E3 Journal of Biotechnology and Pharmaceutical Research Vol. 4(5), pp. 81-88, May 2013
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 © 2013 E3 Journals

Full Length Research Paper

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

Nosheen Aslam[1*], Iftikhar Hussain Bukhari[1], Shahgufta Kamal[1], Jeveria Rehman[1], Afzal Waheed[2]

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

Accepted 17 March, 2013

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

*Corresponding author. Email: afzal_nosheen@yahoo.com.

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^{ε}-(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 A_{1c} 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 andprevious 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 dialysed against dist. H_2O 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 performed by using alkaline phosphatase enzyme and para nitrophenyl phosphate as a substrate, following the procedure of Zhang et al. (2005) slight changes was done according to laboratory conditions and lipid profile (Artiss and Zak, 1997; Friedewald et al., 1972; Lopes-Virella et al., 1977). The ranges, means±SD, correlation values and significance of differences in means were calculated by ANOVA following (Steel et al., 1997).

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; Winocour et al., 1992; Wu and Monnier 2003). In diabetes extracellular

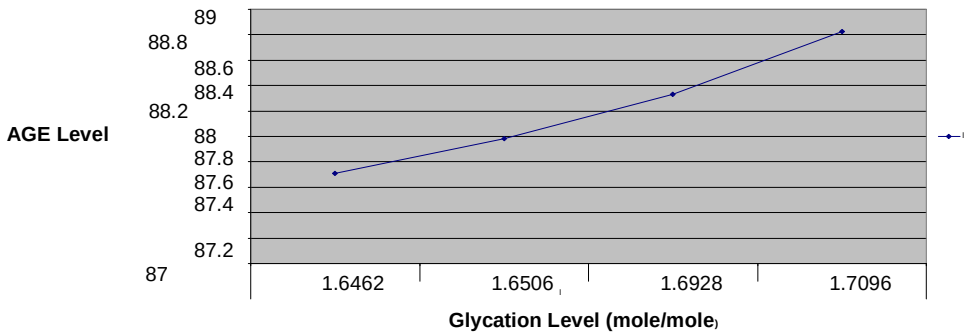

Figure 1. Correlation between AGEs and glycation level in diabetic cardiovascular disease patients.

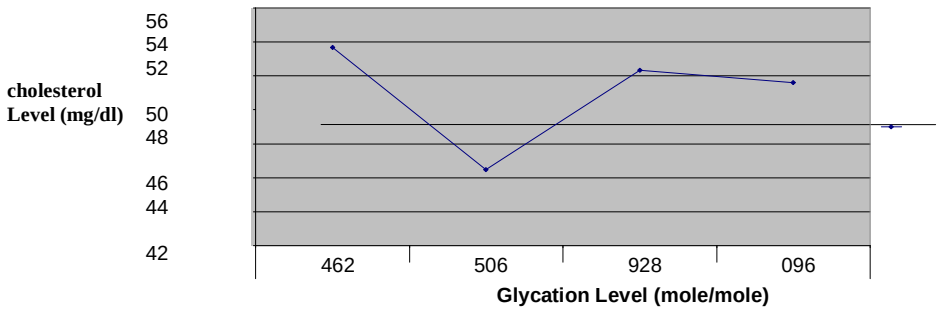

Figure 2. Correlation between cholestrol and glycation level in diabetic cardiovascular disease patients.

trapping of plasma fats by more rapidly accumulating glycated 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 cholestrol level in diabetic cardiovascular diseased subjects ($r=0.003459$) was recorded (Figure 2).

The values of HDL were lower in all groups of

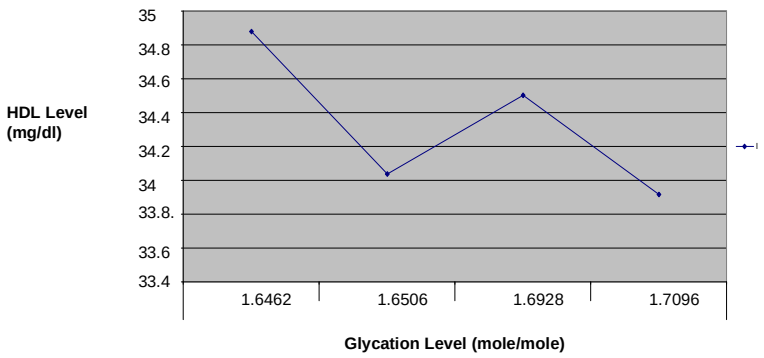

Figure 3. Correlation (r=-0.012532) between HDL and glycation level in diabetic cardiovascular disease patients.

