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Evaluation level of serum vitamin B12 in Iraqi patients with diabetes mellitus type 2, who used the metformin drug as a hypoglycemic agent

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Abstract: Diabetes mellitus type 2 is characterized by insulin resistance, which can be combined with relatively decrease secretion of insulin hormone in the body. Metformin is usually recommended as a first-line treatment for diabetes mellitus-type 2, as it has a significant role in decrease mortality. This study aims to evaluate the B12 levels in Iraqi patients with type 2 diabetes, who were using the metformin drug in short-, medium- and long-term periods. The study included 202 patients, who were classified into four groups, according to their use of the Metformin drug as a hypoglycemic agent, while the fourth group did not use metformin and was termed as 'the standard group'. The results showed that the levels of vitamin B12 in patients using Metformin drugs in short-, medium- and long-term periods were, (444.4±17.21)pg/ml, (403.0±20.34)pg/ml and (261.7±14.70)pg/ml, respectively, compared to the standard group (469.7±26.37)pg/ml. The conclusion of this study was that a significant reduction was seen in the level of vitamin B12 in patients using the Metformin drug for medium- and long-term periods compared to the standard group, who did not use the Metformin drug.

Keywords: Metformin, hypoglycemic agent, diabetes mellitus type 2 and vitamin B12

INTRODUCTION

Diabetes mellitus is a condition in which the pancreatic gland does not create enough insulin or does not respond properly to it, resulting in excessively high blood glucose (sugar) levels (Deshmukh & Jain, 2015). Hyperglycemia is a major symptom, and it can cause long-term problems in the body, as well as cause damage to certain organs, including the eyes, nerves, heart, kidneys and blood vessels (Mellitus, 2005). Diabetes mellitus (DM) may be classified into two types: Insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). The insulin hormone is required for therapy, hence, this categorization is based on it (VandenBerg & Webber, 2019). Insulin resistance is a feature of diabetes mellitus Type 2, which can be paired with a reduction in the insulin hormone output in the body. Insulin receptors are thought to be involved in the impaired insulin responsiveness of the tissues in the body. Reduced insulin sensitivity is the most common anomaly in the early stages of type 2 diabetes (Mezza *et al*., 2019). A variety of treatments and medicines that boost insulin hormone sensitivity or limit glucose production by the liver can be used to treat high blood sugar (Petersen *et al*., 2005). Metformin is commonly used as a first-line therapy for diabetes mellitus type 2, as there is strong evidence that it reduces mortality (Rojas & Gomes, 2013). Metformin belongs to a group of drugs known as Biguanides. Metformin lowers the amount of sugar absorbed from the diet as well as the quantity of sugar produced by the liver (Snyder & Berns, 2004). Metformin also inhibits the body's sensitivity to insulin, a natural hormone that regulates blood sugar levels. Metformin is not commonly used to treat type 1 diabetes (a condition in which the pancreas does not synthesize the insulin hormone and therefore, cannot control the level of glucose in the blood) (Rahimi *et al*., 2021). Vitamin B12 is an essential nutrient. It is crucial for nerve tissue health, brain activities and red blood cell formation. Vitamin B12 is sometimes referred to as cobalamin (Nawaz *et al*., 2020). When vitamin B12 levels are too low, cobalamin deficiency can occur. This can result in permanent neurological problems (Herrmann & Geisel, 2002). B12 deficiency has been linked to serious neurological problems, including peripheral neuropathy. Vitamin B12 deficiency is a prevalent problem among youngsters and the elderly (Hunt *et al*., 2014). Other reasons that might induce vitamin B12 insufficiency, in addition to a low food intake, include gastric bypass surgery and disorders linked with gastrointestinal (GI) malabsorption (Fernández-Bañares *et al*., 2009). The goal of this study is to assess the B12 levels in Iraqi patients with type 2 diabetes, who are taking metformin for short, medium, or long periods of time.

