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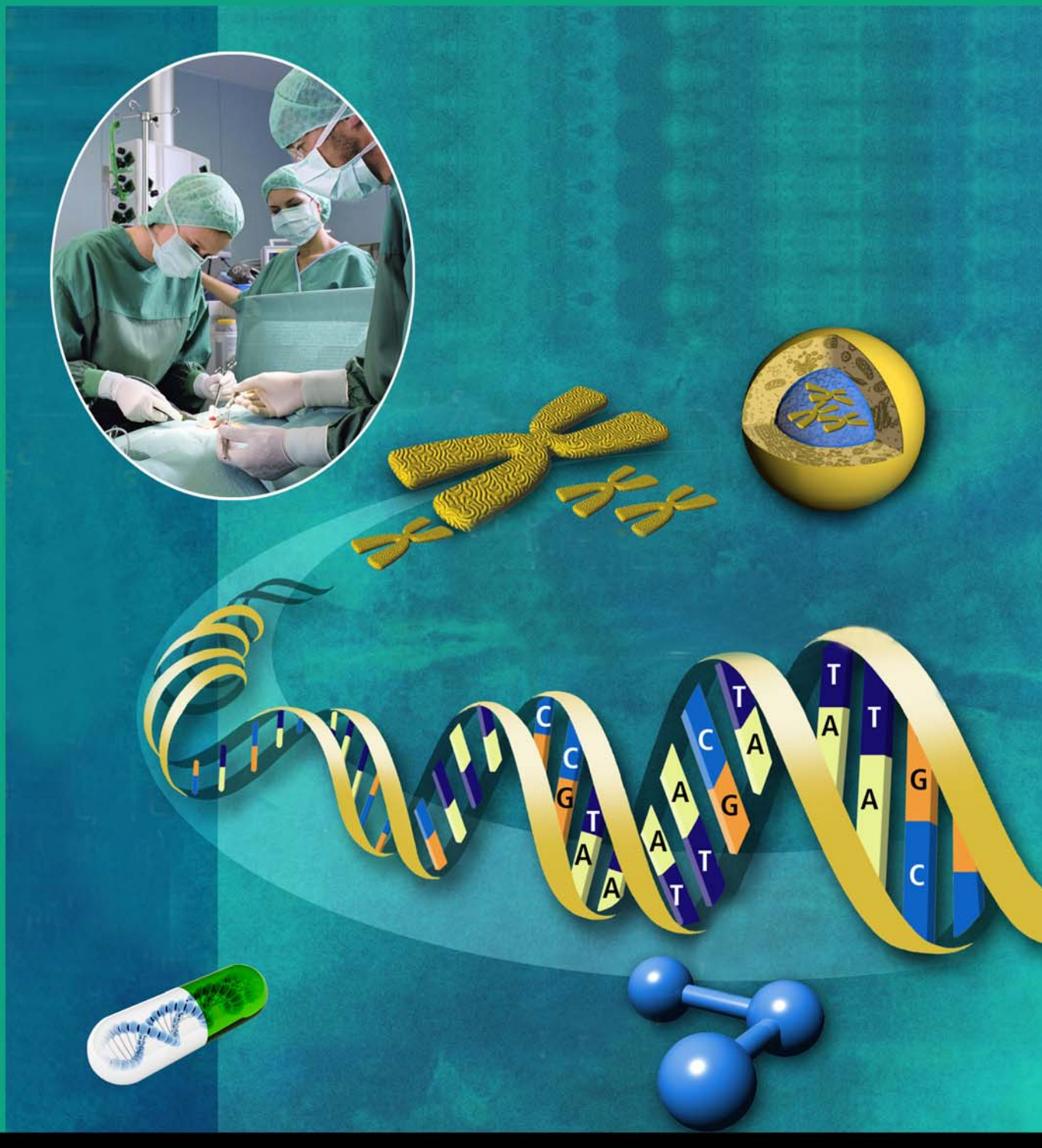

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Research Paper

DNA DAMAGE AND OBESITY IN DIABETIC PATIENTS

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Objective: Increased oxidative stress in accumulated fat is an important pathogenic mechanism for obesity-associated metabolic syndrome. The increased free radicals can cause cellular damage and contribute to the pathogenesis of diabetes. Method: The alkaline single cell gel electrophoresis assay was used to determine DNA damage in peripheral blood leukocytes of 35 subjects with diabetes and 18 age- and sex-matched controls. The patient group differed from control subjects for general (BMI) and central adiposity (waist hip ratio, waist circumference). Diabetic patients included those on treatment (n=18) and yet to start treatment (n=17). Results: DNA damage in both, male and female patients was statistically higher (p=0.000) compared to that in respective controls. There were no gender differences however. Though DNA damage was higher in untreated patients, yet was not significantly different from treated patients. Pearson correlation analysis revealed significant association of waist circumference (central adiposity) with damage index(r=0.331,p=0.052). Conclusion:As an increase in DNA damage is an initial step in carcinogenesis and if unrepaired can lead to cancer and cause age-related disorders, the patients in the present study may be also similary susceptible and require its management.

