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تأثير استخدام الميتفورمين على مستوى فيتامين ب 12 في المرضى الذين يعانون من داء السكري من النوع 2 في منطقة

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الملخص

الميتفورمين، هو خط العلاج الأول لمرض السكري النمط الثاني، يرتبط بسوء امتصاص فيتامين ب 12، وبالتالي يمكن أن يؤدي تطور نقص فيتامين ب 12 أو قصوره إلى ظهور مضاعفات خطيرة مثل الاعتلال العصبي أو فقر الدم في المستقبل. داء السكري من النوع 2هو أكثر أنواع مرض السكري شيوعًا، ويتميز بارتفاع السكر في الدم ومقاومة الأنسولين ونقص الأنسولين النسبي. الهدف من الدراسة: - تهدف هذه الدراسة الى معرفة تأثير استخدام الميتفورمين على مستويات فيتامين ب 12 وبعض القيم الدموية لدى مريضات السكري من النوع 2. الطريقة: - اشتملت دراستنا على 81 امرأة مصابة بداء السكري من النوع 2 تتراوح أعمارهن بين 33 و66 عامًا تم تقسيمهن إلى مجموعتين: المجموعة الاولى نساء يتناولن الميتفورمين كان عددهن 51 امرأة. المجموعة الثانية نساء يتناولن الأدوية المضادة للسكري (المجموعة الضابطة) وكان عددهن 30 إمرة. تم جمع البيانات من المشاركات عن طريق الاستبيان ونتائج الاختبارات المعملية، شملت هذه الاختبارات عد الدم الكامل، قياس فيتامين ب 12 ومقايسة الجلوكوز. النتائج: - أظهرت نتائج دراستنا وجود فروق ذات دلالة إحصائية في متوسط كرات الدم الحمراء والهيموجلوبين بين مجموعة الميتفورمين ومجموعة التحكم، ولكن لم تكن هناك فروق ذات دلالة إحصائية فيPCV ، MCV، MCH وMCHCبين المجموعتين.

كان متوسط تركيز فيتامين ب 12 (571.61 ± 171.59 بيكوغرام / مل) أقل معنوياً في مجموعة الميتفورمين مقارنة بمجموعة التحكم (693.93 ± 106.71 بيكوغرام / مل) (p <0.05) ولم يظهر أي ارتباط بين مدة استخدام الميتفورمين بالسنوات ومستويات فيتامين ب 12 في المصل 0.114 - = (r، 0.424 p =)؛ ولم تكن هناك علاقة بين استخدام جرعة الميتفورمين وفيتامين B12 في الدم 0.056- = (r، 0.699 = p).. نستنتج من هذه الدراسة أن الميتفورمين له دور في نقص فيتامين ب 12 لدى النساء المصابات بداء السكري من النوع 2.

الكلمات المفتاحية:

السكري
مقاومة الانسولين
ميتفورمين
النوع الثاني
فيتامين ب 12

The effect of metformin use on vitamin B12 level in patients with type 2 diabetes mellitus in Brack

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Keywords:

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Type 2
Vitamin b12

ABSTRACT

Background: - Metformin, a first-line treatment for type 2 diabetes (T2DM), is associated with malabsorption of vitamin B12, and therefore vitamin B12 deficiency or insufficiency can lead to serious complications such as neuropathy or anemia in the future. T2DM is the most common type of diabetes and is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency.
Objective: - The current study aimed to study the effect of metformin treatment on some blood values and vitamin B12 in type 2 diabetic patients.
Methods: - Our study included 81 women with T2DM, ages 33 to 66. The women were divided into two groups: women taking metformin (n = 51) and women taking antidiabetic drugs (the control group) (n = 30).
Data were collected using questionnaires and laboratory analyses of blood samples. The samples were tested for complete blood count, vitamin B12 measurement, and glucose assay.

