



Original article

Interrelationship between oxidative stress, DNA damage and cancer risk in diabetes (Type 2) in Riyadh, KSA

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is the most widely known type of disorder of the endocrine system marked by hyperglycemia resulting either due to deficiency of insulin and/or resistance. Persistent hyperglycemia induces oxidative stress and is suggested to play a prominent role in the pathophysiology underlying T2DM. Besides, oxidative stress can result in DNA damage leading to high cancer risk. Current study aimed to evaluate status of oxidative damage, damage to DNA and cancer biomarkers in regard to increased glucose in T2DM patients and to correlate the glycemic state with cancer. A total of 150 subjects consisting of control (50) and T2DM patients (100) were enrolled. Additionally, three tertiles were created among the two groups based on levels of HbA1c (Tertile I = 5.37 ± 0.34 , n = 50; Tertile II = 6.74 ± 0.20 , n = 50; Tertile III = 9.21 ± 1.47 , n = 50). Oxidative stress parameters including malondialdehyde (MDA) and antioxidant enzymes were measured. Damage to DNA was analyzed by measuring the levels of DNA damage adduct-8 hydroxy deoxy Guanosine (8-OHdG). To detect cancer resulting from oxidative stress, cancer biomarkers CEA, AFP, CA125, CA-15, CA19-9, prolactin were measured in these subjects. All measurements were analysed by SPSS software. Levels of MDA and antioxidant enzymes altered significantly in T2DM group at $p < 0.001$ and $p < 0.05$ level of significance. Significant DNA damage accompanied with elevated levels of CEA, CA19-9 and decreased CA125, AFP and prolactin were noted in T2DM group. CA 19-9 and CEA levels increased at $p < 0.05$, whereas levels of prolactin decreased significantly ($p < 0.001$) in T2DM group compared to control. Additionally the mean values of DNA damage adduct 8-OHdG differ significantly at $P < 0.01$ between the two groups. However, no significant correlation in oxidative stress parameter, antioxidant enzymes, DNA damage and neither with the highest tertile of HbA1c ($>7.5\%$) was noted. Based on the results obtained in the present study, we conclude that there is considerable change in oxidative stress and DNA damage in T2DM patients. Hence, assumption that the oxidative stress could cause cancer in T2DM as a result of hyperglycemic state was not speculated in this study.

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1. Introduction

Diabetes mellitus type 2 (T2DM) designated by multiple etiologies is portrayed by chronic hyperglycaemia resulting due to impairment in metabolism of major biomolecules oftenly due to

lack of insulin production and action and is frequently symptomized by glycosuria, polydipsia and polyuria (Ngaski, 2018). T2DM is a fastest increasing disease worldwide and has become a major challenge to the health care providers (Guariguata et al., 2014). It is estimated that around 18.5% of the adult has diabetes in KSA (IDF, 2017). Glycated hemoglobin (HbA1c), a major determinant of hyperglycemia arises due to overload of glucose in blood (Koenig et al., 1976). Chronic hyperglycemia leads to devastating effects in diabetic subjects. One such array of events that occur is oxidative stress that constitutes a major link in the onset and succession of T2DM (Fiorentino et al., 2013). Loss of balance in the levels of oxidants and antioxidants is termed as 'Oxidative stress'. Lipid peroxidation (MDA; malondialdehyde) a symbolic of cellular damage results due to raised levels of free radicals or

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ROS (reactive oxygen species). ROS are maintained within limits under normal physiological conditions by scavenging systems of antioxidants and antioxidant enzymes. The imbalanced redox status arose due to hyperglycemic state leads to damage to biomolecules like lipid, peptides including DNA. Damage to DNA is known to be associated as a cause in varied diseases including cancer (Halliwell, 1994).

ROS, the molecular oxidants are known to trigger the development of cancer as DNA is the probable target of the oxidative attack. Apurinic DNA, oxidized nitrogenous bases, excision of ss or ds DNA are few of the examples of oxidatively induced DNA damages. Free radical can act on the nitrogenous bases and chromatin leading to altered gene expression. Similar array of events could occur in tumour suppressor genes and trigger cancer (Sova et al., 2010; Lee and Chan, 2015). Therefore it is proposed that diabetes subjects are at risk of different types of cancer affecting the major organs like stomach, liver, lung, pancreas, colorectum, breast and other sex organs (De Beer and Liebenberg, 2014). Among different types of oxidative damage to DNA, 8-Hydroxydeoxyguanosine (8-OHdG) is a universal marker measurable by ELISA technique. Association of diabetes with cancer and the alarming number of diabetes among adult population is an eye opener to all diabetologists to explore and find new preventive measures that could reduce the morbidity/mortality risk in these patients.

