Study of the relationship of calcium ions, erythrocyte membrane AChE and some related ions with type 2 diabetes mellitus

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Abstract

The study was designed to understand the relationship between calcium ions, erythrocyte membrane AChE activity and some related ions like iron, copper, zinc, and selenium with type 2 diabetes mellitus.

Sixty patients suffering from type 2 diabetes mellitus (30 males, and 30 females) aged between (34)to(65) years with a mean age of (44.11 ± 12.03) were included in this study. The diabetic patients were diagnosed on the basis of WHO criteria. All patients having a history of duration of disease more than one year and no one of them have a hypertension.

The control group comprised of (60) healthy individuals (30 males and 30 females), aged between (30) to (66) years with a mean age of (45.1± 11.87). The control subjects were selected from the staff of Al-Basrah General Hospital and from the staff and the students of various colleges of Al-Basrah University. They had no history of diabetes mellitus or any other disease.

The results of this study showed significant elevations ($P< 0.001$) in erythrocyte membrane AChE activity in type 2 diabetic patients as compared to control group. Serum calcium was significantly decreased in patients with diabetes type 2. The study showed also

significant elevations (P< 0.001) in iron stores; represented by serum ferritin, and serum copper and a significant decrease serum zinc and selenium of diabetic type 2 patients.

Regression analysis showed a significant negative correlation (P< 0.01) between serum calcium and AChE in males. Non-significant negative correlations between serum calcium and each of AChE, and serum iron of females and with the serum copper and ferritin of males. Serum calcium showed significant positive correlations with both serum zinc and selenium, and a non-significant positive correlations with serum ferritin of females and serum iron of males.

Conclusion can be made that a neuronal shock (stroke) causes elevations in the exocytosis of the neurotransmitter ACh, as a result of high calcium influx into the neurons, and in the same time a shock causes calcium influx into muscle cells in order to do its contraction. The high influx of calcium enhances intracellular oxidation processes and may be complicated to diabetes type 2.

الخلاصة

صممت هذه الدراسة لمعرفة نوع العلاقة بين كل من ايونات الكالسيوم وانزيم الاسيتايل كولين استريز AChE لكريات الدم الحمر وبعض الايونات ذات العلاقة مثل الحديد والنحاس والخارصين والسلينيوم مع مرض السكري النوع الثاني.

شملت الدراسة 60 مرضا بالسكري النوع الثاني, نصفهم من الذكور, تراوحت أعمارهم بين 34و 65 سنة, ومتوسط اعمارهم (44.1 ± 12.03). أما مجموعة السيطرة وعددهم 60, نصفهم من الذكور, تراوحت اعمارهم بين 30 و 66 سنة, ومتوسط اعنارهم (45.1 ± 11.87), فقد تم اختيارها من بين منتسبي مستشفى البصرة العام وطلبة ومنتسبي كليات جامعة البصره, ليس لديهم تاريخ في السكري او أي مرض اخر.

وقد أظهرت النتائج وجود ارتفاعات معنوية (P < 0.001) في مستويات كل من AChE لدى مرضى السكري مقارنة بمجموعة السيطره, بينما ميوى الكالسيوم قد انخفض بشكل معنوي. وقد أظهرت الدراسة كذلك وجود ارتفاعات معنوية (P < 0.001) في مستويات كل من, مخزونات الحديد (المتمثلة بالفرتين) وفي النحاس, بينما كانت هناك انخفاضات معنوية في مستويات كل من الخارصين والسلينيوم لدى مرضى السكري.

كما أظهرت نتائج التحليل الانحداري وجود علاقة سالبة معنوية (P < 0. 01) بين كالسيوم مصل الدم وAChE لدى الذكور. وعلاقة سالبة وغير معنوية بين الكالسيوم وكل من AChE والحديد لدى الاناث, ومع النحاس والفرتين لدى الذكور. كما أظهر الكالسيوم علاقة موجبة معنوية مع كل من الخارصين والسلينيوم, وموجبة لكنها غير معنوية مع كل من الفرتين عند الاناث والحديد عند الذكور.

وبهذه النتائج يمكننا الاستناج بأن الصدمة العصبية تؤدي الى تدفق الكالسيوم الى داخل الخلايا العصبية من اجل اطلاق الناقل العصبي الاسيتايل كولين ACh منها, وفي الوقت ذاته فأن الصدمة العصبية تؤدي الى تدفق الكالسيوم الى داخل

الخلايا العضلية من أجل تقلصها, وأن هذا التدفق الزائد للكالسيوم الى داخل الخلايا يحفز عمليات الاكسدة فيها, والتي ربما تتطور الى مرض السكري.

