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Correlation Between Metformin and Vitamin B12 level among type II Diabetics in Southern Gaza

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الملخص

الإرتباط بين عقار المتفورمين وفيتامين ب12 في مرضى السكري النوع الثاني من جنوب غزة

يعتبر المتفورمين(الجليكوفاج) هو الأكثر شيوعا وتأثيراً في علاج مرض السكري من النوع الثاني, والذي يقلل امتصاص فيتامين B12. **الهدف:** تحديد مستويات فيتامين B12 عند مرض السكري من النوع الثاني الذين يعالجون بالمتفورمين في جنوب غزة. **الطرق والأدوات:** دراسة وصفية شملت 80 مشاركا مقسمين بالتساوي إلى مجموعتين: (مجموعة مرضية) تتكون من 40 مريضا يعانون من مرض السكري من النوع الثاني الذين يعالجون بالميتفورمين. و(مجموعة ضابطة) تتكون من 40 شخصا يعانون من مرض السكري من النوع الثاني ولا يعالجون بالميتفورمين, وتتراوح أعمارهم بين 50−94. وقد تم الحصول على البيانات من المقابلة الشخصية للمرضى في المستشفيات وإجراء الفحوصات مثل مستويات فيتامين B12 وتعداد الدم الكامل. **النتائج:** ظهر نقص ذو دلالة إحصائية عند P=0.008 في مستوى فيتامين B12 في 30% من مرضى السكري الذين يأخذون المتفورمين، ولم يظهر نقص في المجموعة الضابطة. وهناك ايضا ارتباط عكسي بين الجرعة ومستوى فيتامينB12(r=−0.490) كذلك يوجد ارتباط عكسي بين مدة تناول المتفورمين ومستوى فيتامينB12 (r=0.513). أظهر تحليل صورة الدم انخفاضا احصائيا في عدد خلايا الدم الحمراء والبيضاء عند مجموعة المرضى مقارنة مع المجموعة الضابطة p=0.001 اما مستوى MCV وسمك توزيع خلايا الدم الحمراء (RDW) فقد كانا مرتفعين احصائيا في مجموعة المرضى مقارنة مع المجموعة الضابطة (p=0.000) كذلك مستويات الهيموجلوبين (p=0.149) والهيماتوكريت (Hct)، (p=0.106) ومتوسط هيموجلوبين خلايا الدم الحمراء(MCH) (P=0.435) ونسبة احتواء خلايا الدم الحمراء على الهيموجلوبين(MCHC) (P=0.903) والصفائح الدموية(PLT) (P=0.636) لم تظهر أى فروق احصائية. **الاستنتاج:** النقص في مستوي فيتامين B12 يحدث بشكل مستمر في مرضى السكري من النوع الثاني والذين

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يعالجون بالمتفورمين هذا النقص يعتمد علي مدة استخدام الميتفورمين ومقدار الجرعة. هناك تأثير سلبي واضح على معظم مكونات الدم بسبب استخدام الميتفورمين.

الكلمات المفتاحية: الميتفورمين – السكري – فيتامين B12

Abstract

Metformin is the cornerstone in the treatment of diabetes and is the most frequently prescribed as the first line therapy for individuals with type 2 diabetes. **Objective:** To determine the correlation between vitamin B_{12} and metformin in diabetes mellitus type II patients treated by metformin in Southern Gaza.

Methodology and tools This case control descriptive study comprised of 80 diabetic participants divided equally into two groups: experimental group, 40 diabetics patients taking metformin, and control group, 40 diabetics not taking metformin, aged between 50-95years old. Interviews were conducted. Serum vitamin B12 and CBC were measured. Data were analyzed using SPSS version 20. **Results:** VitaminB_{12} deficiency was present in 30% of the cases and non apparent in the control group had deficient vitaminB12. There were statistically significant negative association between dose and vitamine B12. Besides, there was statistically significant negative association between duration and vitamine B12, by means of an increase in duration will lead to decrease in vitamin B12 level. Blood rofile analysis showed a statistically significant positive association between vitamin B12 and Hb, WBC , RBC and PLT; vitamin B12 decrease will lead to a decrease in Hb, WBC, RBC and PLT at (be less than 0.05). **Conclusion:** Vitamin B_{12} deficiency occurs more frequently in patients with type 2 diabetes with longer duration of metformin use and in those taking larger amounts of metformin. There was a clear side effect of metformin use on most of blood indices.

