Serum haematological and biochemical indices of oxidative stress and their relationship with DNA damage and homocysteine in Pakistani type II diabetic patients

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Abstract: *Diabetes mellitus* (DM) is a heterogeneous metabolic disorder characterized by chronic hyperglycaemia, higher glycated hemoglobin (HbA1C) as well as protein. Oxidative stress can cause damage to leukocytic DNA and enhancement of homocysteine (Hcy) level in sera of type 2 diabetic patients. Haematological and biochemical parameters are severely affected by oxidative stress, which results in damages to DNA and Hcy in these patients. Eighty DM patients and 80 normal subjects, after having their consent, were selected for the present study. Leukocytes were characterized for DNA damage by comet assay kit while, blood plasma was taken into account for biochemical indices using commercial test kits. Results indicated that DNA damage was strongly linked with erythrocyte sedimentation rate (ESR) ($P \leq 0.01$), glycated hemoglobin (HbA_{1C}) ($P \leq 0.0001$), glycated serum protein ($P \leq 0.005$), cholesterol ($P \leq 0.011$), triglycerides ($P \leq 0.001$), albumin ($P \leq 0.001$), creatinine ($P \leq 0.006$), urea ($P \leq 0.007$) and ALT ($P \leq 0.02$), and negatively associated with packed cell volume (PCV) ($P \leq 0.002$) and hemoglobin ($P \leq 0.001$). Homocysteine was strongly linked with ESR, HbA_{1C}, glycated protein ($P \leq 0.002$), cholesterol ($P \leq 0.016$), triglycerides ($P \leq 0.0001$), albumin, creatinine, urea, ALT and AST in diabetic patients. Hyc and DNA damages both were negatively linked with total hemoglobin and PCV. Both of these even in their normal range may have a role in the endothelium damage. Nutritional intervention to lower down Hyc and DNA damages in the Pakistani population may mitigate their effect and guarantee in maintenance of a healthy nation.

Keywords: Cholesterol, Glycated hemoglobin, DNA damage, Homocysteine, Packed cell volume.

INTRODUCTION

Population based studies of different diseases and biochemical profiles suggest involvement of genetic variations among different ethnic groups. Genetic variations and nature of environment strongly influence biochemical composition of individuals. These variations play a key role in the metabolic disease diversity in different geographic regions worldwide.

Diabetes mellitus (DM) is a metabolic syndrome of several etiologies categorized by chronic hyperglycaemia with disorders of carbohydrate, protein and fat metabolism resulting from deficiencies in insulin secretion, insulin action, or both (WHO, 2004). During diabetes, chronic hyperglycemia causes higher production of free radicals particularly reactive oxygen species (ROS) from glucose auto-oxidation, hemoglobin and protein glycosylation in all tissues (Peter *et al.,* 2012, Yamada *et al.,* 2012). Free radicals are produced as by-products of usual cellular metabolism; though, some conditions are known to interrupt the balance between ROS production and cellular defense mechanisms. Under this difference diabetic patients are at high threat of developing erectile dysfunction (Peter *et al.,* 2012) cardiac disease and hyperlipidemia hypertension (Yamada *et al.,* 2012). DM is a major global health problem and its prevalence was expected to be 2.8% in 2000 and will increase to 4.4% in 2030 (Shaw *et al.,* 2010, Herman and Zimmet, 2012). Countries like Pakistan, there are a diversity of geographical ethnic groups, socioeconomic conditions and dietary habits, which offer a natural epidemiological laboratory where the epidemiology of conditions like hypertension and DM can be studied. Pakistan has 7 million people with diabetes and currently it is 8th in the world according to WHO estimation of prevalence of diabetes (WHO, 2004, Herman and Zimmet, 2012).

Measurement of glycated haemoglobin (HbA1C) has been shown as an index of the blood glucose concentration over the previous several weeks and can be used in the diagnosis and management of diabetic patients (Geberhiwot *et al.,* 2005) describing how HbA1c measurement may help avoid unnecessary oral glucose tolerance tests (OGTTs) for diagnosis of type 2 diabetes.

Diabetes is linked with the incidence of well-characterized risk elements for coronary heart disease