Introduction

1- Cholinesterases:

True and pseudo cholinesterases were known. These were of the E. C. commission names: E.C.3.1.1.7 and E.C.3.1.1, 8 respectively [1].

The main function of AChE is believed to be the termination of the action of neurotransmitter acetylcholine (ACh) at the cholinergic synapses [2]. The arrival of an action potential at the presynaptic membrane of neuron triggers the opening of 'voltage-gated Ca^{2+} channels', which transiently raises the local [Ca^{2+}]. The resulting influx of extracellular Ca^{2+}, in turn, stimulates the exocytosis of ACh into the synaptic cleft. At the postsynaptic site, M_1 receptors transduce signals through a pathway involving diacylglycerol (DAG), inositol-1, 4, 5-trisphosphate (IP_3) and a Ca^{2+}-dependent protein kinase (PKC) [3, 4].

2- Diabetes Mellitus:

Two types of diabetes were known, these were IDDM and NIDDM. Individuals with a fasting plasma glucose of 3.5- 5.5 mmol/l are considered as healthy, above this figure and greater than 7 mmol/l they are considered as diabetic [5].

Type 2 diabetes, is a term used for individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. [6]. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation, lipid peroxidation and protein glycosylation [7, 8]. Several conditions were known to disturb the balance between ROS production and cellular defense mechanism and cause oxidative stress [7, 8, 9].

3- Calcium and Diabetes

Insulin secretion is a calcium-dependent biological process [10], and an elevation in calcium is required for insulin secretion [11]. In juvenile diabetic patients serum calcium is decreased with increased urinary excretion [12]. It was reported that elevated cytosolic free calcium and reciprocally reduced extracellular ionized calcium levels were observed in type 2 diabetic patients [13].

4- Calcium and Oxidative Stress

Oxidative stress causes Ca^{2+} influx into the cytoplasm. Rising Ca^{2+} concentration in the cytoplasm causes Ca^{2+} influx into mitochondria and nuclei. In mitochondria Ca^{2+} accelerates and distrups normal metabolism leading to cell death. In nuclei Ca^{2+} modulates gen transcription and nucleases that control cell apoptosis. Both in nuclei and cytoplasm Ca^{2+} can regulate phosphorylation/ dephosphorylation of proteins and can modulate signal transduction pathway as a result [14].

5- Serum Iron in Diabetes

There is a considerable current interest in the relationship between insulin and iron pool in the body. Insulin influences the iron uptake and storage by increasing the cell surface transferrin receptors [15], reciprocally iron influences the insulin activity by interfering with glucose uptake and utilization [16]. Iron causes hyperinsulinemia by decreasing the insulin uptake and metabolism by hepatocytes [17]. Diabetes type 2 showed a significant increase in serum ferittin [18, 19] and in serum free iron [18] as compared to healthy control. Iron in its free form is known to induce oxidation of biomolecules through "Heber-Weiss" and "Fenton' reactions by producing harmful hydroxyl radicals [20].

6- Zinc and Diabetes

Zinc is essential for the formation of both stored and active form of insulin. It may also be responsible for the conformational changes that allow insulin to bind to its receptors for activity. In addition, It has been suggested that zinc may be involved in the development or progression of both type 1 and type 2 diabetes [21- 23] . On the other hand, it was reported that zinc metabolism is also adversely affected by diabetes. High levels of zinc are eliminated in urine and absorption is impaired in both type 1 and type 2 diabetes and serum levels of zinc are (20-30) % lower as a result [21]. Zinc protects cell from oxidative damage. Low levels of zinc may lead to damage the retinas (retinopathy), the nervous system (neuropathy), the kidneys (nephropathy) or cardiovascular system [21, 24].

7- Copper and Diabetes

Data regarding dietary intake and blood concentrations of copper and glucose are conflicting. While, glycation was enhanced in dietary copper deficiency, copper supplementation reduced glucose levels [25, 26].

Other studies found that, circulating copper concentrations were not different[27], or were greater only in diabetic patients with chronic complications [28]. Indeed, in the presence of overt diabetes with chronic complications, the associated chronic low-grad inflammatory state might be responsible for an increase in blood copper concentrations [29].

8- Selenium and Diabetes

In addition to its pro-oxidant and antioxidant effects, selenium has been reported to have a strong antidiabetic and insulin-mimetic effects [30, 31]. In animal experiments, selenate decreased the activity of the enzyme tyrosine phosphatase, a negative regulator of insulin [30]. The antidiabetic effect of selenium, however, is limited to selenate at very high doses and does not hold for any other form of selenium [32].

Aim of The Study:

This study is directed to accomplish the following aims:-

1- The measurement of the levels of erythrocyte membrane AChE of patients with type 2 DM and a healthy control group, to find whether there are significant differences between their levels or not.