Key words: Metformin, Diabetes Type-2, Vitamin B12

1-Introduction:

Metformin (Glucophage) is a popular and highly effective oral diabetes drug used to help management of Type 2 diabetes. This drug works by lowering the amount of glucose made by the liver and by making the body's cells more sensitive to insulin. Metformin also has some other beneficial effects that may lower blood lipids; (cholesterol and triacylglycerols) and, in

some people, promote a small amount of weight loss **(Ting et al., 2006; Mazokopakis and Starkis, 2012**; **and Ko Shi et al., 2014).**

Metformin is a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes **(Kirpichnikov et al., 2002).** It is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality, which is a major cause of death in patients with type 2 diabetes **(Pyorala et al., 1987).** There are, few disadvantages to the use of metformin. Metformin does, however, induce vitamin B-12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency **(DeFronzo, 2009; Bauman et al., 2000 ;Ting et al., 2006**; **Bell, 2010; and Aroda et al 2016**). In addition, metformin treatment has been reported to be associated with decreased folate concentration, although the mechanism of this effect has not been elucidated **(Carlsen et al., 1997).** Decreases in both folate and vitamin B-12 concentrations might, in turn, result in an increase in homocysteine concentrations , an independent risk factor for cardiovascular disease, especially among individuals with type 2 diabetes **(Boushey et al., 1995; Nygård et al., 1997 and Welch et al., 1998).**

Vitamin B_{12} or cyanocobalamin is relatively large and complex water-soluble vitamin. The molecular weight of vitamin B_{12} is equal to 1355.4 **(Watanabe, 2007).** Vitamin B_{12} represents all potentially biologically active cobalamins **(Russell and Alpers, 2002)**. Animal foods but not plant foods are considered to be the major dietary sources of vitamin B_{12} **(Ball, 1998).** Vitamin B_{12} is synthesized only by certain bacteria **(Schneider and Stroinski, 1987).**

Most of the current global clinical practical recommendations including those of the American Diabetes Association, the European Association for the Study of Diabetes, and the Korean Diabetes Association, propose that metformin, if there are no contraindications, should be initiated with concurrent lifestyle modifications at initial diabetes diagnosis **(American**

Diabetes Association, 2009; Committee of Clinical Practice Guidelines, 2011; and WHO, 2014).

Most of the side effects of metformin are mild and usually include gastrointestinal symptoms, such as abdominal distress, soft stools, and diarrhea **(American Diabetes Association,(2006)**. Serum vitamin B_{12} levels have been reported to be inversely associated with the dose and duration of metformin use **(Tomkin, 1971; Ting, 2006; De Jager, 2010 and Marwan et al., 2016)**. Previous studies reported that an average of 10 to 30% of patients exhibited malabsorptive vitamin B_{12} deficiency **(Bauman et al., 2000; Lael Reius et al., 2012 and Marwan et al., 2016).** Although the clinical significance of vitamin B_{12} deficiency related to metformin treatment is debatable, monitoring for vitamin B_{12} has been recommended for patients with type 2 diabetes, especially those on long-term metformin treatment. Clinically, vitamin B_{12} deficiency could lead to altered mental status, megaloblastic anemia, and neurological damage **.**Unfortunately, peripheral neuropathy due to vitamin B_{12} deficiency may be confused with diabetic peripheral neuropathy (**Bell, 2010 and Pierce et al.,, 2012**). The progression of neurologic damage due to vitamin B_{12} deficiency can be stopped by early detection and treatment with cobalamine supplementation**((Lindenbaum et al., 1988)**. As metformin has been prescribed worldwide and treatment periods increased, the prevalence of metformin-induced vitamin B_{12} deficiency may have also significantly increased. However, the relationship between metformin use and vitamin B_{12} deficiency in the Asian population has not been widely investigated

Significance Diabetes mellitus type II is a common disease in the Palestinian community and considered as one of the health problems among patients in Southern Gaza. Metformin seems to be an etiologic factor in vitamin B_{12} deficiency. This study brought into focus this health problem.

