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Original Article

Cross-sectional correlates of oxidative stress and inflammation with glucose intolerance in prediabetes

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ABSTRACT

Background: Prediabetes is a condition in which blood glucose level is above the normal but below the diagnostic value of diabetes mellitus. Besides progression to diabetes mellitus, prediabetic subjects are at risk for cardiovascular disease (CVD). It is associated with oxidative stress and inflammation and therefore this research was conducted with the aim to evaluate the risk of cardiovascular disease in prediabetic subjects by measuring the markers of oxidative stress and inflammation and their possible correlation with glucose intolerance.

Materials and methods: A total of 400 human subjects were recruited for the present cross-sectional study. Of them, 200 were prediabetic subjects and 200 were age and gender-matched control subjects. Blood samples were collected from all participants and analyzed for 8-hydroxy-2′-deoxy-guanosine (8-OHdG), malondialdehyde (MDA), reduced glutathione (GSH) and high sensitivity C-reactive protein (hs-CRP).

Results: The markers of oxidative stress i.e. 8-OHdG and MDA were found to be significantly increased in prediabetic subjects as compared to control subjects except GSH, which was significantly reduced in prediabetic subjects. Similarly, hs-CRP (a marker of inflammation) was significantly increased in prediabetic subjects compared to controls. On correlation analysis, 8-OHdG, MDA and hs-CRP were significantly and positively correlated with glucose intolerance in prediabetes whereas GSH showed significant negative correlation with glucose intolerance.

Conclusion: In conclusion, markers of oxidative stress and inflammation should be taken into consideration while evaluating the risk for CVD in prediabetes since these markers were well correlated with glucose intolerance in prediabetic subjects.

1. Introduction

Prediabetes represents an elevation of plasma glucose level above the normal range but below clinical diabetes and considered as leading risk factor for type 2 diabetes mellitus [1]. It is often considered as gray area between normal blood glucose and diabetic levels. Prior to the development of diabetes mellitus, insulin sensitivity was reduced for a period of 13 years with a greater decline observed 5 years before the onset of type 2 diabetes and it has been reported that around 70% of the people with prediabetes will eventually develop diabetes [2]. Besides progression to diabetes mellitus, prediabetic individuals are at high risk for stroke, coronary artery disease and peripheral vascular disease [3]. The prevalence of prediabetes is increasing across the globe and according to International Diabetes Federation, the prevalence of prediabetes was reported to be 318 million in the year 2015 and expected to increase to 482 million by the year 2040 and if untreated will progress to diabetes and associated complications [4].

Increase in blood glucose levels lead to the generation of excess free radicals via several biochemical pathways and induce oxidative stress [5,6]. Oxidative stress can be defined as an imbalance

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between the production and elimination of reactive oxygen species (ROS), in favor of the former and subsequently excessive levels of these ROS can cause damage to macromolecules namely, proteins, carbohydrates, lipids and DNA [1] and is now recognized as being the cause and consequences of cardiovascular disease [7]. Among the many types of ROS-induced oxidative modifications, 8-hydroxy-2′-deoxy-guanosine (8-OHdG) has been widely used as a sensitive marker of oxidative DNA damage and is formed when a hydroxyl group can be added to the eighth position of the deoxy-guanosine molecule under the condition of oxidative stress [8]. In addition, several biomarkers of lipid peroxidation have been studied to monitor the production of ROS. Malondialdehyde (MDA) is the principal and one of the most important studied end-products of lipid peroxidation and is often considered as a surrogate marker of oxidative stress [9]. The products of DNA damage and lipid peroxidation can potentially be used as biomarkers for early diagnosis of disease, monitoring the progression of the disease and determining the efficacy of therapy [1].