Keywords: Body mass index, Comet assay, DNA migration length

INTRODUCTION

The global prevalence of diabetes in 2010 was nearly 300 million adults with a projected prevalence in 2030 of nearly 440 million because of the obesity epidemic and aging population (Business Wire, 2011). Among the diagnosed diabetic cases, type 2 diabetes affects 95% (Andreassi *et al*., 2011). The phenomenal increase in even developing countries is a cause for concern in view of the increased morbidity and mortality. Among its complications are microvascular and macrovascular diseases and a considerable risk of several types of cancers including those of the pancreas, liver, breast, colorectal, urinary tract and female reproductive organs (Vigneri *et al*., 2009); also over 80% of type 2 diabetic patients are obese. In fact, both diabetes mellitus (DM) and obesity are characterized by hyperinsulinemia and higher cancer incidence. Obesity, hyperglycemia and increased oxidative stress may also contribute to increased cancer risk in diabetes. There is an

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increase in reactive oxygen species in diabetes; rather its onset is also associated with oxidative stress (Baerlocher and Lansdorp, 2003; Fenton *et al*., 2001). The simultaneous increased generation of free radicals and decline of antioxidant defense systems in the diabetic condition can cause damage to cellular organelles and macromolecules including nucleic acids. Prevention by early detection can assist in prognosis and in the reduction of progression of complications in DM. The alkaline version of the Single Cell Gel Electrophoresis (SCGE) assay can be used to detect DNA damage caused by double strand breaks, single strand breaks and alkali-labile sites and so assist in disease management and forestall progression since DNA damage and DNA repair play a major role in carcinogenesis. The present study was hence undertaken to assess DNA damage in peripheral blood leukocytes (PBL) of individuals (n=53) compromising those who were diabetic (n=35) and compare to that in normal healthy controls (n = 18) using the Single Cell Gel Electrophoresis (SCGE/comet) assay (Singh *et al*., 1988).

MATERIALS AND METHODS

Subjects

Diabetic patients were contacted from local hospitals where they were undergoing or yet to start treatment and had been diagnosed by the attending physicians as type II diabetes mellitus cases. None of these individuals suffered from related co-morbidities viz. hypertension and/or cardiovascular disease. Sex-, age- matched healthy subjects from the general population who met the same inclusion criteria and were not on any medication or supplements comprised the control group.

Voluntary informed written consent was obtained and the study was cleared by the Institutional Ethics Committee. General and demographic information from each participant was recorded on a pre-designed questionnaire and specific queries pertaining to diabetes, dietary and life style preferences were also recorded. An assessment for obesity of each subject was made from anthropometric measurements viz. height, weight, waist circumference, hip circumference (HC), taken using standard methodology (Weiner and Lourie, 1981) so as to calculate the Body Mass Index (BMI) and Waist Hip Ratio (WHR). The WHO (2004) criterion on the basis of body mass index (similar to (Misra *et al*., 2009)) was followed for the classification of obesity (BMI $\geq$ 25.0kg/m^2). Abdominal obesity respective cut-offs for waist hip ratio (WHR) and waist circumference (WC) for females were 0.80 and 80 cm and 0.90 and 85cm in males (Snehalatha *et al*., 2003).

Blood Pressure Measurements

The systolic and diastolic pressure of each subject was noted with the help of a sphygmo-manometer. Blood pressure readings were noted thrice for each subject at an interval of ten minutes after the subject had rested and the mean value was recorded. All the subjects were normotensive (<140/90 mmHg).

The Alkaline Single Cell Gel Electro-phoresis (SCGE) Assay

Finger-prick blood samples (approximately 200µl) were collected in heparinized microtubes from each individual, brought to the laboratory on ice and processed within 3-4 h of collection. Before carrying out the SCGE assay to assess DNA damage, the number of viable and non-viable cells in an individual's blood sample was checked using