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(CHD), including specific defects of plasma lipids and lipoproteins concentration (Prakash, 2012). DM causes disorders of lipid profile, particularly a higher exposure to lipid peroxidation, which is liable to higher prevalence of atherosclerosis (Mietus-Snyder *et al.,* 2012), a major problem of DM. Higher oxidative stress and deficiency in antioxidant capacity, detected in both clinical and experimental DM, are believed to be the etiology of chronic diabetic complications (Shaw *et al.,* 2010). Consequence of oxidative stress in the pathogenesis of diabetes is proposed due to non-enzymatic protein glycosylation, auto-oxidation of glucose, reduced glutathione metabolism, modification in antioxidant enzymes, lipid peroxidation, reduced ascorbic acid levels and enhanced level of plasma homocysteine (tHcy) (Huang *et al.,* 2010). Hcy concentrations in population have been associated completely with blood pressure, uric acid, creatinine, and/or triglyceride (Mietus-Snyder *et al.,* 2012). Type1 diabetic patients are probable to have standard (Ndrepepa *et al.,* 2008) or relatively low Hcy (Noll *et al.,* 2012). Concentration of Hcy has been shown to link with the presence of diabetic peripheral neuropathy and autonomic neuropathy in patients with type1 diabetes (Shaikh *et al* 2012). Increased quantities of oxidative impairment to DNA, lipids and proteins have been identified in the blood of diabetic patients and their incidence is reliant on the development of diabetes complications (Lodovici *et al.,* 2010).

Many studies have indicated various demographic aspects, biochemical parameters in various ages, gender and in ethnic groups while stating intriguing observations between these groups. Therefore, a comprehensive study was designed to elaborate changes in metabolism with diabetes in young and old individuals with gender in general population of Faisalabad, Pakistan.

MATERIALS AND METHODS

In this prospective study, 160 adolescents and old subjects were selected. All cases and controls were enrolled in conversant permission. The following study was approved by The University Ethical Committee and the experiment was conducted in accordance with the Declaration of Helsinki. The qualified subjects were separated into female and male groups and further divided into four groups for young and old categories (young male controls, young male patients; young female controls, and young female patients; old male controls, old male patients and old female controls and old female patients). A total of one hundred and sixty (160) blood samples were collected between 06-09 h in the morning in a cold test tube from normal and diabetic patients. Tubes were centrifuged at 769 × g for 15 minutes. Serum was isolated and kept in to a small aliquot at -20°C till investigation. Another sample with anticoagulant using heparin (1 %) was also taken for glycated haemoglobin (HbA_{1C}) and proteins, packed cell volume, erythrocyte sedimentation rate and haemoglobin.

Complete physical analysis of every subject i.e. controls and patients were done by one of the attending physicians in Allied Hospital, Faisalabad, Pakistan. Following factors were documented:
1) Body mass index (BMI; kg/m^2) was measured with following formula: BMI = weight (kg)/height (m) ^{2}, 2) Body temperature (°F), with the help of thermometer, 3) Blood glucose, 4) Diabetes management whether requiring medical or dietary therapy, 5) LDL-cholesterol, 6) Hypertension was taken into account by a systolic blood pressure ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg or anti-hypertensive medication, 7) HDL-cholesterol, and 8) Triglycerides were measured in the diagnostic laboratory using commercial test kits.

Design

Subjects were considered to be diabetic if the fasting blood glucose level was 7.77 mmol/L (140 mg/dl) or more. Subjects with cholesterol level equal or higher than 5.69 mmol/L (220 mg/dl) were considered as hyperlipidemics. BP (cutoff values for systolic and diasystolic BP:≥ 130 mm Hg and ≥ 90 mm Hg, respectively) of controls and patients was measured using manual sphygmomanometers after 10 min of rest in a quiet room. Cut-off values of all risk factors were taken from published clinical values.

All recruited subjects were asked to complete a questionnaire, having standard demographic data (table 1) with questions on their immunization status, fever, history, medication and nutritional status. Diabetic patients and healthy subjects were not on any medication just having routine diet. Efforts were made to match the patients and controls for any possible confounding variables, such as age, gender, and socioeconomic condition. Study subjects were from urban area and were not exposed to any pesticide during the study.

Analytical methods

Serum glucose test was performed by using commercially available kit (Biocon, Germany Lot # H 265) using a spectrophotometer. Cholesterol was determined using kit of Randox Laboratories limited, United Kingdom (Cat. No. HN 1530). Commercially available test kits were used to determine Triglycerides (Cat. No. HN 1530; Randox Laboratories Ltd. Ardmore, Diamond Road, United Kingdom). HDL-cholesterol was measured by using commercially available kit (Cat No. D1H20-400, 34400 Okmeydani – Istanbul, Turkey).

Albumin, globulin and serum proteins were also determined by using commercially available kit (Bio Rays). Plasma homocysteine (Hcy) was calculated by homocysteine microtiter plate assay using enzyme–based kit (Diazyme). Creatinine was analyzed by using

Biosystem BTS 330 kit. Concentration of urea was measured by using commercially available kit (Cat No. UR 222; Randox Laboratories Ltd. Ardmore, Diamond Road, United Kingdom). Serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined following the Reitman and Frankel colorimetric method using a Randox kit (Cat No. AL 146).