The interrelation between pancreatoma and diabetes is intricate due to existence of two forms of diabetes with different pathophysiologies. Type 1 DM association with pancreatic cancer is notified as unrelated etiologies by many researchers. While some study have reported absence of correlation between diabetes and pancreatomas (Hjalgrim et al., 1997; Frye et al., 2000), numerous other researchers suggested higher risk of pancreatic cancer due to insulin resistance in diabetes subjects (Wang et al., 2003). However, the link between T2DM and cancer is still debatable. Recently, a meta-analytic study reported 1.2 fold increase risk of breast cancer among T2DM (Vigneri et al., 2009). Hence there is need for an hour to assess the levels of few prominent cancer biomarkers as prognostic tools in diagnosis of different types of cancer in T2DM patients. Currently number of cancer biomarkers (CBs) are available to be used in detection or diagnosis of possible cancer risks. Few of the circulating CBs like Carcinoembryonic antigen (CEA), alpha-feto protein (AFP), CA125 are of prime significance in cancer research. CEA is one of the most commonly used cancer marker (Park et al., 2011). It is expressed at multiple sites including the pancreas, lung, prostate, ovary, breast and colon (Malati, 2007). Tumor biomarker CA 19-9 overexpression has been documented in patients with pancreatoma and cancer of biliary tract in previous reports. CA 19-9 also is raised in other types of cancers like gastric, oesophageal, colorectal, hepatocellular and ovarian cancers (Locker et al., 2006; Perkins et al., 2003). Another significant cancer marker which is used in diagnosis of cancer of liver, testicles and ovary is alpha-feto protein (AFP) (Li et al., 2017). Recently, a tumor marker plasma cancer antigen (CA)-125 related to heart failure following myocardial infarction is identified (Sekiguchi et al., 2017). There also exists a strong link between glucose levels and increased risk for breast carcinogenesis (Dong and Qin, 2011).

So far, no reports have described the interrelation ship of CEA, AFP, CA125, CA-15, CA19-9 with oxidative stress and DNA damage in T2DM subjects. To our knowledge, the correlation between oxidative stress, cancer biomarkers with DNA damage as a consolidated study has not been reported and role of cancer biomarkers most prominently has not yet elucidated. T2DM is a diversified disease with varying levels of increased glucose. Our goal in this case controlled study was not just to compare between normal and diabetes subjects but instead diabetic individuals with HbA1c above cut off of 6.5% were compared to normal non diabetic patients with glycated Hb < 5.7%.

In above perspective and scarcity of information, the current study attempts to a) analyse the oxidative stress markers, antioxidant enzymes and some prominent tumor markers b) to ascertain relationship of oxidative stress markers with DNA damage and cancer risk. To abolish gender based bias in analysis of cancer markers, comparison in both gender was also accomplished.

2. Methodology

Four hundred and twenty volunteers participated in the study. Depending upon the baseline parameters and physician diagnosis 150 subjects were selected by stratified sampling method and grouped into two groups using cut off value of HbA1c 6.5% as per American Diabetic Association (2014)-Group A (control, n = 50) and group B (T2DM, n = 100). Further, subjects were divided into 3 HbA1c tertiles (Tertile I = 5.37 ± 0.34 , n = 50; Tertile II = 6.74 ± 0.20 , n = 50; Tertile III = 9.21 ± 1.47 , n = 50). Of the total studied population, there were 57 males and 93 females aged 18–90 years. The present prospective study was carried out in Department of Clinical laboratory Sciences, College of Applied Medical Sciences, King Saud University and Prince Sultan Military Medical City, Riyadh, KSA. Hospital ethics committee approved the study. Written informed consent from all patients prior to sample collection was obtained.

Inclusion criteria: Normal healthy non-diabetic subjects with HbA1c below 6.5% were included as controls and patients diagnosed with Type 2 diabetes with HbA1c above 6.5% were included under T2DM group.

Exclusion criteria: Patients with Type 1 diabetes, females with pregnancy or lactation, cardiovascular damage, cancer, liver disease, chronic kidney disease, history of chronic alcohol abuse, history of cancer, stroke or organ transplantation were excluded from the study.

2.1. Sample collection and investigations

Whole blood (fasting) was sampled by venipuncture. Blood, plasma and serum were collected separately and EDTA-coated tube were used for HbA1C analysis. Basic laboratory parameters like HbA1c, FBG and lipid were measured with the fully automatic analyzer (Roche Cobas C-702, Germany).