the cell-viability test. All samples with 80-90% viable cells were processed for the alkaline SCGE assay using a slightly modified version of the technique (Singh *et al*., 1988) in the use of locally available chemicals, pre-coated slides and silver staining (Nadin *et al*., 2001). The technique required the embedding of individual cells in a thin agarose gel on a microscope slide. The blood samples (30 μl in 0.8% low melting point agarose solution in PBS) were sandwiched between low melting point and normal melting point agarose layers. The cellular proteins were lysed (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, DMSO,Triton X-100; pH 10.0). The DNA was allowed to unwind under alkaline conditions (300 mM NaOH,1 mM EDTA Na_2H_2, pH $\geq$ 13) and subjected to electrophoresis (20 min, 0.7 V/cm, 300 mA) to enable any DNA fragments or damaged DNA to migrate away from the nucleus. After neutralization, the cells were stained with silver nitrate ($AgNO_3$) solution. The slides were coded and scored blindly, first under low magnification (10X) and then at 40X using a binocular microscope (Olympus No: ID00212, model: CH20BIMF 200). Two slides were made from each sample and 50 cells (25/slide) were scored per individual. A calibrated ocular micrometer fitted into the eyepiece of the microscope was used to measure extent of DNA damage. DNA migration length was calculated as the difference between length of the comet and radius of the comet head. The number of cells with tails (Damage Frequency; DF) was also recorded for each subject, categorized manually into class 0 (no tail) to class IV (almost all DNA in tail) with arbitrary scores assigned to each (from Type 0 = 0 to Type IV = 4) and the sum of products was used to calculate the Damage Index (DI) as arbitrary scores match image analysis for DNA percentage in tail (Collins, 2004).

Statistical Analysis

Mean DNA migration length in μm was calculated by taking the average of the measurements obtained for all the cells/sample using an ocular micrometer. DNA damage in both, patient and control groups, was then statistically analyzed using the Student's t-test since the data were observed to be parametric i.e. variables showed normal distribution. The Chi square (χ^2) test was performed to check the demographic parameters of normal and diseased individuals. Regression Analysis and Analysis of variance were conducted to check whether confounding factors had any effect on DNA damage and all these tests were performed using the SPSS package (version 16.0).Values were taken statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The study group (n=50) comprised 35 diabetic patients and 18 age-matched control individuals. Demographic information and anthropometric measurements of diabetic and control individuals are presented in Tables 1 and 2. Male patients were aged 24-79 y (mean 46.32 ± 1.70y) with BMI 25.20-36.80 kg/m^2 (mean 28.41 ± 0.54 kg/m^2) and WHR 0.87-1.10 (mean 0.97 ± 0.01). The males in the control group were between 22-57y (mean 39.25 ± 3.08y) with BMI 17.65-23.45 kg/m^2 (mean 20.98 ± 0.48 kg/m^2) and WHR 0.80-0.88 (mean 0.85 ± 0.00).Female patients were aged 24-59y (mean 42.20 ± 4.54y) with BMI 25.50- 37.60 kg/m^2 (mean 30.51±1.64 kg/m^2) and WHR 0.83-0.97 (mean 0.89±0.01). Females in the control group were in the age range of 22-40y (mean 28.83 ± 2.96 y), with mean BMI of 19.54±1.00 kg/m^2 and WHR of 0.83 ± 0.02 kg/m^2. The χ^2-test was performed on the attributes of male and female diabetic and control

Table 1: General and Demographic Information of the Study Group

Characteristics		Range	Patient Group	Control Group	χ^2	P-value
Age(y)		≤30	6	7	1.975	0.159
		>30	29	11		
Height(cm)		145-165	18	12	0.589	0.4428
		166-186	17	06		
Weight(kg)		40-70	09	18	23.359	0.0001
		71-101	26	-		
Hip Circumference (cm)		80-115	29	18	1.982	0.1592
		116-151	06	-		
Waist Circumference*(cm)	Males	<85	-	06	11.466	0.0007
		≥85	25	06		
	Females	<80	01	03	1.422	0.2330
		≥80	09	03		
Waist Hip Ratio**	Males	<0.9	02	12	25.395	0.0001
		≥0.9	23	-		
	Females	<0.8	-	01	0.071	0.7897
		≥0.8	10	05		
Body Mass Index*(kg/m^2)		<25.0	-	18	48.635	0.0001
		≥25.0	35	-		
Sex		Male	25	12	0.002	0.9667
		Female	10	06		
Obesity Onset		Childhood	22	-	1.829	0.1763
		Adult	13	-		
Diet Hist.		Veg	14	18	15.467	0.0001
		Non-Veg	21	-		
Mobile Phone usage		Yes	20	12	0.140	0.7078
		No	15	06		
Alcohol Drinking		Yes	10	-	4.610	0.0318
		No	25	18		
Socio-economic status		Middle	33	18	0.074	0.7850
		Lower	2	0		
Diabetic Patients		On treatment	18	0	0.000	1.0000
		No treatment	17	0		

Note: P-values in bold are significant (P ≤ 0.05); * classified according to WHO (2004); and Misra *et al.*, (2009); ** classified according to Snehalatha *et al.* (2003).

