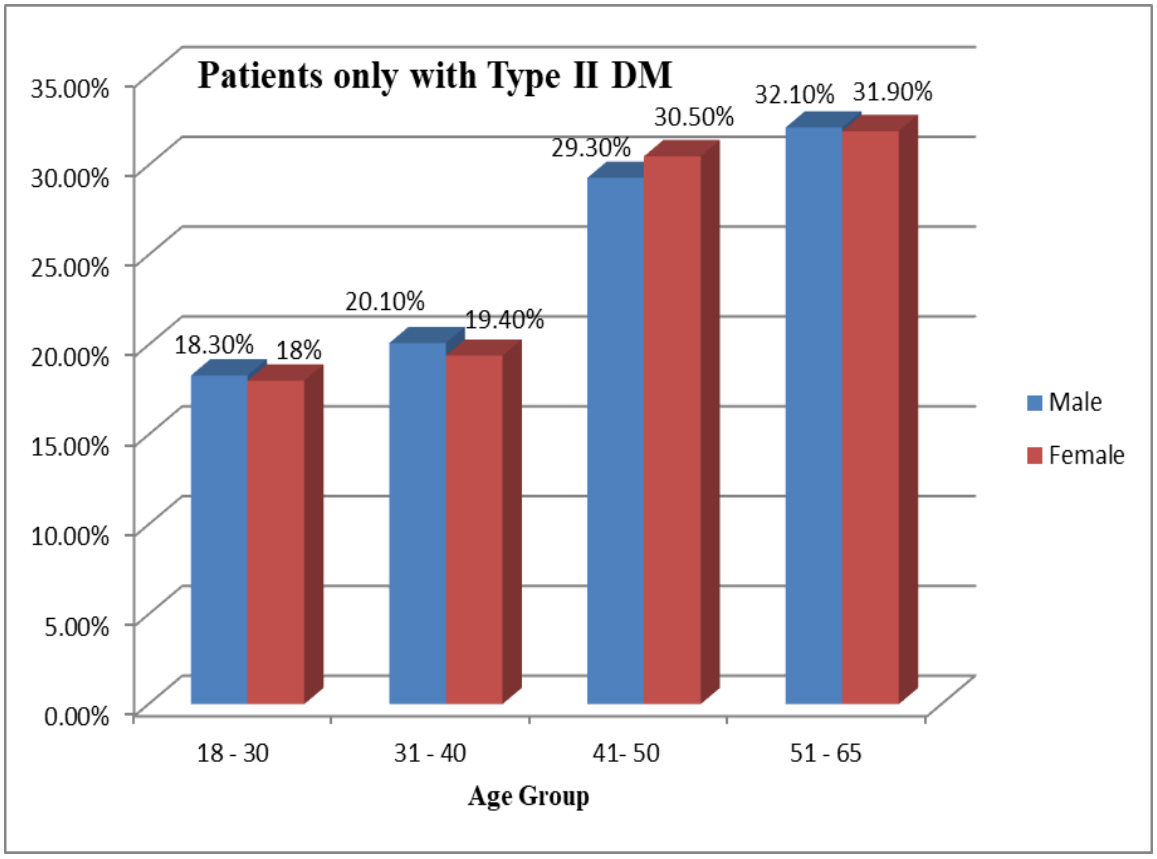

Fig.2 Represents Patients only with Type II DM, either Gender According to their Age Group.

Patient's distribution on Type II DM with co-morbid conditions:

Patients diagnosed with Type 2 DM with co-morbid conditions (123 males, 83 females). Patients with Type 2 DM and HTN include 72 males and 37 females. Patients with comorbid conditions, including Type 2 DM, HTN and IHD, were 40 males and 36 females. Patients with Type 2 DM and various comorbid conditions were 11 males and 13 females.