2- To evaluate serum [Ca^{2+}] of type 2 DM and healthy control group in order to know whether there are changes in calcium homeostasis and to understand the expected role of calcium mediation in both DM and neurodegenerative diseases like neuronal shock.

3- Several biochemical variables related to oxidative stress in DM where taken in consideration in this work such as, iron, ferritin, copper, zinc, and selenium to point the changes in their levels and to find whether correlation exist between each of these variables and calcium.

Materials and Methods:

The study was conducted in Basrah Governorate, Basrah General Hospital, and Science College of Basrah University. Sixty patients suffering from type 2 diabetes mellitus (30 males, and 30 females) aged between (34)to(65) years with a mean age of (44.11 ± 12.03) were included in this study. The diabetic patients were diagnosed on the basis of WHO criteria [33]. All patients have a history of duration of disease more than one year and no one of them have a hypertension.

The control group comprised of (60) healthy individuals (30 males and 30 females), aged between (30) to (66) years with a mean age of (45.1± 11.87). The control subjects were selected from the staff of Basrah General Hospital and from the staff and the students of various colleges of Al-Basrah University. They had no history of diabetes mellitus or any other disease.

The activity of erythrocyte membrane AChE was determined colorimetrically in accordance to Ellman's Method [34], which is the most popular method as it is sensitive for kinetic and enzymological studies. The principle of this method is the measurement of the rate of production of the thiocholine as the acetylthiocholine is hydrolyzed. The rate of hydrolysis of acetylthiocholine by a red cell suspension at pH 7.2 is measured at 412 nm by the reaction of thiocholine with DTNB to give the yellow 5-thio-2-nitrobenzoate anion (molar absorptivity, $13.6x10^6$ $L.mol^{-1}cm^{-1}$). The activity is expressed per litter of packed red cells [35].

Serum calcium was determined by Human Kit (Human Gesellschaft for Biochemica and Diagnostica mbH Max-Planck-Ring21-D-65205 Wiesbaden – Germany). Serum iron concentrations was

determined by Human Kit (Human Gesellschaft for Biochemica and Diagnostica mbH Max-Planck-Ring21-D-65205 Wiesbaden – Germany). Whereas, serum zinc concentrations was determined by LTA- Kit (Via Milano, 15/F-20060 Bussero (Milano) Italy. Serum copper concentrations was determined by Randox Kit, CU 2340 (Randox laboratories Ltd., Ardmore, Antrim, UK, BT29, 4QY).

Serum selenium was determined by the hydride generation atomic absorption technique. By this technique selenium ion (Se^{+4}) was converted to selenium hydride (SeH_4) by a reducing agent. Sodium borohydride $NaBH_4$ was used for this purpose. The resultant hydride was then carried to the absorption quartz T-cell of the atomic absorption spectrophotometer by an inert gas. Argon gas was used in this study. The absorbance of the atomic selenium was measured against a blank of argon gas, and the concentration of selenium was then determined from a standard curve made previously [36].

Lipid peroxidation end product malondialdehyde (MDA) in serum was determined by colorimetric thiobarbituric acid (TBA) method. Under the acid and heating condition of the reaction, the lipid peroxides break down to form MDA, which complexes with TBA to form a coloured red compound that can be measured spectrophotometrically at 535 nm [37, 38].

Serum ferritin concentration was determined by using a VIDAS Ferritin Kit, REF 30 411, bioMerieux SA, France and VIDAS instrument.

Results and Discussion

1- The Effect of DM on Serum Calcium Levels:

Table (1) shows a significant decrease in serum calcium levels of males ($p < 0.001$), and females ($p < 0.05$) patients with DM comparing with control groups.

Table-1: Serum calcium levels in relation to sex in type-2 diabetic patients and healthy control groups.

Serum calcium(mmol/l)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	**Control**	30	2.397	0.069	0.012	2.371	2.423	2.2	2.5
	Type2DM	30	2.293**	0.065	0.011	2.268	2.317	2.2	2.42
Females	**Control**	30	2.374	0.076	0.014	2.346	2.403	2.25	2.5
	Type2DM	30	2.328*	0.069	0.012	2.302	2.354	2.1	2.45

**** P < 0.001** *** P < 0.05**

More than 99% of the calcium of the body is present in the skeleton in the form of "hydroxyapatite" [$Ca_5 (PO_4)_3 OH$], the remaining 1% serves as a number of important functions unrelated to bone structure. It was reported that type-2 diabetes mellitus associated with a higher bone mineral density, low bone turnover, and increased bone fracture risk in black and white women [39]. Furthermore, a recent study in an Arabic female population showed osteoporosis to be more common among postmenopausal females with type-2 diabetes [40].

