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Increased lipid peroxidation and erythrocyte glutathione peroxidase activity of patients with type 2 diabetes mellitus: Implications for obesity and central obesity

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ABSTRACT

Aim: Both diabetes and obesity are a proxidant state by increased generation of reactive oxygen species. Obesity and central obesity is common among most of patient with type 2 diabetes. We aimed to assess the effect of obesity on GPx and SOD activity and lipid peroxidation of patients with type 2 diabetes mellitus, prediabetes and normoglycaemic healthy controls.

Methods: Hundred and forty seven type 2 diabetic patients (T2D), forty seven prediabetics (PDM) and hundred and six normoglycaemic controls (NGC) were recruited. T2D, PDM and NGC individuals were further clustered on the presence of obesity and central obesity. Erythrocyte GPx and SOD were determined using enzymatic reagent kits. Lipid peroxidation marker, malondialdehyde (MDA) was measured using thiobarbituric acid reactive substances (TBARS) assay.

Results: T2D had higher GPx activity and plasma MDA compared to PDM and NGC. PDM showed higher SOD activity than T2D and NGC. Obese and centrally obese T2D had higher GPx activity and plasma MDA with markedly reduced SOD activity.

Conclusion: Obesity and central obesity has further triggered the oxidative stress in T2D as indicated by higher plasma MDA and lower SOD activity. However, rise of GPx activity in parallel to the MDA among obese and centrally obese T2D suggest the induction of "reductive stress".

1. Introduction

Oxidative stress, imbalanced state of excess reactive oxygen species (ROS) and endogenous antioxidant defense is implicated in obesity, diabetes, cardiovascular diseases and atherogenic processes (Alba FS et al., 2011). Obesity mimics the oxidative stress in diabetes. Oxidative stress biomarkers tend to rise among obese individuals and related with the BMI and percentage of body fat, LDL oxidation and triglyceride levels (Pihl et al., 2006). Several studies have shown that presence of high body fat and central obesity could lower the antioxidant defense markers in individuals (Chrysohoou C et al., 2007; Hartwich J et al., 2007).

Glutathione peroxidase (GPx) and superoxide dismutase (SOD) are two major endogenous cytoprotective antioxidant enzymes present in humans. Expression of endogenous antioxidant enzymes, particularly GPx and SOD may alter the intracellular insulin signaling cascade favoring the cellular damage (Chen et al., 2003; Evans et al., 2003; Ceriello and Motz, 2004). Therefore, these two enzymes can be considered as key indicators of oxidative stress (Kumawat et al., 2013; Likidlilid et al., 2010). However, the previous findings on GPx and SOD activities in diabetics were controversial (Kumawat et al., 2013; Kaji et al., 1985; Kesavaulu et al., 2000; Kimura et al., 2003; Jandric-Balen et al., 2004; Fugita et al., 2011; Surapaneni and Venkataramana, 2007).

Obesity has shown to increase the lipid peroxidation and depleted levels of GPx and SOD (Olusi, 2002; Agarwal and Singh, 2017). Elevated levels of SOD (Michiels C et al., 1994) and GPx (Lavoie et al., 2011) were also reported among obese T2D. However, the contribution of obesity towards the GPx and SOD activities of diabetics still has not been completely identified. Therefore, present study was conducted with the objective of assessing the effect of obesity on GPx and SOD activities and lipid peroxidation of T2D.

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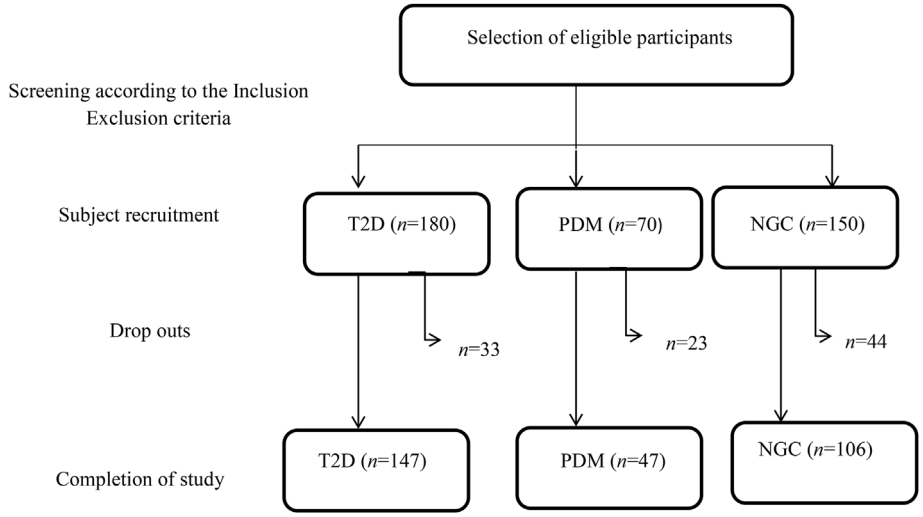

Fig. 1. Flow diagram of subject recruitment.
NGC-Normoglycaemic controls; PDM- Prediabetics; T2D- Individuals with Type 2 diabetes.