Table.3 Type II DM with various co-morbid conditions

PATIENT'S TYPE II DM WITH CO-MORBID CONDITIONS	Males (n= 123)	Females (n= 83)
TYPE II DM WITH HTN	72 (58.5%)	37 (44.5%)
TYPE II DM WITH HTN,IHD	40 (32.5%)	36 (43.3%)
TYPE II DM WITH VARIOUS COMORBID CONDITIONS	11 (8.9%)	13(15.6%)

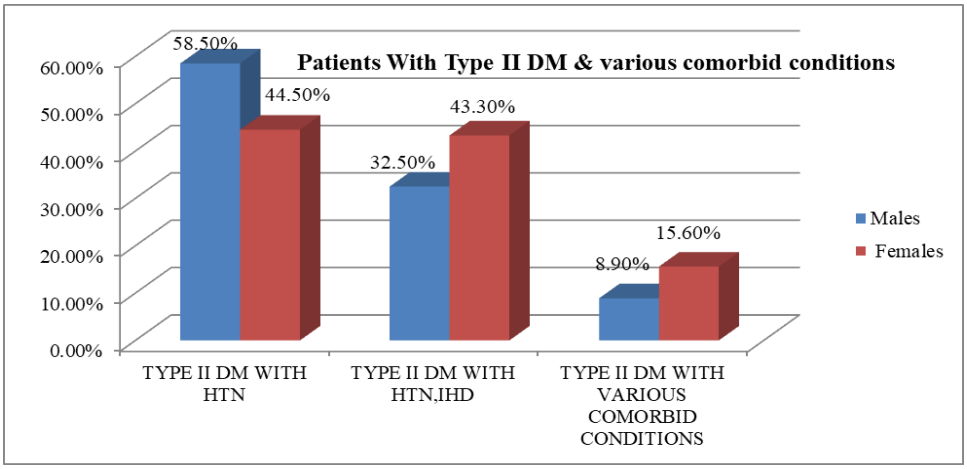

Fig. 3 Represents Patients with Type II DM & various comorbid conditions

PRESCRIPTION OF VARIOUS ANTI DIABETIC AGENTS

Various categories of oral hypoglycemic agents are prescribed, since Metformin (n = 387) is the first line choice of drug, which was mostly prescribed rather than other drugs, followed by Glimepiride (n = 136), prescribed along with metformin, followed by Vidagliptin (n = 87), Pioglitazone (n = 27), Volibose (n = 102), and Insulin (n = 36).

Table. 4 Prescribed various types of Anti-Diabetic agents and Insulin

Type of Oral Hypoglycaemic agents	Number of drugs prescribed
Metformin	387 (100%)
Glimepiride	136 (35%)
Vidagliptin	87 (22.4%)
Voglibose	102 (26.35%)
Pioglitazone	27 (6.9%)
Insulin Human Actrapid	28 (7.2%)
Insulin Human Mixtard	08 (2.06%)

Metformin exerts its glucose-lowering effect primarily by decreasing hepatic glucose production through suppression of gluconeogenesis and enhancing insulin suppression of endogenous glucose production and, to a lesser extent, by reducing intestinal glucose absorption and possibly improving glucose uptake and utilization by peripheral tissues, such as skeletal muscle and adipose tissue. A recent study has found evidence that metformin and antagonize the action of the counter-regulatory hormone glucagon to suppress hepatic glucose production (41).

