



Effect of Acetylcholine Activity on some Blood Parameters in Diabetic Patients

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Abstract

This study was carried out on 160, diabetic patients (equal sex) who investigated in Babagurgur diabetes center in K1 Hospital-Kirkuk and 20 healthy non-diabetic individuals from 10/11/2018 to 10/11/2019. Their mean age was 54.18 ± 8.27 years. Fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) estimation were done for subjected individuals to confirm diabetes. Quantitative estimation of acetyl cholinesterase was done by using the enzyme linked immunosorbent assay (ELISA) kit. Serum levels of cholesterol, triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were estimated. Results: There was a significant ($p < 0.05$) increase in mean values of FBG and glycated hemoglobin patients when compared with the same parameters in control group which were 14.6 mmol/L and of 9.6 %, respectively. From the total 160 diabetic patients only 24 showed decreased in Acetylcholine esterase activation when compared with control group. Lipid profiles; cholesterol, and triglycerides levels, showed significant ($p < 0.05$) increase, while HDL and LDL level showed non-significant ($p > 0.05$) variation. Conclusion: It is concluded that glycemic control are important risk factors related to hyperlipidemia in the current DM population and may inhibits acetylcholine esterase.

Key Words: Diabetes Mellitus, Blood Parameters, Acetylcholine, Acetylcholine Esterase Activity.

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Introduction

Diabetes mellitus (DM) is a complicated metabolic disturbance that may modify cell membranes properties and organizations [1]. Its prevalence has been increasing steady everywhere the globe and quick turning into an outbreak in poorly developed countries, with variety the amount the quantity of individuals affected predictable to twice the current number every decade alongside raised ageing population, thereby adding to the already existing load for healthcare suppliers. Untreated DM can cause severe long-term complications include peripheral vascular disease, neuropathy, nephropathy, foot ulcers, and retinopathy. [2,3].

Although there was obvious decrease in the mortality rate from cardiovascular disease (CVD) in many countries worldwide over past years, the epidemic of cardiovascular disease will be a rising Asian public health challenge with growing

urbanization [4]. Diabetes mellitus induces hypercholesterolemia and significantly increases the likelihood of atherosclerosis.

From the available data on 20 Arab countries with more than twenty million were diabetic. Our country comprises about 9.3% of diabetes in the Middle East. The pattern of lipid components in Iraqi patients and its relationship with plasma glucose levels below the cut? point for the diagnosis of diabetes is less clear.

Acetylcholinesterase is a hydrophobic integral enzyme in erythrocyte membrane, and is an acceptable means for studying changes in because of action of various factors. [5]. Acetylcholinesterase (AChE) activity inhibition has been widely studied red blood cells, plasma and tissues in workers [6-8].

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In the previous investigation [9], we demonstrated the alterations in lipid profiles in diabetic patients and correlation between these changes and HbA1c, and no records were estimated to elucidate acetylcholine esterase activities in diabetic patients. So present study was aimed to clarify the effect of acetylcholine in diabetic patients.

Material and Methods

Sample Collection

Sample Collection: One hundred sixty patients (equal sex) with hyperglycemia and twenty-four healthy individuals were clinically proven by specialists in Babagurgur Diabetes Center in K1 Hospital-Kirkuk-Iraq during a period from 10/11/2018 to 10/5/2019. Their mean age was 54.18 ± 8.27 years.

Complete information includes the age, gender, duration of diabetes; body weight and height of both male and female individuals were noted according the form. Venous blood was drawn from each of subjected individuals via venipuncture. The collected 5 ml of blood divided into 2 parts, 4ml was drawn in anticoagulant free tube and centrifuged (3000xg) for ten minutes for serum separation.

The separated serum was pipetted into clean Eppendorf's tube for estimation of fasting blood glucose (FBG) and lipid profiles; cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein and acetyl cholinesterase. The remainder 1 ml of blood kept in anticoagulated (K3 EDTA) container and used for the estimation of glycated hemoglobin.

Methods: Type -2 DM diagnosed on the basis of the American diabetes association (ADA) 2015 guidelines [10].

HbA1c was estimated by using AFIAS HbA1c (Boditech Med Incorporated –Republic of Korea) a fluorescence immunoassay for the quantitative determination of HbA1c while TC, TG, HDL and LDL estimated by using kit {ASSEL S.r.L via Barsanti 13/A -00012 Guidonia (Rm)}[5].

Quantitative estimation of acetyl cholinesterase was done by using the enzyme linked immunosorbent assay (ELISA) kit (RelAssay Diagnostics) based on the principle of biotin double antibody sandwich technology.

Statistical analysis: Data obtained were analyzed

using the Excel program version 10 package. The significance of invariant differences was assessed by student's t- tests and all values were two-sided. A p-value < 0.05 = statistically significant.

Results

The age and body mass index (BMI) of 160 diabetic patients were 54.18 ± 8.27 years, 32.6 ± 6.39 Kg/m², respectively. The results showed that there was significant (p<0.05) increase in the tests of evaluation of diabetic blood sugar; FBG was 13.16 ± 4.3 mmol/L vs 5.3 and HbA1c of 9.39 ± 1.59 vs. 5.4 in control group (Table 1).

Regarding lipid profiles, mean values of both of Cholesterol and triglycerides levels showed significant (p<0.05) increase which were 6.26 vs. 4, 3.51 vs.1.3 mmol/L, while HDL and LDL showed un significant (p>0.05) differences in mean values. Mean value of HDL decreased from normal values of control group and LDL increased, which were, 0.96 vs. 1.2 and 2.43 vs. 1.9 mmol/L, respectively.