Martin *et al.* (2000) technique was used to asses DNA damage with the help of comet assay. Cells with no DNA damage had an intact nucleus without a tail, while cells with DNA damage had a comet-like look. Ocular micrometer (μm) was used to measure the length of DNA migration in the comet tail and was considered as an estimate of DNA damage. The comet images were classified usually into five categories starting from zero (0) indicating undamaged and 4 as completely damaged cells (Zhao *et al*., 2006).

Malondialdehyde (MDA) levels were calculated by using thiobarbituric acid reactive species using spectrophotometer at 532 nm (Najafi *et al.,* 2012). Packed cell volume (PCV) was determined by the method described by Benjamin (Benjamin, 1978). Erythrocyte sedimentation rate (ESR) was measured by Westergen method as described by (Benjamin 1978). Haemoglobin concentration was determined by cyanmethemoglobin method. The absorbance was measured on a spectrophotometer (Hitachi U-2001) at 540 nm (Benjamin, 1978). Glycated Hb (HbA_{1C}; %) was measured using the glycosal® test which uses boronate affinity chromatography to separate the glycated haemoglobin fraction from the non-glycated fraction. Calibration of instruments was carried out by the reagents supplied by their manufacturers.

Statistical and sensitivity analyses

Collected data were subjected to calculations of Mean ± SD. Differences between healthy and diabetics (young male and female; old male and female subjects) were analyzed by two way analysis of variance (Steel *et al.,* 1997). To assess the relationship between blood characteristics and DNA damage or homocysteine profile (μM/L), comparison of the correlation coefficients provided a viable estimate of the strength of the association between different anthropometric data and biochemical profiles with DNA damage or homocysteine content. All data were tested by two-tailed t test, and *P* values ≤ 0.05 were considered statistically significant.

RESULTS

In male and female, body weight and BMI were significantly higher ($P \leq 0.01$) in diabetic subjects as compared with healthy ones (16.75-22.98 kg/m^2) and was an independent variable in both females and males, young and old (table 1). Overall mean systolic blood pressure (SBP) was significantly ($P \leq 0.001$) higher in female diabetic patients, followed by old diabetic males and diabetic young males and females. Similarly diastolic blood pressure (DBP) was significantly ($P \leq 0.001$) higher in diabetic patients followed by their respective normal subjects. This parameter was the highest in old male diabetic patients, followed by female diabetic patients.

In the present study, PCV and haemoglobin (Hb) reduced significantly ($P \leq 0.001$) higher in diabetic patients as compared to that in normal healthy individuals (table 2). In response to diabetes, erythrocytes sedimentation rate and glycated Hb (HbA_1c;%) were significantly ($P \leq 0.001$) higher in diabetic patients (the highest being in female diabetic patients followed by male diabetic patients). Among young diabetic patients profile of HbA_1c in both male and female diabetic patients was the same. Similarly, glycated HbA_1C was significantly ($P \leq 0.001$) higher in diabetic patients. While the highest glycated concentrations were noted in both old female and male diabetics followed by young diabetic males and females respectively.

Serum glucose concentration was significantly higher in old female diabetic cases ($P \leq 0.01$) as compared to their old and young females and male control counterparts respectively (table 3). Mean serum cholesterol and triglyceride levels were significantly elevated ($P \leq 0.01$) in old female and male diabetic patients than those in young female and male diabetic cases as compared to normal individuals (table 2). HDL-cholesterol was considerably higher in both old and young normal males, followed by normal females and diabetic ($P \leq 0.01$) cases (old group). They were followed by normal male and female (young group). Young diabetic group (both male and female) had significantly ($P \leq 0.01$) lower HDL-C with respect to this variable. On the other hand, LDL-cholesterol was significantly higher ($P \leq 0.01$) in old diabetic female and males, and diabetic young females followed by normal individuals.

The total serum protein concentration were higher in young diabetic male cases, followed by old male diabetic patients. Young normal females and diabetic patients and old diabetic females had statistically same content of total protein. Serum albumin was significantly higher ($P \leq 0.001$) in diabetic old female and male patients followed by young diabetic patients. All normal subjects had significantly lower ($P \leq 0.0001$) content of albumin. Globulin was significantly higher ($P \leq 0.001$) in normal young females followed by young diabetic females, young normal males, young diabetic females, old diabetic males, normal old females and diabetic old females.