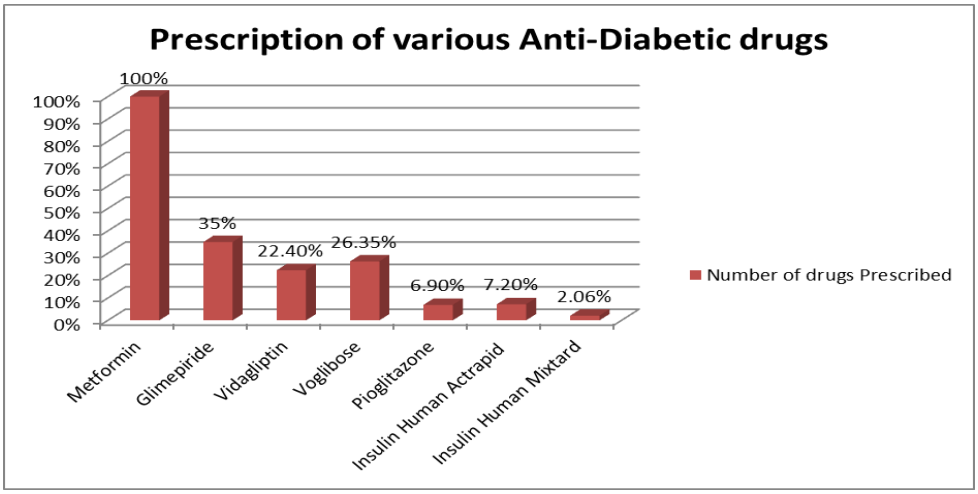

Fig. 4 Represents various types of Anti-Diabetic agents prescribed.

DISTRIBUTION OF SUSPECTED ADRS

During the treatment 16 Patients experienced ADRs include Diarrhoea and Abdominal discomfort in which 6 Males and 10 Females. 20 Patients experienced Metallic taste in which 11 Males and 09 Females. 14 Patients experienced Anorexia in which 08 Males and 06 Females. 24 Patients experienced Hypoglycaemic condition in which 16 Males and 08 Females.

Table. 5 Represents Patients Experienced ADRs during Treatment

SUSPECTED ADRs	MALES (n=41)	FEMALES (n=33)
Diarrhoea and Abdominal discomfort	6 (2.5%)	10 (6.4%)
Metallic taste	11 (4.7%)	09 (5.8%)
Anorexia	08 (3.4%)	06 (3.8%)
Hypoglycaemic	16 (6.8%)	08 (5.1%)

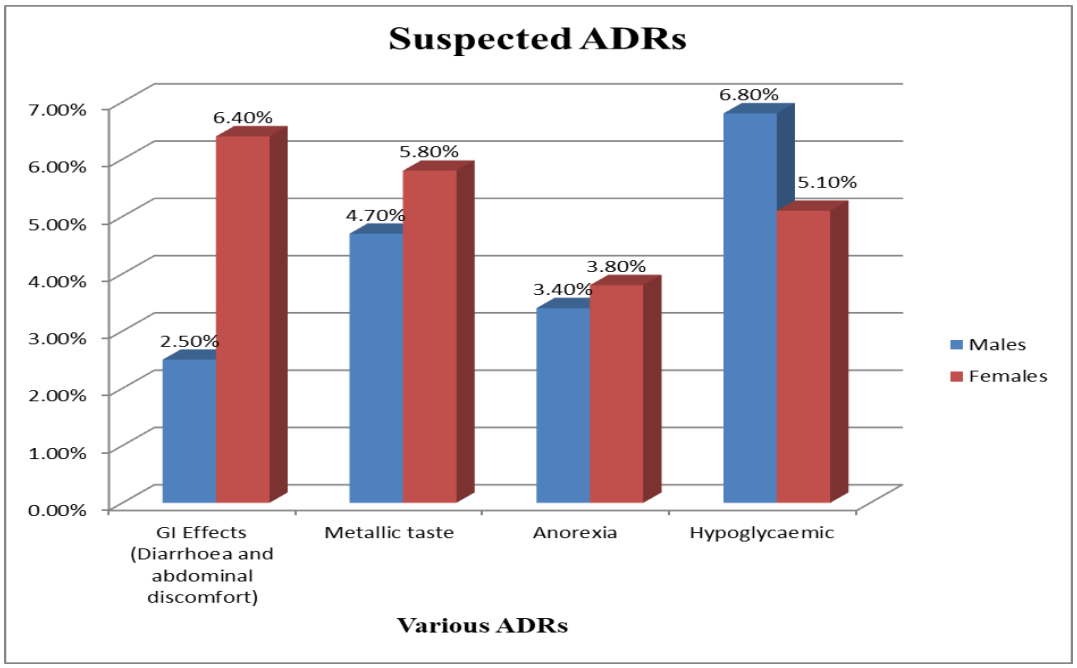

Fig. 5 Represents various ADRs experienced during therapy.