Objective The general goal of the study is to determine the correlation between metformin, vitamin B_{12} level and blood indices, in type-2 diabetics.

2-Materials and methods: Study design and samples: The present study is descriptive case control study. The population of the study includes patients suffering from Diabetes mellitus type 2 aged over 50 years old in Southern Gaza. The sample of the study consisted of 80 patients divided equally into two groups; case group, consisting of 40 patients with Diabetes mellitus type 2 taking metformin , and control group, consisting of 40 diabetics not taking metformin. The study was conducted in the main public hospitals: Nasser Hospital, Gaza European Hospital in Khanyounis, and Al- Najar Hospital in Rafah. The study was carried out during the period of February-June 2016.

Inclusion and exclusion criteria: Known patients with Diabetes mellitus type 2**.** Age between 50-93 years. Patients with renal or liver diseases, endocrine disorders, malabsorption syndrome, gastrectomy, non-vegetarians, Pregnant women, tobacco and drug users like- Antacids, vitamin B12 supplements (either oral or parenteral within last 2-3 years), Fibrec Acid Derivatives, Thiozolidinediones, insulin were excluded **(Twinkal et al., 2016).**

Ethical considerations and permissions: Each patient is given informed consent to get approval for his or her patient participation in this study and the objective of the study is explained to patients. **Data collection:** An interview was used for filling in questionnaires delete designed for matching the study need of the study population. All interviews were conducted face to face by the researchers. **Blood Collection:** All blood samples were collected in a lavender top tube (EDTA) and serum taken from patients, and sent to the laboratory within 2 hours. EDTA tube were collected and tested during 2 hours. Hemolysed sample is rejected, and sample tubes were allowed to reach room temperature (15–30°C) before performing the assay.

Complete blood count (CBC) CBC was performed on all samples including: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin

concentration (MCHC), red cell distribution width (RDW), and platelets (PLT) using cell dyne 1800 (Germany) for analysis(**Heymann et al., 1977)**
Biochemical analysis Determination of Serum Vitamin B_{12} Vitamin B12 is determined quantitatively using ELISA **(Snow, 1999).**

Expected Values for Vitamin B12: Adult: 200 –835 pg/ml, level of ≤ 200 pg/ml considered as low.

Statistical analysis: Data were analyzed using SPSS/ PC, Statistical Package for the Social Sciences, version 20.0. Simple distribution of the study variables and the cross tabulation were applied. Chi-square was used to identify the significance of the relations, associations, and interactions among various variables. Pearson's correlation test was applied. The results in all the above mentioned procedures were accepted as Statistically significant when the p-value was less than 5% ($p<0.05$). SPSS program version 20.0 was used for correlation graphs plotting

3-Results and Discussion

Socio-demographic characteristics of study participants: Table (1) showed the socio demographic characteristics of the study population. These results indicated that there were statistically insignificant differences between the two groups in their socio-demographic characteristics.

Variable	Category	Case Freq. (%)	Control Freq. (%)	df	Chi Square	*P* value
Age (years)	50 – 60	13 (32.5)	18 (45.0)	3	3.556	0.314
	61 – 70	15 (37.5)	9 (22.5)			
	71 - 80	9 (22.5)	7 (17.5)			
	81 - 93	3 (7.5)	6 (15.0)			
	Total	40 (100.0)	40 (100.0)			
Level of education	Primary	13 (32.5)	14 (35.0)	2	0.074	0.964
	Secondary	14 (35.0)	13 (32.5)			
	University	13 (32.5)	13 (32.5)			
	Total	40 (100.0)	40 (100.0)			

Place of residency	City	13 (32.5)	13 (32.5)	2	0.297	0.862
	Camp	14 (35.0)	12 (30.0)			
	Village	13 (32.5)	15 (37.5)			
	Total	40 (100.0)	40 (100.0)			
Place of treatment	PHC	13 (32.5)	14 (35.0)	2	0.074	0.964
	EGH	14 (35.0)	13 (32.5)			
	Private	13 (32.5)	13 (32.5)			
	Total	40 (100.0)	40 (100.0)			
Exercise	No	23 (57.5)	27 (67.5)	1	0.853	0.356
	Yes	17 (42.5)	13 (32.5)			
	Total	40 (100.0)	40 (100.0)			

Table (1): Socio-demographic characteristics of study participants

*The samples were collected in Nasser hospital ,EGH and AL Najar hospital.

