

Article

Study the Impact of Glucose-6-phosphatase Activity in Type 2 Diabetic Patients and Non Diabetic Counterparts

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Abstract: Glucose-6-phosphatase (G6Pase), an enzyme found mostly in the kidneys and the liver, acting significant role of supplying glucose through starvation. This study includes (84) subjects, their age ranged from (40 to 54) years. (20) subjects were healthy chosen as control group and (64) patients with type 2 diabetes mellitus were divided into three groups according to their type of anti diabetic therapy : (23) newly diagnosed group without therapy (Group1), (20) with metformin therapy (Group2) and (21) with metformin plus glibenclamide therapies(Group3). The study found that G-6-Pase activity is increased, thereby leading to an increase in endogenous glucose production (EGP) in patients with type 2 diabetes and, therefore FPG will increase. The result found that increasing G-6-Pase activity will increase the concentration of glucose in the blood and that will increase the long-term glycemic control (HbA1c%).

Keywords: glibenclamide therapies; glycemic; Glucose-6-phosphatase

1. Introduction

The vital functions of the liver and, to a smaller degree, of the kidney cortex is to offer glucose during conditions of starvation. Glucose is formed from gluconeogenic precursors in both tissues, and in the liver also from glycogen. In cooperation gluconeogenesis and glycogenolysis result in the creation of glucose 6-phosphate (Glc-6-P), which has to be hydrolysed by glucose-6-phosphatase (G6Pase) previously being liberated as glucose into the circulation. G6Pase plays thus a critical role in blood glucose homeostasis. The study of the Coris [1] revealed that glycogen is corrupted by phosphorolysis to glucose 1-phosphate, suggesting that the phosphate must be cleared at a later stage to form free glucose. De Duve and co-workers [2] presented that the liver contains a phosphatase that can be partially cleansed by precipitation at pH 5 and acts precisely on Glc-6-P. Cell fractionation studies later exposed that G6Pase is related with the endoplasmic reticulum [3]. Additional major progresses were the result that G6Pase deficit is responsible for glycogen storage disease type I (GSD I) [4] and the hypothesis, put forward in 1975 by Arion and co-workers [5], that G6Pase has its catalytic site oriented towards the lumen of the endoplasmic reticulum, and that it requires transporters for Glc-6-P, glucose and Pi. Absence of Glc-6-Ptransport was then revealed to be the cause of a variant of GSD I called GSD Ib [6]. In continuation of previous studies [7–16], herein we are reporting study on Glucose-6-phosphatase (G6Pase). The study found that G-6-Pase activity is increased, thereby leading to an increase in endogenous glucose production (EGP) in patients with type 2 diabetes and, therefore FPG will increase. The result found that increasing G-6-Pase activity will increase the concentration of glucose in the blood and that will increase the long-term glycemic control (HbA1c%).

2. Materials and Methods

2.1. Patients and Control

Sixty-four patients with type 2 diabetes mellitus were selected according to convenient non-random one and carried out by consecutive pooling of diabetic patients attending the National center of Diabetes in (AL-Mustansiria University) during the period from November 2014 to March 2015. The present study included 84 subjects were divided into four groups Group1: newly diagnosed (without therapy) (23), group 2: with metformin therapy (MT.)(21), group 3: with metformin plus glibenclamide therapy (MT. plus Glib.)(20), and 20 Healthy subjects were included in the study as a control group. Age, Duration of diabetes, BMI, and FPG were evaluate in the Sera of Type 2 diabetic subjects and control, Patients with renal failure, Cushing syndrome or hepatic diseases were excluded from the study after the clinical evaluation. Patients taking oral hypoglycemic agents other than metformin or glibenclamide and those taking drugs that may affect the results of the study had also been excluded. The sample for the assay were taken early in the morning between (8.30 and 11.00 A.M) while both patient and healthy subjects were relaxed and fasting for (12-14) hours. A careful history was obtained from patients including age, duration of diabetes, duration of taking treatment, family history, weight and height, type of treatment, other diseases and smoking. All patients were clinically examined, Pregnant patients were not enrolled. Evaluation of each patient is done by detecting the body mass index (BMI), levels of fasting plasma glucose (FPG) and glucose-6-phosphatase activity (G-6-Pase).

2.2. Collection of Blood samples

From each subjects, 10 mL of blood were obtained by vein puncture, using a 10 ml disposable syringes. The blood sample was divided into two aliquots; 2 & 8 ml. The first aliquot blood was dispensed in a tube containing Ethylene Diamine Tetra acetic Acid (EDTA), this blood mixed gently and used for HbA1c estimation, While the second aliquot was dispensed in a plain tube and left to clot at room temperature (25 °C), and then separated by centrifuge at (3000 rpm) for (10 min) to collect serum and stored in the deep Freeze (-20 0 C) until the assay day. The parameters measured be enzymatic methods: glucose kit supplied by Spinreact, Spain, Glucose-6-phosphatase Enzyme from Cusabio, Chinam, Glycated hemoglobin (GHb) from Infopia, Korea,

2.3. Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Packages for Social Sciences- version 17.1) and Microsoft Office Excel (Microsoft Office Excel for windows; 2007). Data were analyzed by using One Way Analysis of Variance (ANOVA) to calculate the p-value for healthy and other patients groups. Student T-test was done also to compare means between groups. Pearson test was used to test the correlation between the assessed parameters. The results were presented as mean \pm standard error. Statistical significance was considered at the level of ($p \leq 0.01$) and ($p \leq 0.05$).