Creatinine was significantly higher ($P \leq 0.001$) in old female diabetic patients followed by old male diabetic patients, young male diabetic and young female diabetic patients, normal young and old males. Almost similar

Table 1: Anthropometric parameters of normal and diabetic patients with gender and age

Parameters	Young				Old			
	Male		Female		Male		Female	
	Normal n = 20	Diabetic n = 20	Normal n = 20	Diabetic n = 20	Normal n =20	Diabetic n = 20	Normal n = 20	Diabetic n = 20
Weight (kg)	70.17 ±1.14[d]	72.00 ±0.73[c]	47.67 ±1.05[g]	57.50 ±1.26[f]	78.50 ±1.09[b]	82.50 ±1.09[a]	56.67 ±0.96[f]	59.83 ±1.56[e]
Body Mass Index (BMI; kg/m^2)	22.33 ±0.92[f]	27.75 ±0.51[a]	21.83 ±0.53[f]	24.15 ±0.65[e]	24.83 ±0.53[d]	26.62 ±0.18[b]	22.50 ±0.62[f]	25.53 ±0.45[c]
Systolic Blood Pressure (SBP; mm/Hg)	127.83 ±2.67[d]	134.33 ±2.01[c]	127.67 ±1.47[d]	134.17 ±2.57[c]	136.83 ±3.97[b]	138.17 ±3.13[b]	128.50 ±2.11[d]	145.17 ±2.30[a]
Diastolic Blood Pressure (DBP; mm/Hg)	82.83 ±0.70[d]	88.83 ±0.30[c]	79.33 ±1.40[e]	89.17 ±0.83[c]	87.17 ±0.60[c]	97.67 ±0.71[a]	83.00 ±0.81[d]	94.00 ±0.85[b]

Table 2: Haematological profiles of normal and diabetic patients with age and gender

Parameters	Young				Old			
	Male		Female		Male		Female	
	Normal n=20	Diabetic n=20	Normal n=20	Diabetic n=20	Normal n=20	Diabetic n=20	Normal n=20	Diabetic n=20
Packed Cell Volume (%)	38.33 ±0.98[a]	36.00 ±0.81[b]	38.67 ±0.67[a]	37.17 ±0.70[a]	37.83 ±0.40[a]	35.50 ±0.56[b]	38.00 ±0.51[a]	34.00 ±0.51[b]
Erythrocyte Sedimentation Rate (ESR; mm/hr)	2.50 ±0.50[g]	6.62 ±0.46[c]	1.50 ±0.19[h]	5.75 ±0.56[d]	4.00 ±0.32[f]	23.75 ±0.72[a]	4.87 ±0.22[e]	22.87 ±0.67[b]
Haemoglobin (mg/dL)	12.31 ±0.23[a]	11.25 ±0.08[d]	12.30 ±0.09[a]	11.00 ±0.09[e]	12.10 ±0.17[b]	10.03 ±0.18[f]	11.89 ±0.19[c]	9.95 ±0.19[f]
Glycated Hb. (HbA_{IC}; %)	4.05 ±0.27[f]	7.96 ±0.29[c]	3.46 ±0.43[g]	7.95 ±0.22[c]	5.40 ±0.22[d]	9.30 ±0.18[b]	5.08 ±0.09[e]	9.83 ±0.19[a]
Glycated Serum Protein (%)	6.11 ±0.16[e]	7.71 ±0.12[c]	5.68 ±0.20[f]	7.05 ±0.08[d]	4.91 ±0.24[g]	9.06 ±0.14[a]	5.08 ±0.16[f]	8.46 ±0.28[b]

Data are shown as means ± SD. *P*-values were 0.01 to 0.0001. Similar alphabets in a row do not differ significantly at $P \geq 0.05$

trend was observed in the case of serum urea content. It was significantly higher ($P \leq 0.001$) in old female diabetic patients, followed by old male patients, diabetic young females and males; and the least was recorded for normal old females and males.

In the present study, ALT was significantly higher in old female diabetics, followed by old male diabetic, diabetic young females (table 3), and normal old males. AST in serum was significantly ($P \leq 0.01$) higher in old male and female diabetic individuals. In each category of normal and diabetic individuals, diabetics carried significantly ($P \leq 0.0001$) higher load of AST. Serum homocysteine was significantly higher in diabetic patients as compared to that in healthy individuals. DNA damage in diabetic patients was also significantly higher ($P \leq 0.0001$) in male and female patients with age (table 4; fig. 1).

The correlation analyses between DNA damage or Hcy and all studied parameters in diabetic and normal subjects have been given in tables 1 and 3. DNA damage in diabetic patients was strongly dependent ($P \leq 0.0001$to 0.02) on all studied parameters (table 4) except PCV and hemoglobin where it was negatively correlated. Serum homocysteine was correlated positively with all studied parameters (table 5) in diabetic patients ($P \leq 0.0001$ to 0. 006), except BMI and diastolic blood pressure. In control subjects, Hcy was highly dependent on BMI ($P \leq 0.01$), SBP ($P \leq 0.041$) and DBP ($P \leq 0.029$) but was not dependent on any other parameters.