Table (2) shows the distribution of health problems in experimental and control groups. This result reflected statistically insignificant association between taking metformin and vomiting, but those with diabetes and taking metformin are 3.0 times at higher risk of suffering from vomiting compared with those in control group. Also it reflects insignificant association between taken metformin and diarrhea, but those delete taking metformin are 1.758 times at higher risk of suffering from diarrhea compared to those in control group. Results of this work reflect statistically insignificant association between metformin and weakness, and that diabetic patients taking metformin are 4.030 times at higher risk of suffering from weakness compared to controls.

Variable	Category	Case Freq. (%)	Control Freq. (%)	df	Chi Square	*P* value
Vomiting	No	30 (75)	36 (90)	1	3.117	0.077
	Yes	10 (25)	4 (10)			
	Total	40 (100.0)	40 (100.0)			

Diarrhea	No	24 (60)	29 (72.5)	1	1.398	0.237
	Yes	16 (40)	11 (27.5)			
	Total	40 (100.0)	40 (100.0)			
Weakness	No	33 (82.5)	38 (95.0)	1	3.130	0.077
	Yes	7 (17.5)	2 (5.0)			
	Total	40 (100.0)	40 (100.0)			

Table (2): Health problems in cases and control

Table(3a) shows that 30% of cases and none of controls had low vitamin B12 level (P=0.000). This result reflects statistically significant association between metformin ingestion and vitamin B12 level, also; diabetics taking metformin are 2.429 times at higher risk of suffering from vitamin B12 deficiency compared to those in control group.

Vitamine B_{12} level	**Case N (%)**	**Control N (%)**	**CI(mean) (95% interval)**	***P* value**
Low	12 (30.0)	0	2.429 (1.828 – 3.226)	0.000 *
Normal	28 (70.0)	40 (100.0)		
Total	40 (100.0)	40 (100.0)		

Table (3a): Comparison of Vitamine B_{12} between the two groups

*= Significant at 0.05. The range of 1.828 – 3.226 represents the times of high risk of suffering from vitamin B12 deficiency within cases compared to control with a mean of 2.42 times.

Table (3b) shows that there are statistically significant differences at $p<0.05$ in vitamin B12 level between experimental group members (mean rank=33.66) and control group (mean rank= 47.34), z value was –2.632, P value 0.008. this means; vitaminB12 level was significantly lower among cases compared to control members.

Vitamin B12 level	**Case Mean rank**	**Control Mean rank**	**Z score**	***P* value**
	33.66	47.34	- 2.632	0.008 *

Table (3b): Differences in Vitamin B_{12} between the two groups (Mann Whitney test). *= significant at 0.05

Blood profile analysis: Table (4) shows that WBCs and RBCs are significantly lower among cases compared to controls. Furthermore, MCV and RDW levels are significantly higher among cases compared to controls. On the other hand, there are statistically insignificant differences between the groups in levels of Hb (P=0.149), HCT(P=0.106), MCH (P= 0.435) , MCHC (P= 0.903) and PLT (P=0.636).

