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Influence of glycation on low density lipoprotein in diabetic cardiovascular disease patients

Nosheen Aslam¹*, Iftikhar Hussain Bukhari¹, Shahgufta Kamal¹, Jeveria Rehman¹, Afzal Waheed²

¹Department of Chemistry, Government College University Faisalabad 38000, Pakistan.
²Directorate of Medical Science, Government College University Faisalabad 38000, Pakistan.

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Cardiovascular disease (CVD) is the major cause of premature death in individuals' glycated low density lipoproteins (LDL). The potential importance of LDL glycation was observed as an atherogenic modification in diabetic patients. For this purpose the advanced glycation end products (AGEs) levels in all the four groups of cardiovascular diseases patients were analyzed to establish whether glycation is the underlying defect in cardiovascular diseases and this has implications for the development of prevention and treatment strategies. A total of 200 male patients having cardiovascular diseases with type 2 diabetes between the ages of 40-60 and 50 healthy subjects were enrolled in this study. The subjects were divided into five groups. Glycated LDL, which can participate in many of the cellular processes leading to atherosclerosis, generally circulates at higher concentration in diabetic people with cardiovascular diseases. Glycated LDL is the major fundamental factor associated with cardiovascular diseases in the local human population.

Key words: Advanced glycation end products (AGEs), low density lipoproteins (LDL), high density lipoproteins (HDL), cardiovascular diseases (CVD).

INTRODUCTION

Key factors crucial to the formation of AGEs include the rate of turnover of proteins for glycoxidation, the degree of hyperglycemia, and the extent of oxidant stress in the environment (Singh et al., 2001). If one or more of these conditions is present, both intracellular and extracellular proteins may be glycated and oxidized (Brownlee et al., 1985). Advanced glycation end products (AGEs), also known as glycotoxins, are a diverse group of highly oxidant compounds with pathogenic significance in diabetes and in several other chronic diseases (Goldin et al., 2006). AGEs are created through non enzymatic reaction between reducing sugars and free amino groups of proteins, lipids, or nucleic acids. This reaction is also known as the Maillard or browning reaction (Vlassara and Uribarri, 2004). The formation of AGEs is a part of normal metabolism, but if excessively high levels of AGEs are reached in tissues and the circulation, they can become pathogenic (Ulrich and Cerami, 2001). The pathologic effects of AGEs are related to their ability to promote oxidative stress and inflammation by binding with cell surface receptors or cross-linking with body proteins, altering their structure and function (Huebschmann et al., 2006). The AGE formation process, or the Maillard reaction, begins from Schiff bases and the Amadori product, a 1-amino-1-deoxyketose, produced by the reaction of the carbonyl group of a reducing sugar, like glucose, with proteins, lipids, and nucleic acid amino groups (Garay et al., 2005, Rojas and Morales 2004). During Amadori reorganization, these highly reactive intermediate carbonyl groups, known as α-dicarbonyls or oxo aldehydes, products of which include 3-deoxyglucosone and methylglyoxal, accumulate (Baynes and Thorpe, 1999). Such buildup is referred to as “carbonyl stress”. The α-dicarbonyls have the ability to react with amino, sulfhydryl, and guanidine functional groups in proteins (Lo et al., 1994). The reaction results in denaturation, browning and cross-linking of the
targeted lipids (Frye et al., 1998). In addition, the α-dicarbonyls can react with lysine and arginine functional groups on proteins, leading to the formation of stable AGE compounds, such as N-(carboxymethyl) lysine, which are non fluorescent AGES (Ahmed et al., 1986). It also form in vitro from LDL incubated with copper ions and glucose and therefore are believed to be both lipid and protein adducts (Sakata et al., 2001, Imanaga et al., 2000a). Once AGES are formed, they are nearly irreversible (Schmidt et al., 1999; Abordo et al., 1999; Fu et al., 1996).