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Results: - The results of our study showed that there were significant differences in mean RBCs and hemoglobin between the metformin group and the control group, but there were no statistically significant differences in PCV, MCV, MCH, and MCHC between the two groups. The mean vitamin B12 concentration (571.61 ± 171.59 pg/mL) was significantly lower in the metformin group compared to the control group (693.93 ± 106.71 pg/mL) ($p < 0.05$). No correlation was shown between the duration of metformin use in years and vitamin B12 levels in serum ($r = -0.114$, $p = 0.424$); there was no correlation between metformin dose use and serum vitamin B12 ($r = -0.056$, $p = 0.699$). We conclude from this study that metformin plays a role in vitamin B12 deficiency in women with type 2 diabetes.

Introduction: -

Diabetes mellitus (DM) is one of the most frequent public health concerns in both developed and underdeveloped countries [1].

DM is defined as a group of metabolic disorders characterized by the presence of hyperglycemia due to defects in insulin secretion, insulin sensitivity, or both. In addition, the effects of DM cause many organs to malfunction [2, 3].

Type 2 diabetes mellitus (T2DM), also known as "non-insulin-dependent diabetes" or "adult-onset diabetes," is the most common type of diabetes, accounting for 90–95% of all diabetes cases [4, 5]. T2DM is a complex metabolic and endocrine disorder caused by the interaction of genetic and environmental factors that cause varying degrees of insulin dysfunction in peripheral tissues as well as pancreatic beta cells [6].

According to the International Diabetes Federation (IDF), type 2 diabetes mellitus affected 463 million people worldwide in 2019 and is expected to reach 700 million by 2045 [7, 8].

Metformin, a drug commonly sold under the trade name "Glucophage," is an important prescription medication used for the management of diabetes [9]. Diabetes affects around 380 million individuals worldwide, with about 120 million of them on metformin [10].

American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), and the International Diabetes Federation (IDF) recommend metformin as the first choice of therapy for glycemic control [11, 12].

The mechanisms of action of metformin are distinct from those of other oral antihyperglycemic medications. Metformin lowers blood glucose levels by reducing hepatic glucose synthesis (gluconeogenesis), lowering glucose absorption in the intestine, and improving insulin sensitivity by enhancing peripheral glucose uptake and utilization [11, 13, 14]. The mechanisms by which metformin contributes to weight loss can be explained by its effects on lowering insulin resistance, lowering leptin and ghrelin levels after glucose administration, and inducing lipolytic and anorexic effects [15].

Vitamin B12 is a water-soluble vitamin that is produced in nature by microorganisms. It is also called cobalamin (Cbl), and it has two biologically active forms: methylcobalamin and adenosylcobalamin. It works with many other B vitamins to carry out key functions in the human body [5, 16, 17]. Liver, eggs, meat, cheese, fish, and milk, as well as fortified cereals and supplements, are the main sources of vitamins. The liver, followed by the kidneys and the heart, is a particularly good supplier of cobalamin [18].

The mechanism underlying metformin-induced vitamin B12 deficiency is unknown [19]. Several mechanisms have been proposed to explain the vitamin B12 deficiency observed in patients with type 2 diabetes taking metformin therapy, including changes in small intestinal motility, which promote bacterial overgrowth and B12 consumption by bacteria, and changes in intrinsic factor (IF) levels, which may impair vitamin B12 absorption [18], changes in bile acid metabolism and reabsorption, resulting in impaired enterohepatic vitamin B12 circulation. Increased vitamin B12 accumulation in the liver leads to altered vitamin B12 tissue distribution and metabolism [20].

Current evidence suggests that metformin affects calcium channels, resulting in decreased calcium-dependent membrane activity in the ileum and malabsorption of vitamin B12 bound to intrinsic factors. A measurable decrease in serum vitamin B12 concentration can occur as soon as 3–4 months after starting metformin therapy, whereas symptomatic deficiency takes up to 5–10 years due to large body stores in the liver that are not quickly depleted [9, 21].

Material and methods

This study was conducted on 81 women attending Brack General Hospital and Fazzan Laboratory during the period from November 2021 to August 2022; their ages ranged between 33 and 66 years, and they were classified into two groups: 51 women who were treated with metformin, and 30 women with diabetes who were not treated with metformin (control groups).