2. Materials and methods

2.1. Subjects

One hundred and forty seven (147) individuals with Type 2 Diabetes Mellitus (T2D) (mean age: 47.6 ± 8.3 years), 47 prediabetics (PDM) (mean age: 45.7 ± 8.8 years) with impaired fasting glycaemia between 6.1 and 6.9 mmol/L (WHO Expert Consultation, 2006) and 106 normoglycaemic controls (NGC) (mean age: 44.2 ± 8.2 years) were recruited in this cross sectional study. Details of the subject recruitment are given in Fig. 1.

T2D, PDM and NGC individuals with known history of heart disease, liver disease, kidney diseases or other endocrine diseases (except diabetes for T2D) and those who were ill for more than seven days during the last three months were excluded from the study. Smokers, regular users of alcohol (more than 2 times per week), those who follow weight loss regime, use antioxidant supplements (vitamin and minerals) and pregnant and lactating mother were also excluded. NGC those who had fasting glucose level < 6.1 mmol/L were included in the study (WHO Expert Consultation, 2006).

T2D patients, who were clinically diagnosed and verified by a general physician, recruited from the Diabetic clinics of the Government Hospitals in North Western Province of Sri Lanka. None of the T2D was on insulin treatment during the study period. PDM and NGC were enrolled from the Government and Corporate sector institutes in North Western Province after screening for fasting plasma glucose and anthropometric measurements.

The study protocol was approved by the Ethics Review Committee of Sri Lanka Medical Association (ERC/07/007). Written informed consent was obtained from each participant after providing the detailed description of the study. The study was designed in compliance with the Declaration of Helsinki.

2.2. Procedures and measurements

2.2.1. Medical and health history

Health and medical history of study participants were obtained using an interviewer administered general lifestyle questionnaire.

2.2.2. Physical measurements

Standing height and weight were measured in indoor light clothing using a stadiometer and digital weighing scale respectively. Waist circumference was measured using a non-stretchable tape at the point of umbilicus. Hip circumference was measured at the widest part of buttocks at the inter-trochantric level using a flexible tape.

2.2.3. Systolic and diastolic blood pressure

Seated systolic (SBP) and diastolic (DBP) blood pressures were measured in the non-dominant arm using a digital sphygmomanometer (Omron Ltd, Singapore) after resting for 10 min prior to the measurement.

2.2.4. Sample collection and storage

Ten milliliters of blood sample was drawn from the ulna vein through venipuncture after 12–14 h of fasting by a trained phlebotomist. Whole blood was kept frozen at −80 °C for later analysis of erythrocyte Glutathione Peroxidase (GPx) and Super Oxide Dismutase (SOD). Plasma separation was carried out by centrifugation at 4000 rpm at −10 °C for 10 min. Plasma was stored frozen at −80 °C for antioxidant and lipid peroxidation (LPO) analyses. For the other parameters, whole blood and separated plasma aliquots were kept frozen at −20 °C.

2.3. Biochemical analysis

2.3.1. Erythrocyte glutathione peroxidase (GPx)

Erythrocyte GPx level was measured using a commercially available reagent kit (RANSEL RS 505, Randox Laboratories, UK). This method is based on the early work of Paglia & Valentine in 1967 (Paglia and Valentine, 1967). Briefly, haemolyzed blood samples were diluted with the reagent buffer provided with the kit. Absorbance of the samples was read at 340 nm using a spectrophotometer (Model: UV/Visible Auto PC Scanning spectrophotometer; Labo Med, Inc, Germany Model) at 1 min intervals up to 3 min. Activity of the GPx was expressed as units per gram of haemoglobin per minute.

2.3.2. Erythrocyte Super Oxide Dismutase (SOD)

Erythrocyte SOD activity was measured using a commercially available reagent kit (RANSOD SD 125, Randox Laboratories, UK) based on the method described by Wooliams et al. in 1983 (Wooliams JA et al., 1983). Erythrocytes were washed and centrifuged for four times with 0.9% NaCl solution followed by sample dilution to attain percentage inhibition fell between 30 and 60%. Standard curve was developed using the SOD concentrations and % inhibitions. Activity of the SOD was expressed as units per gram haemoglobin per minute.