MATERIALS AND METHODS

This cross-sectional study was carried out in Samawah city - Iraq, between April 2020 and June 2021. Informed consent was obtained from all individual participants included in the study with Ethical approved reference No. (UAM /EC/5/2020). It included 202 patients with diabetes mellitus type 2, where the patients having gastrointestinal

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disease or taking vitamin B12 supplements were excluded. The participants were divided into four groups. The first group, called the 'standard group' (Sta. G), included 33 patients with diabetes mellitus type 2, who did not use the metformin drug. The second group included 61 patients with diabetes mellitus type 2, who were using metformin for a short time, less than six months and was called the 'short-term group' (ST G).

The third group included 49 patients with diabetes mellitus type 2, who had been using metformin for one to three years and was called the 'medium-term group' (MT G). The fourth and final group included 59 patients who had been using metformin for three or more years, called the 'long-term group (LT G).

Blood sample collection

Blood was collected in the General Al-Hussein Hospital in Samawah-Iraq. The consent form was signed by all human volunteers. Data were collected from patients with diabetes mellitus type 2, including: Gender, age and type of hypoglycemic drugs used. Blood samples (2ml each) were collected from human volunteers (N=202) with diabetes mellitus type 2. This was centrifuged at 1600rpm for 10 minutes and the serum was transferred into a new tube and used to determine the level of serum vitamin B12 (Abed *et al*., 2019; Sugihara *et al*., 2017).

Laboratory test

The CL-900i equipment, Mandray, was used to perform a vitamin B12 test, based on the competitive immunoluminometric assay concept (China). The test had to be carried out on the MAGLUMI Fully-auto chemiluminescence immunoassay (CLIA) analyzer and could be utilized for samples ranging from 50.0-2000.0pg/ml (Abed *et al*., 2022; Sharifmousavi & Borhani, 2020).

STATISTICAL ANALYSIS

Statistical analysis were done using one-way ANOVA and t-test with p value ≤0.05. In addition, all results, curves, tables and diagrams of this study were carried out using Microsoft Excel 2010 and GraphPad prism statistical program (version 6).

RESULTS

This cross-sectional study included 202 patients, classified into four groups according to their use of Metformin, as in fig. 1, where the first groups Sta. G, ST G, MT G and LT G represented. 17%, 30%, 24% and 29%, respectively.

The result of 202 patients showed no significant difference according to the age of the patients in the groups, as shown in fig. 2 and table 1, where the mean age of the standard group (Sta. G) was 43.03±2.267 years, and that of the short-term group using metformin (ST G) was 45.56±1.735 years, whereas, that of the medium-term group using metformin (MT G) was 47.88±1.856 years and of the group with long-term use of metformin (LT G) was 46.63±1.767 years.

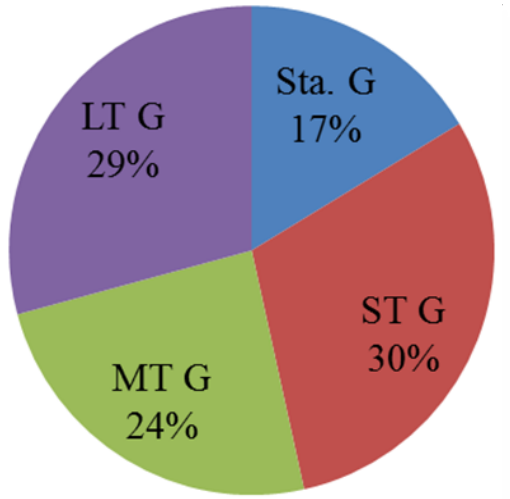

Fig. 1: Percentage of samples distribution in groups where Sta. G: Standard group, ST G: Short term using of metformin, MT G: Medium term using of metformin, LT G: Long term using of metformin.

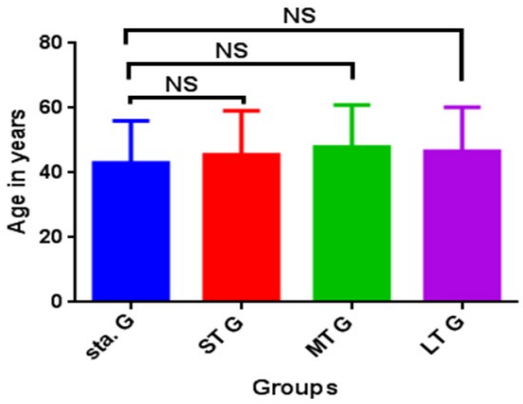

Fig. 2: Mean age of groups, Sta. G: Standard group, ST G: Short term using of metformin, MT G: Medium term using of metformin, LT G: Long term using of metformin, P value ≤0.05, S: significant, NS: Non-significant.

