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Biochemical Profile Indicative of Insulin Resistance in Non-diabetic and Diabetic Cardiovascular Patients

NOSHEEN ASLAM[1], MUNIR AHMAD SHEIKH, KHALIL-UR-REHMAN AND HAQ NAWAZ†
Departments of Biochemistry, University of Agriculture, Faisalabad, Pakistan
†*Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan*
[1]Corresponding author's e-mail: afzal_nosheen@yahoo.com

ABSTRACT

This study was aimed at assessment of some biochemical profiles associated with cardiovascular complications in non-diabetics and those suffering from diabetes type 2 in order to detect insulin resistance as an underlying cause. A total of 400 blood samples comprising of patients with (*n*=200) and without type 2 diabetes (*n*=200) were tested for different biochemical parameters following standard methods. The glycation, advanced glycosylation end products, glucose, insulin, C-peptide and LDL were lower in non-diabetic compared with diabetic subjects having a history of cardiovascular complaints. A positive correlation was found between the level of glycation and AGEs, glucose, HbA1c, C-peptide, cholesterol and LDL in non-diabetic and diabetic groups. There was, however, a negative correlation between the level of glycation and HDL in non-diabetic subjects with cardiovascular disease. Simultaneous presence of high levels of insulin, glucose and glycation suggested insulin resistance in diabetics as well as non-diabetics at risk of diabetes and associated cardiovascular complications. Therefore, disturbances in the biochemical profile and morbidity among all the groups of the present study were mainly attributed to insulin resistance. Diabetes and associated cardiovascular complications in the context of insulin resistance have been discussed.

Key Words: Insulin resistance; Biochemical profiles; Diabetes type 2

INTRODUCTION

Insulin helps to transport glucose into the body cells where it is required for the production of energy (Courtney & Olefsky, 2007; Jafri *et al*., 2011). Liver, fat and muscle cells of insulin resistant individuals; however, do not give response to insulin properly. Consequently, more insulin is produced leading to failure of the pancreas, and excessive glucose accumulates in the blood causing diabetes. Therefore, many people with insulin resistance have high levels of both insulin and glucose circulating in their blood at the same time. Low insulin sensitivity with selective loss of release of insulin identify the individuals who are at increased risk of developing type 2 diabetes (Reaven, 1995; Prato *et al.,* 2005**)**, a risk factor of carotid arteriosclerosis (Fox *et al.,* 2006; Zachary, 2007; Cade, 2008).

Typical findings in insulin resistant patients are low HDL, high triglycerides and normal to slightly increased LDL. This happens, because fat cells are affected by insulin resistance resulting in some enzymes to break fats, releasing fatty acids which then go to the liver (Walcher & Marx, 2004). The fatty acids enhanced the production of triglycerides, which then enter the circulation while simultaneously increasing the secretion of carrier protein low density lipoprotein (LDL) (Hodgkinson *et al*., 2008; Ahmad *et al*., 2012). When the triglycerides are in blood carried by VLDL, there is transfer of cholesterol ester from HDL to the triglyceride and VLDL leave VLDL and HDL is replaced by the cholesterol ester (Fonseca, 2003). In the same time, newly formed VLDL triglyceride is exchanged for ester of cholesterol in LDL and produces small dense lipoproteins. This bidirectional transfer of triglyceride leads to elevated triglyceride levels. Thus, HDL is easily broken down and excreted by the kidney (Montagnani *et al*., 2002). In this way, HDL level is reduced. Such lipid changes can cause deposits of fatty plaque in arteries and results in the CVD (Reaven *et al.,* 2004).

Accumulation of fatty deposits lead to the narrowing of blood vessels (e.g., atherosclerosis) obstructing blood supply to the vital organs like brain, heart, etc. (Kolodgie *et al.,* 2003) and causing stroke, cardiac failure, etc. Nevertheless, glucose plays a crucial role by glycating the proteins in whole body including LDL. Therefore, any disturbance in the normal metabolism of glucose and/or glycation, particularly that resulting from altered insulin functionality has serious consequences. In developing countries like Pakistan, hardly ever regular medical check ups are carried out until and unless the condition gets complicated due to underlying causes such as insulin resistance. The present paper describes some biochemical profiles associated with cardiovascular complications in non-diabetics and those suffering from diabetes type 2.