2.3.3. Lipid peroxidation

Plasma Malon Di Aldehyde (MDA) concentration was measured as a marker of lipid peroxidation using Thiobarbaturic acid reactive substances (TBARS) (Jentzsch et al., 1996). Briefly, 200 μL of heparinized plasma was first mixed with Butylated Hydroxy Toluene (BHT) and plasma proteins were precipitated by adding Tri Chloro Acetic acid (TCA). Then the Thiobarbaturic acid (TBA) was added to the mixture and incubated on a water bath set at 95 °C for 45 min. The resulting chromogen was extracted using the n-butanol and saturated NaCl solution. Organic mixture was separated by centrifugation. Intensity of the upper organic layer was measured using a spectrophotometer (Model: UV/Visible Auto PC Scanning spectrophotometer; Labo Med, Inc, Germany) at 535 nm and 572 nm against the blank. MDA level of the samples was determined using the linear standard curve developed by 0.5–32 μM of 1,1,3,3-tetraethoxypropane.

2.3.4. Fasting plasma glucose, lipids and insulin levels

Fasting plasma glucose and plasma lipid levels were assessed using enzymatic reagent kits. Fasting serum insulin level was measured according to the Sandwich ELISA method using a kit. Insulin resistance was derived using Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

2.4. Data analysis

T2D, PDM and NGC individuals were clustered for the presence of obesity, obese (+) Ob, (BMI $>$ 25 kgm^{-2}) and non-obese (−) Ob (BMI $<$ 25 kgm^{-2}) (WHO expert consultation, 2004) and central obesity, centrally obese (+) COb (W: H ratio $>$ 0.85 for females and W: H ratio $>$ 0.9 for males), non-centrally obese = (−) COb (W: H ratio $<$ 0.85 for females and W:H ratio $<$ 0.9 for males) (WHO expert consultation, 2008).

2.5. Statistical analysis

Data were checked for normality using Kolmogorov-Smirnov test and variables were log transformed when necessary. All variables were expressed as mean ± SD (standard deviation). One-way ANOVA was used to obtain the differences in variables among three study groups. Tukey's multiple comparison was used to determine significant differences between means. Spearman correlation coefficient was used to obtain the relationships between variables. Chi square test was used to compare the proportions. A value of $P < 0.05$ was considered as statistically significant. All the statistical analysis was conducted using the SPSS software (Statistical Package for Social Sciences), version 16.

3. Results

The three study groups of T2D, PDM and NGC were in the similar ages and had the similar gender distribution (male: female ratio of 4:5) with female dominance. Nearly half of the study participants have achieved the secondary education. Majority (60%) of them were employed and had a mean family income of 25,000 LKR (< 145 US$) per month.

Average clinical duration of the T2D was 5.0 ± 4.6 years. Nearly 99% of T2D were on oral hypoglycaemic agents (OHA) for glycaemic control. Only 1–2% reported that they were on dietary and physical activity (life style) modifications for glycaemic control. None of the T2D was on insulin treatment. Biguanides (Metformin) was the main type (93%) of OHA used. In addition, 46% of the diabetics used the Sulfonylureas (Tolbutamide, Glibenclamide, Diaonil) along with Biguanides. None of the PDM was on OHA for glycaemic control. However, only 61% of T2D and 54% of PDM were on proper glycaemic control ($HbA_1c < 7.0\%$, T2D; HbA1c < 6.5%, PDM). Thirty one (31%) percent of diabetics were hypertensive and on antihypertensive drugs. Presence of dyslipidemia among T2D was 20% and all were on lipid lowering drugs (statins). Twenty three (23%) of PDM and 12% of NGC had dyslipidemia and they were not on lipid lowering drugs.

According to the BMI (BMI $>$ 25 kgm^{-2}), 45% of T2D, 38% PDM and 48% of NGC were obese. The prevalence of central obesity was higher among T2D (87%) than PDM (70%) and NGC (67%). T2D had significantly ($P < 0.05$) higher SBP than PDM and NGC (127 ± 19 mmHg vs 121 ± 17 and 121 ± 16 mmHg).

Biochemical profile revealed that T2D had significantly ($P < 0.05$) higher fasting plasma glucose (FPG) (T2D, 7.9 ± 2.8 mmol/L vs PDM, 5.9 ± 1.2 mmol/L; NGC, 5.1 ± 0.6 mmol/L) and insulin resistance (HOMA-IR) (T2D, 3.6 ± 4.0 vs PDM, 2.7 ± 2.8 and NGC, 2.0 ± 2.5) over PDM and NGC study groups. Glycated haemoglobin concentration (HbA1c) of the T2D and PDM groups was significantly ($P < 0.05$) higher than the NGC group (T2D, 6.7 ± 1.5% and PDM, 6.7 ± 1.0% vs NGC 5.1 ± 0.7%). T2D and PDM had significantly ($P < 0.05$) higher plasma TAG compared to NGC (T2D, 1.6 ± . 0.78 mmol/L and PDM, 1.6 ± 0.85 mmol/L vs NGC, 1.30 ± 0.62 mmol/L)

