Determination of Apoptosis Marker in Saudian Diabetic Patients

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Abstract: Diabetes mellitus is characterized by not only increased production of reactive oxygen species but also, sharp decrease in antioxidant defense which leads to more oxidative stress. A key aspect of the apoptotic agent is oxidative stress. We suggested that the Fas-mediated apoptosis may be involved in type 2 diabetes and its complications. So we assessed the levels of Fas and FasL in type 2 Saudi diabetic patients as a marker for apoptosis. This study was performed with 143 patients with type 2 diabetes mellitus matched in age and sex with 106 normal control subjects enrolled from Prince Maged Ben Abdel Aziz diabetic center-Al Madina, S.A. were studied. Investigation for Fas, FasL, fasting sugar, glycated hemoglobin, lipid profile and liver functions were applied for control and diabetics. Results revealed that, mean serum levels of FasL were significantly increased in diabetic patients (183.48±28.55 vs. 165.44±23.54 pg/ml) (p<0.05) while mean Fas levels were 146.52±28.78 vs. 144.53±23.91 pg/ml not different in comparison with control subjects (p>0.05). The levels of fasting glucose, glycated hemoglobin, ALT and triglyceride showed significant increase while the level of HDL showed significant decrease in comparison with control subjects (p<0.001). Significant positive correlation existed between FasL and ALT and Age (r = 0.268, p>0.05). Also a positive correlation existed between Glycated hemoglobin (HbA1c) %, triglycerides (TG), cholesterol and fasting glucose (Glu) in diabetic patients (r = 0.289, r = 0.392 and r = 0.249 p< 0.05) respectively, while a negative correlation was found between Glucose, triglycerides (TG) and HDL in diabetic patients (r = -0.205, r = -0.434, p< 0.05). Atherogenic Index of Plasma (AIP=Log TG/HDL) predicts cardiovascular risk. In this study 45% of our diabetic patients had increased risk and 11% had intermediate risk. In conclusion, this study emphasized a positive association between the apoptosis marker FasL and T2DM especially with elevated ALT. FasL were increased in diabetic subjects with higher ALT levels, suggesting that this protein may be a novel marker of liver injury. Elevated ALT can be considered as a marker of nonalcoholic fatty liver disease (NAFLD) so it can be used in detection of T2DM associated with (NAFLD). Development of new treatment strategies aiming to reduce sFasL, may play an important role in the management of T2DM associated with (NAFLD).

Key words: Type 2 diabetes mellitus • Apoptosis • Fas and FasL

INTRODUCTION

Diabetes mellitus (DM) as a disease was first distinguished around 3000 years ago by the ancient Egyptians and Indians who illustrated some clinical characteristics very similar to what is now known as DM. [1,2] DM is defined as “a group of metabolic diseases distinguished by hyperglycemia resulting from deficiencies in insulin secretion, insulin action or both. The chronic hyperglycemia is correlated with disturbances in carbohydrate, fat and protein metabolism and can generate long-term damage, dysfunction and failure of various organs [3].

In 2014, 9% of adults 18 years and older suffered from diabetes. According to WHO reports 1.5 million deaths reported in 2012 were due to diabetes. Low and middle income countries contribute to more than 80% diabetes related deaths. 90% of the diabetic patients worldwide are of Type 2 diabetes mellitus (T2DM) which is a result of excess body weight and physical inactivity [4-6].
The levels of T2DM in Saudi Arabia have been assessed to be among the highest in the world, it is among the top 10 countries globally with the ultimate expected prevalence of diabetes in 2030 (22.3%) [7]. According to WHO the 7th principle reason of death in 2030 will be diabetes [8].

The disease is correlated with serious complications which affect health and productivity. More than 50% of the diabetic patients die due to cardiovascular disease (primary heart disease and stroke). T2DM is the single most widespread cause of end stage renal disease which needs either dialysis or kidney transplantation. People with T2DM have a risk of lower limb amputation that may be more than 25 times greater than that seen in those without the disease. T2DM is also a major cause of adult loss of vision due to retinal damage [9].