The oxidative stress depends not only on the overproduction of ROS but also the deficiency of antioxidant defense mechanism [10]. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage [11] and its activity is known to reflect the altered redox balance of affected fluids, tissues, or organs in several pathological states. Therefore, antioxidant concentrations or measures of their activity have been used to estimate the amount of oxidative stress [12]. Reduced glutathione (GSH) is the most important endogenous antioxidant and plays important role in detoxification of ROS and prevents ROS induced cellular damage and thus providing protection against oxidative stress [13]. Moreover, GSH acts as a substrate to glutathione peroxidase-1 (Gpx-1), whose deficiency has been shown to be associated with increased risk for the development of coronary artery disease and cardiovascular events [14].

Low-grade systemic inflammation has considered as a key player in the pathogenesis of type 2 diabetes [15,16] and cardiovascular disease [17,18]. High sensitivity C reactive protein (hs-CRP) is an acute phase reactant protein with a short life of around 18 h, is considered as a potential biomarker of subclinical inflammation and has been shown to be increased in a hyperglycemic state [19]. It is produced and released by the liver under the stimulation of cytokines such as tumor necrosis factor α and interleukins 1 and 6 during inflammation and has shown to affects the process of atherothrombosis, hence considered as a powerful risk marker for cardiovascular disease [20].

The study regarding the role of oxidative stress and inflammation for prediction of cardiovascular disease in prediabetes is very limited. Hence, we embarked on a study to evaluate the risk of cardiovascular disease in prediabetes by measuring the markers of oxidative stress and inflammation and their possible correlation with glucose intolerance.

2. Materials and methods

2.1. Study design and inclusion criteria

This cross-sectional study was conducted in the Department of Biochemistry, Gajra Raja Medical College, Gwalior, India. The study population consists of total 400 human subjects, of them, 200 were prediabetic subjects, aged between 20 and 55 years of either sex and 200 were age and gender-matched control subjects. The participants were selected from general population, and those who were at risk of developing diabetes (who had at least one of the main risk factors for diabetes-first degree relative with diabetes, BMI $\geq$25 kg/m^2, women who were diagnosed with gestational diabetes mellitus, women with polycystic ovary syndrome, persons who are physically inactive, and other clinical conditions associated with insulin resistance, for example, severe obesity, acanthosis nigricans, etc.) in Gwalior City through a predesigned screening questionnaire.

The prediabetic subjects had been diagnosed on the basis of the American Diabetes Association (ADA) guidelines as:

a) Fasting plasma glucose level 100–125 mg/dL (Impaired fasting glucose, IFG) and
b) 2-h plasma glucose (after giving 75 gm of glucose) level 140–199 mg/dL (Impaired glucose tolerance, IGT) [21].

Those with a normal range of blood glucose level had been selected as the control group (Fasting plasma glucose (FPG) < 100 mg/dL, 2-h plasma glucose (2h-PG) concentration after giving 75 gm of glucose <140 mg/dL).

The study protocol was reviewed and approved by the Ethics Committee of Gajra Raja Medical College, Gwalior, India (Ref. No: 286/Bio/MC/Ethical, Approval date: 3 March 2017), and informed consent was obtained from people that participate in this study.

2.2. Exclusion criteria

Subjects with type 2 diabetes mellitus, clinical cardiovascular disease, renal disease, hepatic disease, pulmonary tuberculosis, acute or chronic inflammatory disease, gout and arthritis, prolonged illness, subjects who were on antioxidant supplements, subjects not willing to give consent or refuse to participate in the study, and patients receiving medicines known to interfere with glucose metabolism and inflammation were excluded from the present study.

2.3. Anthropometric measurements

Both weight and height were measured in light clothes and without footwear, using the standard apparatus. The weight was measured using calibrated electronic weighing scales prior to eating in the morning and height was measured to the nearest centimeter using a portable stadiometer. Waist circumference (WC) was measured using an anthropometric tape at a level on the skin midway between the mean point of iliac peak and the inferior border of the last rib at the level of the umbilicus while in a standing position at the end of gentle expiration. Hip circumference (HC) was measured over the widest part of the gluteal region at the level of pubic tubercle in standing position. Body mass index (BMI) of the participants was calculated as body weight (kg) divided by the square of height (m^2), BMI=Weight (Kg)/[Height (m)]^2. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference (cm) by hip circumference (cm).

