Assessment of IL-6, MDA, GSH and Serum Electrolytes in Diabetic Patients

Ali M. A. Al-Kufaishi1*, Abtisam F. Al-Shukry2 and Noor J.T. Al-Musawi3

1Al-Furat Al-Awsat Technical University/College of Health and Medical Techniques, Department of Medical Laboratory Techniques, Kufa, Iraq 31003.
2Al-Furat Al-Awsat Technical University, Kufa Institute, Health Community Department, Najaf, Iraq.
3University of Babylon / DNA research center, Iraq.
*Corresponding Author E-mail: Kuh.ali@atu.edu.iq

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The public health community has been paying a lot of attention to metabolic syndrome, sometimes known as diabetes mellitus (DM). In many cases for the patients with type 2 diabetes mellitus characterized by an uncontrolled rise in blood sugar (hyperglycemia) brought on by an inability of the body’s cells (such as muscles) to detect and utilize the circulating insulin, a condition known as insulin sensitivity or insulin resistance, or by an insulin deficiency. (caused by pancreatic beta cell damage or decreased insulin release). These cases are accompanied by systematic inflammation in the long term, and as a result of the immune effect, there will be an increase in the levels of lipids peroxidation and thus a decrease in the levels of antioxidants.

The study includes an evaluation of inflammatory levels and oxidative stress through an assay of IL-6, malondialdehyde, glutathione, and serum electrolyte levels in the patients with uncontrolled type 2 diabetes mellitus compared with the control. The case-control study, where ablood sample collections from patients with uncontrolled type 2 diabetes mellitus and control apparently healthy. The samples were analyzed by ELISA, spectrophotometers, and electro-analyzer apparatus. It was found that patients with uncontrolled type 2 diabetes have lower-grade inflammation, which is the reason for the significant increase in interleukin-6 (215±13 Pg/mL) compared with healthy (50±10 Pg/mL). Also, the high levels of free radicals and peroxides can be expressed by the increase in malondialdehyde, as well as a significant reduction in the levels of glutathione value. Patients with uncontrolled type 2 diabetes suffer from electrolyte disturbances due to excessive urine caused by high osmolality and metabolic acidosis due to high ketone bodies and consequently high potassium levels (hyperkalemia) (6.7±0.59 mmol/L).

Uncontrolled high diabetes in type 2 patients leads to high inflammatory levels in the body, and this is the reason for increase interleukin 6 levels. Inflammations caused by the immune effect resulting from diabetes lead to the synthesis of higher levels of free radicals, leading to the oxidation of lipids and a decrease of glutathione concentration.

Keywords: Diabetes, IL-6, GSH, MDA, Oxidative Stress.
circulating insulin, a condition known as (insulin sensitivity) or (IR Insulin Resistance)\(^1\)\(^2\)\(^3\).

The primary kinds of diabetes are (diabetes mellitus type 2 T2DM), which is primarily carried on by tissue resistance to circulation insulin present in the blood, and (diabetes mellitus type 1 T1DM), an auto-immune disease characterized via the indiscriminate death of -pancreatic beta cells\(^4\). Individuals with diabetes mellitus are susceptible to severe consequences from associated conditions such as hypertension, hyperlipidemia, neuropathy, obesity, chronics kidney disease (CKD), and cardiovascular disease (CVD)\(^5\). Among the detrimental consequences of diabetes on the bodily systems that are currently gaining substantial attention in the fields of public health and medical research are a number of diabetic illnesses, including diabetic retinopathy, neuropathy, and nephropathy. Meanwhile, it’s also noteworthy that patients with diabetes who also have kidney, nerve, and vascular issues frequently experience similar diseases \(^6\)\(^7\).

**Interleukin-6g(IL-6)**

Interleukin-6g(IL-6) functions as an activating cytokine to regulate insulin output. Insulin is one of the most important hormones for regulating the amount of glucose in the body. Its effects are influenced by secretion, cellular sensitivity, and clearing. Numerous studies have examined how IL-6 affects insulin secretion and levels \(^8\). Due to its direct or indirect effects on insulin release through the increase of excess lipid production and glucose homeostasis, interleukin-6 functions as an inflammatory marker. Furthermore, it’s possible that the IL-6 gene contributes to T1DM susceptibility \(^9\).

Moreover, IL-6 may actually harm pancreatic cells by promoting the growth of B lymphocytes and triggering killer T cells. Inflammation-mediated cytokines, such as TNF-alfa and IL-1\(^\beta\), which are produced from macrophages and trigger the production of IL-6 as a consequence of many biological processes, are crucial for the onset of type 1 diabetes \(^10\). By functioning as an activating cytokine, interleukin-6 regulates insulin secretion. Previous research has shown that low concentrations of IL-6 can increase insulin release, whereas high amounts reduce insulin synthesis. Additionally, the vulnerability to T2DM may be influenced by the IL-6 gene \(^11\).