Oxidative stress resulted from hyperglycemia may play an important role in cellular damage. Increase in free radical production and formation of reactive oxygen species can be caused by high glucose levels. The failure of the beta cell’s adaptive response to high glucose levels may precede dysfunction. The Fas-FLIP (FLICE-like inhibitory protein) pathway involves a series of factors which are upregulated in response to high glucose levels and which result in greater beta-cell differentiation, proliferation and function. However, in chronic hyperglycaemia, this adaptive pathway may eventually fail resulting in deterioration in beta-cell function and reduced survival [10]. The possibility of reduction in some vascular dysfunction related to diabetes with anti-oxidant treatment, such as Vitamin E, has been proposed in animal studies, but the change in development or progression of retinopathy or any other microvascular complications related to diabetes have not yet appeared with antioxidant treatment [11].

Other than producing more reactive oxygen species, hyperglycemia, one of the main clinical indicators of diabetes mellitus, weakens antioxidative mechanism by scavenging substances and enzymes [12]. Oxidative stress can be defined as a potentially damaging imbalance between antioxidants and the level of prooxidants. The progress of chronic vascular complications due to the contribution of advanced glycation end products may be enhanced because of lipid peroxides [13-15]. Proteins and Lipids chemical alteration by ROS is hypothesized to participate in pathogenesis of diabetic complications [16, 17]. Base alteration and breaking of DNA strands is also done by ROS [18]. The increased mitochondrial deletions is due to the increase in the transformation of deoxyguanosine (dG) to 8-hydroxydeoxyguanosine (8-OHdG) in DNA caused by ROS [19].

The rise in cellular damage (proteins, lipids and nucleic acids) occurs when the defense mechanism cannot resist the buildup of ROS. Eventually the cell dies due to necrotic or apoptotic mechanism [20]. The crucial part of the apoptotic agent is oxidative stress. Severely impaired DNA leads the cell to undergo apoptosis [21].

Apoptosis, which is the programmed cell death, is a physiological process noticed in different organs and cells which may also occur in pathological situations. Till date several apoptosis mechanisms have been described [22] The Fas system, which is the most common explored pathway, comprised of Fas Ligand (FasL), which is a type II transmembrane glycoprotein and Fas antigen (Fas/Apo-1/CD95), which is a type I transmembrane glycoprotein receptor [23]. Apoptosis is activated in the target cells by cross-linking of Fas by FasL [24]. Many studies have showed the significance of Fas-mediated apoptosis in tumorgenesis [25] and in a number of cell types including cellular components of the vessel wall [26]. Proteins produced by cells involved in atherosclerotic lesions, involving soluble Fas (sFas) and soluble Fas ligand (sFasL), circulate in small, but measurable amounts. Soluble Fas is made by alternative messenger RNA splicing capable of encoding a soluble Fas molecule missing the transmembrane domain, whereas sFasL is discharged in serum from membrane-bound FasL processed by a metalloproteinase. It has been demonstrated that Fas and FasL are expressed in atherosclerotic lesions and the Fas/Fas ligand system is related to the apoptotic and inflammatory responses present in atherosclerotic plaques [27].

The main objective of this work was assessing the serum levels of Fas and FasL in Saudi diabetic patients, as a marker for apoptosis and comparing the results with those obtained in normal controls.

**MATERIALS AND METHODS**

**Patients:** One hundred and forty three patients with type 2 diabetes mellitus matched in age and sex with one hundred and six normal control subjects enrolled from Prince Maged Ben Abdel Aziz diabetic center-Al Madina, S.A. were studied. Exclusion criteria used was for both diabetic and control subjects:
Hypertension (defined as blood pressure. 150/90 mmHg).
Hypercholesterolemia (defined as LDL-cholesterol 75th percentile for age and sex),
Tobacco use within the past 5 years,
Current use of insulin, anti-oxidants or hormone replacement therapy
Laboratory evidence of renal, hepatic, or hematological abnormalities.