The inclusion criteria are:

The duration of treatment with metformin for not less than one year

Exclusion criteria:

Patients with newly diagnosed type 2 diabetes, patients with pernicious anemia, pregnant women, type 1 diabetes mellitus, chronic diseases, inflammatory bowel disease, vegetarians, sepsis, severe infection, or cancer were excluded, cirrhosis of the liver or heart failure or kidney failure, or if they are taking oral vitamin B12 and calcium supplements, histamine-2 receptor blockers (H2 blockers), or proton pump inhibitors (PPIs). Individuals with a history of treatment with vitamin B12 injections in the past 6 months were also excluded.

Data were collected using questionnaires and laboratory analyses of blood samples. 5 ml of blood was collected from each of them. 2 ml into an EDTA tube for a complete blood count (CBC), and the remaining 3 ml was collected in a plain tube for the determination of vitamin B12 and blood glucose levels. CBC is estimated by the BC-3000Plus Auto Hematology Analyzer; Mindray, glucose check by photometer 4040; and vitamin B12 assay by the Roche Diagnostic Cobas e411 Immunoassay System—a fully automated, random-access, software-controlled system for immunoassay analysis.

Statistical analysis:

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistics software (version 26; IBM). The data were expressed as the mean ± standard deviation. Independent t-tests were used to detect differences between groups, and Pearson's correlation coefficient analysis was used to determine the correlations between serum vitamin B12 and metformin use. A p-value of less than 0.05 was considered statistically significant.

Results: -

The study group was divided into two groups: 51 women treated with metformin and 30 women with diabetes not treated with metformin who were enrolled as control groups. Their ages ranged from 33 to 66 years, with a mean of 54.08 ± 7.69 years. After conducting the required tests, the results were as follows:

The independent t-test was used to compare between the two groups in different parameters. No statistical difference was found between them in age, fasting blood sugar, and duration of diabetes mellitus as shown in Table (1).

Table 1: shows the Distribution of age, fasting blood sugar, and duration of diabetes.

	Metformin group (N=51)	**Control group (N=30)**	**P- value**
	(mean ± SD)		
Age(years)	54.12±7.74	54.00±7.74	**0.949**
Fasting blood sugar (mg/dL)	162.38±48.59	170.95±67.85	**0.547**
Duration of diabetes	4.45±4.02	4.70±4.02	**0.789**

(Years)			

The data in Table 2 shows the different blood variables between the study groups, and the results showed that there were statistically significant differences in the average RBCs and hemoglobin between the metformin group and the control group, where the p-value was < 0.05. Moreover, PCV, MCV, MCH, and MCHC showed non-significant changes in the metformin groups compared to the control group, where the p-value was >0.05.

Table 2: shows various hematologic variables among study participants.

Variable	Metformin group (N=51)	Control group (N=30)	P-value
	(mean ± SD)		
RBC (10^{12}/L)	4.42±0.59	4.65±0.39	**0.035***
HGB (g/dl)	12.36±1.63	13.07±1.12	**0.041***
HCT (%)	38.02±4.88	39.69±3.30	**0.099**
MCV (f L)	85.92±7.09	85.12±5.62	**0.597**
MCH (pg)	28.22±2.83	28.20±1.68	**0.974**
MCHC (g/dL)	32.83±1.38	32.89±1.13	**0.845**

The results in Table 3 show that the average vitamin B12 in the metformin group was 571.61±171.59 and in the control group was 693.93±106.71. The difference in the average levels of vitamin B12 in the serum of the two groups was statistically significant (the value of P = 0.001).

Table3: shows mean serum vitamin B12 levels in groups.

	Metformin group (N=51)	Control group (N=30)	P- value
Serum Vitamin B12 (pg/mL)	571.61±171.59	693.93±106.71	**0.001****

Table 4 shows the distribution of vitamin B12 levels based on the duration of the patient's use of the drug metformin. The majority of the 32 patients (63%) had used metformin for less than 5 years, and 19 (37%) had used metformin for 5 years or more. The mean levels of vitamin B12 at less than 5 years and 5 years and over were 613.68 ± 163.52 and 500.77 ± 165.21, respectively.

