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THE EFFECT OF METFORMIN ON B12, LDL, AND LIVER ENZYMES LEVEL IN DIABETIC PATIENTS IN IRAQ

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Аннотация: Background: Diabetes mellitus is a heterogeneous group of disorders characterized by hyperglycemia due to an absolute or relative deficit in insulin production or action. The chronic hyperglycemia of diabetes mellitus is associated with end organ damage, dysfunction, and failure, including the retina, kidney, nervous system, heart, and blood vessels. Metformin (dimethylbiguanide) has become the preferred first-line oral blood glucose-lowering agent to manage type 2 diabetes.

Method: In private laboratories located in Baghdad, Iraq, a prospective study was carried out between September 2023 and January 2024. Men and women under the age of 70 who had recently been diagnosed with type 2 diabetes mellitus and who were on metformin monotherapy and gave their informed agreement to participate in the study comprised the study population.

Results: The study recruited 50 patients with type 2 diabetes mellitus; patients were receiving metformin as their anti-hyperglycemic therapy. The mean age of the patients was 57±9, 30.8% were illiterated, 94.2% were married, 78.8% were non-smokers, and 53.8% were hypertensive. There was a significant difference in B12, LDL, AST, and ALT level after three months of treatment, P value (0.0001).

Conclusion: There was a significant difference in B12, LDL, AST, and ALT level after three months of treatment with metformin.

Keywords: Type 2 diabetes mellitus, Metformin monotherapy, Hyperglycemia, B12 levels, LDL cholesterol..

Introduction

Diabetes Mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar). The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin [1]. Symptoms often include frequent urination, increased thirst, and increased appetite. If left untreated, diabetes can cause many complications [2].

The most important potentially modifiable risk factors for poor diabetes control were dyslipidemia, physical inactivity, and poor adherence to the management protocol. Enhancing the education of patients

and their healthcare providers on these factors is crucial to improving the patients' control status.(3)Although diabetes mellitus type II (DM) is known to affect bone mineral density, at the present time this particular relationship is not clear.(4) Despite advances in management of diabetes mellitus, it remains one of the major causes of morbidity and mortality, yet diabetic nephropathy is one of the main complications of diabetes mellitus, all patients with microalbuminuria had normal blood pressure, and normal glomerular filtration rate, so we can detect the patients early before they develop overt nephropathy, hypertension and impaired renal function.(5)

Metformin is a widely used oral glucose-lowering drug and is recommended as a first-line drug in recent treatment guidelines of the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD). The management of T2DM requires aggressive treatment to achieve glycemic and cardiovascular risk factor goals. In this setting, metformin, an old and widely accepted first line agent, stands out not only for its antihyperglycemic properties but also for its effects beyond glycemic control such as improvements in endothelial dysfunction, hemostasis and oxidative stress, insulin resistance, lipid profiles, and fat redistribution.[6] However, over the last decades several observational studies and meta-analyses have reported a significant association between long-term metformin therapy and an increased prevalence of vitamin B12 deficiency. Vitamin B12 is a water-soluble vitamin that is mainly obtained from animal-sourced foods. At the cellular level, vitamin B12 acts as a cofactor for enzymes that play a critical role in DNA synthesis and neuroprotection. Thus, vitamin B12 deficiency can lead to a number of clinical consequences that include hematologic abnormalities.(7) Findings suggested that metformin treatment reduces circulating low-density lipoprotein-cholesterol LDL-C levels by suppressing the expression of the pro-protein convertase subtilisin/kexin type 9 (PCSK9) and enhancing LDLR expression; hence the potential therapeutic utility of MF in hypercholesterolemia.(8) Studies shown that metformin induces a reduction in serum levels of liver transaminases, anthropometric and metabolic parameters in patients with non-alcoholic fatty liver disease without distinction between diabetic and non-diabetic patients [9].

Methods

Study design

A prospective study was conducted at private laboratories during the period between September 2023 and January 2024.

Ethical approval

The study was approved by the Scientific Committee of the College of Medicine /University of Baghdad. Verbal consent was obtained from the participating patients before data collection.

Study population

The study population included men and women less than 70 years of age who were newly diagnosed with type 2 diabetes mellitus, who were receiving metformin monotherapy, and consented to participate in the study. The exclusion criteria were patients who were diagnosed to have acute or chronic liver diseases or hematological diseases before starting therapy.

Data collection

Sociodemographic data (age, education level, social status and smoking status) and clinical data were collected during the face-to-face interviews with the patients in the laboratory.

Assessment of B12, low-density lipoprotein-cholesterol and liver enzymes

The liver enzymes including (ALT) and (AST), B12, and LDLC were performed in private laboratory before starting treatment and was repeated after three months of treatment to assess the effect of metformin, on these parameters.

