Abstract

In this study 298 patients suffering from diabetes mellitus type 2 (150 male, and 148 female) aged between 35 to 67 years with a mean age of (45.11±12.03) were included. The study was carried out from first of November 2009 to the day 31 of May 2010. The samples were obtained from Al-Kassim hospital and Merjan Teaching Hospital in Hilla city. The study included three groups:

Firstly: 149 patients with type 2 diabetes mellitus treatment with Daonil. Secondly: 149 patients with type 2 diabetes mellitus treatment with Glucophage. Thirdly: 83 apparently healthy subjects were chosen as healthy people.

The results show a significant increase p<0.05 in Acetylcholinesterase activity of patients treated with daonil and glucophage ≤5 years when compared with control group, while AChE activity found to be highly significant increase p<0.001 in patients treated more than five years when compared with ≤5 years and control groups. No differences were found in blood AChE activities between subgroups of each sample used in this study.

Corrected total calcium and ionized concentrations in DM type 2 treated with daonil and glucophage groups ≤5 and more than five years found to were highly significantly increased p<0.001 when compared with that of the control, while there was a non-significant difference p>0.05 between the males and females when compared in the same group (control, daonil and glucophage) in all duration. A significantly increased p<0.001 in the corrected total calcium concentration of DM patients treatment with daonil (more than five years) when compared with that of the patients treatment with glucophage.

A significant increase p<0.05 in magnesium concentrations in diabetes mellitus patients (daonil, and glucophage) ≤5 years when compared to control group. On the other hand a highly significant increase p<0.001 of magnesium concentrations were found in sera of DM patients more than five years when compared to DM patients ≤5 years and control group. No significant difference were found between males and females in control group and patients groups.

A significant decreased p<0.05 in total antioxidant (TAC) concentration in DM patients treatment ≤5 years with daonil and glucophage was found when compared with control groups. A highly significant increase of total antioxidant p<0.001 in sera of TCA of DM type 2 patients treated (6-15y) when compared with that of healthy control group and ≤5 years. No significant difference was found in the males and females of DM type 2 patients treated with daonil and glucophage (≤5y or 6-15 y) when compared with control group P>0.05.
Introduction

Diabetes mellitus is a heterogeneous metabolic syndrome with wide variations in its presentation, clinical course and complication.[1]

Type 2 diabetes mellitus is similar to type 1 and other forms of diabetes in that it is defined by high levels of plasma glucose and is associated with many long-term complications caused or enhanced by hyperglycemia and related metabolic abnormalities. Usually involving excess weight and insulin resistance. In these patients, the pancreas makes insulin initially, but the body has trouble using this glucose-controlling hormone. Eventually the pancreas cannot produce enough insulin to respond to the body’s need for it. Type 2 diabetes is due to a combination of lifestyle and genetic factors. [2,3]

Type 2 diabetes is characterized by elevated fasting blood glucose levels secondary to insufficient insulin action.[4] Other signs and symptoms of diabetes onset may include weight loss, fatigue, frequent urination, blurred vision, increased thirst or hunger, and slow-healing wounds or sores.[5]. The diagnostic criteria for determining diabetes have recently been changed in order to increase the sensitivity of the test. Currently, diabetes is diagnosed by a fasting glucose of 126 mg/dl or a random glucose of 200 mg/dl.[6]. In general, the goals of treatment of type 2 diabetes are the same as for type 1. For a typical patient, these include an HbA1c level of less than 7%, fasting and preprandial glucose levels between 90 and 130 mg/dL, and peak postprandial glucose values below 180 mg/dL.[7]. All patients with diagnosed diabetes should learn about the disorder itself, its natural history and complications, and the range of therapies available. All should learn about self-measurement of blood glucose (SMBG) and obtain the necessary equipment.[8]

Sulfonylureas (SUs) are most effective in patients who have had diabetes for less than 10 years and can
still secrete considerable amounts of insulin. Although initial doses of SUs directly stimulate secretion of insulin, long-term treatment mainly potentiates the effects of glucose (and other stimuli such as amino acids) on insulin secretion, allowing adequate insulin levels at lower glucose levels. The result is a predominant reduction of fasting plasma glucose FPG, typically 50 to 70 mg/dL, with very little effect on postprandial increments.[9]