From the total 160 diabetic patients only 24 showed non-significant p>0.05 decreased in acetylcholine esterase activation when compared with control group (figure 1).

There was a significant (p<0.05) increase in mean values of FBG and glycated hemoglobin patients when compared with the same parameters in control group which were 14.6 mmol/L and of 9.6 %, respectively.

From figure 1, it is very obvious that there is an increase in mean values of FBG and glycated hemoglobin (HbA1c) in patients who showed a decrease in acetylcholine esterase activation.

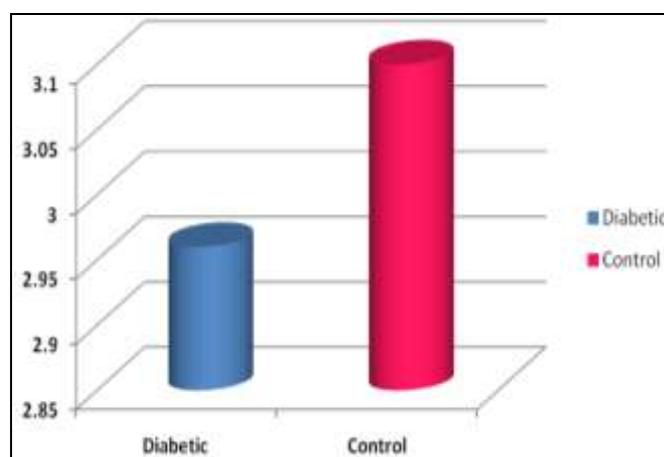


Figure 1. Mean Values (ng/mL) ± SD of Acetylcholine esterase activities in diabetic patients and control group

Table 1. Mean values \pm SD of estimated parameters obtained from all diabetic patients and control group

Parameters	Control Group	Patients with normal AchE	Patients with abnormal AchE
Number	24	160	20
Age (year)	53.2 \pm 9.9	54.18 \pm 8.27	32.5 \pm 5.4
FBG mmol/L	5.3 \pm 0.5	13.16 \pm 4.3*	14.6 \pm 5.8*
HbA1c (%)	5.4 \pm 0.42	9.39 \pm 1.6*	9.6 \pm 1.9*
Cholesterol mmol/L	4.0 \pm 0.63	6.26 \pm 1.02*	5.2 \pm 1.1*
Triglyceride mmol/L	1.3 \pm 0.38	3.51 \pm 1.34*	4.0 \pm 2.0*
HDL mmol/L	1.2 \pm 0.18	0.96 \pm 0.39	1.1 \pm 0.8
LDL mmol/L	1.9 \pm 0.35	2.43 \pm 0.56	2.3 \pm 0.6

* = statistically significant (P<0.05)

Discussion

According to official statistics issued by the Iraqi Ministry of Health in its annual report for 2013 that 2.9% of the deaths for the year was due to the diabetes disease, and we are expected to have, in that year specifically more than 1.5 million cases of the disease, on the other hand, That Iraq ranks 9th in the Arab world and 30 in the world with injuries up to 10.2% of the total. Iraq has a large population of diabetic patients with a vast geographical distribution and significant variations in the types of [11,12]

Our present study reported that there was significant increase in the glycated hemoglobin, FBG and lipid profiles alteration in diabetic patients than in normal individuals. These parameters along with increase in age and poor glycemic control are important factors to be considered when assessing the risk of CVD in the current T2DM in Kirkuk population in Iraq.

It is well established that serum lipid profiles are worse in diabetic than in non-diabetic subjects [13,14]. Our previous work reported that FPG and glycated hemoglobin levels in individuals with hyperlipidemia were higher than individuals with normal serum lipid levels [9]. These findings confirm our results that showed that hyperlipidemia observed in diabetic patients who have elevated FBG levels. Acetylcholine activities

was in the sera of diabetic patients when compared with the values obtained from control group, but the difference was non-significant ($p>0.05$). Acetylcholine (Ach) as a neurotransmitter is produced at the end of intrapancreatic parasympathetic neuron which innervated by cranial nerve (X) a neurotransmitter. So, acetylcholine play a key role in glucose homeostasis through its action on mAChRs and increases insulin releasing via vagus activation [15]

Adult onset diabetes mellitus is a complex metabolic disorder associated with increased oxidative stress (OS), which alter the properties and organization of cell membranes. OS in diabetes has also been suggested to be responsible for the induction of cognitive deficits. Cholinergic neurotransmission is a crucial process underlying cognitive function in which cholinergic function is performed by brain derived Acetylcholinesterase (AChE). Accumulation of free radicals resulted from acetylcholine esterase activity inhibition leads to lipid peroxidation [16]. Free radicals may argue oxidative stress resulting in modification in antioxidants or oxygen free radical scavenging enzyme system [17]. From the above results it was concluded that diabetes mellitus may cause a significant decline in AChE activity and effects on blood parameters. These effects may be through oxidative damage caused by elevating lipid peroxide levels. Now existing evidence advises that the immune cell cholinergic system makes a critical contribution to the regulation of immune function. Cytokine synthesis (e.g., IL-2 in T cells and TNF- α in macrophages) initiation and termination seem to be triggered by the cholinergic signals produced by immune cells.

Conclusion

From the above results it was concluded that FBG and glycated hemoglobin in diabetic patients may be attributed to a decrease to a decrease in AChE activity. A significant increase levels of lipid profiles is correlated with FBG and glycated hemoglobin.

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Conflict of Interest

"The authors declare that there are no conflicts of interest regarding the publication of this manuscript".

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