Table 4: shows the distribution of mean vitamin B12 levels according to the duration of metformin use in years.

Duration	Metformin group (N=51)		
	N	%	Mean±SD
< 5 Years	32	63%	613.68±163.52
≥ 5 years	19	37%	500.77±165.21
			P value =0.022*

Pearson correlation analysis was done to demonstrate the relation between metformin use duration and blood vitamin B12 levels and demonstrated no statistically significant correlation between them (r = - 0.114, P = 0.424) as shown in Figure (1).

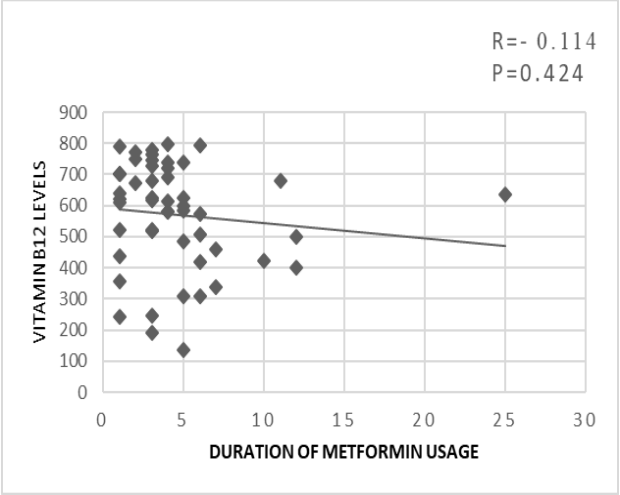

Figure 1: shows the correlation between the duration of metformin use in years and serum vitamin B12 levels.

The data presented in Table 5 showed that there were no significant differences in the average levels of vitamin B12 based on the dose taken, where the p-value was p >0.05, as the average levels of vitamin B12 in the serum of those taking metformin <1000 mg and ≥1000 mg were 580.30 ± 185.42 and 562.59 ± 159.25, respectively.

Table 5: shows the distribution of mean vitamin B12 levels according to the dose of metformin.

Doses (mg)	Metformin group (N=51)		Mean±SD
	N	%	
<1000	26	51%	580.30±185.42
≥1000	25	49%	562.59±159.25
			P value =0.717

Figure 2 shows that there was no significant correlation between metformin dose use and serum vitamin B12 (r = -0.056, P = 0.699).

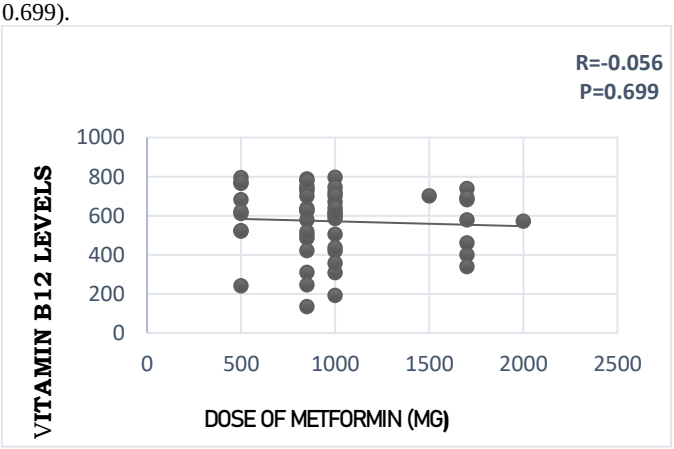

Figure 2 shows the relationship between metformin dose and vitamin B12 level in the blood.

Discussion: -

Metformin is the first and most commonly prescribed drug for the treatment of type 2 diabetes [22]. It is one of the medications used to lower blood sugar levels and improve insulin sensitivity. It can be used alone or in combination with other medications and there are several proposed mechanisms by which it works, one of which, interferes with the absorption of vitamin B12, resulting in a vitamin B12 deficiency [23].