T2D (+) Ob, T2D (−) Ob subgroups had the highest ($P < 0.05$) plasma FPG than PDM and NGC subgroups with and without obesity (T2D (+) Ob, 8.1 ± 3.0 mmol/L; T2D (−) Ob, 7.5 ± 2.5 mmol/L vs PDM (+) Ob, 6.1 ± 1.4 mmol/L; PDM (−) Ob, 5.7 ± 0.9 mmol/L, NGC (+) Ob, 5.1 ± 0.4 mmol/L and NGC (−) Ob, 5.0 ± 0.7 mmol/L). T2D and PDM subgroups with and without obesity had significantly higher ($P < 0.05$) HbA1c level compared to NGC subgroups (T2D (+) Ob, 6.7 ± 1.5%; T2D (−) Ob, 6.8 ± 1.5%; PDM (+) Ob, 6.7 ± 1.0%; PDM (−) Ob, 6.6 ± 1.1% vs NGC (+) Ob, 5.1 ± 0.5%; NGC (−) Ob, 5.1 ± 0.8%). T2D (+) Ob, T2D (−) Ob and PDM (+) Ob had significantly higher HOMA-IR than the PDM (−) Ob, NGC (+) Ob and NGC (−) Ob subgroups (T2D (+) Ob, 4.0 ± 4.6; T2D (−) Ob, 3.3 ± 3.5; PDM (+) Ob, 4.1 ± 3.2 vs PDM (−) Ob, 1.8 ± 1.0; NGC (+) Ob, 2.2 ± 2.1 and NGC (−) Ob, 1.8 ± 2.9). T2D (−) Ob and NGC (−) Ob subgroups had the highest plasma HDL levels than other subgroups (T2D (−) Ob, 0.99 ± 0.20 mmol/L and NGC (−) Ob, 0.95 ± 0.20 mmol/L vs T2D (+) Ob, 0.87 ± 0.30 mmol/L; PDM (+) Ob, 0.87 ± 0.20 mmol/L, PDM (−) Ob, 0.90 ± 0.20 mmol/L and NGC (+) Ob, 0.92 ± 0.30 mmol/L).

T2D (+) COb, T2D (−) COb, PDM (+) COb and PDM (−) COb subgroups had significantly higher ($P < 0.05$) FPG and HbA1c than NGC (FPG, T2D (+) COb 8.0 ± 2.8 mmol/L; T2D (−) COb, 7.1 ± 3.1 mmol/L; PDM (+) COb, 6.0 ± 1.2 mmol/L; PDM (−) COb, 5.9 ± 1.4 mmol/L vs NGC (+) COb, 5.1 ± 0.6 mmol/L and NGC (−) COb, 5.0 ± 0.44 mmol/L, HbA1c, T2D (+) COb, 6.8 ± 1.6%; T2D (−) COb, 6.4 ± 1.3%, PDM (+) COb, 6.5 ± 1.1%; PDM (−) COb, 7.1 ± 0.8% vs NGC (+) COb, 5.0 ± 0.7% and NGC (−) COb, 4.7 ± 0.8%). T2D (+) COb and PDM (+) COb subgroups had significantly higher ($P < 0.05$) HOMA-IR and plasma TAG levels than NGC (+) COb and NGC (−) COb subgroups (HOMA-IR, T2D (+) COb, 3.9 ± 4.2; PDM (+) COb, 2.9 ± 3.2 vs NGC (+) COb, 2.1 ± 2.4; NGC (−) COb, 1.8 ± 2.7, TAG, T2D (+) COb, 1.7 ± 0.8 mmol/L; PDM (+) COb, 1.7 ± 0.9 mmol/L vs NGC (+) COb, 1.5 ± 0.8 mmol/L and NGC (−) COb, 1.3 ± 0.6 mmol/L).

Lipid peroxidation marker, MDA was significantly ($P < 0.05$) higher among both T2D (6.4 ± 5.1 μmol/L) and PDM (6.0 ± 5.7 μmol/L) than NGC (4.7 ± 2.9 μmol/L) Fig. 2 (a). Once the study groups were further sub-grouped for obesity (Fig. 2 (b), T2D (+) Ob (7.4 ± 6.3 μmol/L) and PDM (+) Ob (7.0 ± 6.8 μmol/L) had significantly ($P < 0.05$) higher plasma MDA concentration over other subgroups with and without obesity (T2D (−) Ob, 5.5 ± μmol/L; PDM (−) Ob, 4.2 ± 2.1 μmol/L; NGC (+) Ob, 4.8 ± 2.5 μmol/L; NGC (−) Ob, 4.5 ± 2.5 μmol/L). T2D (+) Ob and PDM (+) Ob subgroups had significantly ($P < 0.05$) higher MDA than their respective non-obese subgroups (T2D (+) Ob, 7.4 ± 6.3 μmol/L vs T2D (−) Ob (5.5 ± μmol/L; PDM (+) Ob, 7.0 ± 6.8 μmol/L vs PDM (−) Ob, 4.2 ± 2.1 μmol/L) (Fig. 2 (b). Similar to the obesity subgroups, T2D (+) COb and PDM (+) COb had significantly (P < 0.05) higher plasma MDA than other subgroups with and without central obesity