Skeletal muscle weakness and morphological alterations in neuromuscular junctions was documented in diabetes. All of these alterations could be related to alterations in Ca^{2+} mobilization across muscle membrane in diabetes [41]. It has been proposed that disturbances in the intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan that elevates cytosolic free Ca^{2+} concentration in pancreas B-cells [42].

The normal range of serum calcium concentration (2.2- 2.6 mmol/l. About 45%

of serum calcium is bound to serum protein, 5% is complexed with anions (e.g., phosphate, bicarbonate, and citrate), and 50% is ionized. The ionized fraction affects cellular function [33].

It was reported that, serum calcium is low in juvenile diabetic patients [12] and not affected in type 2 DM [43], or non-significantly elevated in postmenopausal[44] and elderly type-2 diabetic women [45] due to the release of calcium from bone tissues[46], which was in compatible with our results.

On the other side, it was reported that elevated cytosolic free calcium [Ca_i^{2+}] and reciprocally reduced extracellular ionized calcium ion levels are observed in males with type-2 diabetes mellitus compared to control group [13]. This result is in agreement with our results.

In addition, low calcium intake is consistently found to be inversely associated with incident type-2 diabetes mellitus, and a high intake of calcium and vitamin D was linked with a lower risk of type-2 diabetes [47,48]. These studies support the results of this study.

The study show also a significant negative correlation between serum calcium levels and fasting blood sugar for both males ($r = -0.5$, $p < 0.001$), and females ($r = -0.269$, $P < 0.05$).

2- Calcium and Oxidative Stress in DM:

Increased levels of oxidative stress, that was recognized in diabetes mellitus and responsible for β-cells destruction and the disruption of cellular calcium homeostasis are believed to contribute to neuronal dysfunction and degeneration in many different age-related neurodegenerative conditions including Alzheimer's disease, Parkinson's disease, Huntington's disease, and stroke [49,50] through the following mechanisms which were illustrated in figure (1):

1- Impairment of the membrane transporters promotes membrane depolarization and calcium influx through glutamate receptor channels and voltage dependent calcium channels. In addition, subtoxic levels of membrane lipid peroxidation impair function of signal transduction pathways for neurotransmitters and growth factors. For example, coupling of muscarinic acetylcholine receptors and metabotropic glutamate receptors to the GTP binding protein GQ11 perturbs signaling by these neurotransmitters [14,50]. In addition, calcium mostly enters the cytoplasm from the endoplasmic reticulum (ER), or sarco-plasmic reticulum (SR) in muscles, by the action of the a second

messenger inositol-1, 4, 5-triphosphate (IP_3). IP_3 stimulates the opening of the endoplasmic calcium channels. It is produced by the hydrolyzing action of phospholipase-C (PLC), which is activated by ligand-receptor interactions to hydrolyze phosphatidylinositol-4, 5-bisphosphate (PIP_2) [4, 14]

2- Nervous shock may be resemble neuronal degeneration in Alzheimer's disease and stroke, that cause abrupt extracellular signals and causes a transient rise in the cytosolic [Ca^{2+}] through the sudden stimulation of the phosphinositide cascade by the neurotransmitter acetylcholine, which in turn opens the endoplasmic calcium channels by their interactions with the produced IP_3 [4, 14, 50, 51].

3- Increased cytoplasmic calcium levels disturb mitochondrial function leading to increased production of superoxide anion radical ($O_2^{\cdot-}$). If it is not converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD), due to unbalance between free radical production and enzymatic and non-enzymatic antioxidant systems, it will be converted by Fenton's and Haber-Wiss's reactions into hydroxyl radical, a potent inducer of membrane lipid peroxidation [14, 42].

4- Calcium ion promotes free radical production by activating the enzyme nitric oxide synthase (NOS) leading to the production of nitric oxide (NO), a free radical that can interact with superoxide to form the toxic compound peroxynitrite [50; 52; 53].

5- The free radicals produced by the nitration of peroxynitrite as well as hydroxyl free radicals act as oxidant agents to convert the membrane lipids to lipid peroxide [50].

6- In the presence of influenced nucleotide peroxidase, the superoxide free radical causes the conversion of pyrimidinic nucleotides by multi-steps reaction into alloxan. The produced alloxan induces calcium influx from extracellular and intracellular sources, which in turn participate in elevated cytosolic [Ca^{2+}] [42].

7- Alloxan was known as; a diabetogenic substance has the ability to destroy the insulin secretion by destroying the β-cells of pancreas. The resultant hyperglycemia goes auto-oxidation [54].