The current study found no significant differences in age, diabetes duration, and FBS. This finding was supported by a study conducted in Qatar [24]. According to some recent epidemiological

studies, T2DM is also associated with increased erythrocyte osmotic fragility [25]. Long-term metformin treatment, on the other hand, has been linked to vitamin B12 deficiency and anemia in T2DM patients [26, 27].

Our results showed a decrease in the concentration of hemoglobin the and number of red blood cells in the metformin group, and these results were in agreement with Fakkar et al. (2022) [28]. Protein oxidation and hyperglycemia in diabetics induced an increase in the formation of lipid peroxides that may lead to RBCs hemolysis [29] and subsequently, a decrease in RBC count and hemoglobin levels.

There were no statistically significant differences between the mean MCV, MCH, MCHC, and PCV, with a p-value >0.05. Results were similar to a study in India, where they found no differences in mean MCV [30]. This differs from a study in Nigeria, which showed that there are significant differences between mean MCV and mean MCH [31]. The classic form of anemia caused by vitamin B12 deficiency is megaloblastic anemia (MCV > 100 fl). However, the average MCV level observed in our subjects was not >100 fl and previous reports have indicated that up to 30% of disorders responsive to vitamin B12 have a normal MCV [32, 33]. Also, the masking of macrocytic blood expression in megaloblastic anemia by coexisting with thalassemia, iron deficiency, and chronic diseases has been widely reported [33, 34]. As a result, examination of red cell distribution width and reticulocyte index, in combination with careful examination of blood via peripheral blood smear, may be useful in differentiating anemia associated with vitamin B12 deficiency from anemia due to other factors [35].

Previous research found a relationship between low serum B12 levels and metformin use [23, 36, 37]. Long-term metformin therapy has been shown to increase the risk of peripheral neuropathy, cognitive decline, and macrocytic anemia [38]. Vitamin B12 is an important nutrient for health. It can play a significant role in the development of red blood cells and the function of the nervous system and brain. A deficiency of vitamin B12 may enhance the severity of diabetic neuropathy [39]. In this study, mean vitamin B12 levels in the study group were 571.61 ± 171.59 pg/mL, and in the control group were 693.74 ± 106.71 pg/mL. The difference in vitamin B12 between the two groups was statistically significant (p = 0.001), deficiency of vitamin B12 is more common in patients receiving metformin compared to patients not receiving metformin. [28]. These results were in agreement with Muhammad and Al-Karim (2021) and Razada et al. (2017), who found significant differences between the two groups [40, 41]. metformin delays the absorption of glucose, it has an impact on bacterial overgrowth and small bowel motility [42]. Also, the decrease in B12 absorption caused by metformin may be due to digestive alterations that cause the binding of the B12-intrinsic factor (IF) complex [43].

The study showed that there were statistically significant differences between the metformin group with a duration of less than 5 years and the group with a duration of 5 years or more, where p = 0.02, and this study agreed with Agarwal P et al. Where they reported a similar significant association between the two groups for average vitamin B12 in diabetic patients for more than 5 years, while they found that there were no statistically significant differences between the exposure dose of metformin and vitamin B12 [44], and this is what was obtained in this study.

The current study also showed that there was no significant association between the duration of metformin use and serum vitamin B12 levels (r = -0.114, P = 0.424). In addition, there was no significant association between increased doses of metformin and serum vitamin B12 (r = -0.056, P = 0.699), which was similar to a study by Romero and Lozano, where results showed no association in duration and metformin dose [45], And the results contradicted other studies, where they found that there is a relationship between the duration of metformin use and the dose taken for vitamin B12 deficiency [46, 47].

Conclusion: -

In conclusion, in our study, the vitamin B12 level was lower in type 2 diabetic patients on metformin compared to those on other oral anti-diabetic drugs. As a result, we suggest that vitamin B12 levels be assessed annually after initiating metformin therapy in patients with type 2 diabetes who are on long-term treatment with metformin, even in the absence of hematological.

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