Metformin is the only biguanide extensively used these days, and has become the first-line oral drug in type 2 diabetes. Metformin is used to prevent progression of glucose intolerance and to avoid atherogenic dyslipidemia. The glucose-lowering potential of guanides was first described in medieval times when extracts of Galega officinalis (goat’s rue or French lilac) were used as treatment of diabetes in Europe.[10] The mechanism of action of metformin in humans by not acceleration endogenous glucose production.[11,12,13]. In patients with type 2 diabetes metformin has been shown to inhibit endogenous glucose production in most.[14,15], but not all studies to various degrees (from a nonsignificant ~10% up to a significant ~30%).[15] This could largely be accounted for by inhibition of gluconeogenesis although an additional inhibitory effect of metformin on glycogen breakdown is likely.[16,17]. Metformin decreases hepatic glucose production, mostly through inhibiting gluconeogenesis [18,19]. Because it requires the presence of insulin to be effective and because plasma insulin levels decrease during its use, metformin may be considered a hepatic insulin sensitizer. Hypoglycemia almost never occurs with metformin mono therapy. Metformin also decreases plasma triglyceride and low density lipoprotein (LDL) cholesterol levels and some times increases HDL cholesterol levels. In addition, plasma plasminogen activator inhibitor-1 (PAI-1) activity declines.[20]

Diabetic neuropathy (DN) is one of the most common and most distressing late complication of diabetes mellitus. It affects nearly 50% of diabetic patients it has varied presentations and treatment is not very helpful in many of the cases.[21] Neuropathic complications are divided into autonomic dysfunction and sensory dysfunction. Sensory complications include paresthesias and the loss of sensation in the extremities, leading to an increase in serious foot problems in diabetics. Autonomic complications include sexual dysfunction, gastrointestinal disturbances, bladder dysfunction, and postural hypotension.[22]

Painful diabetic peripheral neuropathy (PDN) is a significant cause of pain and distress in patients with diabetes mellitus (DM).[23]. It affects patients with both Type 1 and 2 DM. Poor blood glucose control is a prominent risk factor. The overall incidence is 20-24%. The prevalence increases as the disease progresses, with approximately 50% of patients developing PDN 25 years after initial diagnosis of DM.[24] It is best defined as the presence of symptoms and/or of peripheral nerve dysfunction in diabetes after the exclusion of other causes (malignancy, chronic alcoholism, nutritional deficiency, infections, iatrogenic, etc.)[25]. Painful diabetic peripheral neuropathy is a type of neuropathic pain (i.e., caused by damage or dysfunction of the nervous system) Since a long time ago it has been known that the risks of cardiovascular disease CVD are increased for diabetes patients. The classic concept has been that macrovascular disease is a diabetes
specific complication, but it has also been discussed whether both type 2 diabetes and CVD stem from the same etiologic causes in form of genetics and environment [26].

Acetylcholinesterase (AChE; EC 3.1.1.7.) is an enzyme participating in cholinergic neurotransmission. It breaks down acetylcholine which terminates the neurotransmission process [27]. The main function of AChE is believed to be the termination of the action of neurotransmitter acetylcholine (ACh) at the cholinergic synapses by hydrolyzing it. Occurrence of cholinesterases are occur in two forms: pseudo-cholinesterase which present in the liver, pancreas, heart, and the white mature cells of the brain, serum, as well as in plants; While, the true or red cell cholinesterase or acetycholinestrase occurs in erythrocytes, lung, spleen, nerve cell endings, and in the gray matter of brain [28].

AChE activity is inhibited by many compounds. The number of known inhibitors is rather extensive. Two main types of inhibitors can be distinguished from a practical point of view: toxins and drugs [29]. From a mechanistic point of view, the inhibitors are compounds with different structural motives as they can bind to the esteratic part of the active site by esterification of serine hydroxyl, or interact with the alpha anionic part of the active site, the aromatic gorge and the peripheral anionic site [30]. The assay of acetylcholinesterase (AChE) activity plays an important role in diagnostic, detection of pesticides and nerve agents [31].

Calcium, the king of minerals is the fifth most common element and the most prevalent cation found in the body which has a very important role to play in skeletal mineralization, blood coagulation, neuromuscular conduction, maintenance of normal tone and excitability of skeletal and cardiac muscle, stimulus secretion of exocrine glands and preservation of cell membrane integrity and permeability, particularly in terms of sodium and potassium exchange [32].