DISCUSSION

Every population has its own attributes like gene flow, gene frequency etc. A quarter of the population of Pakistan would be classified as overweight or obese with the use of Indo-Asian specific BMI cutoff values (Faisal *et al.,* 2010). Body weight as well as BMI were significantly higher ($P \leq 0.001$) in diabetic as compared to normal healthy individuals. Likewise, systolic as well as diastolic blood pressure was higher in old, male and diabetic individuals. Being overweight or obese was independently associated with having hypertension, diabetic and raised serum cholesterol concentration (Ramachandran *et al.,* 2010). Reports from Asian countries supported the use of cutoff value of BMI of 23 kg/m^2 or even lower for identification of subjects with hypertension and diabetes in Indo-Asian populations (Shaw *et al.,* 2010; Grill, 2006; Hegab *et al.,* 2012).

Table 3: Serum biochemical parameters of normal and diabetic patients with age and gender

Parameters	Young				Old			
	Male		Female		Male		Female	
	Normal n = 20	Diabetic n = 20	Normal n = 20	Diabetic n = 20	Normal n = 20	Diabetic n = 20	Normal n = 20	Diabetic n = 20
Glucose (mmol/L)	7.05 ±0.08^{d}	7.80 ±0.27^{c}	6.65 ±0.13^{e}	8.31 ±0.16^{b}	7.41 ±0.11c	10.35 ±0.15^{a}	7.45 ±0.09^{c}	8.33 ±1.41^{b}
Cholesterol (mmol/L)	5.25 ±0.12^{f}	6.38 ±0.09^{d}	5.35 ±0.11	6.77 ±0.09^{b}	5.57 ±0.08^{d}	7.50 ±0.08^{a}	6.65 ±0.08^{c}	7.53 ±0.07^{a}
Triglyceride (mmol/L)	1.13 ±0.08^{g}	2.05 ±0.08^{d}	1.33 ±0.08^{f}	2.40 ±0.08^{c}	1.78 ±0.04^{e}	3.18 ±0.11^{b}	2.03 ±0.04^{d}	3.38 ±0.14^{a}
HDL-Cholesterol (mmol/L)	3.98 ±0.05^{b}	3.45 ±0.04^{d}	3.67 ±0.03^{c}	3.08 ±0.07^{e}	4.41 ±0.11^{a}	3.46 ±0.19^{d}	3.89 ±0.07^{b}	3.55 ±0.13^{d}
LDL-Cholesterol (mmol/L)	3.00 ±0.05^{f}	3.95 ±0.04^{d}	3.00 ±0.03^{f}	4.09 ±0.07^{c}	3.21 ±0.12^{e}	4.47 ±0.20^{b}	3.30 ±0.07^{e}	4.59 ±0.14^{a}
Total Proteins (g/dL)	7.35 ±0.11^{d}	7.77 ±0.09^{a}	7.45 ±0.10^{c}	7.55 ±0.15^{c}	6.73 ±0.04^{e}	7.67 ±0.08^{b}	6.61 ±0.07^{f}	7.53 ±0.05^{e}
Albumin (g/dL)	3.60 ±0.03^{d}	3.91 ±0.06^{c}	3.28 ±0.07^{e}	3.86 ±0.06^{c}	3.60 ±0.04^{d}	4.18 ±0.08^{b}	3.68 ±0.06^{d}	4.33 ±0.11^{a}
Globulin (g/dL)	3.75 ±0.09^{c}	3.85 ±0.08^{b}	4.17 ±0.16^{a}	3.68 ±0.19^{d}	3.13 ±0.07^{e}	3.48 ±0.09^{d}	2.93 ±0.09$^{`f}$	3.20 ±0.12^{e}
Creatinine (mmol/L)	79.17 ±1.44^{e}	93.50 ±0.89^{c}	75.00 ±1.46^{f}	86.67 ±1.02^{d}	73.33 ±1.02^{f}	126.33 ±1.47^{b}	76.33 ±0.91^{f}	143.83 ±2.22^{a}
Urea (mg/dL)	187.83 ±4.35^{e}	353.67 ±1.94^{c}	196.50 ±3.06^{e}	349.83 ±4.19^{c}	246.00 ±2.95d	907.50 ±25.09^{b}	249.00 ±2.69^{d}	942.50 ±17.30^{a}
Alanine Aminotransferase (ALT; U/L)	15.00 ±0.81^{g}	26.83 ±0.60^{e}	18.67 ±0.67^{f}	29.50 ±0.61^{d}	32.83 ±2.30^{c}	52.50 ±1.17^{b}	27.17 ±0.70^{e}	56.33 ±1.20^{a}
Aspartate minotransferase (AST; U/L)	9.83 ±0.54^{f}	13.33 ±1.02^{d}	8.67 ±0.61^{g}	12.33 ±0.61^{e}	14.00 ±1.23^{d}	33.83 ±1.32^{a}	15.17 ±0.83^{c}	27.33 ±0.61^{b}
Homocysteine (μmol/L)	3.68 ±0.29^{e}	8.85 ±0.67^{d}	2.84 ±0.14^{g}	10.91 ±0.51^{c}	5.56 ±0.51^{f}	21.70 ±0.68^{a}	3.58 ±0.13^{e}	20.17± 1.0^{a}
DNA damage (μm)	5.6± 0.5^{g}	13.8 ±1.1^{c}	7.8± 0.5^{d}	14.18± 0.5^{c}	6.64± 1.2^{f}	14.9±0.6^{b}	6.9± 0.4^{e}	20.8± 1.5^{a}