Table 2: Anthropometric Variables of Diabetic Patients and Healthy Subjects

Study Group		No.	WC (cm)	HC (cm)	WHR	BMI (kg/m^2)
Patients	Males	25	104.84***±1.55	107.36**±1.68	0.97***^{a}±0.01	28.41***±0.54
	P value	0.000	0.002	0.000	0.000	
	Females	10	103.3**±5.30	114.70*±5.06	0.89**^{a}±0.01	30.51***±1.64
	P value	0.004	0.018	0.012	0.000	
	Total	35	104.4***±1.83	109.46***±1.92	0.95***±0.01	29.01***±0.61
	P value	0.000	0.000	0.000	0.000	
Controls	Males	12	83.25±1.79	98.00±2.15	0.85±0.00	20.98±0.48
	Females	06	79.00±2.22	94.83±4.40	0.83±0.02	19.54±1.00
	Total	18	81.83±1.45	96.94±2.01	0.84±0.00	20.50±0.47

Note: ***Very highly($P \leq 0.001$), ** highly($P \leq 0.01$), * significant ($P \leq 0.05$) compared to parallel control group (Student's t-test);Values with similar letters are significant; WC-Waist Circumference; HC- Hip Circumference; BMI- Body Mass Index; WHR- Waist Hip Ratio.

groups and matched fully for demography, life style and habits and showed no significant differences for sex, age, dietary pattern and mobile phone usage (Table 1); however, alcohol drinking was absent among control males. Obesity-related data were significantly mismatched as expected. General obesity (BMI) was present in all patients but not in the controls while central adiposity (WHR, WC) was present in most patients and in some controls. Gender-based differences (Table 2) were observed with respect to hip circumference and WHR as these were higher in male compared to female patients (p=0.001). Overall, the patient group was significantly different from controls for obesity indices (WHR, WC and BMI) with the values higher among the diabetics. In fact in literature also, > 80% of type 2 diabetic patients have been observed to be obese (Vigneri *et al*., 2009). Diabetic patients included those on treatment (n=18) and yet to start treatment (n=17). The male patients were mostly doing small-scale business (n=16), half of the females were house wives, two were teachers, two were students and one had her own business. There was no exposure at work place or at home in both the patient and control groups.

Genetic damage in male and female patients was statistically higher (P=0.000) compared to respective controls and the difference between genders was almost similar for DF,DI and DNA migration length (Table 3). On analyzing genetic damage parameters with respect to treatment status (treated vs. untreated), DNA damage was higher in untreated patients but not significantly (Table 4). However, significantly elevated genetic damage in both, treated and untreated patients was observed in comparison to healthy controls (P=0.000).

The relationship of various confounding factors: age, height, weight, BMI,WC, WHR, diet, exercise, mobile phone usage (independent variables) and DNA damage (dependent variable)

Table 3: Genetic Damage in Diabetic Patients and Control Subjects

Group		No.	Damage Frequency (DF) ± SEM (P value)	Damage Index (DI) ± SEM (P value)	$Mean DNA migration length (μm) ± SEM (P value)
Diabetic Patients	Males	25	93.12***±0.98(0.000)	63.32***±3.11(0.000)	30.45***±2.01(0.000)
	Females	10	94.20***±2.15(0.000)	64.70***±5.77(0.000)	30.67***±4.16(0.020)
	Total	35	93.42***±2.15(0.000)	67.71***±2.72(0.000)	30.51***±1.83(0.000)
Controls	Males	12	18.16±1.67	18.16±1.67	14.45±1.56
	Females	06	20.00±2.47	20.00±2.47	15.37±2.76
	Total	18	18.77±1.36	18.77±1.36	14.76±1.35

Note: $ Calculated as an average of individual DNA migration lengths in the group. ***Very highly significant when compared to parallel control group ($P \leq 0.001$, Student's *t*-test).

Table 4: DNA Damage in Treated and Untreated Diabetic Patients

Group Drugs	No.	Cells with tails/ Total cells scored	Damage Frequency (DF) ± S.E.M.	Damage Index (DI) ± S.E.M.	$Mean DNA migration length(μm) ± S.E.M.
Treated# Patients	18	830/900 (P=0.000)	92.00***±1.46 (P=0.000)	58.88***±3.36 (P=0.000)	27.20***±2.26
Untreated Patients	17	805/850	94.94*** ±1.01 (P=0.000)	68.82***±4.07 (P=0.000)	34.02***±2.71 (P=0.000)
Control Subjects	18	169/900	18.77±1.36	18.77±1.36	14.76±1.35

Note: # Diabcure–M: herbal capsules; Dianorm-M: Gliclazide+metformin hydrochloride; Dional -5: Glibenclamide IP; Glycomet –85: Glimepiride+metformin; Glynase–MF: Glipizide + metformin; $ Calculated as an average of individual DNA migration lengths in the group; *** Very highly significant when compared to control group ($p \leq 0.001$, Student's t-test); not significant within patient groups.

was assessed by multiple regression analysis and multivariate ANOVA. In the patient group, Pearson correlation analysis revealed significant association of WC with DI (r=0.331, p=0.050). This parameter was also found significantly associated with genetic damage (DI) by multivariate ANOVA (F value=4.063, p=0.050) and multiple linear regression (r=0.331, p=0.050). In the control group, these confounders were not observed to be significantly associated with damage. An association analysis with type 2 diabetes as outcome and damage index as predictor in logistic regression model however, revealed no significant association (p=0.993) between these when adjusted for age, gender, BMI, diet and exercise.

