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COMPARISON OF OXIDATIVE STRESS PARAMETERS BETWEEN DIABETIC AND OBESE PATIENTS

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ABSTRACT

Oxidative stress and inflammation are implicated in the initiation and development of obesity and diabetes .This study investigated the comparison of important oxidative stress parameters between diabetic and obese patients and it is significantly ($p < 0.05$) showed that the level of oxidative stress is more in obese. Level of Hs-CRP also has been increased in the obese patients. It is revealed that oxidative stress has a main role in this disease.

KEYWORDS: **Obesity, Diabetics, Oxidative stress, Antioxidant,**

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INTRODUCTION

Oxidative damage has been implicated in the pathogenesis of many chronic progressive diseases, such as inflammation, and neurodegenerative disorders. Over the last decade, there has been considerable interest in the role of oxidative stress in vascular disease as well. This interest has been driven by a affluence of data indicating that LDL[1] oxidation is a prominent feature of atherosclerosis .More recently, studies have also suggested that oxidative stress is a feature of many risk factors for premature atherosclerosis, such as diabetes,[2] and obesity. Obesity is an established risk factor for many diseases like diabetes, CVD and metabolic syndrome[3].Oxidative stress can be a consequence and also a trigger for obesity[4] .The inefficient scavenging of ROS in diabetes may countribute toincreased oxidative damage. The mechanism of impaired antioxidant defense in DM is poorly understood. Non enzymatic glycation of antioxidant enzymes with a consequent loss of activity may be one of the causes[5]. In this study we investigate to compare the level of oxidative stress parameters in diabetic and obese patients.

MATERIALS AND METHODS

DPPH was purchased from Sigma Chemical Company, USA. Ascorbic acid and BHT were purchased from SR Laboratories, Mumbai, India. All other chemicals, solvents and reagents were purchased from local suppliers and the chemicals were of highest purity. The study protocol was approved by the ethical committee of Mysore University, India and the consent was taken from all of the subjects. The weight of 20 subjects were recorded by a weight machine with accuracy of $\pm$ 100 gm, models KRPUS (New Delhi, India) and standing height measured without shoes to the nearest 0.5 centimeter with use of commercial meter, with the shoulders in relaxed position and arms hanging freely. Body mass index (BMI) calculated by weight (kg) divided by height in meter square (m^2). Blood was drawn by vein puncture at the Biochem Diagnostic and Research Lab. Whole blood was used for blood glucose estimation immediately. The remaining blood was allowed to clot. The serum was separated by centrifugation and used for assay of High Sensitive C-Reactive Protein (Hs-CRP), total antioxidant capacity and DPPH radical scavenging activity.

Phosphomolybdate method

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using vitamin E as the standard[6]. An aliquot of 0.1ml of the fractions (100 µg) solution was combined with 1.0 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95oC for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against the blank using an UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as rest of the sample. The total antioxidant capacity was expressed as µg equivalents of vitamin E by using the standard vitamin E graph.

Determination of lipid peroxidation

Lipid oxidation in lipoproteins was assessed by spectrophotometric monitoring of conjugated diene formation, according to the method of [7] with slight modification. Briefly, serum (5ul) was diluted with phosphate buffered saline (PBS, 995µl) (5 mM phosphate buffer, 125mM NaCl, pH 7.4). LDL and HDL were diluted 1:50 with PBS. 100 µl of diluted serum sample, LDL or HDL was mixed with 850µl PBS and 50µl of 5µM copper sulfate ($CuSo_4$). The OD of the mixture was monitored at 234 nm every 10 min for up to 90 min. The optical density was plotted against time.

Estimation of serum C-reactive protein (CRP)

With the ultra sensitive C-reactive protein Latex test Kit (Acros organics, Bangalore, India), latex particles coated with antibody specific to CRP aggregate in the presence of

CRP in the sample, forming immune complexes. The immune complexes cause an increase in light scattering, which is proportional to the concentration of CRP in the sample. The light scattering is measured by reading turbidity at 550 nm.