8- Calcium ion can also activate the phospholipase A_2 pathway, leading to the production of arachidonic acid, which is then acted on by lipo-xygenase (LOX) and cyclooxygenases

(COX), again generation free radicals. The relative contributions of these different pathways for increased oxidative stress and perturbed calcium may differ among age-related neurodegenerative conditions [4, 50].

9- Lipid peroxidation, that was distinguished in some age-related neurodegenerative conditions like Alzheimer's disease, due to accumulation of amyloid b-peptide (Ab), impairs the function of membrane ion motive ATPases as well as glucose and glutamate transporters, by a mechanism involving covalent modification of the transport protein by the aldehyde product of lipid peroxidation, 4-Hydroxynonenal (HNE), which in turn promotes membrane depolarization and calcium influx through each of glutamate receptor and voltage-dependent calcium channels [50, 55, 56].

10- The resulted high intracellular glucose levels and the impaired glucose transporters lead to glucose diffusion out of the cell and blood glyction, which in turn increases free radicals production and advanced glycation end products (AGE_s) [57].

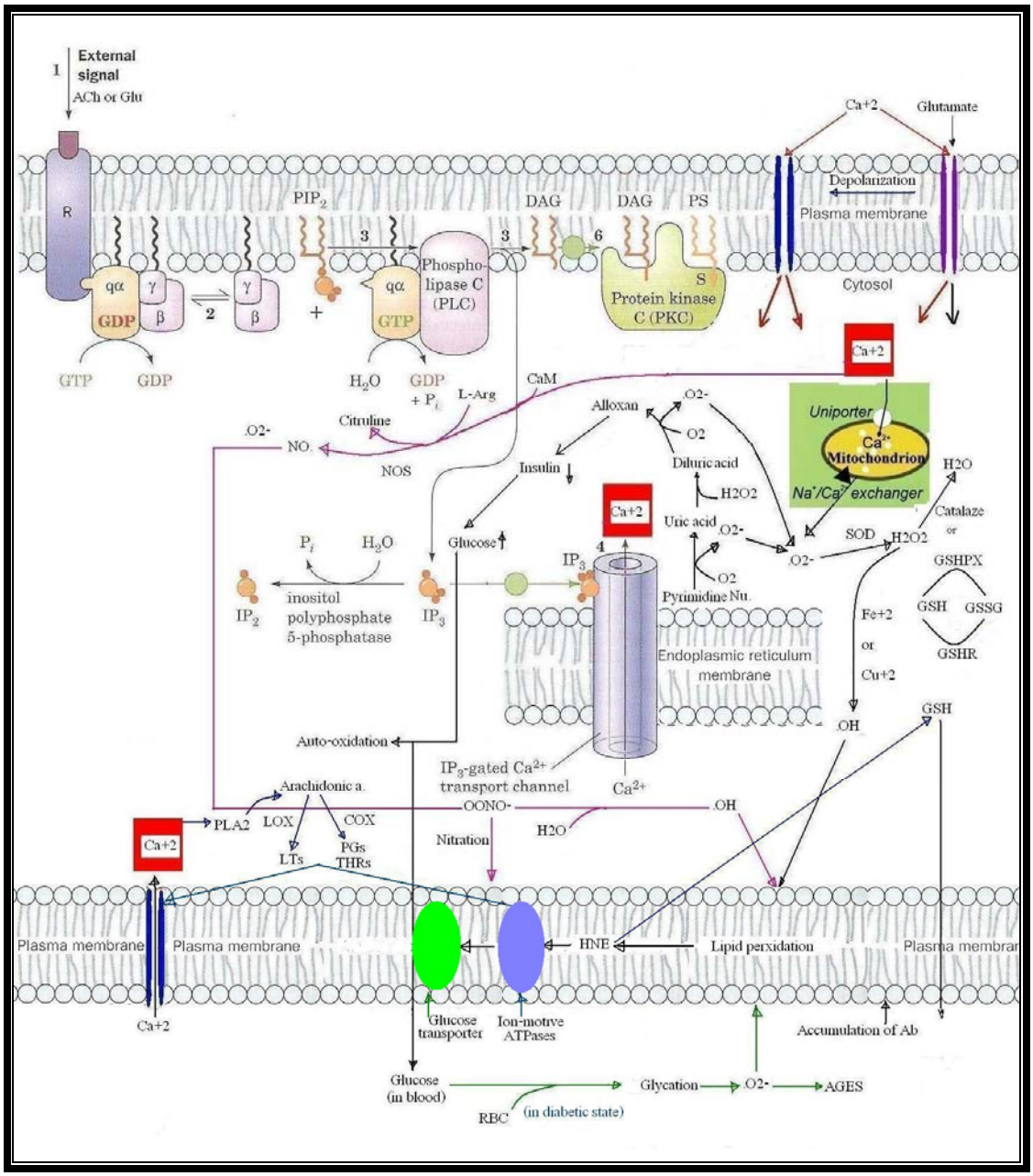

Figure 1: A schematic figure represents our suggestion for the role of Ca^{2+} as a factor mediates neurotransmission, stimulation of events with oxidative stress generation, especially in β-cells, and diabetes mellitus type 2 developments in humans.