The observations of the present study on increased DNA damage in diabetic patients find similarities in literature. Rather, the conditions of diabetes, general obesity (BMI) and central adiposity (WC, WHR) as well as increased weight have separately been documented to show an association with genetic damage. Oxidative DNA damage (8-oxo, 2'-deoxyguanosine levels) in urine and in blood mononuclear cells of Type 2 diabetic patients was significantly raised and increased with increase in oxidative stress (Hinokio *et al*., 1999). On comparing DNA strand breakage in blood samples of diabetic patients, the mean frequency of damaged cells and DNA

migration in insulin-dependent DM patients were lower than in non-insulin dependent DM patients and while Vitamin E supplementation lowered DNA migration length, smoking increased it (Sardas *et al*., 2001). Diabetics with poor glycaemic control and low ascorbic acid (Choi *et al*., 2004) had higher DNA damage than in patients with similar degree of hyperglycaemia and elevated level of ascorbic acid. Peripheral leukocytes, monocytes and T-cells of Type 2 diabetic subjects had significantly increased oxidative DNA damage and significantly decreased telomere length compared to the control group ((Adaikalkoteswari *et al*., 2005; and Sampson *et al*., 2006). The lymphocytic DNA from subjects with type 2 diabetes also had increased susceptibility to oxidative damage (Sampson *et al*., 2001; Andreassi *et al*., 2005; and Hannon-Fletcher *et al*., 2000).

Increased micronuclei frequency and DNA damage was observed with high BMI (Yesilada *et al*., 2006; Violante *et al*., 2003; and Demirbag *et al*., 2005) though controversial results were also documented (Giovannelli *et al*., 2002). On the other hand, urinary levels of 8-hydroxydeoxy-guanosine (oxidative DNA damage) were inversely correlated with BMI (Kasai *et al*., 2001; and Loft *et al*., 1992) and oxidative DNA damage was reported in overweight and obese subjects (Al-Aubaidy and Jelinek, 2011; and Elwakkad *et al*., 2011). Lowered levels of antioxidants and increased DNA damage were documented in obese subjects (Bukhari *et al*., 2010; and Wiegand *et al*., 2010). Chromosomal (Scarpato *et al*., 2011) as well as DNA damage (Tomasello *et al*., 2011) were recently reported to be significantly increased in obese and overweight children and in pre-obese and obese women, respectively. Smoking and being overweight in midlife (irrespective of glucose, cholesterol and blood pressure levels) were observed to be related to shorter leukocyte telomeres in old men (Strandberg *et al*., 2011). Increased central adiposity (as increased WC also observed in patients in the present study) reflects excessive or disproportionate gain of adipose tissue which causes dysfunction at various levels mediated via cellular inflammation from increased concentrations of interleukins, C-reactive proteins,adipocytokines and free fatty acids (Green *et al*., 1994; Hotamisligil *et al*., 1995; Kopelman, 2000; and Boden, 2006) generating free radicals leading to oxidative stress (Curti *et al*., 2011). In fact, the insulin-resistant adipose tissue is also similarly showing chronic inflammation, hypoxia, oxidative stress, etc. (Hotamisligil *et al*., 1995). The association of DI with WC observed in the patients of the present study may well be because the cumulative burden of oxidative stress and inflammation inherent in central adiposity which has impacted the cellular macromolecules and caused damage to DNA (DI).

Type 2 diabetes mellitus is a complex metabolic disorder wherein disturbances of lipoproteins and glucose may induce oxidative and nitrosative stress from increased generation of free radicals in many cell types. The ensuing cellular responses and functional disarray may manifest as neuropathological changes and cardiovascular disease (Allen *et al*., 2005). Complications from obesity can further potentiate/ accelerate cellular dysfunction via inflammatory responses and cytokine production (Green *et al*., 1994; and Hotamisligil *et al*., 1995). Also the pro-inflammatory response from free fatty acids and adipocytokines released from visceral adipose tissue can lead to insulin resistance (Kopelman,

2000; and Boden, 2006). An imbalanced oxidant: antioxidant status with excessive free radicals [39] can well cause oxidative damage to lipids, proteins and nucleic acids. The damage to DNA, if unrepaired, can induce carcinogenesis. In fact, oxidative DNA damage in type 2 diabetes is known to occur (Sampson *et al*., 2001) and was also observed in animal models (Awad *et al*., 2005). This might reflect increased levels of oxidative stress in the obese-type 2 patients, inducing the increased DNA damage observed in this study and in other studies (Sampson *et al*., 2006). High-fat diet intake (as also the diet type of the patients of the present study) was observed to be associated with obesity and accompanied by advanced glycation end products (Li *et al*., 2005).