2.2. Analysis of oxidative stress

2.2.1. Assessment of lipid peroxidation (MDA)

Lipid Peroxidation was measured by MDA Assay Kit, Sigma (Catalog Number MAK085). In brief, serum samples and standard were prepared as per the instructions and read on 96 well titre plate at 532 nm in Microplate reader (IRE96 plate reader, SFRI).

2.2.2. Measurement of activity of superoxide dismutase (SOD)

SOD activity was measured by SOD Assay Kit-WST (19160, Sigma). Appropriate volumes of blank and samples were loaded in 96 well plate with WST Working Solution and enzyme working solution to each well, mix and incubated at 37 °C for 20 min and read at 450 nm using a microplate reader. SOD activity was calculated and expressed as inhibition rate %.

2.2.3. Measurement of activity of catalase (CAT)

Activity of serum catalase was measured by Catalase colorimetric activity kit (E1ACATC, Invitrogen). Serum and standard were prepared according to the kit. 25 µl of standard and serum sample was added to wells in a microtitre plate followed by addition of hydrogen peroxide. After incubating for 30 min, substrate and HRP added and read at 560 nm. SOD activity was expressed as U/ml.

2.2.4. Measurement of activity of glutathione peroxidase (GPx)

Determination of GPx activity was performed using Glutathione peroxidase activity kit (Colorimetric-ab102530, Abcam). GPx assessment involves the consumption of NADPH after its principle reactions. Reduction in levels of NADPH is monitored by absorbance at 340 nm and activity is expressed as mIU/ml.

2.3. Measurement of cancer biomarkers

Circulating cancer biomarkers like AFP, CA125, CA15-3, CA19-9, CEA, prolactin were measured by Roche Cobas e 602 immunoassay analyzer using Sandwich ELISA technique.

2.4. Measurement of 8 hydroxydeoxyguanosine (8OHdG)

ELISA kit (DNA Damage STA 320) from Cell Biolabs was used to quantify 8-OHdG in serum is a competitive enzyme immunoassay and expressed as ng/ml.

2.5. Statistical analysis

The data was shown as mean \pm SD. All studied parameters in the two groups were compared by *t* test and one-way ANOVA and Holm-sidak test were carried out for comparison among the three tertiles. HbA1c tertiles were correlated with lipid profile, oxidative stress parameters, cancer biomarkers and 8-OHdG by Pearson's correlation.

3. Results

The baseline characteristics of the studied groups are depicted in Table 1. The mean values sharing the same superscripts differ significantly at 0.001 level. Both groups include total number of 57 males and 93 females. Mean values of FBG, HbA1c, Tg, HDL-C were significantly increased in T2DM group compared to control. Table 2 shows the mean values of oxidative stress and cancer biomarkers in the aforementioned groups, mean values with different superscripts alter at 0.01 level of significance. Enhanced levels of MDA was observed in T2DM group compared to control at $P < 0.001$. Antioxidant enzymes; catalase, superoxide dismutase, glutathione peroxidase decreased remarkably in T2DM group at $p < 0.05$. Serum levels of CA 19-9, CEA increased whereas levels of AFP and prolactin decreased in T2DM compared to control. Levels of CA19-9, CEA increased significantly whereas a non-significant decrease in the mean values of CA15-3 and AFP was noticed between the groups. Contrarily, the mean values of DNA damage adduct –8 OHdG differ significantly at $P < 0.01$.

Furthermore, to study the correlation between levels of HbA1c, markers of oxidative stress and cancer, the groups were divided into 3 tertiles based on levels of HbA1C. Patients with HbA1c in

Table 1
Comparison of various biochemical indices between the groups.

	Control Group (n = 50)	T2DM group (n = 100)	P value
Male	16	41	
Female	34	59	
Age (years)	49.77 \pm 16.67	56.86 \pm 14.52	
FBG (mg/dl)	5.23 \pm 0.91 ^a	8.91 \pm 4.035 ^a	<0.001
HbA1C (%)	5.37 \pm 0.34 ^a	7.90 \pm 1.62 ^a	<0.001
TC (mg/dl)	4.48 \pm 0.96	4.22 \pm 0.95	0.115 (NS)
TG (mg/dl)	1.37 \pm 0.77 ^a	1.73 \pm 1.06 ^b	<0.05
HDL-C (mg/dl)	1.31 \pm 0.35 ^a	1.18 \pm 0.33 ^c	<0.05
LDL-C (mg/dl)	2.73 \pm 0.78	2.64 \pm 0.9	0.53(NS)

Mean \pm SD values of each group, at P value < 0.001 . Values not sharing same superscripts differ significantly at 0.05 level of significance; NS (not significant).