INVESTIGATIONS OF VITAMIN B12:

Vitamin B12 had been investigated on all recruited patients, in which 39 patients had a low level of Vitamin B12 (<211 pg/ml) among them 16 (6.8%) male patients (mean 185.31± S.D. 7.56) and 23 (14.8%) female patients (mean 184.17± S.D. 10.9), followed by 76 patients who were on the borderline (211–250 pg/ml) among them 33 (14.2%) male patients (mean 220.72± S.D. 6.82), and 16 patients were above the normal level (>911 pg/ml) among them 14 (6.03%) male patients (mean 1578.14± S.D. 406.93) and 2 (1.2%) female patients (mean 991± S.D. 15.55). A vitamin B12 deficit had been observed in patients treated with metformin over a period of 3 to 20 years (mean 15.23 ± S.D. 9.19), and a borderline had been observed in patients who received metformin over a period of 5 to 15 years (mean 9.16 ± S.D. 6.3).

Table. 6 Represents investigations of Vitamin B12

Vitamin B12 (211 – 911 pg/ml)	Male (n= 232)	Mean ± S.D	Female(n=155)	Mean ± S.D
Low (<211 pg/ml)	16	185.31 ± 7.56	23	184.17 ± 10.96
Borderline (211-250 pg/ml)	33	220.72 ± 6.87	43	220.76 ± 6.50
Above Normal (>911 pg/ml)	14	1578.14 ± 406.96	02	991 ± 15.55
Normal (211-911 pg/ml)	169	662.68 ± 171.87	87	647.01 ± 178.20

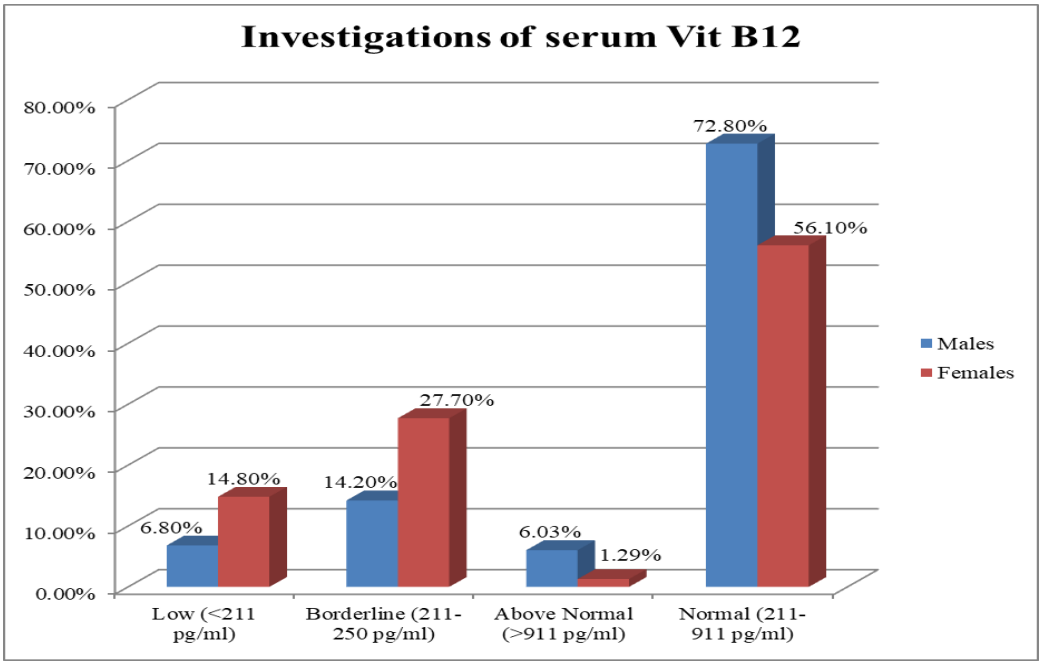

Fig. 6 Represents investigations of Vitamin B12 (Low, Borderline, above normal, normal).