$O_2^{\cdot -}$: superoxide anion; SOD: superoxide dismutase; GSHPX: glutathione peroxidase; GSHR: glutathione reductase; LOX: lipoxygenase; GOX: cyclooxygenase; PG_s: prostaglandins; THR_s: thromboxanes; NOS: nitric oxide synthase; HNE: 4-hydroxynonenal; Ab: amyloid b-protein; ACH: acetylcholine; PIP_2: phosphotidylinositol-4, 5-bisphosphate; IP_3: inositoltriphosphate; LT_s: peptidoleukotrienes; $.OONO^-$: peroxynitrite.

3- The Effect of DM on Erythrocyte Membrane AChE:

Table (2) shows that the activity of the erythrocyte membrane AChE were significantly elevated in both sexes patients with diabetes mellitus type-2 comparing with that of the control groups, and the control males showed higher levels than the control females in both cases.

Table-(2): Red cell-AChE in relation to sex in type 2 DM and controls.

RBC-AChE **(kU/l)**						**95% C.I**		**Range**	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	**Control**	30	11.74	0.79	0.14	11.44	12.03	9.90	12.89
	Type2DM	30	13.12*	2.21	0.40	12.30	13.95	9.13	17.10
Females	**Control**	30	11.12	1.21	0.22	10.66	11.57	9.42	13.00
	Type2DM	30	12.73**	2.25	0.41	11.89	13.57	10.00	16.93

*** P < 0.005 ** P < 0.001**

Several studies were made to determine the activity of the erythrocyte membrane AChE in diabetic patients. Some of these studies showed a significant decrease in the enzyme activity of diabetic type 1 [58], and type 2 [59].

On the other hand, another studies showed that the activity of the red cell enzyme was elevated in islets of Langerhans of diabetic rats [60], erythrocyte membrane AChE of type 1diabetic patients[61] and type 2 DM [62, 63]. These findings are well-matched to the results of this study, that were illustrated in table (2). It was suggested that, abnormal dynamic properties of the erythrocyte membrane in

diabetic patients may play a major role in the described changes of the enzyme activity [61].

We can interpret the increased enzyme activity of diabetic type-2 patients in accordance to the suggestion of Rao , et al, 2007, "DM type-2 is a low-grade systemic inflammation and acetylcholine has anti-inflammatory actions" [60]. So the estimated increased enzyme activity was enhanced by the induced high concentrations of the anti-inflammatory acetylcholine in diabetic patients.

Erythrocyte membrane acetylcholinesterase (AChE) activity was shown by using the regression analysis to have a negative significant correlation with serum calcium concentrations in males ($r = -0.421$, $p < 0.01$), and a non-significant negative correlation ($r = -0.201$, $p = 0.124$) in females.

4- Blood Iron Status in Type-2 Diabetes Mellitus:

a- serum iron:

Table (3) shows that serum iron concentration is a sex-dependent in both, diabetes type 2 and control subjects, and a non-significant elevation in the serum iron concentration of diabetic patients as compared to control subjects.

A significant increase in a free iron levels (non-transferrin bound) was found in diabetes type 2 under poor glycemic [18]. It was reported that hyperglycemia causes hemoglobin glycation, which releases the iron in the Free State [17].

To our knowledge, dimensioned studies were reported on serum iron concentrations in diabetes. It may be due to non-significant differences in serum iron concentrations between controls and diabetic patients. For example, Lecube, et al, 2004, found a non-significant decrease in serum iron levels of diabetic type 2 patients compared to control. Whereas, Mahdi , 2006, showed that non-significant increase in serum iron concentrations in diabetic type 2 patients compared with control group. Mahdi found also, that serum iron concentration is sex-dependent[64]. These results are in compatible with our results in table (3).

Table-(3): Serum iron concentration in relation to sex in type 2 diabetic patients and control groups

Serum iron(µg/dl)						95% C.I		Range	
sex	group	n	Mean	SD	SE	Lower	Upper	Min.	Max.
Males	Control	30	105.42	18.422	3.363	98.541	112.299	80.00	137.60
	Type2DM	30	107.03	21.716	3.964	98.927	115.144	80.00	138.00
Females	Control	30	90.84	21.134	3.858	82.948	98.731	70.00	126.40
	Type2DM	30	100.20	23.309	4.255	91.496	108.903	56.00	137.00

Using of the regression analysis showed non-significant positive correlation between serum calcium and serum iron concentrations for males ($r = 0.127$, $p = 0.33$), and non-significant negative correlation for females ($r = -0.226$, $p = 0.083$).

b- Serum Ferritin:

Table (4) shows a significant elevation in the serum ferritin concentration of diabetic patients ($p < 0.001$) as compared to the control group.