Consent was taken from every patient before conducted the research.

Methods: Thoroughly, all patients and control were examined clinically and about 5 ml of blood samples was collected from each subject in plain and EDTA tubes and whole blood from the EDTA tubes was used for glycated hemoglobin % (HbA1c) estimation. Serum was isolated by centrifugation (10 min at 13,000 rpm) and serum was tested for fasting sugar, glycated hemoglobin, lipid profile and liver function tests to determine patients with complications and the remaining serum was stored at –80°C till further analysis of apoptotic markers. All these tests were done using the clinical chemistry automated machine Dimension X Pand, Siemens Healthcare Diagnostics Ltd. Frimley, Camberley, UK.

Fas and FasL were determined using Fas enzyme-linked immunosorbent assay (ELISA) kit (Sigma – Aldrich, Saint Louis, U.S.A.), with a minimum detectable dose of 5, 2 pg/ml in serum respectively. All procedures were performed at room temperature, according to the manufacturer’s instructions. Each sample and standard protein was assayed in duplicate. Optical density at 450 nm for FasL and Fas was measured with a spectrophotometric microtiter plate reader (Labsystems iEMS Reader, Helsinki, Finland). A standard curve obtained with FasL or Fas samples provided with the kit was used to determine the FasL and Fas concentration in each sample.

Statistical Methods: The entire data was statistically analyzed using SPSS program version 20.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD and were compared by analysis of variance. Differences between groups were evaluated by Student’s t-test. Spearman correlation coefficients were used to look for an association between all parameters in diabetic group. Data not conforming to a normal distribution (in this study AST measurement) were log-transformed prior to parametric analysis. Differences between groups: control group, controlled diabetics and uncontrolled diabetics were tested using one way analysis of variance (ANOVA) test; p < 0.05 was considered to be statistically significant.

RESULTS

The serum Fas level in diabetic patients was 146.52±28.78 pg/ml, which showed insignificant difference compared to the healthy control group (144.53±23.91 pg/ml). However serum FasL level of 183.48±28.55 vs. 165.44±23.54 pg/ml showed significant higher levels compared to the healthy control group (p<0.02) (Table 1 and Figure 1). The plasma glucose level in diabetic patients was 9.6±3.84 vs.5.16±0.41 mmol/L which showed significant increment difference compared to the healthy control group (p<0.001). The glycated hemoglobin level in diabetic patients was high around 9.04±2.01 %, (Table 1 and Figure 2). The level of ALT in diabetic patients was 40.82±12.63 vs. 34.06±6.24 U/L, which showed significant higher levels compared to the healthy control group (p<0.001) while the AST levels was (22.91±9.57 vs. 20.77±5.56 U/L) which was insignificant compared to the healthy control group (Table 1, 2 and Figure 3).