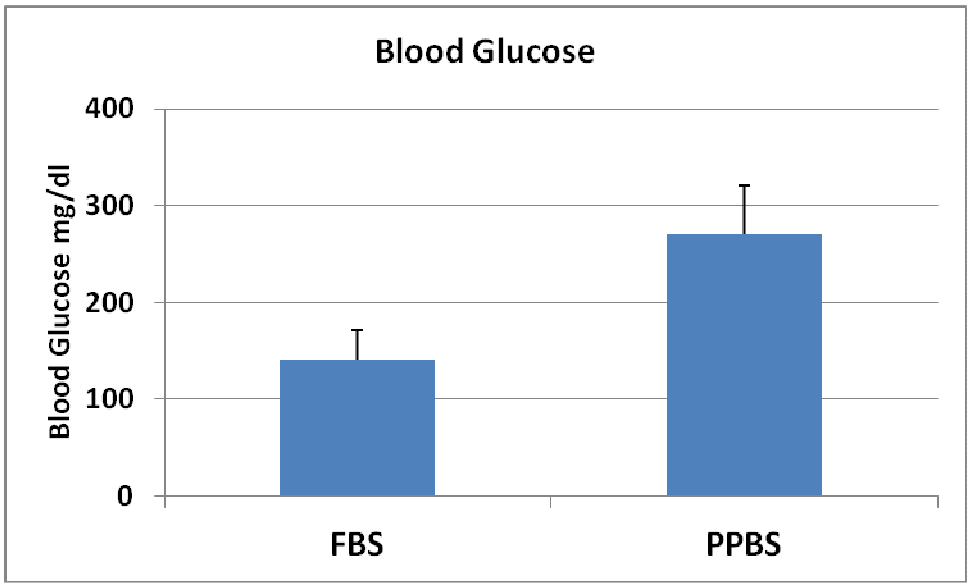
Statistical analysis

Results are expressed as mean ± SD. Means of groups were compared using Students t test.

RESULTS

Figure 1

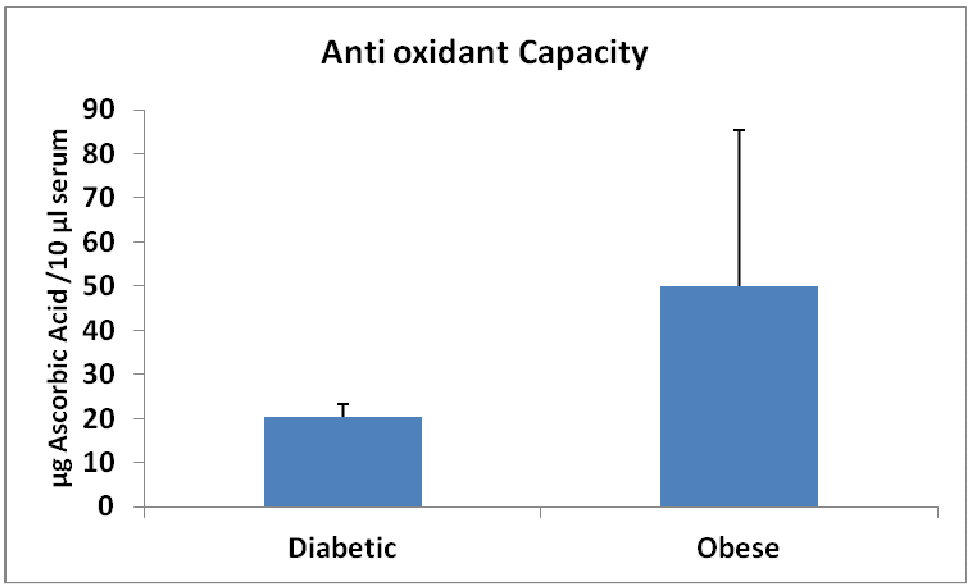
The fasting and post prandial blood glucose of the Diabetic subjects is shown in



The mean blood glucose level was 140±30.2 mg/dl. The range was 103.7 to 176.8 mg/dl. The mean post prandial blood was 270.8±50.1 mg/dl and the range was 200 to 338 mg/dl. The BMI of the obese subjects was 32.82 ± 2.73.

