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Assessment of Fructosamine 3-kinase in Type 2 Diabetic Patients and Its Relation to Some Biochemical Variables

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Abstract

It took over 30 years for first description of the synthesis of fructosamines and this was conducted through non-enzymatic protein-glucose reactions (also known as glycation). Fructosamines in mammalian cells has not been known until recently. It transforms spontaneously into advanced glycation end products. The discovery of fructosamine 3-kinase, which is a novel enzyme, in human reveals the existence of a previously unknown intracellular metabolism of these substances. Protein-bound fructosamines are phosphorylated by fructosamine 3-kinase with high affinity on a third carbon atom of the deoxyfructose moiety and this results in fructosamine 3-phosphates. These later substances are unstable and spontaneously break down into inorganic phosphate and 3-deoxyglucosone, regenerating the unglycated amine in the process. The fact that many prokaryotic and eukaryotic genomes contain proteins connected to fructosamine 3-kinase shows that this 'deglycation' process is not exclusive to mammals. The current study aims at determining the normal values of fructoseamine 3-kinase enzyme in the control group as well as in the group of patients with type II diabetes (the experimental group). The relationship of enzyme activity with some biochemical variables was also studied in patients with type 2 diabetes, due to the association of these variables with high blood sugar, as shown by previous studies. The Research dealt with the investigation of Fructosamine 3-Kinase activity and some biochemical variables for (136) Type 2 diabetes patients. The results indicated that there was significant increase in Fructosamine 3-Kinase activity in type 2 diabetes patients (253 ng/l) in comparison to the control group. The normal value of enzyme in the control group was (181ng/l) and the activity of the enzyme in control group individuals was not affected by age and sex. The research also showed a significant decrease in the level of iron, zinc, chromium and sodium as well as a significant increase in the potassium level in patients with type 2 diabetes compared to the control group individuals. Additionally, the research predicted that there is a significant decrease in the levels of vitamins C and E. Also, it was shown that there was a significant increase in malondialdehyde level and a myeloperoxidase activity in type 2 diabetes patients. Activity of FN3K increased in type 2 diabetes compared to the control group individuals. It was noted that the activity of FN3K in the control group was not affected by age or sex.

Keywords: *Diabetes type 2, Glycation, Fructosamine, Deglycation, FN3K*

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I. INTRODUCTION

Diabetes is one of the most prevalent diseases worldwide. It is a chronic disease that is characterized with high blood sugar levels as a result of changes in insulin secretion or insulin action or both. High blood sugar causes various organs damage and dysfunction as it affects eyes, kidneys, nerves, blood vessels and heart (Mauricio *et al*., 2020). Type 2 diabetes DM T2 accounts for about 90% of diabetes cases and middle-aged or older people are more likely to develop this type of disease. The incidence of this type of Diabetes has recently increased in children mainly due to increased levels of obesity in children (Huo *et al*., 2018). The rise in blood sugar levels enhances the non-enzymatic covalent interactions between sugars with an effective amino group in amino acid lysine in the protein (Zheng *et al*., 2019), which is known as non- enzymatic glycation and it consists of reversible and irreversible reactions that lead to the formation a certain type of free radicals (Ahmad *et al*., 2018). Glycation process passes through three stages: The first stage involves the formation of reversible compounds known as Schiff's bases, which are unstable and that undergo spontaneous ordering processes to form a compound fructoselysine. It is known as an Amador product that represents the second stage. In last stage, these products undergo polymerization processes to form advanced glycation end products, which are highly stable (Alderawi, 2017) and these interactions negatively affect the human body. The body has a special defence mechanism against the excessive build-up of these compounds. This mechanism is known as deglycation

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reactions. Fructosamine-3-kinase enzyme is one of deglycation enzymes and it is considered as a part of cellular repair system to control non-enzymatic glycation reactions, as it works on the phosphorylation of fructoselysine to fructoslysine-3-phosphate. The fructoslysine-3-phosphate decomposes spontaneously to form free lysine, inorganic phosphate and 3-deoxyglucazone (Motshwari, 2018).
Abnormalities in the regulation of peroxide and transitional metal metabolism are presumed to lead to the establishment of the disease as well as its longer term complications. Diabetes mellitus is associated with the oxidative reactions, particularly those which are catalyzed by decompartmentalized transitional metals, but their causative significance in diabetic tissue damage remains to be established.

II. Materials And Methods

A. Samples collection

The samples was selected from Al-Wafaa Specialized Medical Center in Mosul city under the supervision of specialized doctors. A total of 136 patients with ages (35-65) years old who suffer from type 2 diabetes (experimental group) were collected for the period from October to December 2021. The 70 samples of the control group (35-65 years) was selected from the main Blood Bank. Information of the individuals was written down in a special form as shown in the Table 1.