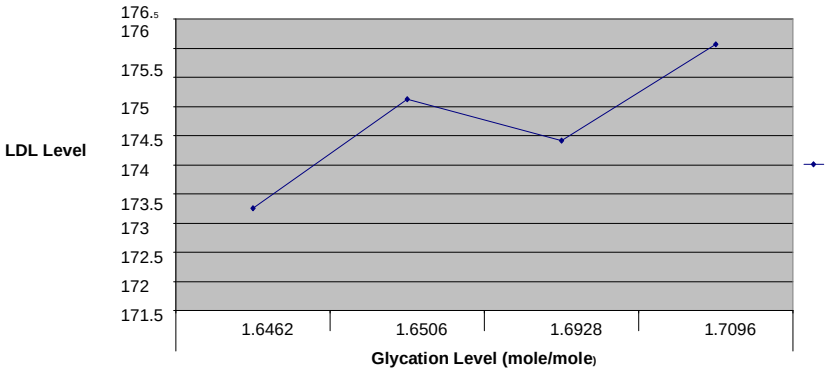

Figure 4. Correlation (r=0.02376) between LDL and glycation level in diabetic cardiovascular disease patients.

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

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

Subjects	1	2	3	4	5	P
Glycation(mole/ mole of protein)	0.48±0.18 0.3-0.9 mole/mole of protein	1.82± 0.30 0.99-2.11	1.75± 0.31 1.11-2.2	1.98± 0.29 1.19-2.3	2.1± 0.31 1.19-2.5	<0.0001
AGEs µg	60.22±7.11 47.52-72.33	89.722± 2.99 79.99-95.6	90.66± 3.88 80.33-96.77	88.98± 3.98 78.45-98.41	91.22± 4.11 84.67-99.55	0.0108
Cholesterol (mg/dl)	174.55±15.75 144-210	255.43± 20.13 225- 291	254.12± 17.75 222-288	256.01± 19.87 212-280	258.45± 20.41 219-298	<0.0001
HDL (mg/dl)	39.11±7.55 32-66	33.99± 5.89 26-49	35.12± 6.02 22-45	33.45± 6.92 23-41	32.71± 8.42 19-29	0.5874
LDL (mg/dl)	105.95±15.99 62-132	172.66± 15.32 145-200	177.32± 20.21 150-212	180.33± 19.32 140-205	182.12± 17.35 152-210	0.8935

1= Healthy control; 2= Diabetic, hyperlipidemic having no cardiovascular symptoms; 3= Diabetic, hyperlipidemic and hypertensive; 4= Diabetic, hyperlipidemic, hypertensive and myocardial ischemia without infarction; 5= Diabetic, hyperlipidemic, hypertensive and previous attack of myocardial infarction.

Table 2. Pair wise comparison within cardiovascular disease due to glycation

Pair wise comparison	Difference	LSD value	Significancy
1 vs 2	1.3	0.298	Sig
1 vs 3	0.38	0.298	Sig
1 vs 4	1.14	0.298	Sig
2 vs 3	-0.92	0.298	Sig
2 vs 4	-0.16	0.298	NS
3 vs 4	0.76	0.298	Sig

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

Abordo EA, Minhas HS, Thornalley PJ (1999). Accumulation of alpha-oxoaldehydes during oxidative stress: A role in cytotoxicity. Biochem. Pharmacol. 58:641-648.

Ahmed MU, Thorpe SR, Baynes JW (1986). Identification of N-ε-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. J. Biol. Chem. 261:4889-4894.

Angelantonio E D, Sarwar N, Perry P (2009). Major lipids, apolipoproteins, and risk of vascular disease. JAMA, 302: 1993-2000.

Artiss JD, Zak B (1997). Measurement of cholesterol concentration. Handbook of lipoprotein testing, Washington: A. C. Press., pp. 99-114.

