The Relevance of Glycosylated Hemoglobin with Oxidative Stress in Insulin Resistant Type 2 Diabetes Mellitus

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Abstract

The present study was conducted to evaluate the relevance of glycosylated hemoglobin with oxidative stress in insulin resistant type 2 diabetes mellitus. To achieve this aim, 93 type 2 diabetic patients of ages 38-84 years were recruited. In addition, 19 apparently healthy individuals with ages 30-60 years, were enrolled as a control group. The concentration of fasting blood glucose (FBG), triglyceride (TG), insulin, glycosylated hemoglobin (HbA1c), malodialdehyde (MDA) and glutathione-S-transferase (GST) were measured in sera of patient and the control groups. Fasting blood glucose and triglyceride levels were estimated by spectrophotometric methods using enzymatic procedures. Insulin level was estimated by enzyme linked immunosorbant assay (ELISA) method. HbA1c level was determined by an ion exchange chromatographic method, while MDA and GST levels were measured by spectrophotometric procedures. Insulin resistance was evaluated by four methods. They include homeostatic model assessment (HOMA), quantitative insulin check index (QUIKI), McAulye (McA), and fasting insulin (FI) methods. Insulin resistance was found in 79 (84.9%), 63 (73.4%), 52 (55.9%) and 35 (37%) out of the 93 diabetic patients by HOMA, QUICKi, McA and FI methods respectively. Type 2 insulin resistant (79) diabetic patients (IRP) that obtained through the HOMA method were assessed for the HbA1c, MDA and GST levels in comparison to the insulin sensitive patients (ISP) as well as to the control group. The analysis of the data revealed a significant increase (p<0.001) of HbA1c levels in the IRP group when compared with those of the control group, while the insulin sensitive group (ISP) couldn’t show significant variation when compared with those of the IRP. The data of MDA failed to give significant variation. However, a significant elevation of GST concentration were observed in the IRP group with respect to those of the control group (p<0.005). On the other hand significant variations could not be obtained among the ISP and IRP. These data suggested that oxidative stress changes are independent on insulin resistance in type 2 diabetes mellitus.
Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose level (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both. The term diabetes mellitus is derived from the Greek words meaning “to run through” (Kojo, 2004). Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations. Free radical production and consequently the oxidative stress may be induced by hyperglycemia via at least four different routes which include increased glycolysis, intercellular activation of sorbitol (polyol) pathway, autoxidation of glucose and non-enzymatic protein glycation (Ahmed, 2005).

Insulin resistance (IR) is a physiological condition where the natural hormone insulin, becomes less effective at lowering blood sugars. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects, depending on dietary conditions. Certain cell types such as fat and muscle cells require insulin glucose uptake, when these cells fail to respond adequately to circulating insulin, blood glucose levels rise (Murano et al., 2008). The subnormal biological response could be due to the inability of plasma insulin to bind to its receptor or the presence of a post-receptor binding defect (Weisberg et al., 2003). IR is associated with a number of diseases including obesity, metabolic syndrome, T2DM, polycystic ovary syndrome and chronic infection (Scott et al., 2005).

Insulin resistance in muscle and fat cells reduces glucose uptake, whereas insulin resistance in liver cells results in reduced glycogen synthesis and storage and a failure to suppress glucose production and release into the blood (Kahn and Flier, 2000). The term glycation is the nonenzymatic reactions that link a sugar to a protein or peptide. The product of glycation is a glycoprotein, or, in the special case of the reaction with hemoglobin, glycohemoglobin, or glycated hemoglobins. In a normal person glucose circulates in the blood. The erythrocytes are freely permeable to glucose and the concentration in the cell is approximately the same as in the plasma. When glucose levels are elevated in plasma, they are proportionately elevated in the erythrocytes (World Health Organization, 2002). Different forms of glycosylated hemoglobin have been identified. HbA1c is the product of hemoglobin reaction with glucose molecule. It is used to assess the glycemic control for the last 6-8 months (World Health Organization, 2002). Malondialdehyde (MDA) is a three-carbon, low-molecular weight aldehyde, produced as a by product of the lipid peroxidation. The latter is enhanced when the level of free radicals and elevates (Sanocka and Kurpisz, 2004). To protect the cells and organ systems of the body against the adverse effects of oxidative stress, humans have evolved a highly sophisticated and complex antioxidant protection systems. They are classified into two categories (Mittler et al., 2004),
The Relevance of Glycosylated Hemoglobin…