The level of LDL in diabetic patients was (2.65±0.9 vs. 2.65±0.65 mmol/L), which showed no difference compared to the healthy control group while the level of HDL in diabetic patients was 1.11±0.29 vs. 1.55±0.4 mmol/L, which were significant lower compared to the healthy control group (p<0.05). The level of cholesterol in diabetic patients was 4.58±1.13 vs. 4.56±0.83 mmol/L, which showed no difference compared to the healthy control group while the level of TG in diabetic patients was 1.8±1.04 vs. 0.73±0.45 mmol/L, which showed significant increment difference compared to the healthy control group (Table 1, 2 and Figure 4). Also a positive correlation existed between Glycated hemoglobin (HbA1c) %, triglycerides (TG), cholesterol and fasting glucose (Glu) in diabetic patients (r = 0.289, r = 0.392 and r = 0.249 p< 0.05) respectively, while a negative correlation found between HDL and Glucose (r = -0.434, p< 0.05 Table 3). The correlation between triglycerides (TG) and High-density lipoprotein (HDL) in diabetic patients show negative significant correlation (r = -0.205, p<0.05 Table 3). A positive correlation existed between triglycerides (TG) and cholesterol in diabetic patients (r = 0.431 p< 0.05, Table 3).
Table 1: Represents the mean concentrations ± standard deviation of lipid profile (LDL, HDL, cholesterol and triglycerides (mmol/L)), amino transferases (ALT and AST,(U/L),glycatedHb % and Fas and FasL (pg/ml) for control group and type II diabetic patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control N= 106 Mean ± SD</th>
<th>Patients N= 143 Mean ± SD</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas (pg/ml)</td>
<td>144.53±23.91(n=36)</td>
<td>146.52±28.78(n=41)</td>
<td>P=0.814</td>
</tr>
<tr>
<td>FasL (pg/ml)</td>
<td>165.44±23.54(n=36)</td>
<td>183.48±28.55(n=41)</td>
<td>P=0.02</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.65 ± 0.63</td>
<td>2.65 ± 0.90</td>
<td>P=0.918</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.55 ± 0.40</td>
<td>1.11± 0.29</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.56 ± 0.83</td>
<td>4.58 ± 1.13</td>
<td>P=0.914</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.73 ± 0.45</td>
<td>1.80 ± 1.04</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.16 ± 0.41</td>
<td>9.60 ± 3.84</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>34.06 ± 6.24</td>
<td>40.82± 12.63</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>20.77± 5.56</td>
<td>22.91± 9.57</td>
<td>P=0.188</td>
</tr>
<tr>
<td>GlycatedHb %</td>
<td>-</td>
<td>9.04 ± 2.01</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Represents the difference in the mean between the three studied groups: Control group, Controlled diabetics and Uncontrolled diabetics, which showed statistically significant difference in ALT, TG, HDL and glucose mean levels in the three studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group n=107 (mean± SD)</th>
<th>Controlled diabetics n=22 (mean± SD)</th>
<th>Uncontrolled diabetics n=121 mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT(U/L)</td>
<td>34.39±7.9</td>
<td>37.23±7.57</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>20.84±5.57</td>
<td>21.86±6.77</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.21±0.70</td>
<td>5.61±0.78</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.56±0.83</td>
<td>4.39±1.17</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.73±0.45</td>
<td>1.34±0.66</td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.65±0.62</td>
<td>2.54±1.05</td>
<td>2.65±0.87</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.55±0.4</td>
<td>1.14±.33**</td>
<td>1.16±0.65**</td>
</tr>
<tr>
<td>Fas (pg/ml)</td>
<td>145.73±26.63(n=36)</td>
<td>150.40±15.65 (n=6)</td>
<td>143.4±27.86 (n=35)</td>
</tr>
<tr>
<td>FasL (pg/ml)</td>
<td>165.44±23.54(n=36)</td>
<td>183.48±28.55(n=41)</td>
<td>183.40±27.24*(n=35)</td>
</tr>
</tbody>
</table>

Using ANOVA test, the mean difference between control and diabetic groups,*=P<0.05, **=p<0.001 and between diabetics groups, |=P<0.05, || =p<0.001