Further, this study also analysed the daily dose of metformin and found that a vitamin B12 deficit had been observed in patients who received the average doses of 1612.8 mg (mean 1612.8 ± S.D. 494.9) and a borderline was observed in patients who received the average doses of 1479.72 mg (mean 1472.9 ± S.D. 353.5). Clinical findings reveal that the dose and duration of metformin use may lead to a deficit in Vitamin B12.

DOSE AND DURATION OF METFORMIN

A total of 256 patients who received daily doses of metformin had a normal level of vitamin B12, and their daily average doses were 1406mg (mean 1406.8 ± S.D. 494.9), which shows there were no huge differences in doses when compared to the low and borderline of the vitamin B12 population. Moreover, the 5- to 15-year (mean 8.45± S.D. 2.82) duration of the metformin use of 256 patients whose vitamin B12 level was quite normal shows that there was no huge difference when compared to the low and borderline levels of the vitamin B12 population.

Table. 7 Represents Daily Doses and Durations of Metformin

Dose in Mg & duration in years (6 months & above)	Vitamin B12 Deficiency (+)	Borderline of Vitamin B12	Normal range of Vitamin B12
Duration of Metformin use (years)	Mean 15.23 ± S.D 9.19	Mean 9.16 ± S.D 6.36	Mean 8.45 ± S.D 2.82
Daily dose of metformin (mg)	Mean 1612.82 ± S.D 464.97	Mean 1472.9 ± S.D 353.55	Mean 1406.85 ± S.D 494.97

DISCUSSION

An observational study was conducted over a period of 54 months in a tertiary care teaching hospital. The purpose of the study is to observe and assess the range of vitamin B12 levels among the Type 2 DM patients who are on the chronic therapy of metformin. Metformin is an euglycemic agent that falls under the biguanides category of oral anti-diabetic agents, which is the first line choice of Type 2 DM. Metformin is a drug that is widely used all over the world and in India among the Type 2 DM populations. Metformin has potential benefits and eventually causes potential side effects like peripheral neuropathy and abdominal discomfort, including bloating, nausea, and a metallic taste. Metformin side effects are usually ignored and are not regularly monitored. Vitamin B12 deficiency

leads to neurological problems like paraesthesia, ataxia, and peripheral neuropathy. Hence, our study described the vitamin B12 levels among the people with type 2 diabetes who were on chronic therapy with metformin. Vitamin B12 was investigated in 387 patients with or without comorbid conditions; among them, 123 males and 83 females had comorbid conditions, and the remaining 109 males and 72 females only had type 2 DM. 30 patients with type 2 DM and hypertension were 17 (16.6%), followed by 21 patients with type 2 DM, HTN, and IHD, followed by 16 patients with various comorbid conditions like COPD, AKI, diabetes ketoacidosis, pulmonary TB, chronic liver disease, alcoholic liver disease, anemia, hypothyroidism, and diabetic foot. Various categories of oral hypoglycemic agents were prescribed; the most prescribed drug was metformin, followed by a combination of glimepiride and metformin, which was the second most prescribed drug, followed by Volibose, Pioglitazone, and Vidagliptin, which were prescribed the least. Investigations of Vitamin B12 had been observed, and based on the patients values, the level of Vitamin B12 had been categorized: 39 patients were on a low level of Vitamin B12 (<211 pg/ml), followed by 256 patients in the normal range of Vitamin B12, followed by 76 patients on the borderline of the Vitamin B12 level (211–250 pg/ml), and 16 patients were above the normal level of Vitamin B12 (>911 pg/ml). Dietary history might be impacted by those found to have above-normal and low levels of vitamin B12. Patients in the age group between 50 and 65 years had a higher rate of low-level Vitamin B12 than other age groups, totaling 20 patients, among them 12 females (Mean 183.08 ± S.D. 13.17) and 8 males (Mean 185.5 ± S.D. 4.3). The study concluded that higher doses with chronic therapy of metformin may interfere with vitamin B12 levels. Some of the patients had an abnormally high value of vitamin B12 due to their diet, which also had some impact on vitamin B12. However, there will be a higher chance of metformin-associated effects on vitamin B12 with high doses of metformin, and more importantly, in patients with comorbid conditions. Moreover, age is also an important factor, as the study reveals that among genders, the male patient population has a higher rate of low-level vitamin B12 than the female population. Patients' age groups between 50 and 65 had a higher rate of low-level vitamin B12 than others, and patients with other comorbid conditions also had low levels and borderline levels of vitamin B12. Therefore, our study concluded that chronic therapy and higher doses of metformin may lead to a deficit in serum Vitamin B12, which requires regular checks on Vitamin B12 among Type 2 DM patients to avoid the risk of Peripheral neuropathy and other complications.