Statistical analysis

The obtained data were entered, coded, displayed, and examined using a computer utilizing the IBM SPSS-29 statistical package (IBM Statistical Packages for Social Sciences, version 29, Chicago, IL, USA) that was made accessible as a data base software program. Simple metrics of frequency, percentage, mean, standard deviation, and range (minimum-maximum values) were used to present the data.(10) The Students-t test was used to determine the significance of the difference between two independent means (quantitative data). When evaluating the significance of differences in percentages (qualitative data), the Pearson Chi-square test (α2-test) was employed, along with Yate's adjustment or the Fisher Exact test if necessary. The P value was taken into consideration for statistical significance if it was equal to or less than 0.05.(11)

Sample size:

Each eligible patient completed the research tool in writing and completed the consent form. The formula (12) was utilized to determine the sample size.

$$n = \frac{Z^2_{1-\alpha/2}\sigma^2}{d^2}$$

In this case, d indicates the precision of the quantitative variable estimate, n is the sample size, α is the first type, Z is the table-based normal distribution index that is taken into account at 5% type-one error (P<0.05), and σ stands for the small variable variance.(13) A first type error in this study has values of 0.05, 96.3, 7.38, and 0.99, correspondingly for z, σ, and d. XXX were taken into consideration as the sample size and were included in the data analysis after accounting for the 10% non-response [14]. For every region in the nation, a sample size of XXX was determined with a design effect of 2, 80% statistical power with a two-sided test, and α = 0.05 to detect a 10% decrease. It was assumed that 30% of people had an irregular legal status at time 1 (the current study) and 20% at time 2 (the subsequent study round) [15].

Results

Sociodemographic and clinical characteristics of the participants

The study included 50 patients with type 2 diabetes mellitus; patients were receiving metformin as anti-hyperglycemic drug. The mean age of the patients was 57±9, 30.8% were illiterated, 94.2% were married, 78.8% were non-smokers, and 53.8% were hypertensive (Table 1).

Table 1: Sociodemographic and clinical characteristics of the participants.

		Metformin	
		No	%
Age (years)	<40years	2	3.8
	40---49	8	15.4
	50---59	23	44.2
	60---69	14	26.9
	=>70years	5	9.6
	Mean±SD (Range)	57±9 (39-76)	
Gender	Male	15	28.8
	Female	37	71.2

Marital status	Married	49	94.2
	Single	3	5.8
Education	Primary & Secondary	36	69.2
	Illiterate	16	30.8
Smoking	Smoker	11	21.2
	Not smoker	41	78.8
Hypertension	Hypertensive	28	53.8
	Not	24	46.2
*Significant difference between percentages using Pearson Chi-square test (χ2-test) at 0.05 level.			
#Significant difference between two independent means using Students-t-test at 0.05 level.			

There was a significant difference (p value < 0.05) in B12 level between base line and after three months of metformin treatment. (Table 2)

Table (2) B12 level before and after three months of treatment

		B12 First visit	B12 After 12 weeks	P value
Total		514.1±158.7 (204-756)	373.4±139.9 (90-650)	0.0001#
Age (years)	<40years	694.5±19.1 (681-708)	560.0±84.9 (500-620)	0.212
	40---49	593.7±124.7 (369-708)	463.3±139.8 (210-580)	0.001#
	50---59	501.4±147.8 (210-756)	356.5±113.9 (90-650)	0.0001#
	60---69	411.3±176.0 (204-681)	271.4±141.2 (100-490)	0.0001#
	=>70years	702.0±	580.0±	-
	P value	0.063	0.009^	
Gender	Male	538.4±123.7 (343-756)	385.6±117.6 (210-650)	0.0001#
	Female	503.7±172.6 (204-756)	368.2±150.2 (90-620)	0.0001#
	P value	0.533	0.723	
-Data were presented as Mean±SD (Range)				
@Significant difference between two independent means using Students-t-test at 0.05 level.				
#Significant difference between two dependent means using Paired-t-test at 0.05 level.				
^Significant difference among more than two independent means using ANOVA-test at 0.05 level.				

There was a significant difference (p value < 0.05) in LDL level between base line and after three months of metformin treatment. (Table 3)

Table (3) LDL level before and after three months of treatment

		LDL First visit	LDL After 12 weeks	P value
Total		186.5±60.9 (99-366)	121.7±46.6 (80-260)	0.0001#
Age (years)	<40years	145.5±16.3 (134-157)	95.0±7.1 (90-100)	0.081
	40---49	154.5±34.3 (116-216)	94.8±7.5 (85-104)	0.004#
	50---59	202.8±69.0 (99-366)	137.5±53.7 (80-260)	0.0001#
	60---69	160.1±24.8 (134-195)	94.0±5.1 (87-100)	0.0001#
	=>70years	253.0±	150.0±	-
	P value	0.142	0.073	
Gender	Male	188.0±57.2 (100-273)	122.4±43.4 (90-200)	0.0001#
	Female	185.8±63.4 (99-366)	121.4±48.7 (80-260)	0.0001#

	P value	0.919	0.950	
-Data were presented as Mean±SD (Range)				
@Significant difference between two independent means using Students-t-test at 0.05 level.				
#Significant difference between two dependent means using Paired-t-test at 0.05 level.				
^Significant difference among more than two independent means using ANOVA-test at 0.05 level.				