Table 3: represents correlation between different variables in diabetic patients

<table>
<thead>
<tr>
<th>variables</th>
<th>ALT</th>
<th>HDL</th>
<th>Triglycerides</th>
<th>LDL</th>
<th>Cholesterol</th>
<th>glucose</th>
<th>Duration of disease</th>
<th>Age</th>
<th>AST</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>-r = -0.17</td>
<td>r = 0.036</td>
<td>r = 0.029</td>
<td>r = 0.101</td>
<td>r = 0.062</td>
<td>r = 0.122</td>
<td>r = 0.628**</td>
<td>r = 0.286</td>
<td>r = -0.17</td>
<td>-r = -0.67</td>
</tr>
<tr>
<td>HDL</td>
<td>r = 0.199</td>
<td>r = 0.144</td>
<td>r = 0.392**</td>
<td>r = 0.325**</td>
<td>r = 0.052</td>
<td>r = 0.191</td>
<td>r = 0.080**</td>
<td>r = 0.087</td>
<td>r = 0.392**</td>
<td>r = 0.199</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>r = 0.036</td>
<td>r = 0.034</td>
<td>r = 0.036</td>
<td>-r = -0.066</td>
<td>-r = -0.031</td>
<td>-r = -0.121</td>
<td>-r = -0.087</td>
<td>-r = -0.087</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>LDL</td>
<td>r = 0.036</td>
<td>r = 0.034</td>
<td>r = 0.036</td>
<td>-r = -0.066</td>
<td>-r = -0.031</td>
<td>-r = -0.121</td>
<td>-r = -0.087</td>
<td>-r = -0.087</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>r = 0.029</td>
<td>r = 0.047</td>
<td>r = 0.047</td>
<td>-r = -0.066</td>
<td>-r = -0.031</td>
<td>-r = -0.121</td>
<td>-r = -0.087</td>
<td>-r = -0.087</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>Glucose</td>
<td>r = 0.101</td>
<td>r = 0.205</td>
<td>r = 0.156</td>
<td>r = 0.249**</td>
<td>-r = -0.121</td>
<td>-r = -0.087</td>
<td>-r = -0.087</td>
<td>-r = -0.087</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>GlycatedHb</td>
<td>r = 0.176</td>
<td>r = 0.067</td>
<td>r = 0.093</td>
<td>r = 0.202**</td>
<td>r = 0.190</td>
<td>-r = -0.070</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>r = 0.062</td>
<td>r = 0.052</td>
<td>r = 0.016</td>
<td>-r = -0.100</td>
<td>-r = -0.130</td>
<td>-r = -0.191</td>
<td>-r = -0.239**</td>
<td>-r = -0.065</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>Age</td>
<td>r = 0.122</td>
<td>r = 0.065</td>
<td>r = 0.191</td>
<td>r = 0.010</td>
<td>r = 0.042</td>
<td>r = 0.239**</td>
<td>-r = -0.128</td>
<td>-r = -0.361</td>
<td>-r = -0.036</td>
<td>-r = -0.036</td>
</tr>
<tr>
<td>Fas</td>
<td>r = 0.031</td>
<td>r = 0.030</td>
<td>r = 0.221</td>
<td>r = 0.040</td>
<td>r = 0.210</td>
<td>r = 0.001</td>
<td>r = 0.104</td>
<td>r = 0.138</td>
<td>r = 0.049</td>
<td>-r = -0.060</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Fig. 1: Represents the means serum level of Fas and FasL (pg/ml) in diabetic and control groups. **=p=0.02
Fig. 2: Represents mean glucose concentration mmol/l and glycated Hb %. **= P<0.01 in control group and diabetic patients.

A positive correlation existed between age of the patients and duration of diabetes or triglycerides. (r = 0.239, r = 0.191 p<0.05 respectively, Table 3). A positive correlation existed between Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes (U/L) in diabetic and control groups. **=p<0.01

Fig. 3: Represents the means serum level of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes (U/L) in diabetic and control groups.

Fig. 4: Represents the means serum concentration of lipids profile (TG, cholesterol, LDL and HDL) in diabetic patients and control group. ***=p<0.01

Fig. 5: Represents the positive correlation between FasL (pg/ml) and Alanine aminotransferase enzymes (U/L) in diabetic groups. *=p<0.05

Fig. 6: Represents the positive correlation between FasL (pg/ml) and Age (years) in diabetic groups. **=p<0.05
Atherogenic Index of Plasma (AIP=Log TG/HDL) predicts cardiovascular risk since AIP < 0.11 - low risk, AIP (0.11 - 0.21) intermediate risk and AIP > 0.21 increased risk. In this study 45% of our diabetic patients had increased risk and 11% had intermediate risk. A positive correlation existed between FasL and the age or the levels of ALT in diabetic patients (r = 0.361, r = 0.286 p< 0.05 respectively) (Table 3, Figures 5 & 6)