Table 1: Groups with mean age of patients samples

Group	Sta. G	ST G of	MT G	LT G
Number of Samples	33	61	49	59
Minimum	25	22	26	21
Maximum	65.00	71.00	71.00	72.00
Mean	43.03	45.56	47.88	46.63
Std. Deviation	13.03	13.55	12.99	13.57
Std. Error of Mean	2.267	1.735	1.856	1.767
Lower 95% CI of	38.41	42.09	44.15	43.09
Upper 95% CI of	47.65	49.03	51.61	50.16

Significant	-	NS	NS	NS

*Sta.G: Standard group, ST G: Short term using of metformin, MT G: Medium term using of metformin, LT G: Long term using of metformin

In addition the results showed that the standard group, included 17 males and 16 females, whereas, the second group (ST G) had 32 males and 29 females, the third group (MT G) included 29 males and 20 females and the last group (LT G) included 35 males and 24 females, as shown in fig. 3.

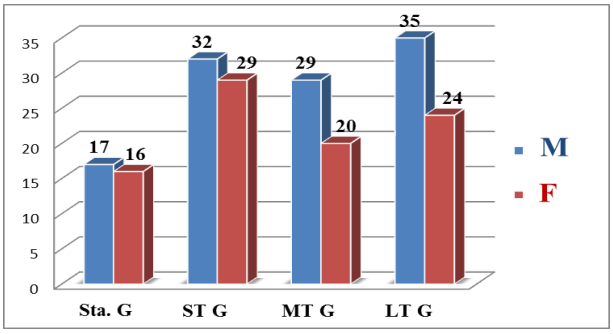

Fig. 3: Classified groups according to gender of patients, M: Male, F: Female.

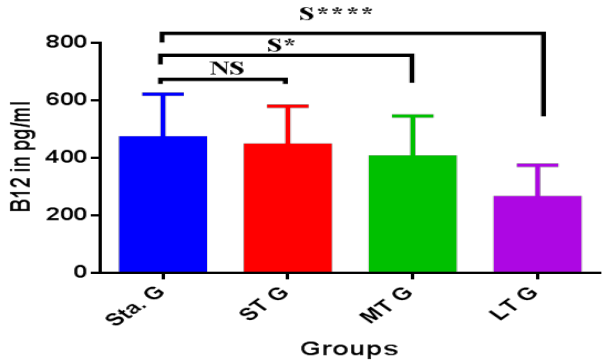

Fig. 4: Serum B12 level in patient that using metformin drug, Sta. G: Standard group, ST G: Short term using of metformin, MT G: Medium term using of metformin, LT G: Long term using of metformin, P value ≤0.0001, S: significant, NS: Non significant

Table 2: Statistical analysis of serum B12 level in the patients of study group

Group	Sta. G	ST G	MT G	LT G
Number of Samples	33	61	49	59
Minimum	90.40	136.1	136.1	101.8
Maximum	754.0	734.3	766.2	564.0
Mean	469.7	444.4	403.0	261.7
Std. Deviation	151.5	134.4	142.4	112.9
Std. Error of Mean	26.37	17.21	20.34	14.70
P value	-	0.4077	0.0459	<0.0001
Significant	-	No	Yes *	Yes ****

*Sta.G: Standard group, ST G: Short term using of metformin, MT G: Medium term using of metformin, LT G: Long term using of metformin.

The result of the serum vitamin B12 level showed no significant difference in *p*-value (0.4077) in the second group (ST G), when compared to the standard group (Sta. G), when the mean of B12 levels were (444.4±17.21) pg/ml and (469.7±26.37)pg/ml, respectively. However, the third (MT G) group showed a significant difference (S*) in *p*-value (0.0459), when the mean level of vitamin B12 in patients was (403.0±20.34)pg/ml, with a high significant difference in the fourth group (LT G), which showed a mean equal to (261.7±14.70)pg/ml and a *p*-value less than 0.001, as shown in fig. 4 and table 2.