Baynes JW, Thorpe SR (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 48:1-9.

Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, Hanssen KF (1999). Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. Diabetes Care, 22:1186-1190.

Berneis KK, Krauss RM (2002). Metabolic origins and clinical significance of LDL heterogeneity. J. Lipid Res., 43(9): 1363-79.

Bohlender JM, Franke S, Stein G, Wolf G (2005). Advanced glycation end products and the Kidney. Am. J. Physiol Renal Physol., 289: 645-659.

Boreggreve SE, Varieas RD, Dullart RP (2003). Alteration in high density lipprotein metabolism and reverse cholesterol transport in insulin resistance and type 2 diabetes mellitus: role of lipolytic enzymes, lecithin: cholesterol acyltransferase and lipid transfer proeins. European journal of Clinical Investigation, 33: 8-12

Brown BE, Rashid I, Van Reyk DM (2007). Glycation of lowdensity lipoprotein results in the time-dependent accumulationof cholesteryl esters and apolipoprotein B-100 protein in primary human monocyte-derived macrophages. FEBS J., 274: 1530-1541.

Brownlee M (1995). Advanced protein glycosylation in diabetes and aging. Ann. Rev. Med., 46: 223-234

Brownlee M, Vlassara H, Cerami A (1985). Nonenzymatic glycosylation products on collagen covalently trap low-density

lipoprotein. Diabetes 34: 938-941.

Buse JB, Tan MH, Prince MJ, Ericken PP (2004). The effect of oral antihyperglycemic medications on serum lipid profiles in patients with type 2 diabetes. Diabetes Obese Metab, 6: 133-156.

Calvo C, Ulloa N, Delpozo R, Verougo C (1993). Decreased activation of lecithin cholesterol acyltransferase by glycated apolipoprotein. Medecine 31(7): 786-789.

Eble AS, Thorpe SR, Baynes JW (1983). Non-enzymatic glucosylation and glucose- dependent crosslinking of protein. J. Biol. Chem., 258: 9406-9412.

Fernandez RJM, Ricart W (2003). Insulin Resistance and Chronic Cardiovascular Inflammatory Syndrome. Endocrine Rev., 24(3): 278-301.

Fluckiger R, Winterhalter KH (1976). *In vitro* synthesis of haemoglobin A_{1c}. FEBS Lett., 71: 356-360.

Fraser DA, Hanssen KF (2005). Making sense of advanced glycation end products and their relevance to diabetic complications. Inter. Diabetes Monitor, 17: 1-7.

Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 28: 499-502.

Friedman EA (1999). Advanced glycosylated end products and hyperglycemia in the pathogenesis of diabetic complications. Diabetes Care, 22(2): 65-71.

Frye EB, Degenhardt TP, Thorpe SR, Baynes JW (1998). Role of the Maillard reaction in aging of tissue proteins. J. Biol. Chem., 273: 18714-18719.

Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR (1996). The advanced glycation endproduct N-[carboxymethyl]-lysine, is a product of both lipid peroxidation and glycoxidation reactions. J. Biol. Chem., 271: 9982-9986.

Gallery P (2001). Advanced glycation endproducts; Free radicals and diabetes. J. Soc. Biol., 4: 387-390.

Garay-Sevilla ME, Regalado JC, Malacara JM, Nava LE, Wrobel-Zasada K, Castro Rivas A, Wrobel K (2005). Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with type 2 diabetes mellitus. J Endocrinol. Invest., 28: 223-230.

Giardino I, Edelstein D, Brownlee M (1994). Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity - a model for intracellular glycosylation in diabetes. J. Clin. Invest., 94: 110-117.

Goh SY, Cooper ME (2008). The Role of Advanced Glycation End Products in Progression and Complications of Diabetes. J. Clin. Endocrinol. Metabolism, 93: 4 1143-41152.

Goldin A, Beckman JA, Schmidt AM, Creager MA (2006). Advanced glycation end products: Sparking the development of diabetic vascular injury. Circulation 114:597-605

Han J, Nicholson AC, Zhou X (2001). Oxidized low density lipoprotein decreases macrophage expression of scavenger receptor B-I. J. Biol. Chem., 276: 16567-16572.

Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ (2007). Advanced glycation end-products (AGEs) and heart failure: pathophysiology and clinical implications. Eur. J. Heart Fail., 9: 1146-1155.

Hedrick C, Thorpe SR, Fu MA (2000). Glycation impairs high density lipoproteins function. Diabetologia 43(3): 312-320.

Hirasawa Y, Sakai T, Ito M, Yoshimura H, Feng Y, Nagamatsu T (2011). Advanced-glycation-end-product cholesterol-aggregated-protein accelerates the proliferation of mesangial cells mediated by transforming-growth-factor-beta 1 receptors and the ERK-MAPK pathway. Eur. J. Pharmacol., 15;672(1-3): 159-168.

Hirayama S, Takako I, Osamu M, Takashi K, Osamu Hanyu H, Utako S, Seiki I, Yoshifusa A, Takashi Miida M (2009). Preβ1-HDL is elevated in the fasting state, but markedly reduced postprandially in poorly controlled type 2 diabetic patients. Clinica Chimica Acta. 401(1-2): 57-62.

Hodgkinson CP, Laxton RC, Patel K, Ye S (2008). Advanced Glycation End-Product of Low Density Lipoprotein Activates the Toll-Like 4 Receptor Pathway Implications for Diabetic Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology, 28:2275-2281.

Huebschmann AG, Regensteiner JG, Vlassara H, Reusch JEB (2006). Diabetes and advanced glycoxidation end products. Diabetes Care, 29: 1420-1432.

Iberg N, Fluckiger R (1986). Nonenzymatic glycosylation of albumin in vivo. Identification of multiple glycosylated sites. J. Biol. Chem., 261 (29): 13542-13545.

Imanaga Y, Sakata N, Takebayashi S (2000a). *In vivo* and *in vitro* evidence for the glycoxidation of low density lipoprotein in human atherosclerotic plaques. Atherosclerosis, 150: 343-355.

Imanaga Y, Sakata N, Takebayashi S, Matsunaga A, Sasaki J, Arakawa K, Nagai R, Horiuchi S, Itabe H, Takano T (2000b). *In vivo* and *in vitro* evidence for the glycoxidation of low density lipoprotein in human atherosclerotic plaques. Atherosclerosis, 150: 343-355.

Imran R, David van Reyk M, Michael JD (2007). Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation *in vitro*, 581(5): 1067-1070.

Jakus V, HrnEiarova M, Sky J, Krahulec B, Rietbrock N (1999). Inhibition of non enzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. Life Sci., 65(18- l9): 1991-1993.

Jasper WL, Hartog, Adriaan AV, Stephan JLB, Andries JS, Dirk van Veldhuisen J (2007). Advanced glycation end-products (AGEs) and heart failure: Pathophysiol. Clin. implications, 9(12): 1146-1155.

Jeppesen J, Hansen TW, Rasmussen S, Ibsen H, Pedersen CT, Madsbad S (2007). Insulin Resistance, the Metabolic Syndrome, and Risk of Incident Cardiovascular Disease. J. Am. Coll. Cardiol., 49: 2112-2119.

Lapolla A, Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, Baccarin L, Laverda B, Fedele D (2007). Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. Diabetes Care, 30: 670-676.

Lo TW, Westwood ME, McLellan AC, Selwood T, Thornalley PJ (1994). Binding and modification of proteins by methylglyoxal under physiological conditions. J. Biol. Chem., 269: 32299-32305.

Lopes-Virella ML, Stone P, Ellis S, Colwell JA (1977). Cholestrol determination in high density lipoproteins separated by three different methods. Clin. Chem., 23: 882-884.

McLellan AC, Thornalley PJ, Benn J, Sonksen PH (1994). Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin. Sci. (Lond)., 87: 21-29.

Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands J-L, Smit A (2008). The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovascular Diabetol., 7: 29.

Miller GJ, Miller NE (1975). Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet, 1:16-19.Miura J, Yamagishi S, Uchigata Y (2003). Serum levels of non-carboxymethyllysine advanced glycation endproducts are correlated to severity of microvascular complications in patients with Type 1 diabetes. J. Diabetes Complications, 17: 16-21

Nahla NY, Handrean S, Valentine C-M, Reena S, Salam H, Philip P, Mohamed ME, Paul ND (2012). High-density lipoprotein impedes glycation of low-density lipoprotein. Diabetes Vascular Dis. Res., 0(0): 1-9.