Majid Kadhum Hussain

enzymatic and non enzymatic antioxidants. One of the enzymatic antioxidants is glutathione-S-transferase (GST) which is involved in the metabolism of divers endo- and xenobiotics. It protects the cells against Oxidative Stress (Wang, 2005). GST is a dimeric enzyme catalyzes the conjugation of GSH to a variety of electrophiles including xenobiotic chemicals and endogenous toxic substances such as ROOH (Seufi et al., 2009). The relevance of oxidative stress with glycosylated haemoglobin is not clear, so that the current investigation is conducted to explore such relationship.

Materials and Methods

The study was conducted on randomly selected 94 type 2 diabetic patients (33 male and 61 female) attending the diabetes mellitus center in Al-Sadder Teaching Hospital in Najaf province. It was carried out from February 2011 to July 2011 in the laboratory of research/Department of Biology in College of Education for Women and the Department of Biochemistry in College of Medicine/University of Kufa. The age of patients was 58.02 ±10.11 y with a range of 38-84 y.

Diabetes mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height, duration of the disease, complications and other diseases. Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e, antioxidants and anti-hyperlipidemic agents were excluded from the current investigation. The study was carried out

A group of 20 apparently healthy subjects (9 males and 11 females) were included as a control group. Their ages were 42.20±7.38 y with a range of 30-60 y. During the dialogue with the volunteers of the control group, they seemed to be free from health problems. The information of the control group were obtained as that of the patients.

Disposable syringes and needles were used for blood collection by vein puncture. Venous fasting blood samples (5 mL) were collected from the patients and healthy volunteers after an overnight fasting. One milliliter of blood was transferred to a tube contained EDTA, it was used for the estimation of HbA1c concentration. The remaining blood sample was transferred to a plastic tube; it was left to clot at 37 °C for 15 min. Blood samples were centrifuged at 3000 xg for 15 min. The sera were separated into three aliquots and stored at -17 °C prior to the determination of the biochemical parameters. Glycosylated hemoglobin concentration was determined by using the kit from Stanbio Laboratory data (2011). Fasting blood glucose concentration was measured by Bablock method (1988).Triglyceride level was estimated by Fossati and Prencipe method (1982). The level of fasting insulin was determined by a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (DeFronzo, 1999). Estimation of insulin resistance was evaluated by four methods, i.e, Fasting insulin concentration (FI), Homeostasis Model Assessment (HOMA) = [glucose (in mmole/L) * insulin (in microU/mL) ] / 22.5, Quantitative Insulin Sensitivity Check Index (QUICKI) = 1/ [log glucose (in mg/dL) + log insulin (in microU/L)], McAuley’s index (McA) = exp [2.63 - 0.28 ln (insulin [in microU/mL] ) - 0.31 ln (triglycerides [in mmole/L])] (Sathiyapriya et al., 2007). Patients were considered as insulin resistant when:

- FI ≥ 12 µU/L.
- HOMA ≥ 2.5
- QUICKI ≤ 0.33
- McA ≤ 5.8

The level of malondialdehyde concentration (MDA) was determined by a method described by Guidet and Shah (1989). Estimation of serum glutathione-S-transferase activity was achieved by Habig
et al., (1974). The data were expressed as mean ± SD unless otherwise stated. Statistical analyses were carried out by using student t-test, ANOVA and Pearson’s correlation analysis through the MINITAB Student-Untitled program. Significant difference was considered when the P < 0.05.