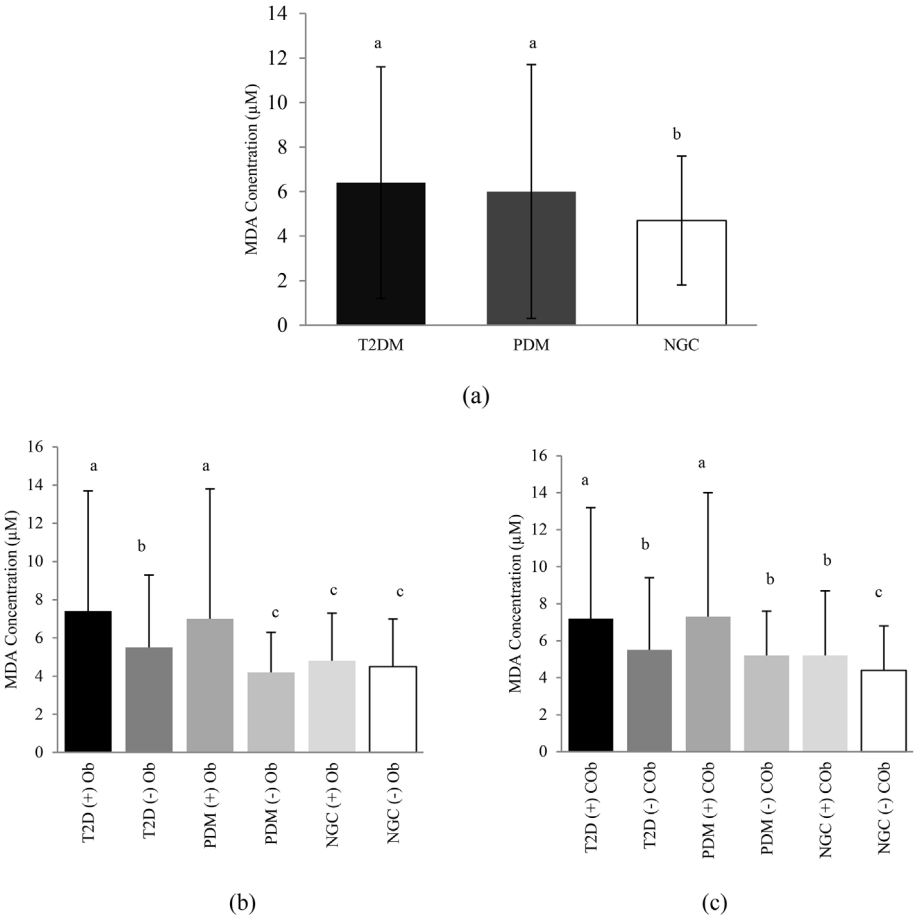

Fig. 2. Plasma MDA level of T2D, PDM and NGC groups (a), obese/non-obese (b) and centrally obese/centrally non-obese (c) sub groups of T2D, PDM and NG groups.
Means with the different superscripts are significantly different at P < 0.05 (Turkey's post hoc test).

(T2D (+) COb, 7.2 ± 6.0 µmol/L; PDM (+) COb, 7.3 ± 6.7 µmol/L vs T2D (−) COb, 5.5 ± 3.9 µmol/L; PDM (−) COb, 5.2 ± 2.4 µmol/L; NG (+) COb, 5.2 ± 3.5 µmol/L and NGC (−) COb, 4.4 ± 2.4 µmol/L) as shown in Fig. 2 (c). Further, T2D, PDM and NGC subgroups with central obesity had significantly ($P < 0.05$) higher plasma MDA than their centrally non-obese subgroups. Erythrocyte GPx activity Fig. 3 (a) was significantly elevated in T2D over the PDM and NGC (326 ± 249 vs 279 ± 203 and 244 ± 159 Ug/Hb per minute; $P < 0.05$). After grouping for the obesity, there was a significantly ($P < 0.05$) elevated GPx activity in T2D (+) Ob than PDM (+) Ob and NGC (+) Ob (342 ± 287 vs 300 ± 219 and 267 ± 147 Ug/Hb per min, $P < 0.05$) (Fig. 3 (b)).