Data are shown as means ± SD. *P*-values were 0.01 to 0.0001. Means followed by similar alphabets in a row do not differ significantly at $P \geq 0.05$

In the present study, packed cell volume (PCV) and haemoglobin (Hb) were significantly lower ($P \leq 0.001$) in diabetic patients as compared to those in normal healthy individuals. In response to diabetes, erythrocytes sedimentation rate was higher in diabetic patients and this increase was observed in young and old individuals irrespective of their group. Altering the diet composition could be empowering method of improving the hyperglycemia of type 2 diabetes without weight loss (Gannon and Nuttal, 2006; Nebeck *et al.,* 2012). Particularly, it has been indicated that metabolic response to carbohydrate diet depends upon the type of carbohydrate and digestible starches which are 100 percent glucose, clearly increase the glucose as well as insulin concentration.

Serum cholesterol, triglyceride, HDL and LDL were significantly higher in diabetic patients, however, cholesterol and triglyceride were significantly higher ($P \leq 0.0001$) in female as compared to male, young and old diabetic patients. The combination of abnormalities, e.g., higher triglyceride and cholesterol, low level of high density lipoprotein, and relatively normal level of LDL in patients has been called the diabetic dyslipidemia (Shaw, 2010). High triglyceride, low HDL-cholesterol and hyperinsulinemia are known as traditional risk factors of DM and explain occurrence of excessive cardiovascular mortality (Shaw, 2010) in different cross-sectional studies [Faisal *et al.,* 2010; Ginsberg *et al.,* 2005).

Chronic hyperglycemia in diabetic patients leads to non-enzymatic glycation of proteins and results in a decrease of lecithin: cholesterol acyltransferase activity and therefore HDL particles may be submitted to complex alteration including compositional modifications [Low *et al.,* 2012; Niranjan *et al.,* 201). Diabetic dyslipidemia includes an overall increase in atherogenic particles and predominance of small dense LDL particles, in addition to easily measurable predictors of LDL size such as triglycerides or LDL-cholesterol and triglyceride to HDL-cholesterol ratio (Hegab *et al.,* 2012; Wagner *et al.,* 2005). Triglyceride to HDL-cholesterol ratio is not superior to non HDL-cholesterol in classifying patients with type 2 diabetes into dyslipidemic phenotype (Gannon and Nuttal, 2006).

In the present study, total proteins, albumin and globulin concentration were found higher with age in diabetic patients. Overall serum total proteins level was higher ($P \leq 0.001$) in young and old diabetic patients as compared to that in normal individuals. Our findings are similar to those of Hegab *et al*. (2012) who also reported a significant increase in total serum protein and serum albumin in diabetic patients with or without cardiovascular complications.

Table 4: Correlation coefficients of anthropometric, haematological profile and biochemical characteristics with DNA damage of lymphocytes of normal and diabetic individuals.

Parameters	Normal DNA (%)	Diabetic DNA (%)
Packed Cell Volume (%)	-	-0.908(0.002)
Erythrocyte Sedimentation Rate (ESR; mm/h)	-	0.836(0.010)
Haemoglobin (mg/dL)	-	-0.970(0.0001)
Glycated Hb (HbA_{IC}; %)	-	0964(0.0001)
Glycated Serum Protein (%)	-	0.874(0.005)
Cholesterol (mmol/L)	-	0.826(0.011)
Triglyceride (mmol/L)	-	0.940(0.001)
Albumin (g/dL)	-	0.928(0.001)
Creatinine (mmol/L)	-	0.862(0.006)
Urea (mg/dL)	-	0.856(0.007)
Alanine Aminotransferase (ALT; U/L)	-	0.789(0.020)

Values in parenthesis are *P*-value for significance

Both glycated hemoglobin and proteins in the present study were also higher ($P \leq 0.001$) with age of diabetic individuals as compared to their control subjects. The advanced glycation end products (AGEs) damage target cells by modification of intra-cellular proteins, extracellular matrix components, their abnormal interaction with other matrix components and with receptors of matrix proteins on cells (Verma *et al.,* 2006; Seino *et al.,* 2008; Emily *et al.,* 2012). They also modify plasma proteins by binding to AGE receptors on endothelial cells, and macrophages including receptor mediated production of reactive oxygen species (Alipour *et al.,* 2012; Dutt *et al.,* 2012). Seino *et al.* (2008) examined the glycaemic control in type 2 diabetes and determined that glycated haemoglobin was correlated positively with age, disease duration and negatively with BMI. Any reduction in HbA_{1C} would reduce the risk of complications.