Since IL-6 can communicate through both liquid (trans-signaling) and cell wall-attached (cis-signaling) IL-6 sensors, it affects inflammatory, immunological, and diverse cells, including fat, skeletal muscle, and pancreatic cells. Elevated IL-6 levels have a substantial impact on diabetes risk, and either by itself or in combination with IL-1\(^\beta\) 30, this cytokine inhibits â cell function \(^12\).

**Oxidative stress (OS)**

Oxidative stress is a major cause of diabetic mellitus. The body produces a few toxic RNS and ROS under normal physiological settings, and its antioxidant defense eliminates both of them quickly before they cause any structural or functional damage. This delicate balance, however, means that cellular damage caused by ROS and RNS occurs often. As a result, damaged molecules need to be replaced or repaired, as shown in vivo in humans and animals \(^13\).

The amount of cellular antioxidant-oxidant balance is compromised by oxidative stress, which increases the buildup of dangerous free radicals and lipid peroxidation byproducts that distort cellular functions and collaborate well with increased inflammatory activities frequently seen in diabetic patients \(^14\). The effects of the condition, including vascular integrity impairment, polydipsia, frequent urination, hyperglycemia, and nervous system dysfunction, may be significantly impacted. In DM, there are more free radicals or less antioxidant activity \(^15\). While severe exposure to oxidants results in disrupted chemical signaling and possibly damage to biomolecules, low exposure causes redox alterations in the particular target molecules that contribute to redox signaling (oxidative eustress). These pathways are modulated and counteracted by adaptive responses. It either promotes the advancement of health or sickness, depending on the result \(^16\).

The mitochondrial electron transport chain produces more ROS when blood glucose levels are elevated in diabetic patients. Moreover, the high reactivity of ROS causes chemical alterations in almost every component of the cell, including lipid peroxidation, DNA and protein modification, and other processes that contribute to the development of diabetic-nephropathy(DN). Since the imbalance among these two categories of particles with respect to the pro-oxidant agents causes oxidative stress, there is a significant need in medical and
scientific fields for accurate and efficient methods in performing a quantitative evaluation of oxidative damage in biological samples, followed by the detection of ROS/RNS and antioxidants. Therefore, research in the biological and pharmaceutical domains, with a focus on DM, greatly benefits from the development of novel ROS/RNS or antioxidant testing techniques 17.

Additionally, it is critical to evaluate these species in various cellular contexts and provide insight into the ensuing pathomechanisms that support the onset and advancement of DM-associated DN.

Antioxidants

On the opposite part of the redox equilibrium, antioxidants have conflicting effects on RNS and ROS. Antioxidants are described as organisms that lessen oxidative stress through the suppression of radical chain reactions inside carbohydrates, lipids, proteins, or DNA, or the degradation of ROS/RNS to reduce reactive species. An organism contains a wide range of active endogenous and exogenous antioxidants. They fall into two categories: non-enzymatic (partially supplied by food intake) and enzymatic (endogenous). The production of low molecular weight molecules (e.g., glutathione, GSH, or bilirubin) and endogenous antioxidants (e.g., superoxide dismutase, SOD) can be accelerated by physical activity, dietary enrichment, or fasting for an extended period 18.

Glutathione

Glutathione is a sulfur-based organic compound identified by the sulfhydryl residues (-SH) in their active sites. Cysteine, which can be readily oxidized, is the main thiol material in the body. A cysteine residue’s oxidation can alter a protein’s functionality. As a result, thiol status measurement could be useful as a mechanistic-based marker. Protein thiols and low molecular weight thiols like cysteine and GSH are examples of thiols found in biological systems. The kind of reactive species active in the oxidative alteration, the electrochemical environment (pH and cofactor availability) and chemical redox state, the pKa-value, and the structural-microenvironment of the targeted-thiol all influence the utilization of thiols as biomarkers 19. Apart from the particular constraints pertaining to thiols, which are exclusively found within cells, the overarching issue is the instability of the oxidation products resulting from protein cysteine residues, necessitating rapid assessment. They can be rapidly reduced by other thiols, thus it’s imperative to treat them right away with an alkylating substance to stop more redox changes. Another drawback of this approach is that variations in GSH levels may not always be the result of oxidative stress but also indicate a nutritional, or metabolic imbalance. GSH transporters may also have an impact on plasma GSH levels, however, Nrf2 cellular mechanisms increase GSH production in response to oxidative stress. Lastly, unless sophisticated HPLC techniques are employed, GSSG quantities are tiny and challenging to assess 20. The latter may be used as a possible diagnostic tool, for example, to quantify the GSH and GSSG ratio from patient plasma using an advanced HPLC approach. Scientists began using this method on patients in 1989. The activity of glutathione synthetase and γ-glutamylcysteine synthetase in the erythrocytes of patients with T2DM was assessed using a basic enzymatic technique. These patients showed a reduction in the GSH/GSSG ratio. Glutathione metabolite measurement provides unique information about the redox state 21,22.