Results and Discussion

Significant elevations of HbA1c (P<0.001), insulin (P<0.001), FBG (P<0.001), Tg (P<0.001) and GST (P<0.002) levels were indicated in the group of patients when compared with those of the control group. However, MDA levels showed an insignificant decrease during a comparable assessment (Table 1).

The increased HbA1c levels reflect the poor metabolic control of diabetic patients (Ikekpeazu et al., 2011). This increase is directly proportional to the blood glucose level at the last 6-8 weeks (Moussa and Romanlan, 2008). Higher Levels of glucose in the blood contributes to more binding and consequent high concentration of glycosylated hemoglobin (Kilpatrick, 2000). The elevated levels of insulin in diabetic patients may be due to the derangement of insulin secretion that may take place during the course of type 2 diabetes, as the pancreas attempts to compensate for the elevated fasting plasma glucose (FPG) concentration. However, as the FPG concentration continues to rise, β-cells are no longer able to sustain their increased rate of insulin secretion and as insulin secretion declines, impaired glucose tolerance (IGT) and eventually overt type 2 diabetes will ensue (Ralph and DeFronzo, 2004). Increased hepatic glucose production (HGP) and decreased muscle glucose uptake further contributes to the state of hyperglycemia. It places further stress on the β-cells and establishes a negative back-loop in which the metabolic control decompensate (glucotoxicity and lipotoxicity) (Abdul-Ghani et al., 2006; DeFronzo, 2009). Consequently this contributes to the β-cells failure and worsening insulin resistance. These results are in agreement with those reported by Kalaivanam et al. (2006) Lebovitz (2006) and Goud et al. (2011). In diabetes mellitus, the persistent hyperglycemia may elevate the reactive oxygen species (ROS) concentration from glucose auto-oxidation and protein glycosylation. This elevation may increase the lipid peroxidation with consequent raised levels of MDA (Kalaivanam, 2006). Such observation was not apparent in the present investigation since MDA level changes seemed to be insignificant. It may be owing to the active compensatory antioxidant system represented by the antioxidant enzymes. This hypothesis may be supported by the significant increase of GST activity in the recruited diabetic patients relative to those of the control group. These results are in consistence with those verified by Velazquez et al., (1991). However, they are in disagreement with those reported by Benrebai et al. (2008) and Lalitha et al. (2010). The evaluation of insulin resistance revealed that 79 out of 93 patients (84.9%) were found to be insulin resistant by HOMA, 73 out of 93 patients (78.49%) found to be insulin resistant by QUICKI, 52 out of 93 patients (55.91%) found to be insulin resistant by McA and 35 out of 93 patients (37.63%) were found to be insulin resistant by FI (Fig.1). However, one healthy person exhibited insulin resistance through the assessment by HOMA, QUICKI and FI methods, but not by McA method (Table 2). This patient was excluded from the control group.