In the present study, diabetic young and old, male and female patients showed significant higher ($P \leq 0.001$) levels of ALT and AST as compared to those in the normal healthy individuals. Serum ALT but not AST was strongly associated with the number of metabolic syndrome i.e. fasting glucose, body mass index and fasting triglycerides (Hanley *et al.,* 2006). Jacobs *et al.,* (2011) suggested that ALT could be incorporated into risk prediction algorithms to identify patients who are likely to develop type 2 DM. Similarly Zhang *et al.,* (2010) suggested a relationship between α-glutamyltransferase (GGT) and ALT, the metabolic syndrome and insulin resistance to serve as a biomarker. Moreover Hanley *et al.,* (2006) suggested that hepatic inflammation may be another possible mechanism by which hepatic enzyme levels are related to diabetic risk. According to Kim *et al.,* (2012) the elevated levels of ALT and GGT within normal range are independent predictors of type 2 diabetes in old men. There have also been a number of studies related to AST and/or ALT and diabetes risk. ALT and AST were independently associated with diabetes in a cohort of male Korean worker (Davies *et al.,* 2001).

In the present study, old female showed a significantly higher ($P \leq 0.001$) damage to their DNA. Also, young and old diabetic patients had damaged DNA significantly higher than that in their control subjects. As DM is not only a complex disease but associated with many other complications including obesity, retinopathy, nephropathy and atherosclerosis (Kaplan and Michael 2012). Therefore, it could be speculated that significant damage to DNA of diabetic patients particularly in old age might have added effect on DNA-damage. It has been reported that several different ROS including hydroxyl radicals, singlet oxygen, peroxyl radicals and perooxynitrite damage DNA in diabetic patients release 8-hydroxy-2-deoxyguanosine (8-HdG) which can be measured in blood and urine of patients (Davies *et al.,* 2001).

The levels of homocysteine (tHcy) are different in different populations that also depends upon their life style, environmental factors and genetic polymorphism of methylenetetrahydrofolate reductase (MTHFR) (Henry *et al*., 2012). tHcy in South London appearances diverse traditional background and genetic polymorphism of MTHFR. tHcy Levels are considerably higher in Bangladesh, Iran, Nepal and Indian populations whites (Akpalu and Nyame 2009). Vegetarian populations have higher levels of tHcy as compared to non-vegetarian population (Nienaber-Rousseau *et al.,* 2013). In contrast, Pakistan had similar tHcy levels to whites while both West Africans and Caribbeans had slightly lower levels (Vimal *et al*., 2013). tHcy levels also depends upon intake of vitamin B, protein, and methionine, some lifestyle factors such as, smoking status, alcohol use, and caffeine use, and other factors like BMI, blood pressure, and anti-hypertensive medication (Van-Guelpen *et al*., 2009).

Yet again, serum homocysteine concentration was significantly higher with age and in diabetic young and old as compared to normal healthy individuals. High levels of homocysteine are believed to promote the formation of oxidation products such as homocysteine

Table 5: Correlation coefficients of anthropometric, haematological profile and biochemical characteristics with homocysteine of normal and diabetic individuals

Parameters	Normal homocysteine (μmol/L)	Diabetic homocysteine (μmol/L)
Body Mass Index (BMI; kg/m^2)	0.990(0.010)	-
Systolic Blood Pressure (SBP; mm/Hg)	0.959(0.041)	0.741(0.035)
Diastolic Blood Pressure (DBP; mm/Hg)	0.971(0.029)	-
Packed Cell Volume (%)	-	-0.913(0.002)
Erythrocyte Sedimentation Rate (ESR; mm/h)	-	0.966(0.000)
Haemoglobin (mg/dL)	-	-0.905(0.002)
Glycated Hb. (HbA$_{IC}$; %)	-	0.926(0.001)
Glycated Serum Protein (%)	-	0.907(0.002)
Glucose (mmol/L)	-	0.804(0.016)
Cholesterol (mmol/L)	-	0.935(0.001)
Triglyceride (mmol/L)	-	0.943(0.0001)
Albumin (g/dL)	-	0.926(0.001)
Creatinine (mmol/L)	-	0.949(0.0001)
Urea (mg/dL)	-	0.976(0.0001)
Alanine Aminotransferase (ALT; U/L)	-	0.918(0.001)
Aspartate Aminotransferase (AST; U/L)	-	0.806(0.016)

Values in parenthesis are p-value for significance

thiolactone and homocysteine disulfides, which have the capacity to damage endothelial cell by excessive sulfation of collagen leading to thrombosis and artheriosclerosis (Geberhiwot *et al.,* 2005). Homocysteine levels are closely related with formation of creatinine. High level of homocysteine in patients can be observed in impaired renal function or in patients with end stage renal diseases (Kaplan and Michael 2012).