Variables	Control (without metformin) (N = 40)	Cases (with metformin) (N =40)	t value	*p* value
	Mean(SD)	Mean(SD)		
Hb	13.617(1.604)	13.080(1.694)	1.457	0.149
WBC*10^3	7.600(1.542)	6.775(1.926)	2.114	0.038
HCT	43.800(4.071)	42.100(5.153)	1.637	0.106
RBC*10^6	4.881(0.106)	4.707(0.300)	3.446	0.001
MCV	85.760(1.321)	89.720(5.691)	-4.287	0.000
MCH	33.770(30.049)	30.030(1.475)	0.785	0.435
MCHC	33.720(1.015)	33.690(0.989)	0.123	0.903
RDW	13.075(0.500)	13.775(0.846)	-4.500	0.000
PLT	285.93(59.996)	297.18(137.265)	-0.475	0.636

Table (4): Differences in CBC parameters between the two groups

Effects of metformin within the case group

Table (5) showsthat there is a high correlation between duration of taking metformin and level of vitamin. B12 (R= 0.894), and that 79.9% of changes in level of vitamin. B12 is explained by duration of taking metformin.

Pearson correlation (R)	R square	P value	Duration
0.894	0.799	0.000	-34.988

Table (5): Changes in B12 related to duration (simple linear regression)

*= Significant at 0.05, R square value represents that 79.9% of changes in vitamin B12 level is due to duration of metformin ingestion and the level of vitamine B12 will decrease by 34mg each year.
Table (6) shows that there is a moderate correlation between the dose of metformin and the level of vitamin. B12 (R= 0.729), and that 53.1% of changes in the level of vitamin. B_{12} are explained by the dose of metformin.

Pearson correlation (R)	R square	P value	Dose
0.729	0.531	0.000	-0.231

Table(6): Changes in B_{12} related to dose (simple linear regression)
*= Significant at 0.05, R square value represents that 53% of changes in vitamin B12 level is due to metformin dose and each one mg of metformin will decrease vitamin B12 level by 0.23mg.

Association between Vitamin B_{12} and selected variables Table (7) and Figures(1, 2, 3 and 4) show statistically significant positive association at p value of <0.05 between vitamin B12 and Hb (r= 0.0.282), vitamin B12 and WBC (r= 0.356), vitamin B12 and red blood cells (r= 0.255); also vitamin B12 and PLT count (r= 0.372).

Variables	**Vitamin B12**	
	Spearman correlation (r)	**P-value**
Hb	0.282	0.01
WBC	0.356	0.01
RBCs	0.255	0.02
PLT	0.372	0.001