Large LDL particles also can be associated with increased coronary disease risk, particularly in the setting of normal or low triglyceride levels. Like small LDL, large LDL exhibits reduced LDL receptor affinity compared with intermediate sized LDL (Zieman, and Kass 2004). Cardiovascular disease (CVD) is the major cause of premature death in individuals with diabetes and is mainly driven by increased arterial atherosclerosis. Increased risk of atherosclerosis is associated with high levels of LDL and, more particularly, with high levels of small dense LDL (sdLDL) (Rizzo et al., 2006). The risk of CVD is increased two to three fold in diabetes, where the typical increase of sdLDL is two to threefold (Tan et al., 2001). Plasma levels of sdLDL correlate with carotid intima-media thickness (Skoglund-Andersson et al., 1999) and are linked to the risk of CVD (Rizzo et al., 2009).

In brief, the finding of this LDL becomes dangerous when it becomes glycated i.e. when sugar molecules become bonded to it (Mclellan et al., 1994). When that happens it is more likely to stick to the artery walls. It is very likely given the correlation between heart attack and A1c that LDL becomes dangerously glycated at a rate that corresponds to the rate at which hemoglobin becomes glycosylated which is what the A1c measures (glycosylation is permanent glycation) (Jasper et al., 2007).

Formation of advanced glycation end products or peroxidation may be involved in glycated LDL-induced alterations in the generation of fibrinolytic regulators (Zhang et al., 1999). Epidemiological studies have demonstrated that the incidence of atherosclerotic cardiovascular diseases correlates positively with low-density lipoprotein (LDL) and negatively with high-density lipoprotein (HDL) (Steinberg and Witztum 2010; Hirasawa et al., 2011).

MATERIALS AND METHODS

A total of 200 male patients having cardiovascular diseases with type 2 diabetes between the ages of 40-60 and 50 healthy subjects, non diabetic with no history of cardiovascular diseases were enrolled in this study. The subjects were divided into four groups as:

1. A group of 50 healthy subjects, non diabetic with no history of cardiovascular diseases.
2. A group of 50 diabetics with hyperlipidemia having no cardiovascular symptoms.
3. A group of 50 diabetic patients having hyperlipidemia and hypertension.
4. A group of 50 diabetic patients having hyperlipidemia, hypertension myocardial ischemia without infarction.
5. A group of diabetic patients having hyperlipidemia, hypertension and previous attack of myocardial infarction.

A total 50 healthy subjects, non diabetic with no history of cardiovascular diseases was taken as control. Samples were analyzed for the following biochemical parameters.

Blood samples of diabetic patients who were clinically diagnosed by Physicians were collected from D.H.Q. Hospital Faisalabad, National Hospital Faisalabad, Chiniot Dialysis Centre Faisalabad, and Allied Hospital Faisalabad, Pakistan. Blood sample from each patient was collected by using sterilize disposable syringe by venopuncture. The blood was transferred into EDTA (ethylenediaminetetraacetic acid; anticoagulant) containing tubes. The samples were mixed gently by tapping and were then centrifuge at 3000 rpm. Plasma fractions were collected and stored at -20°C. Normal plasma was pooled from blood samples of healthy male.

As free glucose is the major hindrance in estimation of glycation level so it was removed by using dialyzing membrane. After dialysis, samples were again placed in 5 mL capped glass tubes at -20°C. Glycated albumin was also dialyzed against dist. H2O at 4°C and samples were stored (for ELISA standard) at -20°C. The samples from all the subjects in above groups were assessed for glycation (Thiobarbituric Acid Method; Fluckiger and Winterhalter, 1976; Furth, 1988). ELISA was used for the determination of glycated albumin (glycation level so it was removed by using dialyzing membrane).