T2D (+) COb had significantly ($P < 0.05$) higher GPx activity over the all other groups with and without central obesity T2D (+) COb, 350 ± 291 Ug/Hb per min vs T2D (−) COb, 300 ± 191 Ug/Hb per min; PDM (+) COb, 292 ± 243Ug/Hb per min; PDM (−) COb, 271 ± 171 Ug/Hb per min; NGC (+) COb, 272 ± 172 Ug/Hb per min; NGC (−) COb, 227 ± 152 Ug/Hb per min, Fig. 3 (c). T2D and NGC subgroups with central obesity showed significantly ($P < 0.05$) higher GPx activity than their respective non-centrally obese group, ((T2D (+) COb, 350 ± 291 Ug/Hb per min vs T2D (−) COb, 300 ± 191Ug/Hb; NGC (+) COb, 272 ± 172 Ug/Hb per min vs NGC (−) COb, 227 ± 152 Ug/Hb per min, (Fig. 3 (c)).

In contrast, erythrocyte SOD activity Fig. 4 (a) was significantly ($P < 0.05$) higher among the PDM (4418 ± 3888 Ug/Hb per min) than NGC (3611 ± 2918 Ug/Hb per min) and T2D (797 ± 560 Ug/Hb per min). Lowest SOD activity was shown by the T2D (+) Ob and T2D (−) Ob subgroups. After grouping the subjects for the presence of obesity, PDM (−) Ob showed the highest SOD activity (4188 ± 3898 Ug/Hb per min). NGC (+) Ob (4150 ± 3199 Ug/Hb per min) showed a significantly ($P < 0.05$) higher SOD activity over the T2D (+) Ob (734 ± 534 Ug/Hb per min), T2D (−) Ob (850 ± 581 Ug/Hb per min) and, NGC (+) Ob (2561 ± 2789 Ug/Hb per min), Fig. 4 (b).

There was a significantly ($P < 0.05$) higher SOD activity among PDM (−) COb (5180 ± 2960 Ug/Hb per min) than T2D (+) COb (690 ± 527 Ug/Hb per min), T2D (−) COb (904 ± 597 Ug/Hb per min), PDM (+) COb (4349 ± 3945 Ug/Hb per min), NGC (+) COb (3091 ± 2555 Ug/Hb per min) and NGC (−) COb (3953 ± 3181 Ug/Hb per min) subgroups. Lowest SOD activity was shown by the T2D (+) COb and T2DM (−) COb subgroups. NGC (−) COb had significantly higher SOD activity over T2DM subgroups, and NGC (+) COb subgroup Fig. 4 (c).

For better understanding of relationship between lipid peroxidation, GPx and SOD activities of obese and centrally obese subgroups, correlations between plasma MDA, GPx and SOD activities are shown in Table 1. BMI and WHR had positive correlations with plasma MDA and GPx and negative correlations with SOD activity among obese and centrally obese T2D (Table 1).

4. Discussion

In the present study, we investigated the effect of obesity on erythrocyte GPx and SOD activities and plasma lipid peroxidation marker, MDA. We found that T2D had higher GPx activity compared to PDM and NGC subgroups. Both T2D and PDM had higher MDA levels than NGC. Similar to our findings, increased GPx activity (Kaji et al., 1985;