2.4. Blood pressure measurements

The systolic and diastolic blood pressures were taken after 10 min of resting by using a standardized mercury sphygmomanometer using standard recommended procedures.

2.5. Biochemical measurements

After at least 10–12 h of overnight fasting, about 8 ml of venous blood samples were collected from all participants under all aseptic precautions and dispensed into 3 different tubes based on analysis to be done. About 2 ml of blood sample was taken into fluoride bulb for estimation of fasting plasma glucose, 2 ml blood was dispensed into an EDTA bulb for determination of reduced glutathione (GSH) in whole blood and remaining 4 ml blood sample was dispensed

into the plain bulb for analysis of 8-OHdG, MDA and hs-CRP. After that, 75 gm of glucose was given orally to each participant and about 2 ml blood sample was collected after 2 h and dispensed into fluoride bulb for determination of 2h-PG during OGTT. The collected blood samples were then centrifuged at 3000 rpm for 10 min in order to get serum/plasma. The serum samples were kept at −20 °C for 8-OHdG, MDA and hs-CRP analysis.

Blood glucose (both FPG and 2h-PG) was determined on fresh plasma sample on Mindray BS-400 chemistry analyzer (Mindray Medical International Ltd., Shenzhen, China) using a commercially available kit from ERBA Diagnostics, Mannheim, Germany on the same day of blood collection. Serum 8-OHdG was estimated by ELISA using a commercially available kit from Cloud-Clone Corp, (Katy, TX 77494, USA). Malondialdehyde (MDA) in serum was estimated using the thiobarbituric acid reacting substance [TBARS] by the method described by Satoh (1978) [22]. Reduced glutathione (GSH) was measured on a fresh sample in whole blood using the 5,5′- dithiobis-2-nitrobenzoic acid (DTNB) reaction by the method described by Beutler et al. (1963) [23]. Serum hs-CRP was measured by ELISA using commercially available kit from Diagnostics Biochem Canada (DBC) (Inc, Dorchester, Ontario, Canada).

2.6. Statistical analysis

All the quantitative variables were presented as mean ± standard deviation (SD). The normality of distribution of variables was checked using the Shapiro-Wilk test. For intergroup comparisons of normally distributed parameters Student Independent Sample *t*-test was used whereas for the intergroup comparisons of skewed data, the Mann-Whitney *U* test was used. For categorical data, a Chi-squared test was used. To assess the possible relationship between studied parameters, the Pearson's correlation analysis was done. A *p* value of less than 0.05 was considered to be statistically significant. All statistical measures were performed using the Statistical Package for Social Science version 20 (IBM, SPSS Statistics 20, Armonk, NY, USA).