Magnesium is the fourth most common cation in the body and the second most common intracellular cation after potassium. The central role of magnesium within the chlorophyll molecule and as a cofactor for the enzymes in the 12-transphosphorylation reactions in photosynthesis makes it probably the most important inorganic element in the production of food and fossil fuel [33]. In addition, it has a fundamental role as a cofactor in more than 320 enzymatic reactions involving energy metabolism and nucleic acid synthesis [34].

Oxidative stress is involved in the process of aging [35] and various chronic diseases such as atherosclerosis [36], diabetes [37] and eye disease [38]. An excessive amount of reactive oxygen/nitrogen species (ROS/RNS) leading to an imbalance between antioxidants and oxidants can cause oxidative damage in vulnerable targets such as unsaturated fatty acyl chains in membranes, thiol groups in proteins, and nucleic acid bases in DNA [39]. Such a state of “oxidative stress” is thought to contribute to the pathogenesis of a number of human diseases [40].

The current study tend to evaluate the role of duration of uncontrolled diabetes mellitus (type 2) patients in less and more than five years and investigate the complication of diabetes mellitus (neuropathy) in different treatment (Daonil and Glucophage) by determination the Acetylcholinesterase erythrocyte membrane, corrected total calcium, ionized calcium, magnesium and total...
Materials and Methods

298 patients suffering from type 2 diabetes mellitus (150 male, and 148 female) aged between 25 to 67 years with a mean age of (45.11±12.03) were included in this study. The study was carried out from first of November 2009 to the day 31 of May 2010. The samples were obtained from Al-Kassim hospital and Merjan Teaching Hospital in Hilla city. The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine /Babylon University.

The diabetic patients were diagnosed on the basis of WHO criteria. The general criteria for all subjects in this study include all patients not suffering from any disease (e.g. Hypertension, asthma, smoker, alcoholism, etc.) and not given any medication only treatment with daonil or glucophage, any subject that have not these criteria are excluded from this study. The study included three groups: Firstly: 149 patients (76 males, 73 females) with type 2 diabetes mellitus treatment with daonil. Secondly: 149 patients (74 males, 75 females) with type 2 diabetes mellitus treatment with glucophage. Thirdly: 83 (42 males, and 41 females) apparently healthy subjects were chosen as healthy people, they were non smoker, alcohols, don’t have any history of chronic diseases. The study samples were serum and blood, samples were aspirated and divided into four parts, and stored at -20 °C until analysis. Determined of acetylcholinesterase erythrocyte membrane AChE activity was done according to [41] determined corrected total calcium was done according to [42] determined Ionized calcium was done according to [43], determined magnesium[44] and total antioxidant capacity was done according to [45] Student's t-test was used to estimate differences between the groups. The differences were considered significant when the probability was (p<0.05) and highly significant at (p<0.001).

Results

1-Determination of erythrocyte membrane Acetylcholinesterase (AChE) activity in blood:

The result revealed an increased of acetylcholinesterase activities in all groups when compared with control group. A significant increased p<0.05 were found in AChE activity of patients treated with daonil and glucophage less than five years when compared with control group, while highly significant increased p<0.001 in more than five years when compared with less than five years and control groups.
No differences were found in blood acetylcholinesterase activities between subgroups of each sample used in this study as shown in figure 1 and 2.

2-Determination of Corrected Total Calcium and Ionized Concentrations in Serum:
From the results in table 1 and figure (3 and 4) corrected total calcium and ionized concentrations in diabetes mellitus(type 2) treated with daonil and glucophage.
glucophage groups less and more than five years found to were highly significantly increased p<0.001, when compared with that of the control and subgroups, while there was a non-significant difference p>0.05 between the males and females when the compared in the same group (control, daonil and glucophage) in all duration.

