ORIGINAL ARTICLE

Oxidant-Antioxidant disturbance in men classified as obese according to the preliminary WHO guidelines for Asians

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Background: Though there are experimental and clinical evidences regarding oxidant-antioxidant disturbance in obese subjects, clinical data supporting the same in Indian male subjects is lacking. The objective of the present study was to verify the oxidative stress status of male subjects classified as obese according to the WHO guidelines for Asians.

Methods: Thirty six obese men with BMI between 25-30 Kg/m² and 30 non-obese men with BMI < 25 Kg/m² were enrolled in the study. Malondialdehyde, reduced glutathione, glutathione peroxidase, catalase, fasting glucose and body mass index were assessed in both the groups.

Results: Plasma MDA and erythrocyte activity of glutathione peroxidase were significantly increased in the obese subjects when compared with controls. The levels of reduced glutathione were significantly reduced in the obese group when compared with controls. Among the obese group, BMI was significantly associated with MDA and glutathione peroxidase. Further among the obese subjects, glutathione peroxidase correlated significantly with MDA. A significant negative correlation was obtained between MDA and GSH in obese subjects.

Conclusion: The data from the present study indicates a significant perturbation of the oxidant – antioxidant status in Indian males considered as obese according to the preliminary WHO guidelines for Asians. The increase in oxidative stress and glutathione peroxidase activity in obesity may contribute towards its pathological complications.

Key words: Obesity, oxidative stress, glutathione peroxidase, malondialdehyde
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Obesity is characterized by increased adipose tissue mass resulting from a chronic energy imbalance between energy intake and energy expenditure (Tataranni, Ravussin 2002). It is widely accepted that both genetic and environmental factors (such as diet) can predispose individuals to the development of obesity (Pi-Sunyer, 2002). Although body mass index (BMI) is the most frequently used index of obesity, it does not reflect fatness uniformly in all populations, and Caucasians have higher BMI than the Asians (Deurenberg et al, 1998). This has promoted the World Health Organization to issue preliminary Asian obesity guidelines, in which it is recommended to consider Asians as obese if their BMI is ≥ 25 kg/m² (WHO, 2000).
The increasing prevalence of obesity in recent decades as a public health problem in both developed and developing countries is of concern, given the comorbidities of this condition. Obesity is recognized as a risk factor for insulin resistance, which can lead to major diseases, such as type 2 diabetes and cardiovascular disease (Tataranni, 2002; Nielsen, Jensen, 1997). A common pathological process associated with these disease conditions is oxidative stress (Evans et al, 2002; Soccio et al, 2005).

The role of oxidative stress and reactive oxygen species (ROS) in the pathophysiology of obesity has been recently the focus of many investigations (Vincent, Taylor, 2006; Atabek et al, 2004; Vincent et al, 2007). Under physiological conditions, ROS produced in the course of normal metabolism are fully inactivated by an elaborate cellular and extracellular antioxidant defence system. Oxidative stress occurs when redox homeostasis within the cell is altered. This imbalance may be due to either an overproduction of ROS or deficiency of an antioxidant system (Yu, 1994). Among these, the enzymes glutathione peroxidase and catalase act as important endogenous antioxidants. Antioxidant enzymes play a crucial role in determining individual risk of developing certain diseases, such as cancer and atherosclerosis (Flora, 2007). Reduced glutathione (GSH), catalase and glutathione peroxidase play important roles in neutralizing the deleterious effects of peroxides (Mates et al, 1999).

Even though a number of studies in the literature have shown the presence of oxidative stress in obese individuals from other ethnic groups (Flora, 2007; Mates et al, 1999; Reitman et al, 2002; Olusi, 2002), little information is available on the activity of glutathione peroxidase, catalase activity and glutathione concentration in obese individuals from the Indian subcontinent. As oxidative stress can be modulated by nutritional, genetic and environmental factors, it was deemed pertinent to study the erythrocyte glutathione peroxidase, catalase activity and reduced glutathione along with lipid peroxides in normotensive, non-diabetic obese Indian subjects and to evaluate their association with BMI. To our knowledge, this is the first study to compare these parameters in obese and non-obese Indian subjects defined using WHO Asia Pacific guidelines.

MATERIALS AND METHODS

Subjects
The study population consisted of 36 obese male subjects with a BMI between 25 to 30 Kg/m² and 30 non-obese age matched subjects (BMI < 25 Kg/m²). Subjects with history of diabetes, hypertension and any infection were excluded from the study. Informed consent was obtained from all individuals after the purpose and nature of the study had been explained. This study was approved by the ethics committee of our institute. Body weight and height of the participants were measured. BMI was calculated as weight in kilogram divided by squared height in meter.