3. Results

Table 1 demonstrates the general characteristics of the studied subjects. Subjects with both the groups were age and gender-matched since there was no difference in terms of age and gender between prediabetic and control subjects. There was significantly increased mean BMI, WC, HC, and WHR in prediabetic subjects compared to control subjects ($29.08 \pm 1.66\ kg/m^2$ vs. $23.42 \pm 0.94\ kg/m^2$, $p < 0.001$; 92.02 ± 3.91 cm vs. 82.51 ± 2.82 cm, $p < 0.001$; 95.99 ± 2.61 cm vs. 95.42 ± 2.42 cm, $p < 0.05$ and 0.96 ± 0.03 vs. 0.86 ± 0.03, $p < 0.001$, respectively). Prediabetic subjects had significantly increased systolic blood pressure (SBP) and diastolic blood pressure (DBP) than the control subjects (127.00 ± 5.66 mmHg vs. 116.30 ± 3.31 mmHg, $p < 0.001$ and 81.87 ± 3.63 mmHg vs. 77.48 ± 3.36 mmHg, $p < 0.001$, respectively). Table 2 demonstrates biomarkers of oxidative stress and inflammation in control and prediabetic subjects. Prediabetic subjects had significantly increased oxidative stress markers namely 8-OHdG (186.29 ± 10.76 pg/ml vs. 126.72 ± 15.94 pg/ml) and MDA (3.28 ± 0.28 nmol/ml vs. 2.47 ± 0.41 nmol/ml) compared to controls except for GSH which was found to be significantly decreased in prediabetes (25.84 ± 5.09 mg/dL vs. 29.61 ± 4.95 mg/dL). Also, there was a significantly increased level of inflammatory marker hs-CRP in prediabetic subjects as compared to control subjects (3112.95 ± 865.60 ng/ml vs. 1492.53 ± 591.79 ng/ml). Table 3 shows the correlation of markers of oxidative stress (8-OHdG, MDA and GSH) and inflammation (hs-CRP) with glucose intolerance (IFG and IGT) in prediabetes. 8-OHdG was positively and significantly correlated with IFG ($r = 0.760$, $p < 0.001$) and IGT ($r = 0.717$, $p < 0.001$). Similarly, MDA was positively and significantly correlated with both IFG ($r = 0.475$, $p < 0.001$) and IGT ($r = 0.476$, $p < 0.001$). The antioxidant GSH was significantly and negatively correlated with IFG ($r = -0.495$, $p < 0.001$) and IGT ($r = -0.368$, $p < 0.001$) both. The inflammatory marker hs-CRP is positively and significantly correlated with IFG ($r = 0.606$, $p < 0.001$) and IGT ($r = 0.455$, $p < 0.001$). Table 4 shows a correlation of 8-OHdG and MDA with GSH in prediabetic subjects. Both 8-OHdG and MDA were significantly and negatively correlated with GSH ($r = -0.456$, $p < 0.001$ and $r = -0.382$, $p < 0.001$ respectively). Table 5 shows a

Table 1
General characteristics of the studied subjects.

Variables	Control Subjects (n = 200)	Prediabetic Subjects (n = 200)
Age (years)	39.07 ± 7.60	39.90 ± 8.84^{NS}
Sex (M/F)	112/88	106/94^{NS}
FPG (mg/dl)	90.19 ± 3.83	116.13 ± 3.62**
2h-PG (mg/dl)	123.65 ± 6.20	150.13 ± 4.16**
BMI (kg/m^2)	23.42 ± 0.94	29.08 ± 1.66**
WC (cm)	82.51 ± 2.82	92.02 ± 3.91**
HC (cm)	95.42 ± 2.42	95.99 ± 2.61*
WHR	0.86 ± 0.03	0.96 ± 0.03**
SBP (mmHg)	116.30 ± 3.31	127.00 ± 5.66**
DBP (mmHg)	77.48 ± 3.36	81.87 ± 3.63**

FPG: Fasting plasma glucose; 2h-PG: 2-h plasma glucose; BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; WHR: Waist to hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ^{NS}Not significant; *Significant at $p < 0.05$; **Significant at $p < 0.001$.

Table 2
Biomarkers of oxidative stress and inflammation in control and prediabetic subjects.

Variables	Control Subjects (n = 200)	Prediabetic Subjects (n = 200)
8-OHdG (pg/ml)	126.72 ± 15.94	186.29 ± 10.76**
MDA (nmol/ml)	2.47 ± 0.41	3.28 ± 0.28**
GSH (mg/dl)	29.61 ± 4.95	25.84 ± 5.09**
hs-CRP (ng/ml)	1492.53 ± 591.79	3112.95 ± 865.60**

8-OHdG: 8-hydroxy-2′-deoxy-guanosine; MDA: Malondialdehyde; GSH: Reduced glutathione; hs-CRP: High sensitivity C-reactive protein; **Significant at $p < 0.001$.

Table 3
Correlation of markers of oxidative stress and inflammation with glucose intolerance in prediabetic subjects.

Parameters	FPG (IFG)		2h-PG (IGT)	
	r-value	*p*-value	r-value	*p*-value
8-OHdG	0.760	<0.001	0.717	<0.001
MDA	0.475	<0.001	0.476	<0.001
GSH	−0.495	<0.001	−0.368	<0.001
hs-CRP	0.606	<0.001	0.455	<0.001

8-OHdG: 8-hydroxy-2′-deoxy-guanosine; MDA: Malondialdehyde; GSH: Reduced glutathione; hs-CRP: High sensitivity C-reactive protein; FPG: Fasting plasma glucose; 2h-PG: 2-h plasma glucose; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance.