Table 1 Corrected total calcium Concentration (mmol/L) in sera of control and patients with diabetes mellitus (type2) treatment with daonil and glucophage (M=males, F=females, Mean Value± SD, **= p< 0.001

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Gender</th>
<th>No.</th>
<th>Mean Value ±SD</th>
<th>RangeValue</th>
</tr>
</thead>
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<tr>
<td>Corrected Total Calcium Conc.</td>
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<tr>
<td>Conc. (mmol/L)</td>
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<tr>
<td>Control</td>
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<tr>
<td></td>
<td>M+F</td>
<td>83</td>
<td>2.29±14.24</td>
<td>2.42-1.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>42</td>
<td>2.30±13.22</td>
<td>2.64-2.11</td>
<td></td>
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<tr>
<td></td>
<td>F</td>
<td>41</td>
<td>2.29±12.76</td>
<td>2.43-2.23</td>
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</tr>
<tr>
<td>DM Patients Treated with Daonil</td>
<td>≤ 5 Y</td>
<td>M+F</td>
<td>76</td>
<td>1.71**±0.76</td>
<td>2.16-1.64</td>
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<tr>
<td></td>
<td>M</td>
<td>39</td>
<td>1.68**±1.24</td>
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<tr>
<td></td>
<td>F</td>
<td>37</td>
<td>1.75**±0.78</td>
<td>1.96-1.24</td>
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<td></td>
<td>6 -15 Y</td>
<td>M+F</td>
<td>75</td>
<td>1.64**±0.55</td>
<td>1.98-1.32</td>
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<tr>
<td></td>
<td>M</td>
<td>39</td>
<td>1.65**±0.83</td>
<td>1.99-1.45</td>
<td></td>
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<tr>
<td></td>
<td>F</td>
<td>36</td>
<td>1.63**±0.56</td>
<td>2.1-1.33</td>
<td></td>
</tr>
<tr>
<td>DM Patients Treated with Glucophage</td>
<td>≤ 5 Y</td>
<td>M+F</td>
<td>76</td>
<td>1.59**±0.55</td>
<td>2.32-1.43</td>
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<tr>
<td></td>
<td>M</td>
<td>39</td>
<td>1.55**±0.83</td>
<td>1.99-1.46</td>
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<tr>
<td></td>
<td>F</td>
<td>37</td>
<td>1.63**±0.56</td>
<td>2.3-1.23</td>
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<td></td>
<td>6 -15 Y</td>
<td>M+F</td>
<td>75</td>
<td>1.45**±0.71</td>
<td>1.93-1.32</td>
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<td></td>
<td>M</td>
<td>39</td>
<td>1.43**±0.16</td>
<td>1.87-1.21</td>
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<td></td>
<td>F</td>
<td>36</td>
<td>1.48**±0.51</td>
<td>2.2-1.43</td>
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</table>

A significantly increased p<0.001 in the corrected total calcium concentration of diabetes mellitus patients treated with daonil (more than five years) when compared with that of the patients treated with glucophage.
**Fig. 3** Ionized calcium conc(mmol/L) in sera of control and patients with DM(type 2) treated with daonil and glucophage ≤ 5 y.

**Fig. 4** Ionized calcium conc(mmol/L) in sera of control and patients with DM(type 2) treated with daonil and glucophage 6-15 y.

**Magnesium Concentration in Serum:**

In our study we found that a significant increase p<0.05 in magnesium concentrations in diabetes mellitus patients (daonil, and compared to control group. On the other hand a highly significant increase p<0.001 of magnesium concentrations in sera of diabetes mellitus patients more than five years (daonil and glucophage) when compared to
diabetes mellitus patients more than five years and control group.

No significant difference were found between males and females in control group and patients groups.

**Table 2** Total magnesium Concentration (mmol/L) in sera of control and patients with diabetes mellitus(type2)treated with daonil and glucophage .