The type 2 diabetic patients were on treatment: Diabcure–M (herbal capsules; n=3), Dianorm-M (Gliclazide+ metformin hydrochloride; n=5), Dional-5(Glibenclamide IP; n=3), Glycomet – 85(Glimepiride + metformin; n=3); and Glynase–MF (Glipizide + metformin;n=3) but none was on subcutaneous insulin alone or in combination with any drug. Among the oral anti-diabetic (Vigneri *et al*., 2009) drug families (sulphonylureas, biguanides, and thiazolidinediones), the prescribed medication comprised sulphonylureas (Glibenclamide, Glipizide, Glimepiride, Gliclazide) and biguanides (metformin), singly or in combination. Non-mutagenic/genotoxic nature of these drugs has been documented (Information on the active ingredient : Metformin Hydrochloride, 2012; Competact INN: Pioglitazone & Metformin, 2012) Investigational Compound Dapagliflozin, 2012; Product Information: Glucovance® metformin hydrochloride and glibenclamide, 2012; and Product Information: Amaryl® (Glimepiride tablets) 2012). However in the literature also significantly elevated levels of DNA damage as in treated vs.untreated subjects of the present study, were reported: as in the neutrophils from diabetic subjects even with acceptable glycaemic control (by treatment) compared to controls (Hannon-Fletcher *et al*., 2000), probably by the anti-oxidant nature of the prescribed drugs (c.f. Sliwinska *et al*., 2008).

The limitations of the present study, besides the small sample size, are that oxidative stress status and oxidative DNA damage were not assessed which can evaluate the cross-talk between DNA damage and oxidative stress. Also whether DNA damage may be a cause or a consequence of type 2 diabetes/obesity requires further separate studies. Despite these constraints, the relevance of the current findings cannot be undermined as the observed DNA damage in diabetic obese patients confirmed the previous findings of genetic instability correlating with diabetes, BMI and overweight.In summary, the study demonstrates that peripheral blood leucocytes from type 2 diabetic, obese patients are characterized by significant DNA damage probably from oxidative stress (given the connection of obesity, ageing and diabetes) initiated via the excessive generation of reactive oxygen species (Ahima, 2009), which could further initiate malignancy (Vigneri *et al*., 2009) regardless of the underlying mechanism. Given the projected prevalence of diabetes because of ageing and the obesity epidemic (Business Wire, 2011), urgent management/intervention strategies for diabetes and /or obesity are required.

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REFERENCES

1. Adaikalkoteswari A, Balasubramanyam N and Mohan V (2005), "Telomere shortening occurs in Asian Indian type 2 diabetic patients", *Diabet. Med.,* Vol. 22, pp. 1151-1156.
2. Ahima R S (2009), "Connecting obesity, aging and diabetes", *Nat.Med.,* Vol. 15, pp. 996-997.
3. Al-Aubaidy H A and Jelinek H F (2011), "Oxidative DNA damage and obesity in type 2 diabetes mellitus", *Eur J Endocrinol* Vol. 164, pp. 899-904.
4. Allen D A, Yaqoob M M and Harwood SM (2005), "Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications", *J. Nutr. Biochem.,* Vol. 16, pp. 705-713.
5. Andreassi M G, Botto N, Simi S, Casella M, Manfredi S, Lucarelli M, Venneri L, Biagini A and Picano E (2005), "Diabetes and chronic nitrate therapy as co-determinants of somatic DNA damage in patients with coronary artery disease", *J. Mol. Med.,* Vol. 83, pp. 279-286.
6. Andreassi M G, Barale R, Iozzo P and Picano E (2011), "The association of micronucleus frequency with obesity, diabetes and cardiovascular disease", *Mutagenesis,* Vol. 26, pp. 77-83.
7. Awad O, Jiao C, Ma N, Dunnwald M and Schatteman G C (2005), "Obese diabetic mouse environment differentially affects primitive and monocytic endothelial cell progenitors", *Stem Cells,* Vol. 23, pp. 575-583.
8. Baerlocher G M and Lansdorp P M (2003), "Telomere length measurements in leukocyte subsets by automated multicolor flow-FISH", *Cytometry A,* Vol. 55, pp. 1-6.
9. Boden G (2006), "Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver", *Current Diabetes Reports,* Vol. 6, pp. 177-181.
10. Bukhari S A, Rajoka M I, Nagra S A and Rehman Z U (2010), "Plasma homocysteine and DNA damage profiles in normal and obese subjects in the Pakistani population*", Mol. Biol. Rep.,* Vol. 37, pp. 289–295.
11. Business Wire (2011), "FDA Advisory Committee Makes Recommendation on Investigational Compound Dapagliflozin", July 19, 2011. Accessed from: *http:// www.businesswire.com /news/home/ 20110719007340/en/FDA-Advisory-Committee-Recommendation-Investigational-Compound-Dapagliflozin*. Accessed on Feb.16, 2012.
12. Choi K M, Lee J, Lee K W, Seo J A, Oh J H, Kim S G, Kim N H, Choi D S and Baik S H (2004), "The associations between plasma adiponectin, ghrelin levels and cardiovascular risk factors*", Eur. J. Endocrinol.,* Vol. 150, pp. 715-718.
13. Collins A (2004), "The comet assay for DNA damage repair*", Mol Biol.,* Vol. 26, pp. 249-260.
14. Competact INN: Pioglitazone & Metformin (2012), *http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_- _Scientific_Discussion/human/000655/WC500097069. pdf.* Accessed 5 Feb 2012.