RESULTS AND DISCUSSION

The glycation level was higher (p<0.0001) in cardiovascular disease patients and there was a significant difference (0.5811) in the glycation level between the four groups of cardiovascular disease patients. The higher level of glycation in cardiovascular disease patients may be attributed to diabetes (Hartog et al., 2007) which adds to the cardiovascular complications (Fernandez and Ricart, 2003; Jeppesen et al., 2007) due to endothelial dysfunction, a precursor for adverse cardiovascular events. Non enzymatic glycation has been well reported in cardiovascular disease patients (Wautier and Schmidt 2004; Thornalley, 2003) due to post translational modification of proteins by the sugars and their de-gradational products (Valcourt et al., 2007). This may also be attributed to the Maillard reaction between sugar and proteins contributing to the increased chemical modification and cross-linking of long lived tissue proteins in diabetes (Westwood and Thornalley 1997; Winicour et al., 1992; Wu and Monnier 2003). In diabetes extracellular
trapping of plasma fats by more rapidly accumulating glycate proteins on connective tissue cannot promote excessive fat accumulation. Low density lipoprotein can be attacked by glycated proteins can contribute to the process of cardiovascular disease because of the infiltration of these particles into the blood vessel wall (Surekha et al., 2007). Low glycation at low glucose levels has also been reported previously (Eble et al., 1983; Younis et al., 2009).

Higher levels of AGEs (p<0.0001) was calculated in cardiovascular diseased patients and there was a significant difference (0.2999) in the glycation level between the four groups of patients. Increased AGE accumulation is closely related to the development of cardiovascular complications in diabetes (Meerwaldt et al., 2008; Giardino et al., 1994). Several lines of evidence suggested that AGEs are related to the development and progression of heart failure in non-diabetic patients as well (Fraser and Hanssen, 2005; Panteghini et al., 1995; Lapolla et al., 2007; Berg et al., 1999, Bohlender et al., 2005; Ryle et al., 1997; Brownlee, 1995). The data evaluated by Sampathkumar et al. (2005) Yamagishi et al. (2007) and Friedman et al. (1999) argued our results as they observed the formation of AGEs or glycation of serum albumin (Gallery, 2001; Goh and Cooper, 2008; Iberg and Fluckiger, 1986) with the chronic exposure to high glucose levels which leads to cardiovascular complications (Miura et al., 2003).

Interestingly, a positive correlation between glycation level and AGEs level in diabetic cardiovascular diseased subjects (r=0.012346) was recorded (Figure 1).

Higher level of cholesterol was calculated (p<0.0001) in diabetic patients with cardiovascular disease as compared to normals. In different stages of cardiovascular disease within groups there was a large variation in the cholesterol levels. Higher level of cholesterol has also been reported in diabetic patients with cardiovascular diseased (Saini et al., 2004; Calvo et al., 1993). Cholesterol is used to estimate the number of low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Jakus et al., 1999). Hypercholesterolemia has been shown to cause cardiovascular dysfunction due to direct action on membrane fluidity, enzyme activities and cation transporters in the endothelial cells, vascular smooth muscle cells and cardiomyocytes (Taylor et al., 2002). Glycation of low-density lipoprotein (LDL) by reactive aldehydes, such as glycolaldehyde, can result in the cellular accumulation of cholesterol in macrophages (Imran, 2007).

A positive correlation between glycation level and cholesterol level in diabetic cardiovascular diseased subjects (r=0.003459) was recorded (Figure 2).

The values of HDL were lower in all groups of
cardiovascular patients compared with the normal subjects. There was also a significant difference between the decreased values of the four groups (0.5874). The decline of HDL values was however, more manifested in diabetics with cardiovascular disease (Rosenson, 2006, Boreggreve et al., 2003). Modification in the function of HDL which is caused by hyperglycemia are the contributor of accelerated atherosclerosis (Hedrick et al., 2000; Miller and Miller, 1975; Owens et al., 2008; Soliman, 2008; Hirayama et al., 2009). HDL showed a protective effect against in vitro non-enzymatic glycation of LDL (Nahla et al., 2012; Robins et al., 2003).