Sample collection:
After subjects had fasted overnight for at least eight hours, blood (5 ml) was drawn and collected in EDTA bottles. Whole blood was used for the estimation of hemoglobin and reduced glutathione. Plasma was collected from the rest of the sample by centrifuging at 5000 rpm for 5 minutes at 4°C. Plasma glucose levels were estimated immediately. Erythrocytes were washed with cold saline 3 times and then were lysed with cold distilled water (1 in 5 dilutions). This lysate was used for the estimation of glutathione peroxidase and catalase activity.
Analytical methods:

Whole blood glutathione was determined as described by Beutler et al (1963). Hemoglobin content of blood was estimated by cyanmethemoglobin method of Drabkin et al (1932). Fasting plasma glucose was measured by fully automated glucose oxidase method in Ciba Corning 550 express plus. Glutathione peroxidase activity in RBC was determined according to the method of Wendell (1981). Catalase was estimated in erythrocyte by the method of Aebi (1984). Plasma MDA measured as TBARS was estimated by the method of Yagi (1984).

Statistical analysis:

Results were statistically analyzed by SPSS version 10. Student’s t-test was used to assess the significance of difference between the groups. All results are presented as mean ± S.D. A 'p' value of less than 0.05 was considered significant.

RESULTS

The clinical characteristics of the subjects are shown in Table 1. As shown in the table the study groups were well matched for age distribution with respect to their control group. There was no significant difference in the levels of fasting blood glucose between the study groups. As shown in Table 1, the levels of whole blood glutathione were reduced among obese subjects when compared with controls. The erythrocyte glutathione peroxidase activity was higher in the test subjects compared with the control group. The levels of whole blood glutathione were significantly reduced among obese subjects when compared with controls. The levels of lipid peroxidation measured as MDA was significantly increased in the obese test group when compared with that of controls. But, there was no significant difference in activity of erythrocyte catalase between the study groups.

When Pearson correlation was performed, glutathione peroxidase correlated significantly with BMI (r = 0.394, p = 0.017; figure 1). Similarly, MDA had a significant association with BMI (r = 0.483, p = 0.003; figure 2). Apart from the significant association with BMI, MDA had a significant correlation with both glutathione peroxidase (r = 0.603, p = < 0.001; figure 3) and reduced glutathione ( r = - 0.466, p = 0.004; figure 4).

Table 1: Anthropometric and biochemical characteristics of the study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n =30)</th>
<th>Obesity (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>35 ± 9</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 1.88</td>
<td>26.97 ± 1.47**</td>
</tr>
<tr>
<td>Waist/Hip ratio</td>
<td>0.89 ± 0.04</td>
<td>0.93 ± 0.02**</td>
</tr>
<tr>
<td>Systolic Blood pressure (mm Hg)</td>
<td>117 ± 5</td>
<td>120 ± 8</td>
</tr>
<tr>
<td>Diastolic Blood pressure (mm Hg)</td>
<td>76 ± 5</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>86.17 ± 17.75</td>
<td>93.30 ± 16.04</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>2.46 ± 1.20</td>
<td>3.26 ± 1.74*</td>
</tr>
<tr>
<td>GSH (mg/g Hb)</td>
<td>3.58 ± 1.26</td>
<td>2.83 ± 1.28*</td>
</tr>
<tr>
<td>Glutathione - Peroxidase (u/g Hb)</td>
<td>44.84 ± 27.93</td>
<td>87.23 ± 42.46**</td>
</tr>
<tr>
<td>Catalase (k/ml)</td>
<td>25.29 ± 12.13</td>
<td>22.22 ± 11.04</td>
</tr>
</tbody>
</table>

* p < 0.05 and ** p < 0.01 compared to controls
Figure 1: Linear regression analysis of glutathione peroxidase and BMI in obesity ($r = 0.40$, $p = 0.017$).

Figure 2: Linear regression analysis of MDA and BMI in obese Indian subjects ($r = 0.483$, $p = 0.003$).
Oxidant-Antioxidant disturbance in men...

Figure 3: Linear regression analysis of MDA and glutathione peroxidase in obese subjects ($r = 0.603$, $p = 0.001$).

Figure 4: Linear regression analysis of MDA and GSH in obese Indian subjects ($r = -0.47$, $p = 0.004$).

DISCUSSION

There is an overwhelming evidence to indicate that oxidative stress, defined as an imbalance between oxidants and antioxidants in favor of the...
former, leads to many biochemical changes and these are important pathological mediators in a wide spectrum of human disease (Vincent, Taylor, 2006). Oxygen free radicals are highly reactive and attack almost every cell component causing damage to the surrounding tissues (Vincent et al, 2007).