Table 2

Status of oxidative stress and cancer markers between the groups.

	Control Group (n = 50)	T2DM group (n = 100)	P value
Lipid peroxidation -MDA (nmol/ μ L)	0.26 \pm 0.05 ^a	0.66 \pm 0.37 ^a	<0.001
Catalase (U/ml)	29.31 \pm 14.8 ^a	23.79 \pm 14.6 ^b	<0.05
Glutathione peroxidase (U/ml)	120.75 \pm 15.77 ^a	76.32 \pm 42.54 ^a	<0.001
Superoxide dismutase (inhibition rate %)	78.81 \pm 9.3 ^a	73.99 \pm 9.08 ^b	<0.01
8OHdG (ng/ml)	6.29 \pm 1.50 ^a	7.31 \pm 2.34 ^b	<0.01
CA125 (U/ml)	12.12 \pm 8.23 ^a	10.0 \pm 5.30 ^b	<0.1
CA15-3 (U/ml)	15.7 \pm 6.74	15.05 \pm 9.54	0.66(NS)
AFP (U/ml)	3.03 \pm 1.88	2.21 \pm 1.9	0.2(NS)
CA19-9 (U/ml)	10.69 \pm 7.4 ^a	17.51 \pm 15.9 ^b	0.005
CEA (ng/ml)	1.83 \pm 0.78 ^a	2.61 \pm 0.68 ^b	<0.05
PRL(ng/ml)	913.04 \pm 202 ^a	241.9 \pm 113.2 ^a	0.001

Mean \pm SD values of each group at P value < 0.001 , $P < 0.05$. Values not sharing same superscripts differ significantly at 0.05 and 0.1 level of significance; NS (not significant).

the range 4.8–5.9% were categorized under tertile I, HbA1C from 6.5 to 7.0 under tertile II and HbA1C > 7.0 under tertile III. The mean values of HbA1c in the three tertiles were-Tertile I = 5.37 \pm 0.34 (n = 50); Tertile II = 6.74 \pm 0.20 (n = 50); Tertile III = 9.21 \pm 1.47 (n = 50). Table 3 depicts the, Pearson's correlation performed between various tertiles of HbA1C with cancer biomarkers. HbA1c in tertile III was strongly correlated with PRL, whereas CA 125, CA19-9, CEA, AFP, CA-15-3 did not correlate with HbA1c in tertile I, II, III. CA 125, CA19-9, CEA, AFP, CA-15-3 increased in tertile II, III compared to tertile I, but none of the cancer biomarkers were correlated significantly with HbA1C levels. However, PRL was found negatively correlated with HbA1C in tertile III. PRL was found to decrease significantly with HbA1C and also differ significantly among the three tertiles of HbA1c at $P < 0.05$ level. The mean values of oxidative stress markers in the three tertiles are shown in Fig. 1.

DNA damage: DNA damage adduct 8OHdG increased significantly in T2DM group compared to control. Raised levels of 8OHdG were positively correlated with HbA1C in T2DM group when compared with control ($r = 0.335$, $p = 0.0006$) (Fig. 5). Correlation of 8OHdG with oxidative stress markers between the two groups is depicted in Table 4. 8OHdG was strongly correlated with glutathione peroxidase (GPx) at $p < 0.0001$. The association of 8OHdG with MDA, CAT and SOD was insignificant between the two groups.

Furthermore, correlation of HbA1c with oxidative stress and damaged DNA adduct-8OHdG in the HbA1c tertiles is shown in Figs. 2–4. In tertile I, the correlation between HbA1c with oxidative stress markers and 8OHdG were non-significant. In tertile II, SOD was negatively correlated with HbA1c at $p < 0.05$. However, with MDA, GPx, CAT, 8OHdG, it was non-significant. In tertile III, HbA1c did not correlate significantly with oxidative stress markers studied including 8OHdG. Table 5 depicts the gender based differences in

Table 3

Pearsons correlation between various tertiles of HbA1c with cancer biomarkers in both the groups.