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Gender</th>
<th>No.</th>
<th>Mean Value ±SD</th>
<th>RangeValue</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Total Magnesium Conc.(mmol/L)</td>
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<tr>
<td>M+F</td>
<td>83</td>
<td>0.82±0.23</td>
<td>1.04-0.62</td>
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<tr>
<td>M</td>
<td>42</td>
<td>0.85±0.21</td>
<td>1.06-0.63</td>
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<tr>
<td>F</td>
<td>41</td>
<td>0.78±0.18</td>
<td>1.02-0.53</td>
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<td><strong>DM Patients Treated with Daonil</strong></td>
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<td>Total Magnesium Conc.(mmol/L)</td>
<td>≤ 5 Y</td>
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<tr>
<td>M+F</td>
<td>76</td>
<td>0.63*±0.16</td>
<td>0.69-0.58</td>
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<tr>
<td>M</td>
<td>39</td>
<td>0.61*±0.21</td>
<td>0.69-0.58</td>
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<tr>
<td>F</td>
<td>37</td>
<td>0.59*±0.17</td>
<td>0.7-0.59</td>
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<td>6-15 Y</td>
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<tr>
<td>M+F</td>
<td>75</td>
<td>0.51**±0.17</td>
<td>0.62-0.51</td>
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<tr>
<td>M</td>
<td>39</td>
<td>0.52**±0.18</td>
<td>0.64-0.59</td>
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<td>F</td>
<td>36</td>
<td>0.51**±0.16</td>
<td>0.63-0.51</td>
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<td><strong>DM Patients Treated with Glucophage</strong></td>
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<tr>
<td>Total Magnesium Conc.(mmol/L)</td>
<td>≤ 5 Y</td>
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<tr>
<td>M+F</td>
<td>76</td>
<td>0.59*±0.12</td>
<td>0.71-0.58</td>
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<tr>
<td>M</td>
<td>39</td>
<td>0.58*±0.23</td>
<td>0.69-0.57</td>
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<td>F</td>
<td>37</td>
<td>0.59*±0.16</td>
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<td>6-15 Y</td>
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<td>M+F</td>
<td>75</td>
<td>0.51**±0.21</td>
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<tr>
<td>M</td>
<td>39</td>
<td>0.51**±0.26</td>
<td>0.62-0.51</td>
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<tr>
<td>F</td>
<td>36</td>
<td>0.52**±0.23</td>
<td>0.66-0.52</td>
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4-Determination of Total Antioxidant Concentration in Serum:
Firstly, the figure 5 represents the results of total antioxidant concentration in diabetes mellitus(type 2)patients treated(≤y) with daonil 1.23 ± 0.82 and glucophage 1.24 ± 0.71 and compared with control groups 1.59 ± 0.72 mmol/dl. The test shows a significant decreased p<0.05 in total antioxidant concentration in diabetes
mellitus patients compared with the healthy control group.

Fig. 5 Total antioxidant concentration conc(mmol/L) ) in sera of control and patients with DM(type 2) treated with daonil and glucophage ≤ 5 y.

Secondly, a highly significant increase in glucose concentration in sera of diabetes mellitus(type 2) patients treated (6-15y) with daonil 0.89 ± 0.87 and glucophage 0.88 ± 0.74 when compared with that of
healthy control group 1.59 ± 0.72 as shown in figure 6.

Thirdly, a highly significant increase p<0.001 of glucose concentration in sera of diabetes mellitus(type 2) patients treatment(6-15y) when compared with that of the patients less than five years.

Finally, No significant was found in the males and females of diabetes mellitus(type 2) patients treated with daonil and glucophage (less than 5 y or 6-15 y)when compared with control group P>0.05  as shown in figure 5 and 6 respectively.

Discussion

The results of this study have shown that there are significant and highly significantly increased in erythrocyte membrane acetylcholinesterase (AChE) activities in duration five and more than five years respectively in patient treated with daonil and glucophage .This study agreement with other studies ,in a study of Abdulkareem show increased in erythrocyte membrane acetylcholinesterase [46].Also several studies showed that the activity of the red cell enzyme was elevated in diabetic patients comparing to control.

Acetylcholinesterase is a key enzyme in cholinergic neurotransmission.

Acetylcholinesterase from erythrocyte membrane is a hydrophobic integral membrane enzyme, which is similar to enzyme forms purified from nervous tissues [47]. Acetylcholinesterase is located in the outer monolayer of the erythrocyte membrane and is a final tool for measuring the structural changes in the membrane under the action of various factor. Diabetes mellitus is an example of a complex metabolic disorder that may alter the properties and organization of cell membranes. We have shown that erythrocytes from diabetic patients exhibit a changed membrane lipid composition[48],decreased membrane fluidity[49], increased susceptibility to hydroperoxide induced oxidation[50],and hyperpolarization membrane [51]. The oxidative stress, well documented in diabetes mellitus, may damage the system of nitric oxide production, leading to insulin resistance and atherosclerosis [52].Earlier we have shown a significant hyperpolarization of red blood cell membranes of diabetic patients [51].The changes of erythrocyte membrane properaties, such as lipid bilayer packing and composition, fluidity and transmembrane potential, during diabetes development may influence the activity of membrane acetylcholinesterase .