Nahla NY, Soran H, Sharma R, Charlton-Menys V, Greenstein A, Elseweidy MM, Durrington PN (2010). Small-dense LDL and LDL glycation in metabolic syndrome and in statin-treated and non-statin-treated type 2 diabetes. Diabetes Vascular Dis. Res., 7(4): 289-295.

Navab M, Reddy ST, Van Lenten BJ (2011). HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. Nat. Rev. Cardiol., 8: 222-232.

Owens D, Stinson J, Collins P, Johnson A, Tomkin GH (2008). Hypertriglyceridemia and its influence on low density lipoprotein. Singapore Med, 49(2): 136.

Panteghini M, Cimino A, Pagani F, Girelli A (1995). Nonenzymic glycation of apolipoprotein B in patients with insulin- and noninsulin-dependent diabetes mellitus. Clin. Biochem., 28: 587-592.

Pasupathi P, Rao YY, Faook J, Saravanan G, Bakthavathsalam G (2009). Oxidative stress and cardiac biomarkers in patients with acute myocardial infarction. Eur. J. Sci. Res., 27(2): 275-285.

Rabbani N, Godfrey L, Xue M, Shaheen F, Geoffrion M, Milne R, Thornalley PJ (2011). Glycation of LDL by Methylglyoxal Increases Arterial Atherogenicity. Diabetes, 60(7): 1973-1980.

Rizzo M, Berneis K, Corrado E, Novo S (2006). The significance of low-densitylipoproteins size in vascular diseases. Int. Angiol., 25: 4-9.

Rizzo M, Pernice V, Frasheri A (2009). Small, dense low-density lipoproteins (LDL) are predictors of cardio- and cerebro-vascular events in subjects with the metabolic syndrome. Clin. Endocrinol. (Oxf)., 70:870-875.

Robins SJ, Rubins HB, Faas FH, Schaefer EJ, Elam MB, Anderson JW, Collins DD (2003). The Veterans Affairs HDL Intervention Trial (VA-HIT): Insulin resistance and cardiovascular events with low HDL cholesterol: the Veterans Affairs HDL Intervention Trial (VA-HIT). Diabetes Care, 26: 1513-1517.

Rojas A, Morales MA (2004). Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life Sci., 76: 715-730.

Rosenson RS (2006). Low and high density lipoprotein cholesterol and cardiovascular disease; risk reduction with statin therapy. Am. Heart J., 151: 556-563.

Ryle C, Leow CK, Donaghy M (1997). Nonenzymatic glycation of peripheral and central nervous system proteins in experimental diabetes mellitus. Muscle Nerv., 20: 577-584.

Saini HK, Arneja AS, Dhalla NS (2004). Role of cholesterol in cardiovascular dysfunction. Can. J. Cardiol., 20(3): 333-346.

Sakata N, Uesugi N, Takebayashi S, Nagai R, Jono T, Horiuchi S, Takeya M, Itabe H, Takano T, Myint T, Taniguchi N (2001). Glycoxidation and lipid peroxidation of low-density lipoprotein can synergistically enhance atherogenesis. Cardiovasc. Res., 49: 466-475.

Sampathkumar R, Balasubramanyam M, Rema M, Mohan C, Premanand M (2005). A novel advanced glycation index and its association with diabetes and microangiopathy. Metabolism, 54: 1002-1007.

Schmidt AM, Yan SD, Wautier JL, Stern D (1999). Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ. Res., 84: 489-497.

Singh R, Barden A, Mori T, Beilin L (2001). Advanced glycation end-products: A review. Diabetologia, 44: 129-146.

Skoglund-Andersson C, Tang R, Bond MG, de Faire U, Hamsten A, Karpe F (1999). LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. Arterioscler. Thromb. Vasc. Biol., 19: 2422-2430.

Sobal G, Menzel J, Sinzinger H (2000). Why is glycated LDL more sensitive to oxidation than native LDL? A comparative study. Prostaglandins Leukot. Essent. Fatty Acids, 63(4): 177-186.