Table 4
Correlation of 8-OHdG and MDA with reduced glutathione (GSH) in prediabetic subjects.

Parameters	Reduced glutathione (GSH)	
	r-value	*p*-value
8-OHdG	−0.456	<0.001
MDA	−0.382	<0.001

8-OHdG: 8-hydroxy-2′-deoxy-guanosine; MDA: Malondialdehyde; GSH: Reduced glutathione.

Table 5
Correlation of markers of oxidative stress and inflammation with anthropometric parameters in prediabetic subjects.

Parameters	8-OHdG		MDA		GSH		hs-CRP	
	r-value	*p*-value	r-value	*p*-value	r-value	*p*-value	r-value	*p*-value
BMI	0.492	<0.001	0.301	<0.001	−0.341	<0.001	0.433	<0.001
WHR	0.391	<0.001	0.227	<0.01	−0.222	<0.01	0.353	<0.001
SBP	0.457	<0.001	0.301	<0.001	−0.254	<0.001	0.429	<0.001
DBP	0.387	<0.001	0.386	<0.001	−0.246	<0.001	0.299	<0.001

8-OHdG: 8-hydroxy-2′-deoxy-guanosine; MDA: Malondialdehyde; GSH: Reduced glutathione; hs-CRP: High sensitivity C-reactive protein; BMI: Body mass index; WHR: Waist to hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 6
Correlation of oxidative stress markers with inflammation in prediabetic subjects.

Parameters	Hs-CRP	
	r-value	*p*-value
8-OHdG	0.566	<0.001
MDA	0.425	<0.001
GSH	−0.369	<0.001

8-OHdG: 8-hydroxy-2′-deoxy-guanosine; MDA: Malondialdehyde; GSH: Reduced glutathione; hs-CRP: High sensitivity C-reactive protein.

correlation of markers of oxidative stress and inflammation with anthropometric parameters in prediabetic subjects. 8-OHdG, MDA and hs-CRP were significantly and positively correlated with BMI ($r = 0.492$, $p < 0.001$; $r = 0.301$, $p < 0.001$ and $r = 0.433$, $p < 0.001$ respectively), WHR ($r = 0.391$, $p < 0.001$; $r = 0.227$, $p < 0.01$ and $r = 0.353$, $p < 0.001$ respectively), SBP ($r = 0.457$, $p < 0.001$; $r = 0.301$, $p < 0.001$ and $r = 0.429$, $p < 0.001$ respectively) and DBP ($r = 0.387$, $p < 0.001$; $r = 0.386$, $p < 0.001$ and $r = 0.299$, $p < 0.001$ respectively). GSH was negatively and significantly correlated with BMI ($r = -0.341$, $p < 0.001$), WHR ($r = -0.222$, $p < 0.01$), SBP ($r = -0.254$, $p < 0.001$) and DBP ($r = -0.246$, $p < 0.001$). Table 6 shows a correlation of markers of oxidative stress with inflammation in prediabetic subjects. 8-OHdG and MDA were positively and significantly correlated with hs-CRP ($r = 0.566$, $p < 0.001$ and $r = 0.425$, $p < 0.001$ respectively) whereas GSH showed a negative correlation with hs-CRP ($r = -0.369$, $p < 0.001$).

4. Discussion

In this cross-sectional study, we have evaluated the risk of cardiovascular disease in subjects of prediabetes by measuring markers of oxidative stress (8-OHdG, MDA and GSH) and inflammation (hs-CRP) and found significant differences in the levels of these markers between control and prediabetic subjects. In addition, we found strong correlation of oxidative stress and inflammation with glucose intolerance in prediabetes.