We propose that change of erythrocyte membrane acetylcholinesterase activity under diabetes mellitus development may represent a mechanism of cell injury promoted by oxidative stress. The oxidant-induced membrane disturbances change the membrane-bound enzyme activity. Rao, et al[53] reported that AChE was found to be higher in islets ' Langerhans in rats made diabetic with streptozotocin compared to control. Tesa, et al, [54]has been found that a significant increase in the enzymatic activity of erythrocyte membrane AChE and a change in its enzymatic properties in type 1 diabetic patients compared with those in normal subjects.On the other hand, several studies showed that the a significant decrease in the erythrocyte membrane AChE in patients with type-2 diabetes mellitus comparing to control group(Rizvi and Zaid, [55].Suhail and Rizvi, [56], reported that the activity of the erythrocyte membrane AChE of patients with type-1 diabetes mellitus was significantly decreased compared to control . They
were attributed this decrease in the enzyme activity to a decreased number of the enzyme molecules on the erythrocyte membrane in the diabetic type I patients.

In a studies of krajewksa, et al., [57]and Singh, et al.[58]has been found the activity of the cell enzyme higher in diabetic patients compared with control. These links are well-matched to the results of this study. The study showed also a non-significant positive correlation between the enzyme activity and the fasting blood sugar in males. Testa, et al., [54] suggested that, abnormal dynamic properties of the erythrocyte membrane in diabetic patients may play a major role in describing changes in the enzyme activity. As a result of the above information., a suggested explanation for the increased enzyme activity in diabetic type-2 its in accordance to Rao, et al., [53]that "diabetes mellitus type 2 is a low-grade systemic inflammation and acetylcholine has anti-inflammatory action. So the estimated increased enzyme activity was enhanced by the induced high concentration of the anti-inflammatory acetycholin in diabetic patients."

In present study, corrected total calcium showed a significant decrease in diabetes mellitus patients treatment with daonil and glucophage more than five years measured $p<0.001$.

Calculated free calcium also showed a significant decrease than ($p<=0.001$). This decreased is probably due to variation in total protein concentration and variable binding of calcium to protein (albumin) in different individuals. Variations in calculated free calcium and corrected total calcium were observed because of the change in total protein concentration especially albumin so calculated parameters (calculated free calcium, corrected total calcium) may not reflect actual calcium status in hypoproteinemic or hyperproteinemic conditions[59]. These findings are in accordance with the work of Thode et al. [60,61]

In present the correlation between measured total calcium and total protein and albumin was considerably positive but weakly correlated with calculated free calcium, while measured free calcium showed a negligible correlation with total protein and albumin. Findings of the present study were also corroborated by the work of Sorva et al. [62]

The reasons for the high prevalence of Mg deficiency in diabetes are not clear, but may include increased urinary loss, lower dietary intake, or impaired absorption of Mg compared to healthy individuals.Several studies have reported increased urinary Mg excretion in type 1 and 2 diabetes [63-66].

In addition, we have recently shown that type 2 diabetics in reasonable metabolic control and without nephropathy absorb dietary Mg to a similar extent as healthy controls, and have similar rates of urinary excretion [67]. Increased urinary Mg excretion due to hyperglycaemia and osmotic diuresis may contribute to hypomagnesaemia in diabetes [64-66]. Several authors have described a correlation between HbA1c and plasma Mg in type 1 diabetics [68,69].

However, no such correlation was found in type 2 diabetes [68,70,71], similar to our results there are a number of reports of low Mg values in various blood cells and tissues associated with normal serum/plasma Mg concentrations [72].It appears therefore that plasma Mg concentration is an insensitive, but highly specific indicator of low Mg status. Of the total Mg in serum, around 55% is present as free ionised Mg$^{2+}$, 15% is complexed to anions (e.g. bicarbonate, citrate, sulfate) and 30% is bound to proteins,
mainly albumin. It could therefore be argued that in diabetics with microalbuminuria, serum Mg might be reduced because of lower serum albumin concentration. In contrast, Corsonello et al. [73] demonstrated significantly lower ionized serum Mg in type 2 diabetic patients with microalbuminuria or clinical proteinuria compared to a normoalbuminemic group. Free ionized serum Mg, however, is not associated with serum albumin levels. Moreover, microalbuminuria should not lower plasma albumin, because plasma contains macro-amounts (35–52 g/L) of albumin. Therefore, we did not exclude subjects with microalbuminuria.