Soliman GZA (2008). Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) level in Egyptian of type 2 diabetic patients. Singapore Med. J., 49(2): 129-136.

Steel RGD, Torrie MJH, Dickey DA (1997). Principles and Procedures of Statistics: A Biometrical approach. McGraw Hill Book Co., Inc. New York, USA. pp. 10-20.

Steinberg D, Witztum JL (2010). Oxidized low-density lipoprotein and atherosclerosis. Arterioscler. Thromb. Vasc. Biol., 30: 2311-2316.

Surekha RH, Srikanh BMV, Jharna P, Ramachandra RV, Dayasagar RV, Jyothy A (2007). Oxidative stress and total antioxidant status in myocardial infarction and renal complications. Singapore Med. J., 48(2): 137-142

Tan CE, Chew LS, Chio LF (2001). Cardiovascular risk factors and LDL subfraction profile in type 2 diabetes mellitus subjects with good glycaemic control. Diabetes Res Clin Pract 51:107–114

Taylor AJ, Kent SM, Flaherty PJ, Coyle LC, Markwood TT, Vernalis N (2002). Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol: A randomized trial comparing the effects of atorvastatin and pravastatin on carotid intima medial thickness. Circulation, 106: 2055-2060.

Thornalley PJ (2003). Glyoxalase I- structure, function and a critical role in the enzymatic defense against glycation. Biochem. Soc. Trans., 31: 1343-1348.

Ulrich P, Cerami A (2001). Protein glycation, diabetes, and aging. Recent Prog. Horm. Res., 56: 1-21.

Valcourt U, Merle B, Gineyts E, Viguet-Carrin S, Delmas P, Garnero P (2007). Non-enzymatic glycation of bone collagen modifies osteoclastic activity and differentiation. J. Biol. Chem., 282(8): 5691-5703.

Vlassara H, Uribarri J (2004). Glycoxidation and diabetic complications: Modern lessons and a warning? Rev. Endocrin. Metab. Disord., 5: 181-188.

Wautier JL, Schmidt AM (2004). Protein Glycation; A Firm Link to Endothelial Cell Dysfunction. Circulation Res., 95(3): 233-245.

Westwood ME, Thornalley PJ (1997). Glycation and Advance glycation end products. In: ColacaoCed. The Glycation Hypothesis. Landes Bioscience, Georgetown, TX, USA, pp. 59-87.

Winocour PD, Watala C, Perry DW, Kinlough-Rathbone RL (1992). Decreased platelet membrane fluidity due to glycation or acetylation of membrane proteins.Thromb

Haemost., 68(5): 577-582.
Wu XL, Monnier VM (2003). Enzymatic deglycation of proteins. Arch. Biochem. Biophys., 419: 16-24.

Yamagishi S, Matsui T, Ueda S, Nakamura K, Maizumi T (2007). Advanced glycation end products (AGEs) and cardiovascular disease (CVD) in diabetes. Cardiovasc Hematol. Agent Med. Chem., 5(3): 236-240.
Younis NN, Soran H, Sharma R, Charlton-Menys V, Durrington PN (2009). Lipoprotein glycation and atherogenesis. Clin. Lipidol, 4: 781–790.
Zhang EY, Swaan PW (1999). Determination of Membrane Protein Glycation in Diabetic Tissue. AAPS. Pharm. Sci., 1(4): 20-24.
Zhang J, Ren S, Sun D, Shen GX (1998). Influence of glycation on LDL-induced generation of fibrinolytic regulators in vascular endothelial cells. Arterioscler. Thromb. Vasc. Biol., 18(7):1140-1148.
Zhang X, Frischmann M, Engel RK, Steinmann K, Stopper H, Niwa T, Pischetsrieder M (2005). Two immunochemical assays to measure advanced glycation end-products in serum from dialysis patients. Clin. Chem. Lab. Med., 43(5): 503-511.
Zieman SJ, Kass DA (2004). Advanced glycation endproduct crosslinking in the cardiovascular system: Potential therapeutic target for cardiovascular disease. Adis Int., 64(5): 459-470
Zoppini G, Negri C, Stoico V (2012). Triglyceride-highdensity lipoprotein cholesterol is associated with microvascular complications in type 2 diabetes mellitus. Metabolism, 61: 22-29.