Circulating ferritin is usually in equilibrium with that in stores [33]. Several studies were performed to know the association between high serum ferritin (SF) levels and the risk of development of diabetes mellitus. Increased iron stores have been found to predict the development of type 2 diabetes while iron depletion was a protective [19, 65, 66]. It was reported that a significant correlation between SF and insulin resistance syndrome (IRS) [67, 68].

Several researchers recorded significant elevations in serum ferritin levels in type 2 diabetic patients compared to control groups [65, 69-71]. The results of this study a compatibility to the above reported results. This study showed a significant correlation ($r = 0.709$, $p < 0.001$) between serum ferritin and the fasting blood sugar.

Table-(4): Serum ferritin in relation to sex in type 2 diabetic patients and control groups

S.ferritin (ng/ml)						95% C.I		Range	
sex	group	n	Mean	SD	SE	Lower	Upper	Min.	Max.
Males	Control	10	109.80	49.49	15.65	74.39	145.2	40.00	170.00
	Type2DM	10	331.05**	186.02	58.82	197.98	464.1	43.26	620.00
Females	Control	10	59.38	27.19	8.59	39.92	78.83	17.00	100.00
	Type2DM	10	302.47**	118.37	37.43	217.79	387.1	134.10	524

** $P < 0.001$

The regression analysis showed a non-significant negative correlation between serum calcium and serum ferritin concentrations in males ($r = -0.124$, $p = 0.6$), and a non-significant positive correlation for females ($r = 0.25$, $p = 0.288$).

5- Serum Copper in Type 2 DM:

Table (5) shows that serum copper concentration is a sex-independent and a significant elevation in the serum copper concentration ($p < 0.01$) of diabetic patients as compared to the control subjects.

Table-(5): Serum copper in relation to sex in NIDDN and controls.

Serum copper(µg/dl)						95% C.I		Range	
sex	group	n	Mean	SD	SE	Lower	Upper	Min.	Max.
Males	Control	30	91.84	17.48	3.19	85.31	98.37	70.00	122.89
	Type2DM	30	106.61*	15.41	2.81	100.86	112.37	85.00	140.00
Females	Control	30	91.98	12.21	2.23	87.42	96.54	80.00	121.50
	Type2DM	30	105.88*	19.33	3.52	98.66	113.10	80.10	145.60

*** P < 0.01**

Copper is an essential trace element, acts as a critical cofactor when incorporated into specific cupro-enzymes that catalyze electron transfer reactions required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, and connective tissue formation [72]. Copper deficiency causes accumulation of arterial lipid peroxides [73], leads to elevated blood cholesterol levels [74].

Copper overload are consistent, however, with oxidative damage to membranes or macromolecules [75]. Higher plasma copper has been found in diabetic with and without complications [28, 64, 74]. The results of this study are in agreement with these studies.

These observations could be explained by the antagonistic effect of the zinc and zinc deficiency, which is taken place in diabetic patients and could greatly increase copper absorption via the gastrointestinal tract [76].

Using of the regression analysis showed a non-significant negative correlation between serum calcium and serum copper concentrations for males (r = 0.182), and no correlation for females (r =0.016).

6- Serum Zinc in Type 2 DM:

Table (6) shows a significant decrease in the serum zinc concentration ($p < 0.001$) of diabetic patients as compared to the control subjects.

Table-(6): Serum zinc in relation to sex in type 2 diabetic patients and control groups.

Serum zinc(µg/dl)						95% C.I		Range	
Sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	94.95	9.28	1.69	91.48	98.41	83.30	111.30
	Type2DM	30	78.96*	6.27	1.14	76.61	81.30	70.00	104.10
Females	Control	30	95.52	12.65	2.30	90.79	100.24	80.00	116.00
	Type2DM	30	80.18*	8.59	1.56	76.97	83.39	70.60	105.00

*** P < 0.001**

Zinc is one of the essential elements that are required to maintain the normal physiological function of all forms of life. It plays a central role in the immune system[77]. Zinc deficiency has been recognized to be associated with chronic illnesses such as diabetes mellitus [78]. It has been suggested that zinc may be involved in the development or progression of both type 1 and type 2 diabetes [21- 23].