Cancer biomarkers	HbA1C		
	Tertile I r (p value)	Tertile II r (p value)	Tertile III r (p value)
CA125	-0.175(0.225)	0.028 (0.84)	0.20(0.15)
AFP	-0.24(0.09)	0.11 (0.44)	0.015(0.9)
CA 19-9	0.123(0.39)	0.03(0.8)	0.0165(0.25)
CA 15-3	0.25(0.07)	-0.17 (0.23)	0.21(0.13)
CEA	0.252(0.07)	0.085 (0.55)	0.17(0.23)
PRL	-0.13(0.34)	-0.25(0.09)	-0.30(0.03)*

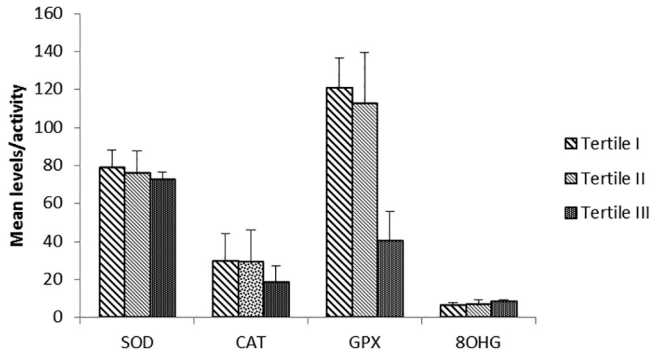


Fig. 1. Status of oxidative stress markers in the three HbA1c tertiles.

Table 4
Correlation between 8OHdG and oxidative stress in study population.

8OHdG	MDA r (p value)	CAT r (p value)	GPx r (p value)	SOD r (p value)
Control	0.19(0.18)	0.02 (0.88)	-0.05(0.7)	0.24(0.09*)
T2DM group	0.01 (0.88)	0.08 (0.40)	0.35(0.0003)**	0.10(0.3)

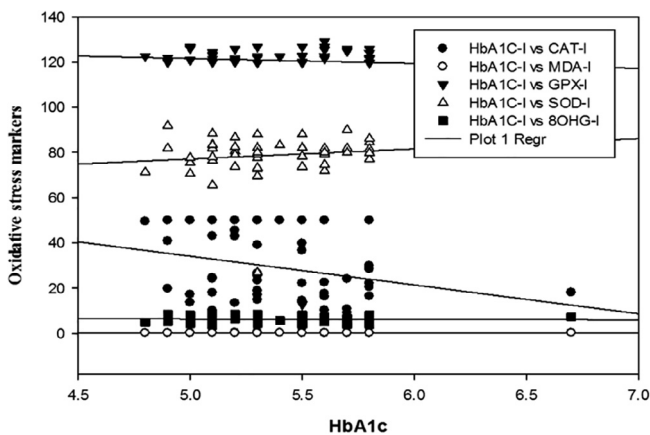


Fig. 2. Correlation graph of oxidative stress and DNA damage marker in tertile I.

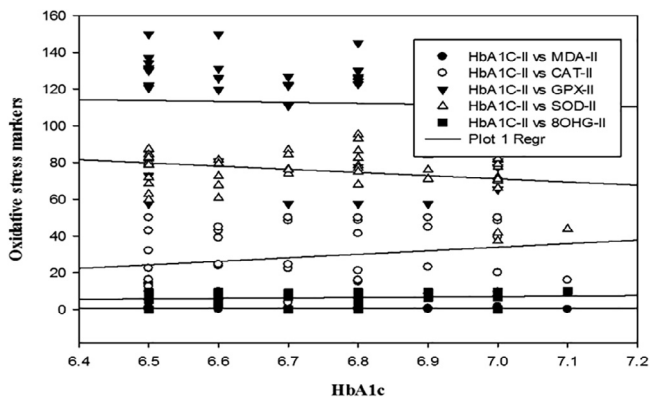


Fig. 3. Correlation graph of oxidative stress and DNA damage marker in tertile II.

the levels of HbA1c and cancer biomarkers in both groups. Levels of cancer biomarkers remained insignificant in both gender. Higher levels of AFP were observed in T2DM females compared to men yet the association between AFP and HbA1c in both gender was insignificant.

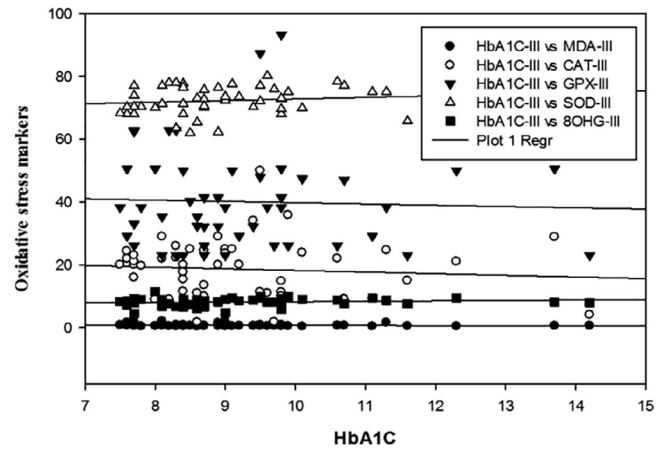


Fig. 4. Correlation graph of oxidative stress and DNA damage marker in tertile III.