REFERENCES

1. Van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. Eur J Cardiovasc Prev Rehabil 2010 May;17 Suppl 1:S3- 8.
2. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care 2011 Jun;34(6):1249-1257.
3. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus present and future perspectives. Nat Rev Endocrinol 2011 Nov 8;8(4):228-236.
4. Lin Y, Sun Z. Current views on type 2 diabetes. J Endocrinol 2010 Jan;204(1):1-11.
5. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 2012 Jun;35(6):1364-1379.
6. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin.Diabetologia 2006 May;49(5):930-936.
7. Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. South Med J 2010 Mar;103(3):265-267.
8. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012 Mar;122(6):253-270.

9. Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res. Clin. Pract. 2019, 157, 107843. [CrossRef] [PubMed]
10. Cho, N.H.; Shaw, J.E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J.D.; Ohlrogge, A.W.; Malanda, B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 2018, 138, 271–281. [CrossRef] [PubMed]
11. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2009, 32 (Suppl. 1), S62–S67. [CrossRef] [PubMed]
12. Katsiki, N.; Ferrannini, E.; Mantzoros, C. New American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD) guidelines for the pharmacotherapy of type 2 diabetes: Placing them into a practicing physician's perspective. Metab. Clin. Exp. 2020, 17, 154218. [CrossRef]
13. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998, 352, 854–865. [CrossRef]
14. Beedholm-Ebsen R, van de Wetering K, Hardlei T, Nexø E, Borst P, Moestrup SK. Identification of multidrug resistance protein 1 (MRP1/ABCC1) as a molecular gate for cellular export of cobalamin. Blood 2010; 115: 1632-1639 [PMID: 19897579 DOI: 10.1182/blood-2009-07-232587]
15. Seetharam B, Li N. Transcobalamin II and its cell surface receptor. Vitam Horm 2000; 59: 337-366 [PMID: 10714245 DOI: 10.1016/s0083-6729(00)59012-8]
16. Ankar A, Kumar A. Vitamin B12 Deficiency. 2020 Jun 7. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [PMID: 28722952]
17. Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, Ueland PM, Yajnik C. Vitamin B12 deficiency. Nat Rev Dis Primers 2017; 3: 17040 [PMID: 28660890 DOI: 10.1038/nrdp.2017.40]
18. Beedholm-Ebsen R, van de Wetering K, Hardlei T, Nexø E, Borst P, Moestrup SK. Identification of multidrug resistance protein 1 (MRP1/ABCC1) as a molecular gate for cellular export of cobalamin. Blood 2010; 115: 1632-1639 [PMID: 19897579 DOI: 10.1182/blood-2009-07-232587]
19. Seetharam B, Li N. Transcobalamin II and its cell surface receptor. Vitam Horm 2000; 59: 337-366 [PMID: 10714245 DOI: 10.1016/s0083-6729(00)59012-8]
20. Ankar A, Kumar A. Vitamin B12 Deficiency. 2020 Jun 7. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [PMID: 28722952]
21. Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, Ueland PM, Yajnik C. Vitamin B12 deficiency. Nat Rev Dis Primers 2017; 3: 17040 [PMID: 28660890 DOI: 10.1038/nrdp.2017.40]
22. Konda M, Godbole A, Pandey S, Sasapu A. Vitamin B12 deficiency mimicking acute leukemia. Pro (Bayl Univ Med Cent) 2019; 32: 589-592 [PMID: 31656431 DOI: 10.1080/08998280.2019.1641045]
23. 23 Kumar N. Neurologic aspects of cobalamin (B12) deficiency. Handb Clin Neurol 2014; 120: 915- 926 [PMID: 24365360 DOI: 10.1016/B978-0-7020-4087-0.00060-7]
24. 24 Langan RC, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. Am Fam Physician 2017; 96: 384-389 [PMID: 28925645]
25. Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? J Diabetes Metab Disord 2013; 12: 17 [PMID: 23651730 DOI: 10.1186/2251-6581-12-17]
26. Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. South Med J 2010; 103: 265-267 [PMID: 20134380 DOI: 10.1097/SMJ.0b013e3181ce0e4d]