DISCUSSION

Vitamin B12 belongs to the water-soluble, B-vitamin family and it is a vital nutrient that must be obtained from one's diet (Lyon *et al*., 2020). Vitamin B12 belongs to the cobalamins family of naturally occurring cobalt-containing compounds, which include a planar corrin ring that binds a single cobalt atom. In mammals, cobalt is crucial as a functional unit of vitamin B12, serving as an enzyme cofactor for two vitamin B12-dependent, enzyme-catalyzed processes (Stover, 2010; Suman & Gretarsdottir, 2019).

The presence of vitamin B12 in older persons has piqued people's curiosity. Apart from the clinical characteristics of B12 deficiency (i.e., irreversible neuropathy and megaloblastic anemia), new data suggest that subclinical B12 insufficiency (i.e., low biomarker status) may play a role in the progression of other chronic illnesses, particularly those affecting the elderly (Carmel, 2011). The threat of developing metformin-associated vitamin-B12 deficiency is significantly influenced by the patient's features such as age, health status, metformin-dose, and length of use (Atabi *et al*., 2021). In the result of this study, fig. 2 shows that there are no significant differences with respect to the age of the participants (patients), which is an important factor that has an effect on the result of this study, because severe deficiency of vitamin B12 often manifests as cobalamin deficiency, anemia, myelopathy and neuropathy and it mainly occurs in elderly people.

Some studies revealed that gender plays an important role in the metabolism of vitamin B12, in that, there appears to be a two-fold greater risk factor for deficiency among men, independent of anemia, homocysteine and folate status. Despite the prevalence of the same level of Vitamin B12 as reported in the neighboring Middle Eastern countries, the gender (sex) differences are surprising (Margalit *et al*., 2018; Naik *et al*., 2011).

Diabetes type 2 is a complicated, chronic, and varied disease. It is one that features a number of pathophysiological anomalies, as well as a variety of predispositions to complications and clinical reactions to

treatment (Wakeman & Archer, 2020). Patients with type 2 diabetes need to be under medical care all of the time, with a high level of self-management, to regulate blood sugar levels, lipid profiles and blood pressure. It also necessitates multifaceted strategies to reduce the risk of acute and long-term complications and diseases, such as retinopathy, neuropathy, nephropathy and cardiovascular disease, which are increased two- to three-fold in these patients and are a leading cause of death (Valensi & Picard, 2011). Metformin is a prescription medication that is used to treat type 2 diabetes. It belongs to a group of drugs known as antidepressants (biguanides). Glucose levels (glucose) in patients with type 2 diabetes mellitus are uncontrolled and rise faster than in healthy people. The medicine metformin does not cure diabetes, but it does assist in bringing the blood glucose (blood sugar) levels down to a healthy range (Sanchez-Rangel & Inzucchi, 2017). Metformin is a medication that must be used for a long time. This makes us question what kind of adverse effects it may have. Metformin can have both moderate and major adverse effects in both men and women (Pernicova *et al*., 2020).

When compared to the standard, the long-term use of metformin resulted in a considerable drop in blood vitamin B12 levels. The mechanism by which metformin lowers the serum vitamin B12 levels is unknown. However, the most plausible explanation is that the medication interferes with the activities of the calcium-dependent membrane in the terminal ileum, which are important for vitamin B12 intrinsic factor absorption (Kim *et al*., 2019). Other studies have looked into other factors, for example, whether the metformin-induced absorption problems may be alleviated by taking multivitamins, which typically include tiny levels of vitamin B12 (2 to 30g/d). A new study suggested that patients using long-term metformin therapy, use B12 vitamin supplements, to prevent vitamin B12 deficiency (Kamrul-Hasan *et al*., 2021; Valdés-Ramos *et al*., 2015).

In the present study a decreased in vitamin B12 occurred with medium- and long-term use of metformin, as compared to the standard group and this results agreement with other studies which prescription of role of metformin drug in reducing serum of Vitamin B12 level in human.

CONCLUSION

This study showed a significant reduced level of vitamin B12 in patients using Metformin in the medium- and long-term periods compared to the standard group that did not use Metformin.

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