Table 5
Gender based differences in levels of cancer biomarkers in study population.

Cancer biomarkers	Control		T2DM	
	Males (n = 16)	Females (n = 34)	Males (n = 41)	Females (n = 59)
HbA1C	5.47 ± 0.24	5.34 ± 0.36	8.18 ± 1.8	7.82 ± 1.44
CA 125	9.5 ± 5.7	13.35 ± 8.98	8.82 ± 3.93	10.88 ± 6.04
CA 15-3	15.62 ± 6.3	15.73 ± 7.02	17.07 ± 3.01	13.42 ± 5.79
AFP	1.77 ± 1.07	3.63 ± 7.05	1.69 ± 1.0	2.50 ± 2.3*
CA 19-9	8.8 ± 4.6	11.55 ± 8.36	17.31 ± 14.42	17.76 ± 17.61
CEA	1.71 ± 0.79	1.67 ± 0.9	2.96 ± 1.68	2.37 ± 1.65

* P < 0.05.

4. Discussion

The present study reports increased levels of FBG, HbA1C, MDA, DNA damage adduct –8OHdG in T2DM patients compared to non-diabetic control. Additionally, increased serum TG and decreased HDL-C was obtained in T2DM patients. Exploring the role of oxidative damage in diabetes, we found significant changes in the concentrations of MDA and antioxidant enzymes in T2DM patients compared to non-diabetic control indicating that increased oxidative damage is responsible for etiology underlying T2DM. It is speculated that oxidative stress developing on account of poor glycemic control could leads to cancer and diabetes patients may pose a higher risk of developing different types of malignancies. Several investigations have rationalized on the association of oxidative stress with cancer in diabetes, yet the underlying cause in T2DM that leads to cancer is not clearly elucidated.

Oxidative damage as mentioned earlier is a result of free radical attack that eventually causes cells and its components to lose their structure and function (Martin et al., 2003). PUL (Polyunsaturated lipids) are prone to the attack by the free radicle, resulting in a cascade with the generation of final products like malondialdehyde (MDA). Elevated levels of ROS is a result of imbalance in the physiology underlying oxidation-reduction cycles in the biological system. Enhanced oxidation occurs due to increased generation of ROS besides poor destruction of the antioxidant system composed of prominent antioxidant enzymes. The concentrations of these antioxidant enzymes critically effect the susceptibility of cells to oxidative stress and may also be linked to the progress of complications in diabetes mellitus. The hyperglycemic state can exacerbate the effects of oxidative damage that substantially leads to diabetes. Hyperglycemic state with decreased antioxidant enzymes in T2DM patients observed in the current study are in line with earlier reports (Ngaski, 2018). On contrary, other investigations suggests an increased levels of SOD and CAT activity in T2DM compared

to healthy controls (Bondor et al., 2015). SOD is catalytically involved in production of the hydrogen peroxide and oxygen from superoxide anion. GPx is a selenoprotein. Haem-containing enzyme, example catalase converts H_2O_2 into water and oxygen and thus neutralizes it. CAT regulates the metabolism of H_2O_2 and eventually diminished levels can exerts deleterious effects on cellular components –lipids, RNA and DNA. Under conditions of CAT deficiency, enhanced oxidation in the beta cell of pancreas could lead to β -cells dysfunction and eventually to diabetes (Ullah et al., 2016). Decreased levels of catalase activity in T2DM subjects could be a consequence of increased enzymatic glycation. Results obtained in the current study are in line with that of Palekar and Ray (2016). Diminished levels/activity of GPx in diabetes may be attributed to the excessive glycation via the polyol pathway (Komosíńska-Vassev et al., 2005).