Low Mg status is common in type 2 diabetics in the Zurich region. Because Mg depletion reduces insulin sensitivity and may increase risk of secondary complications, it may be prudent in clinical practice to periodically monitor plasma Mg concentrations in diabetic patients. If plasma Mg is low, an intervention to increase dietary intakes of Mg may be beneficial. Lower in Mg concentration appeared in obesity and DM2 [74]. Hypomagnesemia in diabetic is usually observed in patients with deficient metabolic control, or associated to the DM chronic complications, according to clinical and epidemiological studies [75, 76].

The underlying mechanisms for Mg deficiency in patients with diabetes have still not been clarified, mainly about the impact in the insulin resistance, in the development of diabetes and its chronic complications [77-80].

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems[81, 82] are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress [82, 83]. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [84].

Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus [85].

In diabetes, reactive oxygen species (ROS) formation is a direct consequence of hyperglycemia [86, 87]. ROS and subsequent oxidative stress are believed to play a key role in the pathogenesis of late diabetic complications and in causing insulin resistance [88]. Earlier Wolff and Dean have suggested that nonenzymatic protein glycation and glucose autoxidation resulted in superoxide and hydroxyl radical generation [86]. The pathways of hyperglycemia-induced cell injury include formation and autooxidation of advanced glycation end products (AGEs) and their interaction with receptors (RAGEs), activation of protein kinase C [89], induction of the polyol pathway [90], increased hexosamine pathway flux, and activation of transcription factors [91]. Antioxidants, especially lipoic acid, were found to improve insulin sensitivity [91, 92].

**Conclusion**

1- Neuropathy (complication of DM) is associated with type 2 diabetes mellitus especially in patients treatment more than five years by daonil and glucophage drugs by
elevated membrane AchE activity in blood samples.
2-The significantly decreased in concentration of corrected and ionized calcium may be due changes in calcium homeostasis which was represented by calcium influx accompanied with this disease.
3-Type 2 diabetes mellitus patients is associated with elevated oxidative stress as indicated by decreased the total antioxidant especially in patients treatment more than five years by daonil and glucophage drugs.
4-The decreased in magnesium concentration in diabetes mellitus patients contributed oxidative stress development is due to inactivation of enzymatic antioxidant defense.
5-Elevation in membrane AchE activity, as a result to high calcium influx into the neurons, this influx of calcium enhances oxidation processes which lead to oxidative stress.(complication).
6-The clinical advantage in biochemical measurement of sera, urine and blood samples in patients with DM may help in earlier follow up (nephropathy and neuropathy) and/or prognosis of these lesions.

References
31. Miroslav Pohanka, , Martina Hrabinova, Kamil Kuca and Jean-Pierre Simonato : Assessment of Acetylcholinesterase Activity Using
Indoxylacetate and Comparison with the Standard Ellman’s Method. Int. J. Mol. Sci. 2011, 12, 2631-2640
46.Abdulkareem M."The role of calcium ion in cholinesterase activity and its correlation with some metal ions in sera with type 2 diabetes mellitus. Thesis, college of science University of Basrah, Dept.of chemistry.2010
47.Ott,P.Diethyl p-nitrophenyl phosphate was the most potent inhibitor in membrane lipid composition Biochim.Biophys .Acta 822,375-392 (1985)
59.Laxmayya Sava, Sandhya Pillai, Umesh More, Dr Alka Sontakke" serum calcium measurement: total versus free (ionized) calcium, Indian Journal of Clinical Biochemistry, 2005, 20 (2) 158-161
83.ROSEN, P., P.P. NAWROTH, G. KING, G. MOLLER, H.J. TRITSCHREV, L. PACKER, The role of oxidative stress in the onset and progression of diabetes and its complication,Diabetes/Metabolism
Research and Reviews, 2001, 17, 189–212.