Low levels of HDL components are related not only with atherosclerosis but also with microvascular complications. (Navab et al., 2011). Our experiments suggest that HDL might indeed be protective against glycation (Figure 3).

The LDL values were lower in normal subjects and higher in cardiovascular diseased patients. There was a large variation (0.8660) in the LDL level in the four groups of cardiovascular diseased patients signifying the anticipated risk of advance complications. Findings of this study are in line with the information (Zoppini et al., 2012; Imanaga et al., 2000b; Buse et al., 2004; Pasupathi et al., 2009; Han et al., 2001). AGE-LDL activates signaling pathway and provoke proinflammatory cytokine creation (Angelantonio et al., 2009; Hodgkinson et al., 2008), which increases the risk of atherosclerosis in diabetics. Similarly, glycation of LDL cholesterol caused by hyperglycemia, contribute to accelerated complications of diabetes like cardiovascular diseases (Brown et al., 2007; Hedrick et al., 2000). LDL which is most closely linked with heart diseases, undergoes more glycation than others (Nahla et al., 2010) and glycated LDL is much prone to oxidation than other native LDL (Sobal et al., 2000; Younis et al., 2009; Berneis and Krauss, 2002; Rabbani et al., 2011) or may be involved in the generation of fibrinolytic regulators (Zhang et al., 1998) (Figure 4 and Tables 1 and 2).

**CONCLUSION**

Hyperglycemia and hyperlipidemia are considered critical to the development of advanced glycation end products. Our findings suggested that cholesterol promotes the
formation of advanced-glycation-end-products-protein. Glycated LDL, which can participate in many of the cellular processes leading to atherosclerosis, generally circulates at higher concentration in diabetic people with cardiovascular diseases. Glycated LDL is the major fundamental factor associated with cardiovascular diseases in the local human population. Therefore, hard work may be made to formulate the strategies intended for the improvement of AGEs sensitivity.

REFERENCES


Table 1. Level of different parameters in cardiovascular diseased patients with diabetes in comparison with the healthy subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycation(mole/mole of protein)</td>
<td>0.48±0.18</td>
<td>1.82±0.30</td>
<td>1.75±0.31</td>
<td>1.98±0.29</td>
<td>2.1±0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AGES μg</td>
<td>60.22±7.11</td>
<td>47.52-72.33</td>
<td>89.72±2.99</td>
<td>90.66±3.88</td>
<td>88.98±3.98</td>
<td>91.22±4.11</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>174.55±15.75</td>
<td>144-210</td>
<td>255.43±20.13</td>
<td>222-288</td>
<td>256.01±19.87</td>
<td>258.45±20.41</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.11±5.55</td>
<td>32-66</td>
<td>33.99±5.89</td>
<td>35.12±6.02</td>
<td>33.45±6.92</td>
<td>32.71±8.42</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>105.95±15.99</td>
<td>62-132</td>
<td>172.66±15.32</td>
<td>177.32±20.21</td>
<td>180.33±19.32</td>
<td>182.12±17.35</td>
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Table 2. Pair wise comparison within cardiovascular disease due to glycation.

<table>
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<tr>
<th>Pair wise comparison</th>
<th>Difference</th>
<th>LSD value</th>
<th>Significance</th>
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<tbody>
<tr>
<td>1 vs 2</td>
<td>1.3</td>
<td>0.298</td>
<td>Sig</td>
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<tr>
<td>1 vs 3</td>
<td>0.38</td>
<td>0.298</td>
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<td>1 vs 4</td>
<td>1.14</td>
<td>0.298</td>
<td>Sig</td>
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<td>2 vs 3</td>
<td>-0.92</td>
<td>0.298</td>
<td>Sig</td>
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<tr>
<td>2 vs 4</td>
<td>-0.16</td>
<td>0.298</td>
<td>NS</td>
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<td>3 vs 4</td>
<td>0.76</td>
<td>0.298</td>
<td>Sig</td>
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