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MATERIALS AND METHODS

A total of 400 male patients with (*n*=200) and without type 2 diabetes (*n*=200) between the age of 40-60 were enrolled in this study and placed in five groups (Table I).

Collection of samples: Fifty blood samples of each of the above categories 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 venipuncture. The blood was transferred into EDTA (anticoagulant) containing tubes. The samples were mixed gently by tapping and were then centrifuged at 3000 rpm. Plasma fractions were collected and stored at -20°C. Normal plasma was pooled from blood samples of healthy male.

Dialysis of plasma samples: For glucose determination, plasma samples were first dialysed as free glucose is the major hindrance in estimation of glycation level (Trinder, 1969). So, it was removed by using dialyzing membrane. Plasma samples were dialyzed against dist. H_2O for 24 h at constant stirring at room temperature. 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.

Parameters: The samples from all the subjects in above groups were assessed for glycation (Thiobarbituric Acid Method; Fluckiger & Winterhalter, 1976; Furth, 1988), advanced glycation endproducts (ELISA; Zhang *et al*., 2005), glucose (standard kit method), HbA1c (Bisse & Abraham, 1985), insulin (ELISA; Clark & Hales, 1994), serum C-peptide (ELISA; Horwitz *et al.,* 1975) and lipid profile (Friedewald *et al.,* 1972; Lopes-Virella *et al.,* 1977).

Statistical analysis: 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, advanced glycosylation end products (AGEs), glucose, insulin, C-peptide and LDL were lower in non-diabetic compared with diabetic subjects in all the groups (Table II). A positive correlation was found between the level of glycation and AGEs (r=0.011236: r=0.00543), glucose (r=0.0013214: r=0.01121354), HbA1c (r=0.0011365: r=0.036987), C-peptide (r=0.0006398: r=0.011214), cholesterol (r=0.002156: r=0.002369) and LDL (r=0.003396: r=0.01145) in non-diabetic and diabetic groups, respectively. There was however, a negative correlation between the level of glycation and HDL in non-diabetic (r=-0.001214) and diabetic (r=-0.011785) subjects with cardiovascular disease.

The higher (*p*=0.9679) levels of insulin in diabetics may be attributed to insulin resistance (Mack *et al.,* 2004; Samaras *et al.,* 2006), which adds to the cardiovasular complications (Fernandez & Ricart, 2003; Jeppesen *et al*., 2007) due to endothelial dysfunction, a precursor for adverse cardiovascular events. Interestingly, a positive correlation between glycation level and insulin level in non-diabetic (r=0.001245) and diabetic cardiovascular diseased subjects (r=0.002565) was recorded. Simultaneous presence of high levels of insulin, glucose and glycation suggested insulin resistance in diabetics as well as non-diabetics at risk of diabetes and associated cardiovascular complications. Therefore, disturbances in the biochemical profile and morbidity among all the groups of the present study are mainly attributed to insulin resistance. Diabetes and associated cardiovascular complications in the context of insulin resistance have been discussed as under.

Non enzymatic glycation has been well reported in diabetics (Austin *et al.,* 1990; Halton *et al.,* 1993; Stoynev *et al.,* 2004) due to post translational modification of proteins by the sugars and their de-gradational products (Argirov *et al.,* 2003). 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 (Fu *et al.,* 1994).

Higher levels of AGEs in diabetic patients may lead to vascular complications (Goh & Cooper, 2008; Peppa & Raptis, 2008). AGEs affect extracellular proteins and activate cytokine production and transcription factors by binding to AGE receptors; and thus, accumulation of AGEs has been predicted to closely correlate with the development of cardiovascular complications (Zieman & Kass, 2004; Meerwaldt *et al.,* 2008). Likewise, chronic hyperglycemia has a central role in complications in diabetics through production of AGEs from glucose (Lee *et al.,* 1998; Thorpe & Baynes, 2003; George & Sivakami, 2004) and/or glycation of human serum albumin (Kobayashi *et al.,* 1991). Low glycation at low glucose levels has also been reported previously (Eble *et al.,* 1983; Winocour *et al.,* 1992). Regarding HbA1c, results are in support of the earlier workers (Singer *et al.,* 1992; Viktrorova *et al.,* 1993; Arun *et al.,* 2002).