Table (7): Correlation of vitamin B12 with study parameters

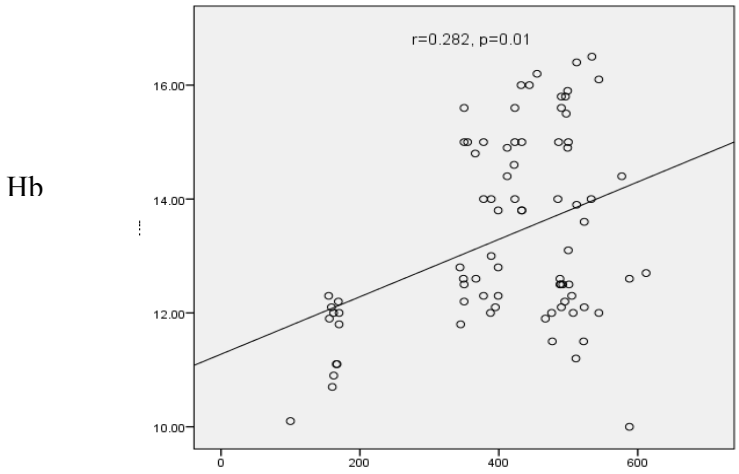


Figure (1) Correlation between Vitamin B12 level and Hb of the study population

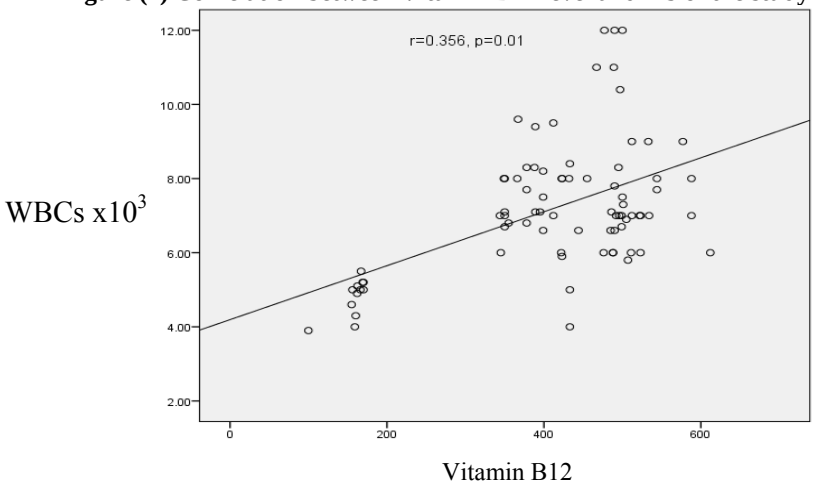


Figure (2). Correlation between Vitamine B12 level and WBC of the study population

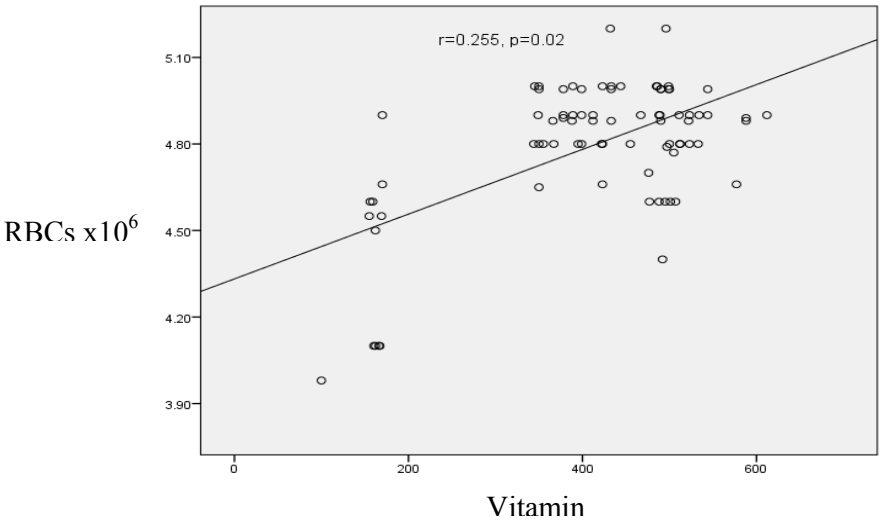


Figure (3). Correlation between Vitamine B12 level and RBC of the study population