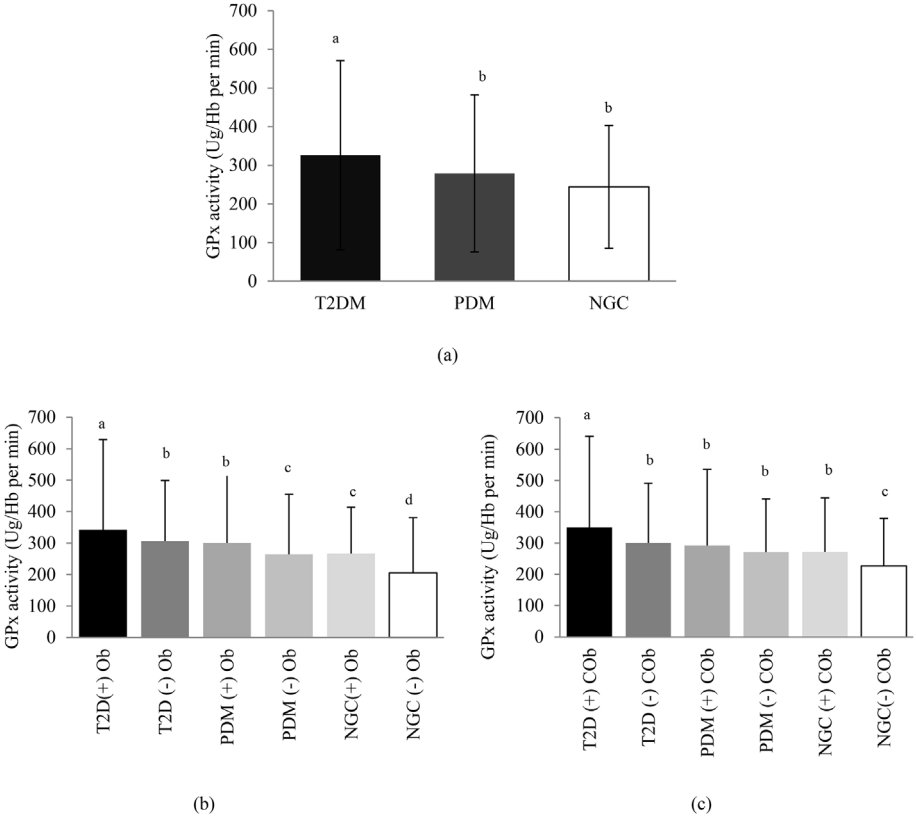

Fig. 3. Erythrocyte GPx activity of T2D, PDM and NGC groups (a), obese/non-obese (b) and centrally obese/centrally non-obese (c) sub groups of T2D, PDM and NG groups.
Means with the different superscripts are significantly different at $P < 0.05$ (Turkey's post hoc test).

Matkovics et al., 1982; Rema et al., 1995) and MDA levels has been previously reported among T2D (Moussa, 2008; Bandeira et al., 2012) and PDM (Dzięgielewska-Gęsiak et al., 2014). PDM showed the higher erythrocyte SOD activity than T2D and NGC in the present study. Higher and lower SOD activity among PDM and T2D (Dzięgielewska-Gęsiak et al., 2014; Domingeus C et al., 1998) particularly higher SOD activity among the T2D at the onset of diabetes has been reported previously (Domingeus C et al., 1998).

In our study, obese and centrally obese T2D subgroups had the highest GPx activity and plasma MDA level. It is well consistent with the previous findings on obese T2D (Colas et al., 2011; Madhikarmi et al., 2013). Therefore, we can speculate that, parallel to the rise of MDA, GPx activity has raised to counteract the deleterious effects of excess lipid peroxidation in obese and centrally obese T2D. Presence of positive correlations between BMI and GPx; BMI and MDA; WHR and GPx and WHR and MDA among obese and centrally obese T2D further verify the above hypothesis. Marked reduction of SOD activity among obese and centrally obese T2D even below the level of T2D study group advocates the presence of excess lipid peroxidation and subsequent inactivation of SOD by excess hydrogen peroxide generated (Fajans, 1995). It is further evident with the presence of negative correlations between BMI and SOD and WHR and SOD among obese and centrally obese T2D, respectively. Obese and centrally obese T2D has the highest FPG and HbA1c levels in the current study. Hence, significant reduction of SOD activity can also be linked to the progressive enzymatic glycation of SOD as previously revealed (Arai et al., 1987).

Excess fat accumulation and accompanied deregulations in adipokine production has shown to contribute to the insulin resistance in obesity (Ikeoka et al., 2010; Jung and Choi, 2014; Hotamisligil GS and Spiegelman, 1993; Uysal KT et al., 1997). Raised GPx activity has shown to be complemented with insulin resistance (Oliveira AC et al., 2009; Tinahones FJ et al., 2009; Michiels C et al., 1994), particularly with the obese individuals with insulin resistance (Oliveira AC et al., 2009; Tinahones FJ et al., 2009; Matsuzawa-Nagata N et al., 2008). Although the mechanism by which the insulin resistance increases the GPx activity is not clearly understood, rise in GPx activity of obese and centrally obese T2D and PDM in present study may be partly contributed by the presence of insulin resistance among them.

Findings on GPx activity among T2D particularly among obese and centrally obese T2D of the present study supports the concept of "reductive stress". Elevated GPx tissue expression through the induction of reductive stress has been previously reported (Zhang et al., 2010). Further, increased tissue expression of GPx appears to be hydrogen peroxide mediated similar to our findings (Wagner et al., 2009). GPx compensates the excess peroxide generation in lipid peroxidation, favoring the subsequent degradation of peroxides thus contributes to the reductive stress in obesity (Matsuzawa-Nagata N et al., 2008; Zhang et al., 2010; Michiels C et al., 1994). Reductive stress expressed through the pronounced GPx activity, suggests either to be one of or the first event in initiating the metabolic alterations underneath the accompanied cardio-metabolic comorbidities of obesity (Zhang et al., 2010).