The anthropometric parameters namely BMI, WC, HC and WHR were significantly increased in prediabetic subjects as compared to controls. Similar to the findings of our study, Ferrannini [24] and Agarwal et al. [25] also reported the significant increased BMI, WC, HC and WHR in prediabetes and suggested that prediabetic subjects have a higher rate of general obesity and central obesity which may predispose prediabetic subjects to an increased risk for CVD. In addition, we also found significantly increased levels of SBP and DBP in prediabetic subjects compared to controls which further increases the risk of CVD in prediabetes.

Oxidative stress and the generation of reactive oxygen species are believed to play an important role in the pathogenesis of atherosclerotic cardiovascular diseases. It is well known that DNA damage frequently occurs in cells exposed to reactive oxygen species [26]. Increased blood glucose leads to the generation of ROS which causes damage to the DNA, including oxidized bases and DNA strand breakage leading to increase in 8-OHdG and destruction of endothelial function resulting in atherosclerosis [27,28]. 8-OHdG is considered as a novel biomarker of oxidative DNA damage in vivo and is the most frequently detected and studied DNA lesion [29]. A significant increase in the level of 8-OHdG was noted in prediabetic subjects compared with control subjects. This result is consistent with the findings of previous studies [28,30,31]. Similarly, Maschirow et al. [32] and Kant et al. [1] found a significant rise in the level of 8-OHdG in prediabetic subjects as compared to controls though they have measured 8-OHdG in urine sample. Furthermore, on correlation analysis, 8-OHdG was found to be significantly and positively correlated with glucose intolerance i.e. both IFG and IGT in prediabetes. The increased level of 8-OHdG in prediabetes may be due to the hyperglycemia-induced generation of reactive oxygen species, which causes damage to the DNA [28]. Increased levels of 8-OHdG indicate an increase in the degree of oxidative stress, affecting tissue function and integrity and hence provides useful information on oxidative stress and disease progression [28,30]. It has been suggested that oxidative DNA damage plays a potential role in the pathogenesis of atherosclerosis, a major cause of cardiovascular disease [26] and formation of atherogenic lesions may be initiated in arterial smooth muscle cells by mutational events after DNA damage [33].

Another important biomarker related to oxidative stress is malondialdehyde (MDA) which is formed as a result of lipid peroxidation caused by ROS activity [34] and is considered as a major player in low-density lipoprotein modification. We found the significantly increased level of MDA in prediabetic subjects as compared to controls. The finding of the current study concurs with the results of previous studies [35,36]. A rise in serum MDA in prediabetic subjects reflects lipid peroxidation which is the consequence of oxidative stress. Also, the increase in the level of MDA significantly and positively correlates with glucose intolerance (with both IFG and IGT) in prediabetic subjects. This may be because of self-oxidation of glucose during hyperglycemia, which could generate free radicals and causing damage to the lipids. Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors [37]. Malondialdehyde (MDA) interacts with lysine residues in apo B-100 of low-density lipoprotein (LDL) to form oxidized LDL. Oxidized LDL plays important role in the induction of atherosclerosis (by stimulating monocyte infiltration and smooth muscle cell migration and proliferation), atherothrombosis (by inducing endothelial cell apoptosis), and plaque erosion (by impairing the endothelial anticoagulant balance), which ultimately leads to the development of cardiovascular disease (CVD) [38].

Besides generating reactive oxygen species (ROS), increased blood glucose level also attenuates anti-oxidative mechanisms by scavenging enzymes and antioxidant substances [39] A number of antioxidants exist in the cells either enzymatic (superoxide dismutase, glutathione peroxidase and catalase) or non-enzymatic (as glutathione and uric acid) as scavengers of ROS, to prevent