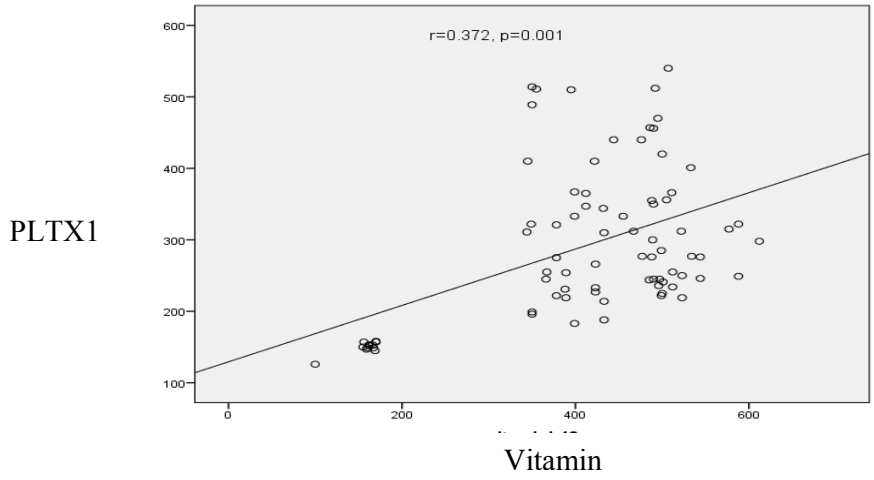
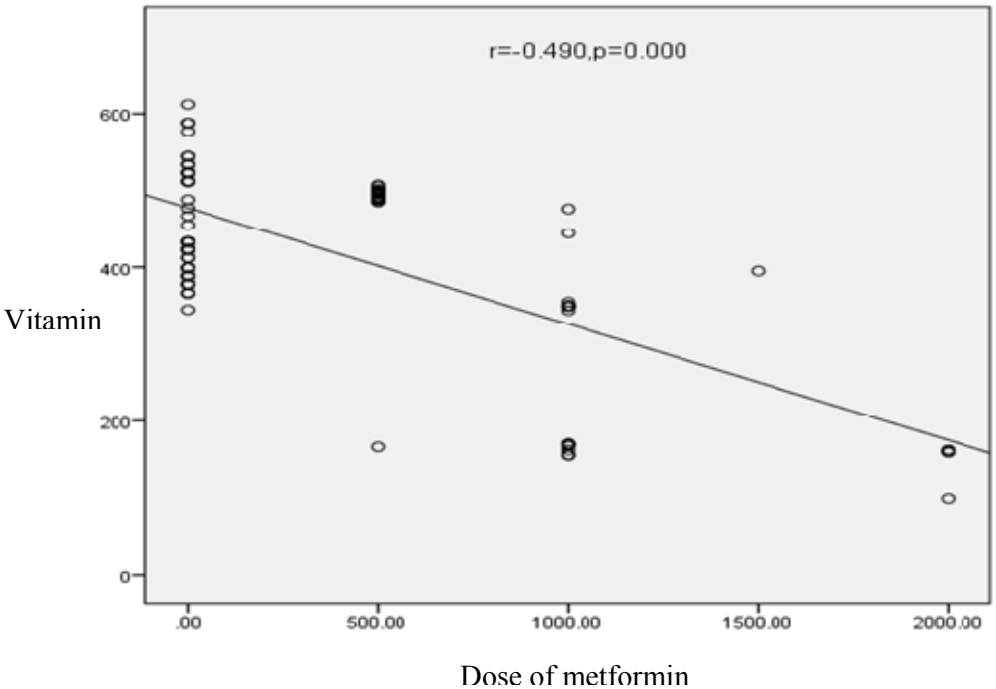


Figure (4). Correlation between Vitamine B12 level and PLT of the study population

Association between Dose and vitamin B12: Table (8) and Figure (5) show statistically significant negative association at 0.000 between metformin dose and vitamin B12 (r= -0.490).

Variables	Dose	
	Spearman correlation (r)	P-value
Vitamin B12	-0.490	0.000

Table(8): Correlation of metformin dose with vitamine B12



Figure(5). Correlation between metformin Dose level and Vitamine B12 in cases

Association between Duration and vitamin B12: Table (9) and Figure(6) show statistically significant negative association between duration of metformin ingestion and vitamin B12 (p=0.000 r= -0.513).

Variables	Duration	
	Spearman correlation (r)	P-value
Vitamine B12	-0.513	0.000

Table (9):Correlation between duration of metformin and vitamine B12

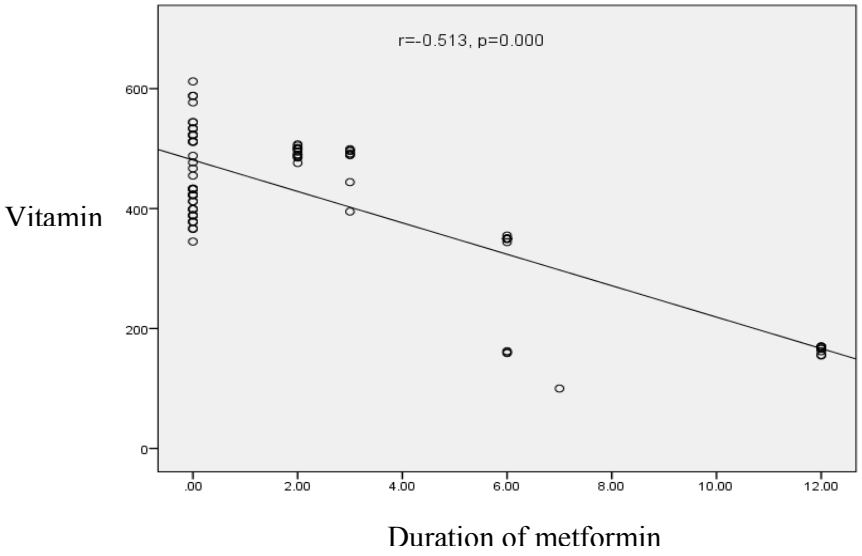


Figure (6). Correlation between duration of metformin administration and Vitamine B12 level

Based on our results, it can be said demonstrated that daily metformin dosage and treatment duration were the most consistent risk factors for vitamin B12 deficiency.