To select insulin resistant patients as accurate as possible in the present investigation, the four indirect methods of evaluation of insulin resistance were examined. The data exhibited high rate of insulin resistance through the HOMA and QUICKI methods (84.9% and 78.49% of diabetics were insulin resistant.)
respectively) and low rate with McA and FI methods (55.91% and 37.63% of diabetics were insulin resistant respectively). Thus the HOMA method was implicated for the selection of insulin resistant diabetics. Two factors have strongly led us for the HOMA implication. The first is the wide use of HOMA in the previous works mentioned in literatures (Young et al., 2006; Jin and Pan, 2007). The second is that 79% of the enrolled patients were overweight or obese. Insulin resistance is a serious mechanism involved in the pathogenesis of type 2 diabetes mellitus in particular those of abnormal weight. Thus the data of the HOMA method was highly suggestive to be used for selection of insulin resistant type 2 diabetics, therefore 79 out of 93 patients were categorized as insulin resistant diabetics The results of the current study are in agreement with those of Amato et al. (2006) and Erus et al. (2007) in regards to the data of HOMA methods. However, they were inconsistent those reported by McAuley et al. (2001) and Lukshmy, et al. (2006). The most satisfactory reasons for the difference may be the patient’s status and the number of the enrolled diabetics. Some authors have mentioned that FI method is also an alternative rational for the evaluation of insulin resistance and could demonstrate comparable results for those of other indirect methods. The present study is inconsistent with such findings and determination of fasting insulin level seemed to be inappropriate for the prediction of insulin resistance accurately (Luksmy et al., 2006) The insulin resistant patients (IRP) contained 79 patients, while the group of insulin sensitive patients (ISP) consisted of 14 patients. The two groups of insulin sensitive and insulin resistant type 2 diabetic patients as well as the insulin sensitive healthy subjects were compared together for the difference of the glycosylated hemoglobin (HbA1c), malondialdehyde (MDA) and glutathione-S-transferase (GST) levels. The results showed a significant elevation (p<0.001) of HbA1c in the IRP when compared with those of the control group, such difference was not demonstrated with respect to those of ISP. The result of MDA estimation failed to give significant variation. On the other hand, data of GST activity pointed out a significant increase (p<0.005) in the IRP when compared with those of the control group. Such difference could not be obtained for IRP with ISP (Table 3). Type 2 diabetes mellitus is partially characterized by elevated fasting serum glucose (FSG), insulin concentration (in most cases), the percentage of HbA1c and decreased insulin sensitivity (Lebovitz, 2006). Insulin resistance is frequently brought on by obesity or being overweight which results in reduction of insulin receptors and impaired post-insulin binding signaling transduction mechanisms (Liese et al., 2005). The response of the pancreas to insulin insensitivity is to increase the blood serum concentration of insulin (Rewers et al., 2004). However, this rise in insulin levels seldom compensates completely for the insulin insensitivity and consequently serum glucose concentrations rise (Cefalu, 2001). When blood glucose concentration rises, HbA1c level will elevate as there is an increased ratio of glucose to hemoglobin concentration, allowing the glycosylation process to occur at a high rate (Mentink et al., 2006). This is the most likely cause of HbA1c increase in IRP when compared with those of the control group. However the insignificant difference between IRP and ISP indicates that HbA1c formation is independent on insulin resistance. The insignificant change of MDA and GST levels in IRP in comparison to the ISP suggests that oxidative stress alteration is independent on insulin resistance in type 2 diabetes mellitus. This observation may be attributed to the racial adaptation, type of the diet or other factors. Some studies have shown decreased levels of antioxidant capacity in diabetes mellitus.
The Relevance of Glycosylated Hemoglobin…

Majid Kadhum Hussain

(Bashan et al., 2009). Furthermore, a decline in cellular antioxidant defense mechanisms, including the glutathione redox system, vitamin C and E has been reported (Evans et al., 2002).

In conclusion, most of type 2 diabetic patients are presented with insulin resistance, glycaemic control seemed to be independent on insulin resistance in type 2 diabetic patients a glycaemic regulation is independent on oxidative stress in insulin resistance type 2 diabetic patients.