As far as C-peptide, it was also reported higher (Stadler *et al.,* 2005; Chailurkit *et al.,* 2007) in diabetics due to higher levels of insulin and glucose intolerance. The insulin resistance causes abnormal metabolism of glucose ultimately leading to increased glycation of proteins. The cholesterol levels were lower ($p<0.0001$) in normal compared with both diabetic and non-diabetic subjects with cardiovascular disease. Large variation in the cholesterol levels within groups indicates different stages of cardiovascular disease. Higher cholesterol has also been reported earlier in insulin resistant subjects (Jakus *et al.,* 1999; Taylor, 2002; Garvey *et al.,* 2003; Surekha *et al.,* 2007). Glycation of plasma protein may contribute to excess risk of developing atherosclerosis diabetic patients causing an increase in the level of cholesterol (Calvo *et al.,* 1993).

The values of HDL were lower in all groups of cardiovascular patients compared with the normal subjects. The reduction of HDL values was however, more

Table I: Details of patients sampled for investigation in the present study

Groups[1]	Non-diabetics	Diabetics
1	Hyperlipidemia having no cardiovascular symptoms	Hyperlipidemia having no cardiovascular symptoms
2	Hyperlipidemia and hypertension	Hyperlipidemia and hypertension
3	Hyperlipidemia, hypertension and myocardial ischemia without infarction	Hyperlipidemia, hypertension and myocardial ischemia without infarction
4	Hyperlipidemia, hypertension and previous attack of myocardial infarction	Hyperlipidemia, hypertension and previous attack of myocardial infarction
5	Non diabetic with no history of cardiovascular diseases as control	

[1]Each group had 50 subjects

Table II: Biochemical profiles of diabetic and diabetic type 2 subjects with cardiovascular complaints in comparison with the healthy ones

Subjects	ND	NDHNCVD	NDHH	NDHHMIS	NDHHMI	DHNCVD	DHH	DHHMIS	DHHMI	"P" value
Glycation level (mole/mole of protein)	0.47±0.17 (0.2-0.8)	0.58±0.14 (0.34-0.81)	0.57±0.13 (0.35-0.81)	0.60±0.13 (0.31-0.84)	0.57±0.13 (0.31-0.84)	1.65±0.24 (0.99-1.99)	1.70±0.29 (0.96-2.03)	1.70± 0.30 (0.98-2.1)	1.71± 0.27 (1.12-2.1)	<0.0001
AGEs (per µg of plasma protein)	59.71±6.38 (46.73-71.45)	67.09±10.56 (47.23-85.46)	66.08± 9.65 (44.66-85.63)	68.09±9.66 (49.62-86.16)	68.61± 9.90 (45.82-86.99)	87.71± 2.76 (79.84-93.56)	88.33± 3.42 (78.47-94.32)	88.00± 3.40 (77.42-94.21)	88.83±2.63 (83.56-93.98)	<0.0001
Glucose (mg/dl)	96.32±12.82 (75-124)	108.62±15.45 (80-131)	107.32±13.26 (80-131)	108.24±14.00 (82-132)	107.48±14.56 (80-136)	164.14±17.76 (130-199)	161.42±20.79 (122-200)	161.64±21.07 (133-210)	167.28±22.17 (133-211)	<0.0001
HbA1c (%)	5.36 ±0.79 (4.2-6.2)	6.38±0.67 (5.2-7.6)	6.01±0.62 (4.8-7.4)	5.90± 0.72 (4.2-7.1)	5.94±0.70 (4.3-7.0)	7.71± 0.93 (7.0-9.8)	7.61±0.78 (6.6-9.3)	7.64±0.78 (7.0-9.5)	7.82±0.80 (6.6-9.5)	<0.0001
Insulin (miu/ml)	17.11±2.97 (10.0-20.5)	18.26±4.12 (10.0-25.0)	17.06±3.75 (9.0-24.0)	18.28±4.11 (11.0-25.0)	17.9 ±3.94 (9.0-25.0)	15.32±4.71 (13.0-23.4)	17.24±6.61 (5.6-29.1)	17.13±5.81 (4.5-28.9)	15.45±6.52 (3.4-32.9)	0.9679
C-peptide (ng/ml)	2.22±0.84 (0.4-3.5)	2.47±0.79 (1.2-3.8)	2.36±0.60 (0.7-3.1)	2.47±0.78 (1.2-3.8)	2.47±0.70 (0.7-3.8)	3.96±0.65 (1.9-5.2)	4.15±0.69 (2.1-5.6)	3.93±1.16 (1.9-5.7)	3.38±1.0 (1.3-5.7)	<0.0001
Cholesterol (mg/dl)	175.58±15.68 (140-200)	262.98±17.64 (201-296)	262.54±13.70 (240-288)	261.64±14.75 (222-288)	264±15.31 (241-307)	253.66±19.66 (223-289)	252.32±16.11 (220-290)	246.48±18.18 (209-278)	251.62±19.67 (216-288)	<0.0001
HDL (mg/dl)	38.84±7.45 (30-65)	38.96±4.84 (30-50)	37.96± 3.25 (31-43)	38.70±4.89 (28-49)	39.14±5.07 (29-48)	34.88±6.04 (25-50)	34.5±6.05 (21-43)	34.04±7.01 (22-42)	33.92±8.32 (20-30)	0.0108
LDL (mg/dl)	106.74±16.46 (60-130)	174.38±15.09 (140-199)	170.14±18.30 (141-197)	176.42±12.70 (151-199)	171.44±16.96 (140-200)	173.26±14.49 (143-198)	174.42±19.30 (140-203)	175.12±17.88 (139-203)	176.06±15.57 (136-204)	<0.0001