This study aims to evaluate the levels of interleukin-6 in patients with type 2 diabetes, which is considered a good indicator of the occurrence of chronic inflammation, which in turn can generate many free radicals that doing lipids peroxidation of cell membranes and fatty acids present in the blood, which are estimated by the MDA. In addition to estimating the level of glutathione, which expresses the strength of antioxidants in the body. As a result of some imbalances in electrolyte concentrations in diabetic patients, they were measured compared to healthy people.

MATERIALS AND METHOD

Sample Collection

Blood serum samples for patients with type 2 diabetes were collected from Marjan Medical City in Babylon Governorate (from December 2022 to April 2023), after obtaining ethical medical approvals from the Babylon Health
Department. Patients who are diagnosed with type 2 diabetes by specialized doctors in addition to their medical history. 5 ml of patients’ blood samples were drawn after a period of fasting (8-12) hours, and samples were drawn from a group of healthy people similar to them in terms of age and gender, which numbered 100 men and 100 women (total samples equal to 200). Excluded criteria: All patients with diabetic nephropathy, cardiovascular disease, pulmonary disease, chronic diseases other than diabetes, and autoimmune diseases such as rheumatoid arthritis were excluded.

**Estimation of the level of lipid peroxidation**

In diabetic patients and healthy control, lipids peroxidation has been measured. This assay was done through the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBARS) in acidic medium.

**Estimation of the level of reduced glutathione (GSH)**

Reduced glutathione (GSH) assay levels in the serum was performed. TCA (400 µL; 20%) and a 100 µL sample of serum were combined, and the mixture was then centrifuged one more (10 min, 4 °C, 10,000 rpm). The supernatants (2.5 mL) centrifuged, then add to (DTNB 2mL;0.6M), and then incubation for10 min at room temperature. At (412 nm absorbance) was finally measured.

**Estimation of IL-6 by ELISA**

Quantified IL-6 in patient serum at room temperature in accordance with instructions from the ELISA kit’s manufacturer. The samples’ levels of IL-6 were measured, computed using the kits’ standard curve, and expressed in (pg/mL).

**Serum Electrolyte Level Estimations**

Sodium, potassium, and calcium were all estimated in the serum using the appropriate commercial kits. Spectrophotometric methods were used in all electrolyte measurements.

**Analytical Statistics**

(Analysis of variance ANOVA) was used to analyze the data, followed by the Tukey post hoc test to compared results across all experimental groups and the results were given as Means SEM. At p 0.05, all statistical differences were deemed significant.

**RESULTS AND DISCUSSION**

Table 1 of the results for age and BMI, as well as the duration of diabetes for patients compared to healthy people.

The samples were taken on the basis of the great similarity between patients and healthy people in terms of age and BMI, so there is no significant difference between them and the reason is to reduce some physiological differences between people. The duration of the disease was also taken into account from the beginning of diabetes until the samples were collected, in order to demonstrate the complications of diabetes in patients and their effect on their levels of oxidation - antioxidants. Long periods of illness, especially for patients with uncontrolled diabetes, may have a negative impact on kidney functions, which was assessed through the electrolyte values mentioned in Table 2.

The high blood sugar values in patients (265±21), as well as the significant difference in the high glycated hemoglobin in patients (12.8±3.4) compared to healthy people (86±7) and (4.23±1.15), respectively, indicate the type of diabetes in patients is the uncontrolled type 2 DM and causes many complications. The most prominent complications