In the present study, prevalence of vitamin B12 deficiency in diabetic patients taking metformin was 30%. These results are quiet close to the prevalence of vitamin B12 deficiency shown in previous studies, which ranged from 5.8 to 30% in patients taking metformin. **(Matthew et al., 2009, Mazokopakis&Starakis, 2012; Reinstatler et al., 2012, Lael et al., 2012, Akinlade et al., 2015, and Marwan et al., 2016**). This wide variation in the reported prevalence could probably be explained by the variability of measurement methods of vitamin B12 levels in the laboratories or may be due to variation in metformin dose and duration of ingestion (**Akinlade et al., 2015)**. The present study shows a clear inverse relationship between the dosage or duration of metformin use and vitamin B12 deficiency in patients with type 2 diabetes. According to correlation analysis, vitamin B12 deficiency was associated with metformin dosage and length of administration, it was not until 2006 that the increased risk of the vitamin B12 deficiency with metformin was rediscovered through a case control study of Chinas patients, which showed a correlation between the dose and duration of metformin use with vitamin B12 deficiency **(Bell, 2010).**

There is no known mechanism of metformin-induced vitamin B_{12} deficiency but some hypotheses included bacterial overgrowth in the small intestine, which has been attributed to diabetes mellitus, changes in small bowel motility, changes in bacterial flora, competitive inhibition or inactivation of vitamin B_{12} absorption, or an effect of calcium on cell membranes **(Greibe et al., 2013, Liu et al., 2014, Niafar et al., 2015, and Aroda et al., 2016).**

The more likely explanation for metformin induced vitamin B12 deficiency is that metformin has an effect on calcium dependent membrane action in the terminal ilium. Absorbtion of vitamin B12-intrinsic factor complex in calcium dependent and metformin interferes with this absorption**.** Dietary calcium supplementation referees metformin induced vitamin B12 malabsorption, which supports the previous hypothesis **(Bell, 2010)**. Chronic metformin use results in vitamin B12 deficiency and the exhaustion of vitamin B12 stores usually occurs after twelve to fifteen years of absolute vitamin B12 deficiency. Vitamin B12 malabsorption is a chronic

complication of metformin therapy, which can result in irreversible neuronal damage. On metformin therapy vitamin b12 levels should be checked on an annual basis **(Pflipsen et al ., 2009; and Bell, 2010**). An alternative and a more practical and cost-effective method to avoid vitamin B12 deficiency would be an annual vitamin B12 injection that would provide more than the annual vitamin B12 needs for every patient on chronic metformin therapy **(Niafar et al., 2015 and Aroda et al., 2016).** Furthermore, a daily multivitamin may protect against B12 deficiency **(Pflipsen et al ., 2009).** Long term use of metformin was associated with biochemical B12 deficiency and anemia. Routine testing of vitamin b12 levels in metformin treated patients should be considered **(Aroda et al., 2016).** Also the result of the present study shows a clear effect of metformin on decreasing of RBCs, WBCs, MCH and Hb levels. Aroda and others, result is in agreement with the present work result of blood indices. This may be due to the role of vitamin B12 in blood indices formation and Rbc's metabolism.

Conclusion The present work recorded that vitamin B_{12} deficiency occurs more frequently in type 2 diabetics with longer duration of metformin use and in those taking larger amounts of metformin. There was no significant association between gender and Vitamin B12 levels among cases and controls.. The present study suggested the need for regular CBC and vitamin B_{12} monitoring in type 2 diabetics receiving metformin.

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