ND= Normal non-diabetic; NDHNCVD= Non-diabetic, hyperlipidemic having no cardiovascular symptoms; NDHH= Non-diabetic, hyperlipidemic and hypertensive; NDHHMIS= Non-diabetic, hyperlipidemic, hypertensive and myocardial ischemia without infarction; NDHHMI= Non-diabetic, hyperlipidemic, hypertensive and previous attack of myocardial infarction; DHNCVD= Diabetic, hyperlipidemic having no cardiovascular symptoms; DHH= Diabetic, hyperlipidemic and hypertensive; DHHMIS= Diabetic, hyperlipidemic, hypertensive and myocardial ischemia without infarction; DHHMI= Diabetic, hyperlipidemic, hypertensive and previous attack of myocardial infarction

pronounced in diabetics compared with non-diabetics with cardiovascular disease. Defect in insulin action and/or the associated hyperinsulinemia may lead to an increase in plasma triglyceride and a decrease in HDL and high blood pressure (Reaven, 1993; Hirayama *et al.,* 2009). Alteration in function of HDL caused by exposure to hyperglycemia could contribute to the accelerated atherosclerosis observed in type 2 diabetes (Hedrick *et al.,* 2000). The LDL values were lower in normal subjects and higher in cardiovascular diseased patients with or without insulin resistance. There was however, a large variation in the level of LDL in all the four groups of cardiovascular patients indicating the projected risk of further complications. Findings of the present study endorse the previous reports (Buse *et al.,* 2004; Chan *et al.,* 2005; Pasupathi *et al.,* 2009). AGE-LDL activates TLR4-mediated signaling pathway and induces proinflammatory cytokine production (Hodgkinson *et al.,* 2008), which partly explains the increased risk of atherosclerosis observed in diabetics. Similarly, alteration (glycation) in function of LDL cholesterol caused by exposure to hyperglycemia could contribute to accelerated diabetic complications like cardiovascular diseases (Hedrick *et al.,* 2000). According to Hodgkinson *et al.* (2008) there is substantial evidence indicating that glycated LDL promotes atherosclerosis.

CONCLUSION

Insulin resistance is the major underlying factor leading to diabetes and cardiovascular diseases in the local human population. Therefore, efforts may be made to devise the strategies aimed at improving insulin sensitivity of the available chemicals/biologicals.

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