5. Conclusions

T2D, particularly the obese and centrally obese T2D experienced the greater oxidative stress as shown by the elevated plasma lipid peroxidation marker, MDA and reduced SOD activity compared to PDM and NGC. Elevated SOD activity in PDM suggests the initiation of antioxidant defense against the systemic oxidative stress. Rise of GPx activity in parallel to the MDA among obese and centrally obese T2D advocates the induction of "reductive stress". Reductive stress indicated by the pronounced GPx activity in our study can be considered as first or one of the initial steps of depositing metabolic alterations of obesity and central adiposity. Further, genetic expression of GPx represents a

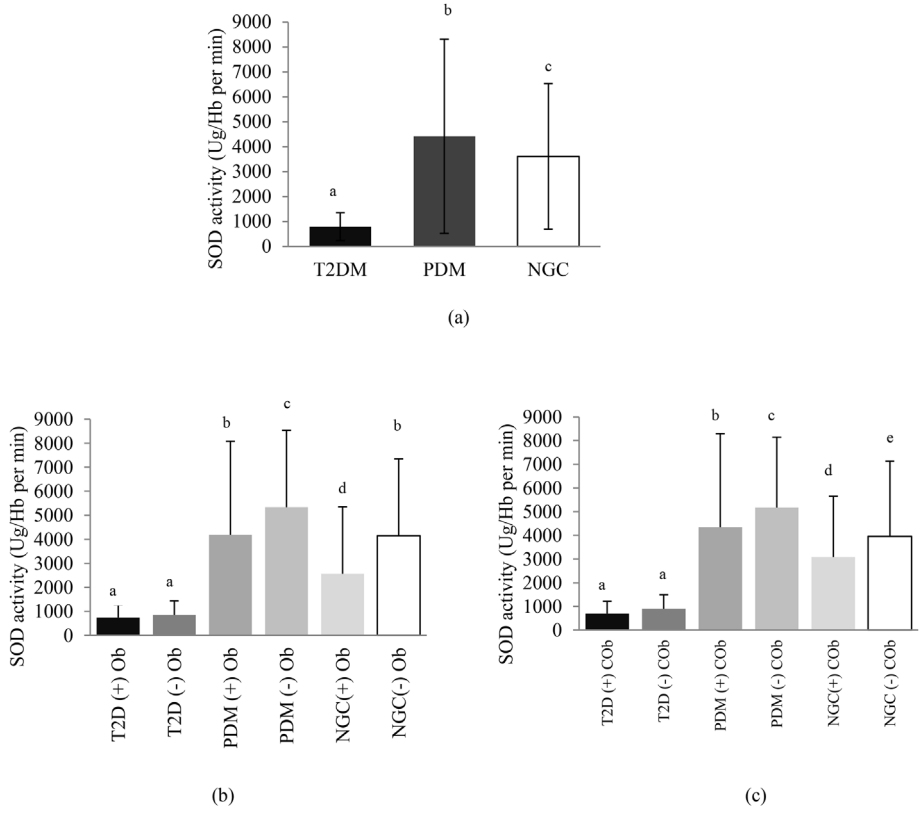

Fig. 4. Erythrocyte SOD activity of T2D, PDM and NGC groups (a), obese/non-obese (b) and centrally obese/centrally non-obese (c) sub groups of T2D, PDM and NG groups.
Means with the different superscripts are significantly different at $P < 0.05$ (Turkey's post hoc test).

Table 1
Correlation coefficient between plasma MDA, GPx and SOD activities in obese and centrally obese T2D subgroups.

	GPx (U/g Hb per min)		SOD (Ug/Hb per min)		MDA (μmol/L)	
	T2D (+) Ob	T2D(+) Cob	T2D (+) Ob	T2D (+) COb	T2D (+) Ob	T2D (+) COb
BMI	n = 66	n = 129	n = 66	n = 129	n = 66	n = 129
	r = 0.23	r = 0.24	r = - 0.17	r = −0.23	r = 0.30	r = 0.33
	P = 0.044	*P* = 0.047	*P* = 0.047	*P* = 0.041	*P* = 0.021	*P* = 0.049
WHR	n = 66	n = 129	n = 66	n = 129	n = 66	n = 129
	r = 0.20	r = 0.40	r = −0.20	r = −0.24	r = 0.21	r = 0.18
	P = 0.043	*P* = 0.021	*P* = 0.049	*P* = 0.047	*P* = 0.049	*P* = 0.043

BMI- Body mass index; GPx-glutathione peroxidase activity; MDA, plasma malondialdehyde, SOD-superoxide dismutase activity; WHR-waist to hip ratio.

great opportunity to study and predict the cardio-metabolic abnormalities of humans. Hence we suggest more comprehensive studies to generate the sequence of events associated with GPx induced reductive stress and later development of metabolic abnormalities of obesity.

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Conflicts of interest

None.

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Appendix A. Supplementary data

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