3. Results and Discussion

Table (1) revealed that the mean G-6-Pase activity and FPG was found to be elevated in diabetic patients compared with control group, and the differences were statistically significant ($p \leq 0.05$). A characteristic feature of type 2 diabetes is increased endogenous glucose production, largely due to increased hepatic glucose production (HGP) [17]. During fasting, hepatic gluconeogenesis is the primary source of endogenous glucose production and the major enzyme responsible for the regulation of gluconeogenesis is glucose-6-phosphatase [18]. Common final pathway of glucose release involves the dephosphorylation of glucose via glucose-6-phosphatase (G-6-Pase) [19]. Hundal et al (2000) found that increased rate of glucose production in the diabetic subjects could be attributed to an increased rate of gluconeogenesis [20]. So our study found that G-6-Pase activity is increased, thereby leading to an increase in endogenous glucose production (EGP) in patients with type 2 diabetes and, therefore FPG will increase. This result agree with

Clore J. et al (2000) [21], who found that hepatic G-6-Pase activity determined from freshly isolated microsomes was significantly increased in the type 2 diabetic patients compared with the control subjects and rates of endogenous glucose production (EGP) were increased in the diabetic patients and were closely correlated with fasting plasma glucose.

Table (1) mean distribution of G-6-Pase and FPG in type 2 diabetic patients and nondiabetic counterparts.

Parameters	Mean \pm SD		P-value
	Control group(N=20)	Diabetic group(N=64)	
G-6-Pase (U/ml)	30.90 \pm 13.52	61.99 \pm 43.41	0.0023*
FPG(mmol/L)	4.67 \pm 0.39	9.00 \pm 2.85	0.0001*

*Significant difference using student's t-test for comparing between two independent means at 0.05 level of significance

3.1. Glucose-6-phosphatase Activity in the Studied Groups Dividing them According to anti-diabetic therapy

Table (2) showed that mean G-6-Pase activity and FPG concentration were found to be elevated in group1 (newly diagnosed group) when compared with group2 (with metformin therapy), group3 (with metformin plus glibenclamide therapy) and control group, and the differences was statistically significant ($p \leq 0.05$). There were a nonsignificant difference between treated groups and control. Abnormally high liver glucose-6-phosphatase occurs in poorly controlled or untreated diabetes mellitus [22]. Some previous studies showed that metformin therapy was normalized glucose-6-phosphatase activity in diabetic rats [23]. Kolawole and Akanji (2014) revealed that in vivo G-6-Pase activity of diabetic rats was significantly increased compared to that of non-diabetic control and treatment of diabetic rats with metformin for 28 days caused a significant decrease in the activity of G-6-Pase compared to diabetic control [24]. Hundal et al (2000) found that metformin treatment in the diabetic human decreased rates of glucose production through a reduction in the rate of gluconeogenesis [20]. This results revealed that using metformin as mono therapy or combination with glibenclamide reduce hepatic glucose production by reducing G-6-Pase activity leading to decrease FPG levels in type 2 diabetic patients.

Table (2) mean values of G-6-Pase activity and FPG in three groups diabetic patients and control group.

Parameter	Mean \pm SD				P-value
	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. plus Glib.	
G-6-Pase(U/ml)	30.90 \pm 13.52	113.95 \pm 25.22 a	31.35 \pm 13.47 d	34.44 \pm 14.94 e	0.0001*
FPG(mmol/L)	4.67 \pm 0.39	12.00 \pm 2.34 a	6.96 \pm 1.30 b d	7.70 \pm 1.23 c e	0.0001*

*significant using ANOVA test at 0.05 level of significance.

- a) indicate significant difference between control and Group1.
- b) indicate significant difference between control and Group2.
- c) indicate significant difference between control and Group3.
- d) indicate significant difference between Group1 and Group2.
- e) indicate significant difference between Group1 and Group3.
- f) indicate significant difference between Group2 and Group3.

Correlation Between serum G-6-Pase Activity and other variable

The present study found that when G-6-Pase activity is increased in patients with type 2 (Table 3) diabetes the endogenous glucose production will increased and therefore FPG will increased. Clore J. et al (2000) found that hepatic G-6-Pase activity was significantly increased in the type 2 diabetic patients and rates of EGP were increased in the diabetic patients and were closely correlated with fasting plasma glucose [24-26]

The result found that increasing G-6-Pase activity will increase the concentration of glucose in the blood and that will increase the long-term glycemic control (HbA1c%).

Table (3) Correlation coefficient between G-6-Pase and study parameters.

Parameters	Control group	Group1 Newly diagnosed	Group 2 MT.	Group 3 MT. Plus Glib.
Age(years)	0.112 NS	0.171 NS	0.341 NS	0.412 NS
BMI(kg/ m2)	0.281 NS	0.251 NS	-0.167 NS	0.054 NS
Duration of diabetic(years)	---	---	-0.072 NS	0.164 NS
Duration of taking treatment(years)	---	---	-0.122 NS	-0.267 NS
FPG(mmol/L)	0.421 NS	0.658**	0.604**	0.600**
HBA1c %	0.322 NS	0.511*	0.466*	0.430 NS

* ($P \leq 0.05$), ** ($P \leq 0.01$), NS: Non-significant.

Conclusion: The result found that increasing G-6-Pase activity will increase the concentration of glucose in the blood and that will increase the long-term glycemic control(HbA1c%).

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