The significant decrease in serum zinc levels of diabetic patients in comparison to healthy control, that was noticed in this study (Table 6), is in agreement with many studies made previously [22, 23, 64,74].

On the other hand Diwan et al, 2006, reported that serum zinc in type 2 diabetic patients were not found to be statistically different as compared to healthy control [79]. Moreover, Zargar , et al, 1998, noticed an elevation in serum zinc level of type 2 diabetic patients [76].

It has been postulated that low levels of zinc in diabetic patients may be due to alterations in metabolism of zinc that decreases it's serum concentration and increases its urinary excretion [22, 76], as well as intestinal absorption of zinc is impaired in diabetic patients [21]. Since zinc plays a clear role in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin in the hexameric form, the decreased zinc, which

affects the ability of the islets cell to produce and secret insulin, might then compound the problem, particularly in type 2 diabetes [21].

Nsonwu AC, et al, 2006, were attributed the higher levels of serum zinc of diabetic females, that was found in their study as compared to diabetic males to hormonal imbalance associated with diabetic state and to the additional seminal loss of zinc in diabetic males [22].

The regression analysis showed a significant positive correlation between serum calcium and serum zinc concentrations for males ($r = 0.434$, $p = 0.001$), and a significant positive correlation for females ($r = 0.227$, $p = 0.032$).

7- Serum Selenium in Type 2 DM:

Table (7) shows that serum selenium concentration was significantly decreased in diabetic patients ($p < 0.001$) as compared to the control subjects.

Table-(7): Serum selenium in relation to sex in NIDDM and Controls

Serum Se(ng/ml)						95% C.I		Range	
Sex	group	n	Mean	SD	SE	Lower	Upper	Min.	Max.
Males	Control	30	121.73	8.01	1.46	118.74	124.72	100	130
	Type2DM	30	83.13*	6.03	1.10	80.88	85.38	72	92
Females	Control	30	119.16	7.04	1.28	116.53	121.79	103	129
	Type2DM	30	82.56*	7.47	1.36	79.77	85.35	70	96

*** P < 0.001**

Selenium, an essential trace element, is involved in the complex system of defense against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins [80, 81]. Because of its antioxidant properties, selenium might thus prevent the development of diabetes. In addition, selenate ($SeO_4^{=}$), an inorganic form of selenium, mimics insulin activity in experimental models [30, 82].

In a cross-sectional analysis of the Health Professional Study, toenail selenium, a biomarker of selenium status, was inversely associated with diabetes [83]. The studies that were made to determine serum selenium levels in patients with diabetes type-2 showed contradictive results. In a probability sample of the U.S. population, high serum selenium levels were positively associated with the prevalence of diabetes [84]. Nsonwu, et al, 2006, found a significant increase in serum selenium of type 2 diabetic females and a significant decrease in type-2 diabetic males. They attributed this gender related differences to hormonal imbalance associated with the diabetic state [22]. In a cross-sectional study of a representative sample of Asian residing in Singapore, the researchers found a non-significant decrease in serum selenium levels of diabetic patients [85].

Other Studies have shown reduced levels of selenium in people with both type1 and type 2 diabetes [24]. The results of this study that was shown in Table (7) are in agreement with some of the above studies.

Since selenium has antioxidant, antidiabetic and insulin-memetic effects [30, 82]. Hence, insulin deficiency in diabetic type-2 patients, which was investigated in this study, is an acceptable result.

The regression analysis showed a significant positive correlation between serum calcium and serum selenium concentrations in males ($r = 0.652$, $p = 0.001$), and females ($r = 0.314$, $p = 0.014$).

Conclusions:

The following conclusions have been abstracted from the present study:

1- Diabetes mellitus type 2 is associated with elevations in erythrocyte membrane AChE activities. These elevations may be due to increased concentrations of their substrate, a neurotransmitter and an anti-inflammatory agent ACh, because DM is regarded a low grad inflammatory disease.

2- The decreased levels of serum Ca^{2+}, that were shown in DM, may be due to changes in calcium homeostasis, which was represented by Ca^{2+} influx accompanied with this disease. This influx of Ca^{2+} in turn, increases ROS production and oxidative stress.

3- The decreased levels of each of Zn and Se in DM contributed in oxidative stress development is due to inactivation of enzymatic antioxidant defense represented in Cu/Zn- SOD and Se- GPx.

4- Type 2 DM is associated with increased levels of trace elements related to oxidative stress development iron and copper.

5- The neuronal shock (stroke) causes elevations in the exocytosis of ACh, as a result of high calcium influx into the neurons, and it causes calcium influx into muscle cells also, in order to its contraction. This influx of calcium enhances oxidation processes, which lead to oxidative stress, and may be complicates to diabetes.

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