Table 1. Results of glycosylated hemoglobin (HbA<sub>1c</sub>), fasting insulin (FI), blood glucose (FBG), triglyceride (Tg), malondialdehyde (MDA) and glutathione-S-transferase (GST) levels in the study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>No</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA&lt;sub&gt;1c&lt;/sub&gt; (%)</td>
<td>Patients</td>
<td>93</td>
<td>10.44 ± 2.74</td>
<td>5.97 - 17.25</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19</td>
<td>5.67 ± 0.99</td>
<td>3.20 - 6.72</td>
<td></td>
</tr>
<tr>
<td>Insulin ( µIU/ml)</td>
<td>Patients</td>
<td>93</td>
<td>13.40 ± 12.50</td>
<td>2.92 - 73.06</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19</td>
<td>5.97 ± 3.09</td>
<td>3.25 - 17.39</td>
<td></td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>Patients</td>
<td>93</td>
<td>204.60 ±73.7</td>
<td>68.07 - 414.64</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19</td>
<td>102.70±16.50</td>
<td>75.80 - 146.18</td>
<td></td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>Patients</td>
<td>93</td>
<td>6.64 ± 4.87</td>
<td>1.29 - 31.41</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>3.27±1.51</td>
<td>1.38 - 7.29</td>
<td></td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>Patients</td>
<td>93</td>
<td>18.60 ± 8.60</td>
<td>2.83 - 103.52</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19</td>
<td>24.00 ± 18.40</td>
<td>7.13 - 34.73</td>
<td></td>
</tr>
<tr>
<td>GST (U/ml)</td>
<td>Patients</td>
<td>93</td>
<td>12.20 ± 8.11</td>
<td>0.00 - 18.75</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19</td>
<td>5.23 ± 8.15</td>
<td>0.00 - 43.52</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Insulin resistance (IR) among type 2 diabetes mellitus.

The number and percentage of patients with insulin resistance evaluated by HOMA, QUIKI, McA and FI methods, Insulin resistance was found in 79 (84.9%), 63 (73.4%), 52 (55.9%) and 35 (37%) out of the 93 diabetic patients by HOMA, QUICKi, McA and FI methods respectively.

Table 2. The Incidence of insulin resistance and sensitivity in diabetic and the control groups

<table>
<thead>
<tr>
<th>Index</th>
<th>Insulin resistant subjects</th>
<th>Insulin sensitive subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control</td>
</tr>
<tr>
<td>HOMA</td>
<td>79 (84.9%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>73 (78.49%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>MCA</td>
<td>52 (55.91%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>FI</td>
<td>35 (37.63%)</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

Table 3. Glycosylated hemoglobin (HbA$_{1c}$), malodialdehyde (MDA) and glutathione-S-transferase (GST) levels in insulin resistant (IRP), insulin sensitive (ISP) type 2 diabetic patient and the control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>No</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA$_{1c}$ (%)</td>
<td>IRP</td>
<td>79</td>
<td>10.38 ± 2.63</td>
<td>5.97-15.81</td>
<td>0.001 A</td>
</tr>
<tr>
<td></td>
<td>ISP</td>
<td>14</td>
<td>10.77 ± 3.35</td>
<td>5.93-17.25</td>
<td>0.001 B</td>
</tr>
<tr>
<td></td>
<td>Contr</td>
<td>19</td>
<td>5.80 ± 1.03</td>
<td>3.20-6.92</td>
<td>NS C</td>
</tr>
<tr>
<td>MDA (μM)</td>
<td>IRP</td>
<td>79</td>
<td>23.90 ±19.50</td>
<td>3.90-103.50</td>
<td>NS A</td>
</tr>
<tr>
<td></td>
<td>ISP</td>
<td>14</td>
<td>24.50 ± 11.40</td>
<td>11.0-39.30</td>
<td>NS B</td>
</tr>
<tr>
<td></td>
<td>Contr</td>
<td>19</td>
<td>18.38 ± 8.78</td>
<td>7.10-26.70</td>
<td>NS C</td>
</tr>
<tr>
<td>GST (U/ml)</td>
<td>IRP</td>
<td>79</td>
<td>11.71 ± 7.63</td>
<td>0.00-43.51</td>
<td>0.005 A</td>
</tr>
<tr>
<td></td>
<td>ISP</td>
<td>14</td>
<td>14.80 ± 10.20</td>
<td>0.00-39.89</td>
<td>0.005 B</td>
</tr>
<tr>
<td></td>
<td>Contr</td>
<td>19</td>
<td>5.21 ± 8.38</td>
<td>0.00-18.75</td>
<td>NS C</td>
</tr>
</tbody>
</table>

A: Insulin resistant patients (IRP) vs control group, B: Insulin sensitive patients (ISP) vs control group, C: Insulin resistant patients (IRP) vs Insulin sensitive patients (ISP).

References

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