15. Curti M L R, Jacob P, Borges M C, Rogero M M and Ferreira S R G (2011), "Studies of Gene Variants Related to Inflammation, Oxidative Stress, Dyslipidemia, and Obesity: Implications for a Nutrigenetic Approach", *Journal of Obesity,* Article ID 497401, p. 31, doi:10.1155/2011/497401.

16. Demirbag R, Yilmaz R, Gur M, Kocyigit A, Celik H, Guzel S and Selek S (2005), "Lymphocyte DNA damage in patients with acute coronary syndrome and its relationship with severity of acute coronary syndrome", *Mutat. Res.,* Vol. 578, pp. 298-307.

17. Elwakkad A, Hassan N E, Sibaii H, Zayat S, Sherif L, Hameed E and Shaheed A (2011), "Relationship Between Obesity and 8-hydroxy-2-deoxy Guanosine as an Oxidative Marker in Obese Adolescents of Giza", *Journal of Medical Sciences,* Vol. 11, pp. 231-235.

18. Fenton M, Barker S, Kurz D J and Erusalimsky J D (2001), "Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries", *Arterioscler Thromb Vasc Biol*., Vol. 21, pp. 220-226.

19. Giovannelli L, Saieva C, Masala G, Testa G, Salvini S, Pitozzi V, Riboli E, Dolara P and Palli D (2002), "Nutritional and lifestyle determinants of DNA oxidative damage: A study in a Mediterranean population", *Carcinogenesis.,* Vol. 23, pp. 1483-1489.

20. Green A, Dobias S B, Walters D J A and Brasier A R (1994), "Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase", *Endocrinology,* Vol. 134, pp. 2581–2588.

21. Hannon-Fletcher M P, O'Kane M J, Moles K W, Weatherup C, Barnett C R and Barnett Y A (2000), "Levels of peripheral blood cell DNA damage in insulin dependent diabetes mellitus human subjects", *Mutat. Res.,* Vol. 460, pp. 53-60.

22. Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, Toyota T (1999), "Oxidative DNA damage in diabetes mellitus: its association with diabetic complications", *Diabetologia,* Vol. 42, pp. 995-998.

23. Hotamisligil G S, Arner P, Caro J F, Atkinson R L and Spiegelman B M (1995), " Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance", *The Journal of Clinical Investigation,* Vol. 95, pp. 2409–2415.

24. Information on the active ingredient: Metformin Hydrochloride (2012), *http://www.masterfarma.com/producto_glucofinn_en.html.* Accessed 5 Feb 2012.

25. Investigational Compound Dapagliflozin Added to Metformin Sustained Reductions in Blood Sugar Levels in Adult Patients with Type 2 Diabetes in Two-Year Study (2012), *http://www.businesswire.com/news/home/20110626005054/en/Investigational-Compound-Dapagliflozin-Added-Metformin-Sustained-Reductions*. Accessed 5 Feb 2012.

26. Kasai H, Iwamoto-Tanaka N, Miyamoto T, Kawanami K, Kawanami S, Kido R and Ikeda M (2001), "Life style and urinary 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage: Effects of exercise, working conditions, meat intake, body mass index,

and smoking", *Jpn. J. Cancer Res.,* Vol. 92, pp. 9-15.

27. Kopelman P G (2000), "Obesity as a medical problem", *Nature,* Vol. 404, pp. 635-643.

28. Li S Y, Liu Y, Sigmon V K, McCort A and Ren J (2005), "High-fat diet enhances visceral advanced glycation end products, nuclear O-Glc-Nac modification, p38 mitogen-activated protein kinase activation and apoptosis", *Diabetes Obes. Metab,* Vol. 7, pp. 448-454.

29. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K and Poulsen H E (1992), "Oxidative DNA damage estimated by 8-hydroxydeoxy-guanosine excretion in humans: influence of smoking, gender and body mass index", *Carcinogenesis,* Vol. 13, pp. 2241-2247.