Through a decade, there has been growing awareness of the impact of DNA damage in chronic diseases and cancer. Probing into the investigations on the role of oxidative stress parameters and DNA damage in T2DM, we observed a significant increase in levels of 8OHdG/8oxodG an indicator of DNA damage in T2DM patients. A similar study by Kakimoto et al. (2002) found raised levels of 8-OHdG in streptozotocin-induced diabetic rats. 8-oxodG and Tg are potential markers/indicators of oxidative stress in different biological species ranging from bacteria to human cancer patients. Particularly 8-OHdG occurrence in steady levels in genetic materials (genomic, mitochondrial DNA and RNA) (Kryston et al., 2011). 8-OHdG constitutes the most profused oxidative products of DNA playing vital role in carcinogenesis. 8OHdG after excision from DNA appear in the blood. Furthermore, 8OHdG has been implicated as an index of oxidative stress in correlation with human cancer (Sova et al., 2010). Besides, Pearson correlation applied between oxidative stress parameters and 8OHdG did not reveal any significant association in T2DM. However, GPx was found to be significantly correlated with 8OHdG in T2DM. Previous investigations by Choi et al. had found DNA damage and HbA1c to be directly linked with each other (Choi et al., 2005). But increased levels of 8OHdG was positively correlated with HbA1c in T2DM compared to control ($r = 0.335, p = 0.0006$) (Fig. 5). Various epidemiological and clinical studies portrayed DNA damage associated with poor glycemic control and its complications, however none had analyze oxidative stress parameters in connection with detected DNA damage in diabetes subjects. With regard to HbA1c levels in three tertiles (range <6.5% to >7.5%) confounding results were obtained in our study. HbA1c did not reveal any significant correlation with oxidative stress parameter, antioxidant enzyme activities and DNA damage.

Association between diabetes and various types of cancer most notably the pancreatic cancer has been perceived. Unraveling the link between HbA1c, oxidative stress and cancer in diabetic subjects, analysis of some cancer biomarkers was performed to elucidate any possible correlation that exists between these markers. Potential cancer markers like CA125, CEA, AFP CA15-3, CA19-9, and prolactin were analyzed in serum of studied population. Serum

values for CA 19-9, CEA increased whereas levels of AFP and prolactin decreased in T2DM compared to control. CA 19-9, CEA was found to increase significantly ($p < 0.05$) whereas PRL decreased significantly at $p < 0.001$ in T2DM patients. Altered levels of CA15-3 and AFP were non-significant. The levels of CA125 were weakly significant among the groups. In contrast to our finding, increased values of CA125, CA15-3 and decreased CEA in T2DM patients was reported by Turgutalp et al. (2013). Homology to the finding of Turgutalp et al. (2013) we observed increased values of CA 19-9 and decreased AFP in T2DM subjects. Verily, elevated values of CA 19-9 obtained in current study are in agreement with earlier studies but conflicting results was observed in HbA1c association with CA19-9 in T2DM subjects (Gul et al., 2011). CA19-9 levels although found to increase in T2DM in our study yet the correlation with HbA1c was not conspicuous. The paramount in the present study was none of the cancer biomarkers exhibited significant association with HbA1c except for PRL. PRL was negatively associated with HbA1c in tertile III ($r = 0.30$) at $p < 0.05$ level of significance. A study by Uygur-Bayramicli et al. (2007) observed increased values of CA 19-9 levels in T2DM patients than controls but analysis of correlation between CA19-9 with glycemic control was not investigated. Also, contradictory evidences on the role of cancer markers in T2DM were reported. Few studies exhibited significant correlation of HbA1c with FBG and CA 19-9 whereas some did not (Benhamou et al., 1991; Banfi et al., 1996). Higher levels of CEA observed in the present work are contradictory to that reported in previous study (Turgutalp et al., 2013).

A previous study in Qassim, KSA involving T2DM females have reported elevated CEA level. The outcome of their study suggested the role of CEA as connecting link between disturbances in metabolism and suggested CEA as an intermediary linking cancer and metabolic disturbances in diabetic subjects (Hasan and Mohieldein, 2015). Nonetheless, no such correlation was noticed in our study. CEA- Carcinoembryonic antigen is a widely known soluble cancer marker (Park et al., 2011). It is a 180 kDa glycoprotein present in large intestine in normal tissues but is overexpressed in different types of malignancies like gastrointestinal, lung, breast and thyroid cancers (Gur et al., 2011). Apart from these, several non-neoplastic conditions like renal or hepatic insufficiency, acute and chronic inflammations, benign cancer etc were found to be associated with higher serum levels of CEA (Ruibal Morell, 1992). In a recent study on Japanese men, CEA was found to be link predominantly with metabolic syndrome (Lee et al., 2011). CA19-9 is identified as a vital diagnostic marker/indicator in different types of malignancies including gastrointestinal, hepatobiliary and urothelial cancer, most notably pancreatoma (Locker et al., 2006; Kim et al., 2009). Similar to our findings, previous report have suggested higher CA19-9 levels in T2DM with no correlation with cancer risk (Ventrucci et al., 2009). Increased CA19-9 could be a sequel of deprived metabolic compensation and poor glycemic control (Shimojo et al., 1990). α -fetoprotein (AFP) is another potential biomarker in identification of hepatic carcinomas. Many epidemiologic studies had firmly investigated on the association between diabetes and pancreatoma. Yet, the question...whether diabetes constitutes as a risk factor for pancreatic cancer or is only the manifestation of pancreatic cancer is a subject of debate. Elevated values of CEA and CA 19-9 observed could be suggestive of some cancer but HbA1c was not found to be a predisposing factor in T2DM. Epidemiological studies had reported increased prevalence of diabetes found in pancreatoma and simultaneously enormous number of cases of pancreatic cancer in diabetic subjects. Thus, the question emerges “Is diabetes a cause or consequence of cancer”?