biological membranes from oxidative damage [40]. The most ubiquitous pool of antioxidants is erythrocyte reduced glutathione (GSH), which detoxify the free radicals and ROS entering the bloodstream. GSH acts as an electron donor in the conjugation reaction driven by glutathione transferase for detoxifying endogenous compounds [41]. In addition, GSH plays an excellent role by keeping up the cellular levels of active forms of Vitamin C and E by neutralizing free radicals, thus protecting the cells from oxidative damage [40]. Our data showed a significant reduction of reduced glutathione (GSH) in prediabetic subjects as compared to controls, which is parallel with the findings of previous studies [42,43]. Decreased level of GSH may be due to its reduced synthesis or it may have been used up in order to counterbalance the free radicals generated in prediabetes due to hyperglycemia. Our result also supports the findings of Nwose et al. [14], who also reported the decreased level of GSH in prediabetes and suggested that the initial phase of response by the erythrocytes commence prior to the establishment of diabetes. In addition, we found a significant and negative correlation between GSH and glucose intolerance (both IFG and IGT) in prediabetes, which confirms the link between hyperglycemia and decreased GSH. Furthermore, a negative correlation was observed between antioxidant GSH and oxidative stress markers MDA and 8-OHdG which indicate the existence of an abnormal balance between the pro-oxidants and protective mechanisms in subjects with prediabetes.

Moreover, it has been reported that inflammation is associated with prediabetes [25,43]. It has been suggested that chronic inflammation plays a causal role in endothelial dysfunction and formation of atherosclerotic plaque, contributing to the development of vascular complications in patients with diabetes [44]. Increased inflammation in the prediabetic state has been indicated in our study by increased concentration of hs-CRP, which is in agreement with the findings of previous studies [45,46]. Moreover, the correlative analysis revealed a strong positive correlation between hs-CRP and glucose intolerance (both IFG and IGT) in prediabetic subjects. These findings suggest the presence of underlying inflammatory milieu before the onset of diabetes mellitus, which predispose prediabetic subjects to an increased risk for the cardiovascular disease since elevated hs-CRP levels are associated with increased risk of cardiovascular disease [47]. Our finding also supports the hypothesis of Pickup and Crook, who stated that the chronic inflammation may act as an initiating factor in the development of type 2 diabetes mellitus [48]. The increased concentration of hs-CRP in prediabetes may be due to decreased insulin sensitivity, which is known to counteract the physiological effect of insulin on hepatic acute phase protein synthesis. Therefore, hepatic insulin resistance could lead to increased synthesis of CRP [49].

When we correlate the markers of oxidative stress and inflammation with anthropometric parameters in prediabetes, then we found significant and positive correlation of 8-OHdG, MDA and hs-CRP with BMI, WHR, SBP and DBP whereas GSH showed significant negative correlation with anthropometric parameters, indicating that obesity and increased blood pressure strongly contributes to the generation of reactive oxygen species which ends up in the induction of oxidative stress and creates the environment of inflammation. Also, in the present study, we have correlated the markers of oxidative stress namely, 8-OHdG, MDA and GSH with inflammatory marker hs-CRP in prediabetic subjects. Pearson's correlation coefficient showed a significant positive correlation of 8-OHdG and MDA with hs-CRP whereas the antioxidant GSH was significantly negatively correlated with hs-CRP in prediabetic subjects. This indicates that oxidative stress goes hand in hand with inflammation due to increased blood glucose in prediabetes. Inflammation in conjugation with a state of elevated oxidative stress participates in foam cell formation in atherosclerosis and hence accelerates the pathogenesis of the cardiovascular disease [50].

The strength of this study is that the subjects were selected on the basis of both fasting plasma glucose and 2-h plasma glucose (after giving 75 g of glucose). In addition, our study was carried out in a large sample size and was adequately powered since the significance of results was high. However, our study has got major limitations because of the cross-sectional nature of the data which limits the inferences about causal relationships between markers of oxidative stress and inflammation, and glucose intolerance.

5. Conclusion

In conclusion, the results of the present study indicate that the prediabetes is associated with oxidative stress (reflected by increased 8-OHdG, increased MDA and decreased GSH) and inflammation (reflected by increased hs-CRP), which put prediabetic subjects to an increased risk for the development of cardiovascular disease. Furthermore, markers of oxidative stress and inflammation were well correlated with glucose intolerance in prediabetic subjects. Hence, these markers should be taken into consideration while evaluating the risk for CVD in prediabetes. However, there is a need for further clinical studies to finally accept the concept.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all the participants included in the study.

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