30. Misra A, Chowbey P, Makkar B M, Vikram N K, Wasir J S, Chadha D, Joshi S R, Sadikot V, Gupta R, Gulati S, Munjal Y P (2009), "Consensus Statement for Diagnosis of Obesity, Abdominal Obesity and the Metabolic Syndrome for Asian Indians and Recommendations for Physical Activity, Medical and Surgical Management*", J. Assocn. Physicians Ind.,* Vol. 57, pp. 163-170.

31. Nadin S B, Vargas-Roig L M and Ciocca D (2001), "A silver staining method for single-cell gel assay", *Journal of the Histochemical Society,* Vol. 49, pp. 1183-1186.

32. Product Information: Amaryl® (Glimepiride tablets) (2012), *http://products.sanofi.us/amaryl /amaryl.pdf.* Accessed 5 Feb 2012.

33. Product Information: Glucovance® metformin hydrochloride and glibenclamide (2012), *http://www.pbs.gov.au/meds%2Fpi%2 Fafpgluco11110.pdf*. Accessed 5 Feb 2012.

34. Sampson M J, Astley S, Richardson T, Willis G, Davies I R and Hughes D A (2001), "Increased DNA oxidative susceptibility without increased plasma LDL oxidizability in type II diabetes: effects of alpha-tocopherol supplementation", *Clin. Sci.,* Lond., Vol. 101, pp. 235-241.

35. Sampson M J, Winterbone M S, Hughes J C, Dozio N and Hughes D A (2006), "Monocyte Telomere Shortening and Oxidative DNA Damage in Type 2 Diabetes", *Diabetes Care,* Vol. 29, pp. 283-289.

36. Sardas S, Yilmaz M, Oztok U, Cakir N and Karakaya AE (2001), " Assessment of DNA strand breakage by comet assay in diabetic patients and the role of antioxidant supplementation", *Mutat. Res.,* Vol. 490, pp. 123-129.

37. Scarpato R, Verola, C, Fabiani, B, Bianchi V, Saggese G and Federico G (2011), "Nuclear damage in peripheral lymphocytes of obese and overweight Italian children as evaluated by the ã-H2AX focus assay and micronucleus test", *The FASEB Journal,* Vol. 25, pp. 685-693.

38. Singh N P, McCoy M T, Tice R R and Schneider E L (1988), "A simple technique for quantification of low levels of DNA damage in individual cells", *Exp Cell Res.,* Vol. 175, pp. 184-191.

39. Sliwinska A, Blasiak J, Kasznicki and Drzewoski J (2008), "*In vitro* effect of gliclazide on DNA damage and repair in patients with type 2 diabetes mellitus

(T2DM)", *Chem.Biol. Interact* Vol. 173, pp. 159-165.

40. Snehalatha C, Viswanathan V and Ramachandran A (2003), "Cut-off values for normal anthropometric variables in Asian Indian adults", *Diabetes Care,* Vol. 26, pp. 1380-1384.

41. Strandberg T E, Saijonmaa O, Tilvis R S, Pitkälä K H, Strandberg A Y, Miettinen T A and Fyhrquist F (2011), "Association of Telomere Length in Older Men With Mortality and Midlife Body Mass Index and Smoking", *J. Gerontol. A Biol. Sci. Med. Sci.,* Vol. 66A, No. 7, pp. 815-820.

42. Tomasello B, Malfa G, Galvano F and Renis M (2011), "DNA damage in normal-weight obese syndrome measured by Comet assay", *Med. J. Nut.Metabol.,* Vol. 4, pp. 99-104.

43. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R (2009), "Diabetes and cancer", *Endocrine-Related Cancer,* Vol. 16, pp. 1103-1123.

44. Violante F S, Sanguinetti G, Barbieri A, Accorsi A, Mattioli S, Cesari R, Fimognari C and Hrelia P (2003), "Lack of correlation between environmental or biological indicators of benzene exposure at parts per billion levels and micronuclei induction", *Environ. Res.,* Vol. 91, pp. 135-142.

45. Weiner J S and Lourie J A (1981), *Human Biology: A Guide to Field Methods*. Practical Human Biology, Academic Press Inc., New York.

46. WHO (2004), "Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies", *Lancet*, Vol. 363, pp. 157-163.

47. Wiegand S M, Keller M, Robl D, L'Allemand D, Reinehr T, Widhalm K and Holl R W (2010), "Obese boys at increased risk for nonalcoholic liver disease: Evaluation of 16390 overweight or obese children and adolescents", *Int. J. Obesity,* Vol. 34, pp. 1468-1474.

48. Yesilada E, Sahin I, Ozcan H, Yildirim I H, Yologlu S and Taskapan C (2006), "Increased micronucleus frequencies in peripheral blood lymphocytes in women with polycystic ovary syndrome" *Eur. J. Endocrinol.,* Vol. 154, pp. 563-568.

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