**DISCUSSION**

Chronic insulin resistance and continuous alteration in β-cell functioning is connected to T2DM [28]. Factors that control the β -cell mass are, a balance of β -cell replication and apoptosis, as well as synthesis of new islets by exocrine pancreatic ducts (neogenesis). The reduction in the production of insulin is a result of decrease in β-cell mass which is due to any kind of disorder of any of the pathways of β -cell formation or enhanced rates of β -cell death [29, 30]

Our results showed increasing rate of apoptosis in type 2 diabetes patients observed by the significant increase in the level of FasL in diabetic patients when compared to control group. A recent very sensibly performed study gave new and persuasive data indicating that the main cause in the reduction of β -cell mass in type 2 diabetics is increased apoptosis and not decreased neogenesis or replication. Due to the fact that new islet formation, which is the leading contributor into the β -cell mass, seems undamaged in type 2 diabetics, hence the reason for the reduced β -cell mass would have to be increased β -cell apoptosis [31].

β -cell loss is specified as an initial process in the pathogenesis of diabetes mellitus due to the fact that IFG (impaired fasting glucose) patients showed a 40% shortage in relative β -cell volume. Patients acquire hyperglycemia when the β-cell mass falls below a critical level and insulin production is no longer able to endure the metabolic demands [31].

A question arises as to, what are the reasons for the increase in apoptosis in the islets of type 2 diabetics? The islet in type 2 diabetes is distinguished by deposits of islet amyloid polypeptide (IAPP) [32, 33–38]. This peptide triggers apoptosis of β -cells [39, 40], especially when it is in the shape of small IAPP oligomers [41]. Furthermore, in our results in the uncontrolled diabetics both glucotoxicity (as shown by increasing the level of fasting blood sugar and the glycated hemoglobin and their positive correlation) and lipotoxicity, as reported in our results by increasing TG and decreasing HDL and the increased TG: HDL ratio (atherogenic dyslipidemia), caused β -cell apoptosis.

Recent studies have further revealed the molecular mechanisms resulting in gluco- and lipotoxic apoptosis in the human β –cell. In cultured human islets different studies have reported the effect of increased glucose concentrations and different free fatty acids (FFAs) on β -cell proliferation, apoptosis and function. The results showed that depending on the dose, β -cell apoptosis was amplified by the extended exposure of cultured human islets to high glucose levels. Furthermore, increased indicators of β -cell apoptosis and decreased β -cell proliferation are caused due to the long-lasting exposure of cultured human islets to palmitic acid (saturated fatty acid). However there is no effect in DNA fragmentation and induced β -cell proliferation with palmitoleic acid and oleic acid (monounsaturated fatty acids). Moreover, apoptosis was prevented and insulin secretion was enhanced by preventing impairment of β-cell proliferation, which was caused by palmitic acid and/or hyperglycemia, when each of the monounsaturated fatty acids were co supplemented [42-45].

Federici et al. [46] reported an over-expression of proapoptotic genes Bad, Bid and Bik and unchanged expression of anti-apoptotic gene Bcl-2 when cultures of human pancreatic islets were exposed to high glucose concentration, thus signifying alteration of the balance towards apoptosis and β-cell death. As a response to continued exposure to hyperglycemia, β -cells generate and produce interleukin (IL)-1β. The resultant stimulated apoptotic pathway (NF-kB activation, Fas upregulation, DNA fragmentation) is suppressed by an IL-1 receptor antagonist. Additionally, reports suggest that IL-1β producing β-cells are found in pancreatic sections of type 2 diabetic patients and not in normal control pancreases [42].

The consequences of prolonged contact with high levels of free fatty acids (FFA) results in overload of lipid in pancreatic cells, diminished insulin production [47,48] and apoptotic cell death [45, 49-51]. In rodents [45, 50] and also in human pancreatic islets [49] apoptosis stimulated by FFA and decreased β -cell proliferation capability were noticed. Lipotoxicity has been recognized for buildup of saturated fatty acids and not linked to contact with unsaturated fatty acids in various studies [45, 52, 53]. Not only saturated fatty acids have harmful influence on the pancreatic β -cell, also raised serum FFAs participate in the pathogenesis of heart disease and the metabolic
syndrome. Unlike adipocytes who keep in lipid droplets, surplus fatty acids as triglyceride, reduced ability of the non-adipose tissues to store lipids in seen. Cell malfunction and/or cell death in non-adipose tissues is caused by the accumulation of extra lipid in hyperlipidemic states. In numerous tissues studied this lipotoxicity seems specific for saturated fatty acids and is improved by unsaturated fatty acids [45, 52–55].