A new insight in the present research was the role of CA-19-9. Increased concentrations of CA 19-9 are known as indicator of cardiac failure, cardioembolic stroke and death (Sekiguchi et al.,

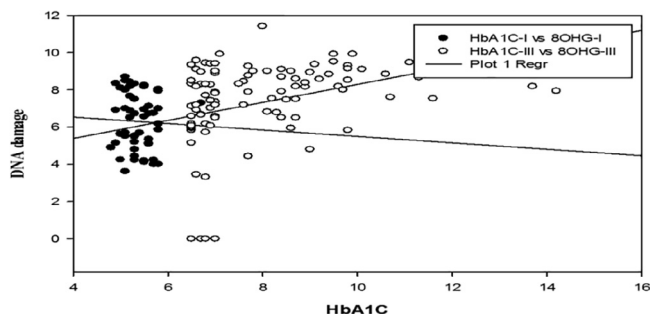


Fig. 5. Correlation graph between HbA1c and DNA damage in control and T2DM.

2017). Raised values of CA 19-9 observed in this study suggests its role as a cardiovascular risk factor during diabetes progression (Braybrook, 2005). Another intriguing finding was the correlation obtained between prolactin (PRL) and HbA1c. During pregnancy and its course, prolactin acts as a regulator in maintaining a normal glucose levels. Previous studies has investigated on the association of PRL and cancer and found higher cancer hazard with low serum prolactin in females (Wang et al., 2016). In a study by Tworoger et al. (2006) greater risk of breast cancer was linked with higher prolactin concentrations. Among the various cancer biomarkers studied, PRL was found to be significantly correlated with HbA1C. However role of PRL in relation to hyperglycemic state in diabetes needs further validation. Based on the results obtained, role of PRL could be suggested/validated as a potent indicator/biomarker in diagnosis of tumor in T2DM subjects under poor glycemic state. In a Cohort study, investigating cancer risk in hyperprolactinemia patients, the risk of total/overall cancer increased in hyperprolactinemic subjects specifically in upper GI and hematopoietic cancers (Berinder et al., 2011).

Furthermore, to assess the gender related differences in these markers and clear the gender bias relating to the cancer markers, the levels of HbA1c and cancer biomarkers were compared between males and females in control and T2DM group (Table 5). Surprisingly, no significant changes in cancer biomarkers among males and females were noticed except AFP. Higher levels of AFP were noticed in T2DM females than men. Nevertheless, non-significant correlation existed between males and females with respect to AFP and HbA1c. The main hallmarks of the study are increased oxidative stress with diverse changes in cancer biomarkers in T2DM patients with no significant association with HbA1c. Evaluation of oxidative induced damage in T2DM patients may assist in speculating and preventing oxidative damage-induced complications. Significant correlation between HbA1c and DNA damage in diabetes may be accounted as a prerequisite for cancer initiation but other factors are probably responsible for cancer development in T2DM patients.

In conclusion data obtained in our study suggests that oxidative stress and some cancer biomarkers are increased in diabetes, but are insignificant with the hyperglycemic state. The exact mechanism how diabetes could result in cancer is uncertain, but it is speculated that modulation or imbalance between the various markers of oxidative damage and damaged DNA observed in the present study play a vital role in understanding the etiology underlying diabetes. The assumption: Oxidative damage and damage to DNA consequently lead to cancer in T2DM was not conspicuous. Nevertheless in accordance with the results obtained in the present work, it could be stated that “diabetic milieu could be sequelae of generated oxidative stress as a consequence of deprived metabolism and poor glycemic control in T2DM”. More comprehensive studies linking cancer with diabetes are needed.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Authors' contribution

M. Abudawood, H. Tabassum involved in conceptualization, data interpretation, preparation of the manuscript; A. Aljohi, B. Almaarik, H. Tabassum in sample collection, biochemical analysis and data collection.

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