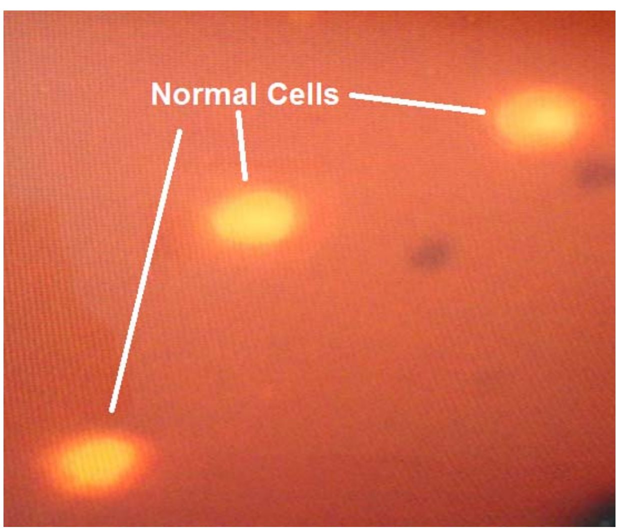

Fig. 1: Shows the zero level comet assay with no tail (Healthy).

DNA damage (table 4) was positively correlated with ESR, glycated Hb, glycated serum protein, cholesterol, triglycerides, albumin, creatinine, urea, and ALT in diabetic individuals. In normal participants, it was not related to any risk factor of diabetes. This study provide a positive correlation of homocysteine with SBP, ESR, glycated Hb, glycosylated serum protein, cholesterol, triglycerides, albumin, urea, ALT and AST in diabetic individuals and confirmed the work of others (Davies *et al.,* 2001). Correlation coefficient of Hyc and DNA damage indicated that both of them were negatively associated with globulin.

Seino *et al.,* (2008) found that hemoglobin concentrations and WBC counts were associated with other components of the insulin resistance syndrome such as body mass index, blood pressure, lipid profiles and fasting plasma insulin levels (surrogate for insulin resistance). For such studies an oral tolerance test for the estimation of glucose, insulin and HbA$_{1C}$ is essential (Dutt *et al*., 2012). Packed cell volume and haemogobin concentration was negatively correlated with homocysteine and DNA damage in diabetic patients (table 4, table 5). Increase in homocysteine and DNA damage may lead to hyperglyceremia, hyperlipidimia, and hyper liver enzyme levels to develop diabetes and needs intervention for their control to decrease disease burden in our population. With regards to nutrition, the average consumption of proteins, fats, and carbohydrates by controls is lower than that in the diabetic's diet (Martin *et al.,* 2000) and same level may be suggested for diabetic individuals.

CONCLUSION

More comprehensive study for understanding the relationships between ROS in diabetic patients to cause cell damage may greatly improve our ability to develop interventions in decreasing oxidative stress in diabetics individuals (Ramachandran *et al.,* 2010). Nutritional strategies to improve total antioxidant response (TAR) may be effective in lowering risk of the toxic effect of ROS. Increase in homocysteine and DNA damage may lead to hyperglyceremia, hyperlipidemia, and hyper liver enzyme levels. We need to eradicate these adverse effects in our population in order to have healthy nation.

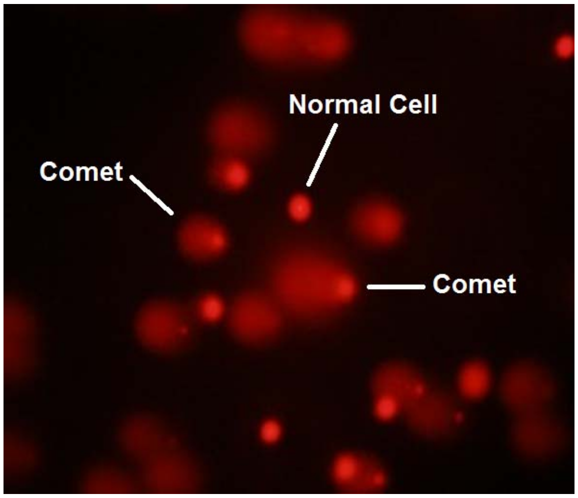

Fig. 2: Comet assay show normal cell (with no tail) and a comet (having tail), which show DNA damage. Tail of comet clearly illustrates the DNA damage. (Healthy and damaged leukocytes in a diabetics patient)

ACKNOWLEDGEMENTS

These studies were supported by Higher Education Commission, Pakistan; Punjab Government and University of Agriculture, Faisalabad. Ethical Committee is thanked for permission to carry out this work. Lab staff and Departmental statistician are thanked for their assistance.

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