The most deleterious impact of oxidative stress is lipid peroxidation, which has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer, and aging (Spiteller, 2007). Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition by oxygen radicals, resulting in the oxidative deterioration of polyunsaturated fatty acids (Cheng, Li, 2007). Several previous studies have reported significant increase in the levels of lipid peroxides in obese subjects of different ethnic groups (Mates et al, 1999; Yesilbursa et al, 2005; Zwirska-Korczala et al, 2003; Mohn et al, 2005). This study demonstrates an elevated concentration of MDA in obese Indian subjects, which reflects in vivo oxidative damage to lipids. Apart from finding increased MDA levels in obese, we found a significant correlation between the levels of lipid peroxides and BMI. This is in agreement with other studies reporting a significant association between MDA and BMI in obese subjects (Zwirska-Korczala et al, 2003; Mohn et al, 2005) and it has also been suggested that obesity on its own is an independent risk factor for plasma lipid peroxidation (Mates et al, 1999).

Among the elaborate antioxidant defence system of erythrocyte, the primary catalytic cellular defense that protects cells and tissues against lipid peroxidation is the glutathione peroxidase enzyme (Arthur, 2000). Glutathione peroxidase is a selenium containing enzyme having single seleno-cysteine residue in each of the four identical subunits, which are essential for enzyme activity. It has been observed that glutathione peroxidase can be rapidly induced in some conditions where cells or organisms are exposed to oxidative stress (Lu et al, 1993). The increased glutathione peroxidase activity of obese subjects in the present study may be interpreted as compensatory mechanism due to increased lipid peroxidation. Because it has been shown that glutathione peroxidase is more potent on a molar basis than catalase and other antioxidant enzymes to protect cells from oxidative stress (Lu et al, 1993), it can be hypothesized that body tends to combat stress by over expressing glutathione peroxidase gene as the first line of defense in obese subjects. However, the marked increase in the activity of this enzyme is not sufficient to protect cells during oxidant exposure, since increased MDA levels indicate that oxidative damage has already occurred. In addition to the increased glutathione peroxidase activity in obese subjects we have also found significant positive association of glutathione peroxidase with BMI and MDA.

McClung et al have reported that transgenic mice over-expressing glutathione peroxidase become heavier than the wild type mice (McClung et al, 2004). It has also been demonstrated that the glutathione peroxidase overexpressed mice developed hyperglycemia, hyperinsulinemia, and elevated plasma leptin concentrations, as well as reduced phosphorylation of Akt (a kinase downstream of the insulin receptor) in both liver and muscle after insulin stimulation. It has been postulated that increased glutathione peroxidase activity might over quench intracellular reactive oxygen species that are required for the insulin signaling (McClung et al, 2004). This finding is also supported by the report of Li et al that β-cell-specific overexpression of catalase and metallothionein in mice results in accelerated...
Oxidant-Antioxidant disturbance in men...

spontaneous diabetes and altered insulin signaling (Li et al., 2006). Thus, it can be speculated that the increased glutathione peroxidase activity seen in obese subjects may act as cofactor in the development of insulin resistance in these subjects.

Catalase is yet another free radical scavenger that is known for its detoxification action against lipid peroxides (Yu, 1994). But, we found no significant alteration in the activity of erythrocyte catalase in obese individuals when compared with controls. It can be concluded that neither the activity nor the expression of this antioxidant enzyme is altered in non-diabetic, normotensive obese subjects.

Among the aqueous-phase non-enzymatic antioxidant defense system, reduced glutathione plays a major role in combating lipid peroxidation (Chaudière, Ferrari-Iliou, 1999). Apart from this, its functions include the detoxification of xenobiotics, carcinogens, regulation of immune function; and maintenance of protein structure, function, and turnover (Chaudière, Ferrari-Iliou, 1999). Reduced levels and altered metabolic turnover of glutathione have been previously reported in obese human subjects (Faber et al., 2002; Cazzola et al., 2004). Our results showed that erythrocyte GSH was depleted in obese subjects who were non-diabetic, normotensive. A significant negative correlation was also observed between GSH and MDA in obese subjects which in turn indicates a severe disturbance in the antioxidant defence mechanisms in these subjects.

To conclude, our results point towards an imbalance in the oxidant / antioxidant ratio in non-diabetic, normotensive obese Indian subjects. These findings lend further support for the implementation of primary prevention programmes for type 2 diabetes and chronic heart diseases. Future epidemiological polymorphic studies to identify candidate antioxidant genes that are altered in obese individual are warranted. This will increase our understanding of the genetic modulation of antioxidant enzymes in these subjects which will be useful for the development of molecular interventions in them. Further studies are also required to define whether dietary or supplemental antioxidants ameliorate these processes.

REFERENCES


Oxidant-Antioxidant disturbance in men...