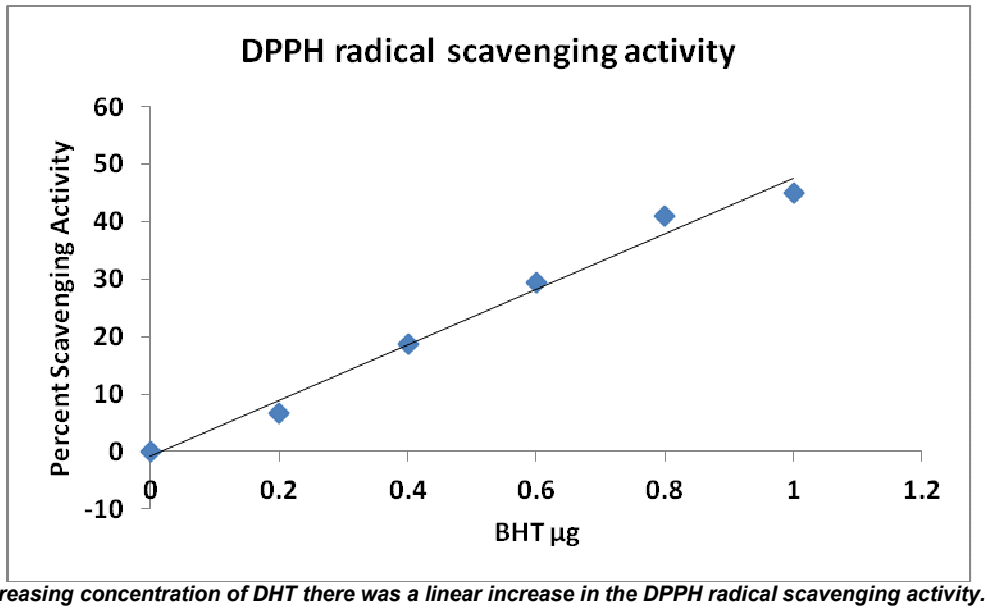
Figure 2

Comparison of antioxidant capacity of diabetic and obese patients in



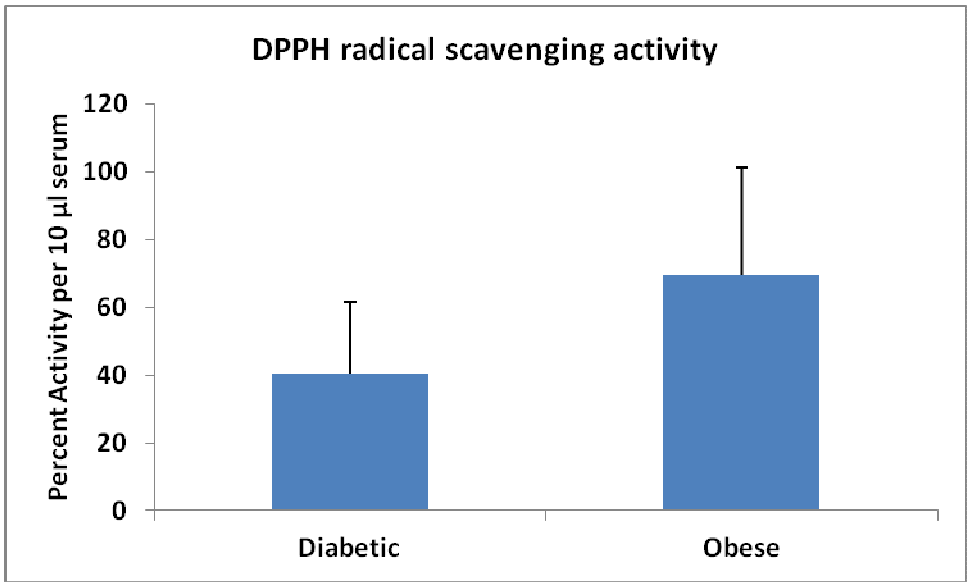
The antioxidant capacity of the serum of diabetics was 20.4±2.8 µg. ascorbic acid for 10µl serum. For the same amount of serum the antioxidant capacity of obese subjects was 50.0±35.4. The difference was statistically significant (P=0.006).