Table 1. The questionnaire form

The questionnaire form	
Age	Name
Weight	sex
BMI	height
Fasting	The type of disease
disease duration	smoking
Family genetic history	and other diseases

A 5 ml of blood was taken from the vein to obtain the serum from it by placing the blood sample in a clean and dry plastic tube. After that is placed it in a water bath at a temperature of 37°C for 10 minutes and then placing it in a centrifuge for 15 minutes to separate the serum (Burtis *et al*., 2015).

B. Methods

Analysis kit (from Sunlong company) was used to measure the activity of fructosamine-3kinase by using ELISA technique. Iron, zinc, chromium and potassium were estimated using atomic absorption spectrometry technique and atomic absorption was measured after determining wavelength of each metal measured. Assay kit (made by Solarbio Chinese company) was used to measure the total antioxidants capacity through using the spectrometry technique. In addition to that, analysis kit (Coral company clinical) was used to measure the sodium by using the spectrometry technique and the activity of myeloperoxidase enzyme was measured using the method of (Kumar *et al*., 2002). Malondialdehyde level in serum was determined using modified method used by (Guidet and Shah, 1989). Moreover, the concentration of E vitamin in blood serum was estimated using a method that is dependent on oxidation and reduction reactions by using spectrometry technique, while the concentration of C vitamin was estimated by using also spectrometry technique (Al-Chalab and Al-Abachi, 2008).

III. Results

The research results demonstrated that the activity value of FN3K enzyme for healthy people (control group) was (181.51 ± 2.82ng/l) and it is not affected by gender (Male, Female) or age (35-44, 45-54, 55≤). This is in agreement with the findings of (Cikomola *et al*., 2017) as shown in Fig. 1 and Fig. 2. Moreover, there was a significant increase in the activity of fructosamine-3-kinase for patients who suffer from type 2 diabetes (Group 2) (253.79±7.39ng/l) compared to the healthy group (Group 1) at probability level of P≤0.001, as shown in Fig. 3.

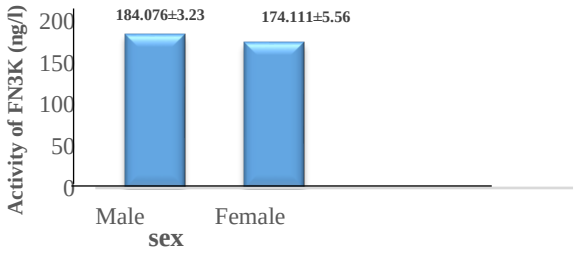

Figure 1: Comparison of Fructosamine-3-kinase activity for the control group according to sex.

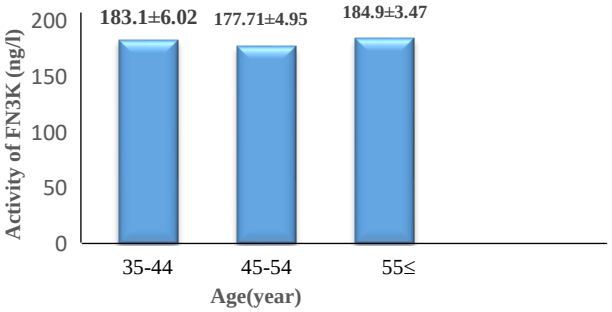

Figure 2: Comparison of Fructosamine-3-kinase activity for the control group according to age.

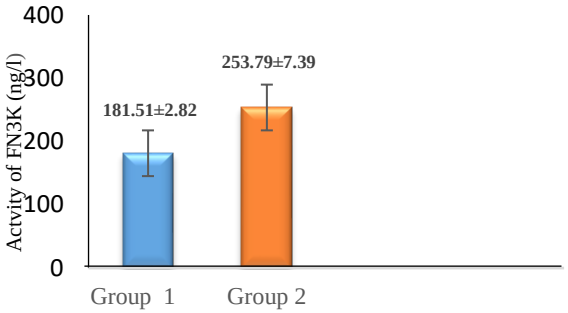

Figure 3: Comparison of fructosamine-3-kinase activity in group1 and group 2

Results of research also indicated that there were a decrease in the levels of iron, zinc, chromium, sodium, E vitamin and C vitamin in patients with type 2 diabetes (experimental group) compared to healthy individuals (control group) and an increase in the levels of potassium and malondialdehyde and the activity of myeloperoxidase in patients suffering from type 2 diabetes compared with healthy individuals (control group), And there was no significant difference in level of total antioxidants capacity of healthy group and type 2 diabetes patients, as shown in Table 2. The relationships between activity of fructosamine-3-kinase enzyme with the essential minerals and vitamins levels and total antioxidants capacity was studied by determining the linear correlation coefficient. Results showed that there was a highly significant inverse relationship between the iron level and FN3K enzyme activity. From the other and there was no significant relationship between the chromium zinc and myeloperoxidase with the enzyme activity. Additionally, results indicated that there was a significant direct relationship between the potassium, sodium, malondialdehyde and E vitamin with the enzyme activity and a significant direct relationship between C vitamin and the enzyme activity.