Our results confirmed a significant increase in FasL and no significant increase in the level of Fas could be clarified on the basis of that Islet β-cells normally express Fas ligand (FasL) constitutively but not the Fas receptor [49, 54]. Furthermore, the fact that increased Fas expression represents an earlier detectable factor than increased FasLin the association with phenotypes like obesity, fat distribution, adipocyte tissue function and insulin sensitivity [55], where in our study diabetic patients had a long history of type 2diabetes and a lot of them was obese uncontrolled diabetics.

ROS which occurs in diabetes also known to increases expression of Fas-ligand on hepatocytes that interacts with normally expressed membrane receptor Fas on the adjacent hepatocytes triggering apoptotic cell death [56]. ROS may also initiate the activation of the transcription factor NF-κB, which leads to increased production of proinflammatory cytokines (TNF-α, TGF-β, IL-6, IL-8) [57]. Furthermore, there are convincing data that inflammatory cytokines (TNF-α, IL-6 and IL-1β) also play an important role in the pathogenesis of NAFLD which is manifested by increased ALT enzymes [58].

Our results showed significant increase in the lipid profile levels with raised rate of apoptosis in the diabetic patients which could be explained on the basis of that besides the inflammation, in atherosclerotic lesions the Fas/FasL/ caspase death pathway is recorded to be stimulated, which could develop out of prolonged increase in oxidized LDL.

It should be noted that the desirable value for TG/HDL ratio has been suggested to be less than 2.5. The TG/HDL ratio gives assessment of the total integrated lipid exposure to the tissue. A combined parameter of TG/HDL ratio is also beneficial for assessing the presence of small LDL, therefore by considering the ratio of TG/HDL it may be possible to differentiate those subjects who are at greater risk of coronary heart disease [59].

45% of our patients had increase risk and 11% had intermediate risk of cardiovascular disease according to the atherogenic index of plasma (log TG/HDL) which indicated cardiovascular risk.

A study discovered that in the chronic stable angina pectoris (SAP) patients Fas/APO1 displayed correlation to be negative with high-density lipoprotein cholesterol (HDL-C) (p<0.05), whereas FasL revealed the correlation to be significantly positive with low-density lipoprotein cholesterol (LDL-C) (p<0.05). Only in the non-stable angina pectoris (NSAP) patients, significant difference (p<0.05) in Fas levels was seen in patients with normal cholesterol levels and patients with higher cholesterol levels. In the NSAP patients the correlation between Fas concentration and diabetes mellitus (p < 0.05) and FasL concentrations and both cholesterol (p < 0.01) and triglycerides (p < 0.01) was detected to be strong [60].

FasL production may contribute to the damaging outcomes in atherosclerotic lesions. Geng et al. [61] described that Fas is expressed in atherosclerotic lesions. Contact of Fas expressing cells with its ligand can produce apoptosis in these cells, causing a decrease of the cell number of the lesions.

Significant raise in plasma triacylglycerol revealed the dyslipidemia noticed in the current diabetic patients, while in comparison to control one concentration of HDL-c was reduced significantly in diabetic groups. These results agree with those of Hallfrisch et al. and Nardelli et al. [62]. The responsible factor believed for significant cardiovascular diseases associated morbidity and mortality is the lipoprotein abnormalities resulting from drop of lipoprotein activity [64]. Glucose autooxidation and protein glycation processes increase the production of ROS in the diabetic state. Lipid peroxidation is triggered by ROS and chemical alterations by cross-linking and fragmentation damage protein [65]. Hence it is believed that oxidative stress participates in the pathological progression of diabetic complications. Chronic type 2 diabetes is supplementary to increased peroxidation of lipid in plasma membranes [66]. The delivery of considerably more free radicals, draining of antioxidant enzymes, production of chromosomal aberration and DNA damage due to diabetes mellitus have been confirmed by results. Increased oxidative damage to DNA because of diabetes has been established by several reports; it increases with aging and in chronic diabetics [67-69].