27. Köbe T, Witte AV, Schnelle A, Grittner U, Tesky VA, Pantel J, Schuchardt JP, Hahn A, Bohlken J, Rujescu D, Flöel A. Vitamin B-12 concentration, memory performance, and hippocampal structure in patients with mild cognitive impairment. Am J Clin Nutr 2016; 103: 1045-1054 [PMID: 26912492 DOI: 10.3945/ajcn.115.116970]
28. Ito H, Ishida H, Takeuchi Y, Antoku S, Abe M, Mifune M, Togane M. Long-term effect of metformin on blood glucose control in non-obese patients with type 2 diabetes mellitus. Nutr Metab (Lond) 2010; 7: 83 [PMID: 21070671 DOI: 10.1186/1743-7075-7-83]
29. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012; 122: 253-270 [PMID: 22117616 DOI: 10.1042/CS20110386]
30. 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; 42: 316- 327 [PMID: 27130885 DOI: 10.1016/j.diabet.2016.03.008]
31. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, Bray GA, Schade DS, Temprosa MG, White NH, Crandall JP; Diabetes Prevention Program Research Group. Longterm Metformin Use and Vitamin B12 Deficiency in the Diabetes Prevention Program Outcomes Study. J Clin Endocrinol Metab 2016; 101: 1754-1761 [PMID: 26900641 DOI: 10.1210/jc.2015-3754]
32. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. Medicine (Baltimore) 2019; 98: e17918 [PMID: 31725641 DOI: 10.1097/MD.0000000000017918]
33. Kancherla V, Elliott JL Jr, Patel BB, Holland NW, Johnson TM 2nd, Khakharia A, Phillips LS, Oakley GP Jr, Vaughan CP. Long-term Metformin Therapy and Monitoring for Vitamin B12 Deficiency Among Older Veterans. J Am Geriatr Soc 2017; 65: 1061-1066 [PMID: 28182265 DOI: 10.1111/jgs.14761]
34. Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med 2015; 10: 93-102 [PMID: 25502588 DOI: 10.1007/s11739-014-1157-5]
35. Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. PLoS One 2014; 9: e100379 [PMID: 24959880 DOI: 10.1371/journal.pone.0100379]
36. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformininduced vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pract 2012; 97: 359-367 [PMID: 22770998 DOI: 10.1016/j.diabres.2012.06.001]
37. Caspary WF, Creutzfeldt W. Analysis of the inhibitory effect of biguanides on glucoseabsorption: inhibition of active sugar transport. Diabetologia 1971 Oct;7(5):379-385.
38. Buvat DR. Use of metformin is a cause of vitamin B12 deficiency. Am Fam Physician 2004 Jan 15;69(2):264; author reply 264, 266.
39. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care 2000 Sep;23(9):1227-1231.
40. Bostom AG, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, et al. Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the Folic Acid for Vascular Outcome Reduction in Transplantation trial. Circulation. 2011;123(16):1763-70.
41. Miller R.A. Chu Q.Xie J.Foretz M. Viollet B. Birnbaum M.J. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature.* 2013; 494: 256-260.