There was also a significant direct relationship between the total antioxidants capacity and the enzyme activity, as shown in Table 3.

Table 2. Results of some variables in type 2 diabetes patients compared to the control group.

Variables groups	Control group Mean ± S.E	Type 2 diabetes Mean ± S.E
Fe(µg/dl)	119±2.45	105±2.91
Cr(µg/l)	0.251±3.37	0.144±2.99
Zn(µg/dl)	67±0.0008	24±0.0008
K(mmol/l)	3.9±0.04	4.9±0.11
Na(mmol/l)	138±0.50	118±0.80
Vit. C(mg/100ml)	0.41±0.004	0.30±0.009
Vit. E(mg/l)	1.19±0.02	0.88±0.049
Malondialdehyde(µmol/L)	0.90±0.08	8.04±0.22
Myeloperoxidase(U/ml)	27.83±0.36	105.5±3.35
Total Antioxidant Capacity(µmol/ml)	1.54±0.02	1.59±0.01

Table 3. linear correlation coefficients of some variables with Fructosamine-3-kinase.

Fe	FN3K	r -0.169
K		r 0.227
Na		r 0.229
Vit. E		r 0.245
Vit. C		r 0.277
Malondialdehyde		r 0.233
Total Antioxidant Capacity		r 0.188

IV. DISCUSSION

Intracellular clearance of deglycation is controlled by FN3K, where the enzyme activity increases in the serum of patients with type 2 diabetes and the reason could be due to the cellular leakage of enzyme in terms of increasing activity of the enzyme in tissues and organs that are exposed to high levels of sugar (Cikomola *et al.*, 2017). Some clinical variables were measured in patients with type 2 diabetes (experimental group) and compared to healthy individuals (control) group. Results showed that there was a decrease in the level of iron in diabetic patients as the high blood sugar is responsible for much damage in human body and thus this results in a lack of nutrients and affects proportion of minerals in cells as the proper absorption of nutrients in the body is impeded (Ahmed *et al.*, 2019). These results are in conformity with the results of (Forte *et al.*, 2013). In addition to that, a decrease in level of chromium also occurs, which is one of basic elements necessary for the normal functioning of insulin and regulation of blood sugar levels and a vital antioxidant for maintaining insulin balance for patients suffering from type 2 diabetes. The reason for decrease in the chromium level is attributed to the high sugar level, as the rise in sugar level is considered an expelling factor for the chromium in the body (Rajendran *et al.*, 2015). These results in conformity with the results of (Basaki *et al.*, 2012). Oxidative stress increases for the patients who suffer from diabetes as a result of the continuous rise in sugar and

enzymes that act as antioxidants and need elements for their efficiency to work, and thus the level of zinc in diabetic patients decreases (Omer, 2018) this is in conformity with (Basaki *et al*., 2012). There has been also a decrease in sodium concentrations in diabetic patients due to the high sugar levels, as this results in an osmotic force that works to attract water out of cells and this, in turn, reduces the sodium concentrations outside the cells (Chuang *et al*., 2020). This is in agreement with (Kannenkeril *et al*., 2019). The results also showed that there was an increase in the level of potassium in the blood that may be attributed to the blood high acidity for the patients suffering from diabetes. Therefore, the hydrogen ions increase and the body works with special mechanisms that endeavors to get rid of the hydrogen ions through exchange with potassium ions and this, consequently, leads to nephropathy Diabetes, due to reducing potassium excretion (Harris *et al*., 2018). The results of research indicated a decrease in the level of E vitamin and C vitamin. The reason may be due to the free radicals increase that are generated for the patients suffering from type-2 diabetes (Al-Ramadhan *et al*., 2015; Iqbal *et al*., 2004) as well as the increase in lipid peroxidation that is represented accumulation of lipid peroxidation products, which increases the activity of myeloperoxidase and the increase malondialdehyde level for patients (Shiu *et al*., 2014; Tangvarasittichai *et al*.,2009) and this result is in conformity with (Fathi *et al*., 2020) .

V. Conclusion

Activity of FN3K increased in type 2 diabetes (experimental group) compared to the control group. It was noted that activity of FN3K in the control group was not affected by age or sex.

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