Lorenz et al. [70] showed that hyperglycemia produces glycation of DNA and reduces the DNA repair process in human endothelial cells. Nishikawa et al. [71] suggested that damage DNA produces urinary 8-OHdG (8-hydroxydeoxycytanosine) which is a valuable marker of early micro- and macro vascular complications in type 2

diabetic patients. An essential part of the apoptotic agent is oxidative stress which is an outcome of diabetes. Diminishing of apoptosis is considered as a vital factor mediating tissue turnover. The Fas/FasL pathway may have a role to play [72].

The relation between raising the level of liver function tests and the increasing in FasL level are shown in many investigations which have described that FasL is implicated in the producing of hepatic inflammation and the speeding up of liver regeneration noticed after partial hepatectomy [73]. Depending on the level of FasL expression and/or the induction of inflammatory responses in the local liver microenvironment studies have suggested that Fas/FasL collaboration in the liver can be injurious/immunodestructive or protective/proliferative. Obstruction of FasL could be an effective, therapeutic intervention to ease the progression of liver damage in particular liver inflammations accompanied with increase of FasL expression, is indicated by the important role of FasL in producing liver damage or liver regeneration. These outcomes propose an important mechanism of cell death may be the Fas/Fasl - dependent connections between liver-infiltrating T cells and hepatocytes, thereby resulting in the stimulation of liver damage [74].

This qualitative difference in stimulating liver inflammation was related to raise FasL expression, as systemic administration of anti-FasL antibody abolished liver inflammation. This implies that hepatocyte damage and liver inflammation may be due to increased expression of FasL on core-expressing CD4 T cells. Apoptosis increases due to stimulation of downstream caspase molecule resulting from the Fas/FasL interaction which produces hepatocyte damage by engaging Fas-associated death domain with caspase 8 [75-77]. The indication that FasL induces inflammation by stimulating chemokine secretion and enabling recruitment of inflammatory cells (macrophages, dendritic cells) is quite interesting [78,79]. Additionally FasL holds the costimulatory function to increase the proliferation of CD8 T cells to a greater magnitude than that of CD4 T cells [80]. Remarkably, block of FasL obliterated the development of hepatocellular carcinoma, thereby signifying the pivot role FasL plays in producing liver cancer by chronic inflammation and stimulating cytokines secretions accompanied with fibrosis development [81].

Liver injury with increased serum AST, ALT and up-regulated liver FasL due to Cerulein induced pancreatitis and is considered a key prognostic marker in acute pancreatitis. Gadolinium significantly reduced the elastase brought rise in FasL and FasL mRNA but had little influence on Fas, i.e. acute pancreatitis brings liver damage and hepatocyte death while increasing FasL [82].

CONCLUSIONS

Our study emphasizes a positive association between the apoptosis marker FasL and T2DM especially with elevated ALT. FasL are increased in diabetic subjects with higher ALT levels, suggesting that this protein may be a novel marker of liver injury. Elevated ALT can be considered as a marker of nonalcoholic fatty liver disease (NAFLD) so it can be used in detection of T2DM associated with NAFLD. β-cell damage in diabetes is attributed to Apoptosis and hence the need to improve understanding shall enable development of therapeutic strategies to prevent β-cell loss as well as diabetes. Development of new treatment strategies aiming to reduce sFasL may play an important role in the management of T2DM associated with NAFLD. Developing dietary and pharmacological strategies with the aim to revolutionize increased β-cell apoptosis in people who are at high risk of developing type 2 diabetes is a possibility for slowing the progression or even preventing diabetic complications.

Conflict of Interest: The authors declared that there is no conflict of interest.

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