There was a significant difference (p value < 0.05) in AST level between base line and after three months of metformin treatment. (Table 4)

Table (4) AST level before and after three months of treatment

		AST First visit	AST After 12 weeks	P value
Total		32.0±20.6 (12-99)	26.7±18.3 (10-87)	0.0001#
Age (years)	<40years	62.0±52.3 (25-99)	53.5±47.4 (20-87)	0.249
	40---49	22.2±4.5 (19-30)	18.5±3.6 (15-25)	0.001#
	50---59	30.1±14.7 (12-66)	25.0±12.4 (10-59)	0.0001#
	60---69	40.4±30.6 (18-99)	33.1±28.7 (15-87)	0.006#
	=>70years	19.0±	17.0±	-
	P value	0.109	0.139	
Gender	Male	27.2±13.9 (12-66)	22.9±12.8 (10-59)	0.0001#
	Female	34.1±22.8 (14-99)	28.3±20.2 (12-87)	0.0001#
	P value	0.335	0.399	
-Data were presented as Mean±SD (Range)				
@Significant difference between two independent means using Students-t-test at 0.05 level.				
#Significant difference between two dependent means using Paired-t-test at 0.05 level.				
^Significant difference among more than two independent means using ANOVA-test at 0.05 level.				

There was a significant difference (p value < 0.05) in ALT level between base line and after three months of metformin treatment. (Table 5)

Table (5) ALT level before and after three months of treatment

		ALT First visit	ALT After 12 weeks	P value
Total		41.3±28.6 (11-139)	40.1±27.9 (11-130)	0.0001#
Age (years)	<40years	52.0±36.8 (26-78)	52.0±38.2 (25-79)	-
	40---49	22.0±3.6 (18-26)	21.2±3.4 (17-25)	0.004#
	50---59	45.2±27.5 (11-139)	43.7±26.3 (11-130)	0.001#
	60---69	44.7±40.0 (14-120)	44.0±40.3 (13-119)	0.047#
	=>70years	18.0±	18.0±	-
	P value	0.388	0.383	
Gender	Male	36.0±13.6 (11-49)	34.8±13.4 (11-48)	0.012#
	Female	43.6±32.9 (14-139)	42.4±32.2 (13-130)	0.002#
	P value	0.450	0.440	
-Data were presented as Mean±SD (Range)				
@Significant difference between two independent means using Students-t-test at 0.05 level.				
#Significant difference between two dependent means using Paired-t-test at 0.05 level.				
^Significant difference among more than two independent means using ANOVA-test at 0.05 level.				

Discussion:

Our study's findings showed that there was a significant difference in B12 level before and after three months of metformin therapy, table (2). In a meta-analysis study results found that the prevalence of vitamin B12 deficiency is greater in patients receiving metformin compared to patients who did not receive metformin.(16) Another study showed that long-term metformin use was associated with an increased risk of B12 deficiency in patients with T2DM, with compounding risk over time.(17)

Regarding LDL results showed that there was a significant difference in LDL level before and after three months of metformin therapy, table (3). Metformin may partially ameliorate HDL dysfunction and reduce LDL modification by inhibiting alpha-dicarbonyl-mediated modification of apolipoprotein residues. (18) In a short-term study of the effect of oral hypogly-caemic drugs on lipoproteins, results showed a 10% fall in LDL cholesterol after 3 months' therapy with metformin.(19) In a cross-over study, the effects of 3 months treatment with metformin on serum lipoproteins in type 2 diabetic patients, Metformin was significantly reduce low density lipoprotein cholesterol.(20)

On another hand our results showed that there was a significant difference in AST and ALT level before and after three months of metformin therapy, table (4,5). In a study to assess the effects of metformin, pioglitazone, and silymarin, results showed that all drugs decrease ALT and AST level. (21) During the diabetic prevention program DPP, serum ALT concentrations were on average lower in individuals randomly assigned to metformin than in those assigned to placebo, even after adjustment for baseline covariates.(22).

Metformin-treated patients showed only a modest, but statistically significant (P=0.04) decrease in AST compared to placebo or diet and exercise, also a statistically significant reduction was observed in ALT in metformin-treated patients.(23) In a double blind randomized clinical trial which was performed on eighty patients who were randomly assigned to metformin and pioglitazone treatments. Administration of either medication significantly led to a decrease in the serum AST, ALT, ALPand an increase in the serum HDL. (24)

Conclusion

Our results demonstrated that there was a significant difference in B12 level before and after three months of metformin therapy. Given the importance of vitamin B12 in nutrition, metformin-induced B12 decrease may be harmful to patients with T2DM. Supplemental vitamin B12 may be advantageous for those on metformin. Also Metformin may partially ameliorate HDL dysfunction and reduce LDL; consequently, the results are salient because cardiovascular disease incidence may be reduced. On another hand there was a significant decrease in AST and ALT level after three months of metformin treatment.

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