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Means ± SD</th>
<th>95% (Confidence Intervals)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Age/Years</td>
<td>Patients</td>
<td>50.37±10.05</td>
<td>45.64</td>
<td>60.16</td>
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<tr>
<td></td>
<td>Control</td>
<td>52.24±8.34</td>
<td>46.17</td>
<td>61.90</td>
</tr>
<tr>
<td>BMI</td>
<td>Patients</td>
<td>23.65±2.19</td>
<td>21.88</td>
<td>24.19</td>
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<tr>
<td></td>
<td>Control</td>
<td>21.47±0.39</td>
<td>20.13</td>
<td>22.12</td>
</tr>
<tr>
<td>Duration/Years</td>
<td>Patients</td>
<td>10.08±3.00</td>
<td>6.30</td>
<td>13.18</td>
</tr>
<tr>
<td></td>
<td>Control</td>
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NF: Not Found
Table 2. The Results of Biochemical Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Means ± SD</th>
<th>95% (Confidence Intervals)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Fasting Blood</td>
<td>Patients</td>
<td>265±21</td>
<td>240</td>
<td>280</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>Control</td>
<td>86±7</td>
<td>78</td>
<td>90</td>
</tr>
<tr>
<td>HbA1C%</td>
<td>Patients</td>
<td>12.8±3.4</td>
<td>9.44</td>
<td>14.32</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.23±1.15</td>
<td>4.01</td>
<td>5.09</td>
</tr>
<tr>
<td>IL-6 (Pg/mL)</td>
<td>Patients</td>
<td>215±31</td>
<td>187</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>52±10</td>
<td>43</td>
<td>60</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>Control</td>
<td>3.19±0.77</td>
<td>3.65</td>
<td>4.11</td>
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<tr>
<td></td>
<td>Patients</td>
<td>8.42±0.75</td>
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<tr>
<td>GSH (mmol/L)</td>
<td>Control</td>
<td>30.07±4.86</td>
<td>26.05</td>
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<td>Patients</td>
<td>20.01±3.25</td>
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<td>S. Na⁺ (mg/dL)</td>
<td>Patients</td>
<td>155±8.04</td>
<td>149</td>
<td>161</td>
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<tr>
<td></td>
<td>Control</td>
<td>147±5.11</td>
<td>145</td>
<td>151</td>
</tr>
<tr>
<td>S. K⁺ (mmol/L)</td>
<td>Control</td>
<td>4.41±0.37</td>
<td>4.21</td>
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<td></td>
<td>Patients</td>
<td>6.7±0.59</td>
<td>5.98</td>
<td>6.95</td>
</tr>
<tr>
<td>S. Ca²⁺ (mg/dL)</td>
<td>Control</td>
<td>9.41±0.61</td>
<td>8.79</td>
<td>10.02</td>
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<tr>
<td></td>
<td>Patients</td>
<td>8.93±0.77</td>
<td>8.36</td>
<td>9.72</td>
</tr>
</tbody>
</table>

Diabetes mellitus was associated in this study by along with a rise in oxidant and decrease in antioxidant increase of cytokines that are pro-inflammatory. According to the study’s findings, the induction of diabetes mellitus causes metabolic imbalance by upregulating the oxidonitrosative (pro-oxidants release), and inflammatory condition in the kidney, and brain cells. This, in turn, causes neuronal cell and kidney damage. By damaging structural elements, the creation of these free radicals may seriously affect renal function. The circumstance may provide an environment that mobilizes and encourages the development of transcription factors that represent diabetic nephropathy and proinflammatory cytokines (such vTNF-α and jIL-6). In a related study on diabetic and kidney diseases, it was shown that diabetes-induced oxidative stress, which is linked to abnormalities in kidney function, is a primary suspect for cellular damage and the development of diabetic kidney disease, or diabetic nephropathy by elevated IL-6 and TNF-α that associated with disturbance of kidney functions.

Through the elevations in the level of HbA1C for uncontrolled diabetics lead to disturbances in their levels of electrolytes were observed, through a significant increase in potassium levels (hyperkalemia) which as a result causes metabolic acidosis, which in turn increases the incidence of inflammation and thus a rise in interleukin-6 levels. It is necessary to follow the values of the glycated hemoglobin (HbA1C) levels in patients to assess their health conditions and their response to medications in order to reduce complications resulting from the development of the disease with a healthy diet and good diet as well as exercise to reduce oxidative stress.

**CONCLUSION**

It is concluded from the study that high levels of glycated hemoglobin are a state...
of uncontrolled diabetes that causes many problems, including the occurrence of low-grade inflammation, which is determined by high levels of interleukin 6, which in turn increases the rise of free radicals and raises levels of lipid peroxidation. In contrast, levels of total antioxidants, represented here by glutathione, decrease. Disturbances in the levels of electrolytes in the blood, including high levels of potassium, and thus the occurrence of metabolic acidosis, resulting from the beginning of kidney deterioration in patients with uncontrolled diabetes or maybe the result of a difference in the acid-base balance within the body resulting from an increase in ketone bodies.

ACKNOWLEDGMENT

We extend my thanks and appreciation to the medical staff of (Marjan Teaching Hospital) for their assistance in completing the researches.

Ethical Approval

The study was carried out after obtaining legal approval from the Iraqi Ministry of Health/Babil Health Department, as well as the patients’ personal approval.

Conflict

There is no conflict with others.

Funding disclosure

There are no sources of funding.

REFERENCES

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