Figure 3
The DPPH radical scavenging activity of BHT is shown in



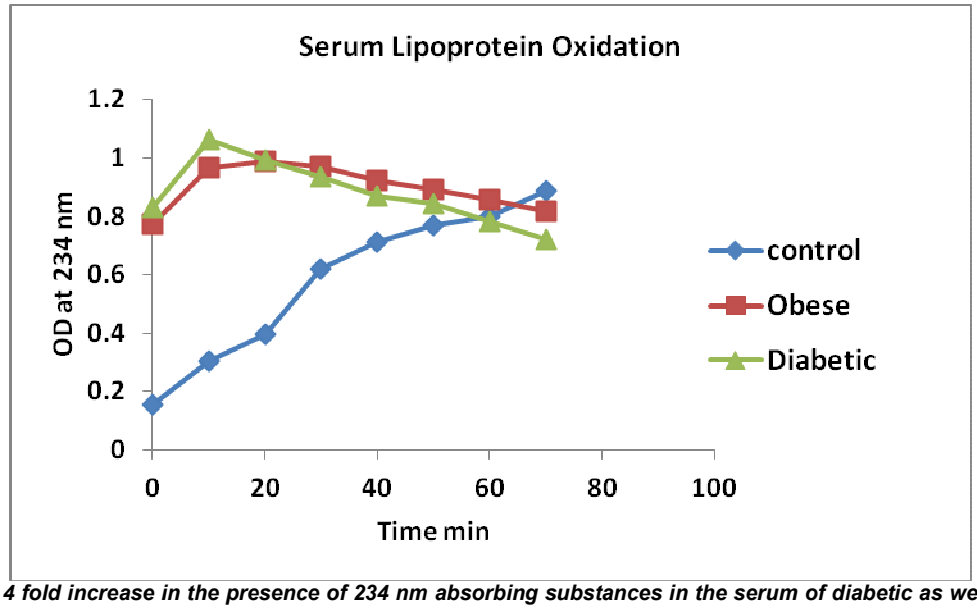
With increasing concentration of DHT there was a linear increase in the DPPH radical scavenging activity.

Figure 4
The DPPH radical scavenging activity of serum from diabetic and obese subjects are shown in



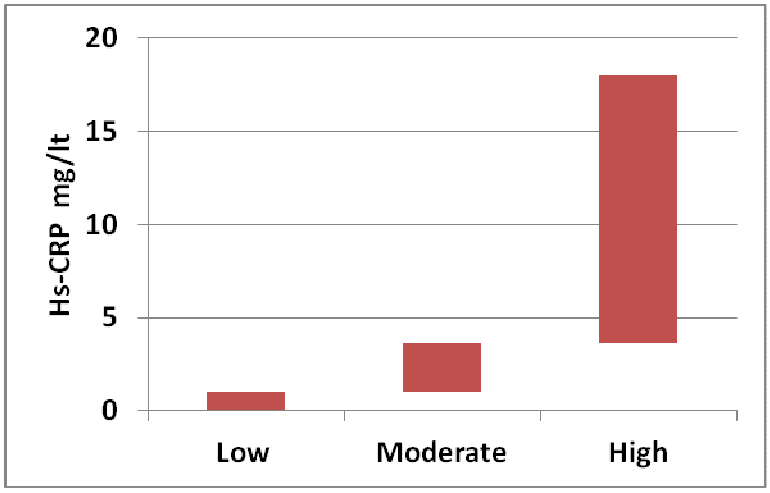
The DPPH radical scavenging activity of 10µl of serum of diabetics was equivalent to 40.5± 21.3 µg of BHT. Whereas the radical scavenging activity of obese subjects was 69.5±32.0 µg of BHT. The difference as were statistically significant (P=0.0146)

Figure 5
Serum lipoprotein oxidation of obese and diabetic subjects is shown in and compared with control subjects.



Initially there was a 4 fold increase in the presence of 234 nm absorbing substances in the serum of diabetic as well as obese subjects. Upon oxidation with cu++ ions, the 234 nm absorption increased with time for the control subjects. Where for both the diabetic and obese subjects, there was an initial increase followed by a decrease in the absorption at 234 nm. There was no significant difference between the diabetic and obese subjects. (P >0.05).

Figure 6
The Hs-CRP of obese subjects is shown in



The mean Hs CRP level was 7.12±5.64 mg/lt. From the graph of ranges of Hs CRP, two subjects had this Hs CRP levels moderately high and 8 had High. There were no subjects with low Hs CRP.

DISCUSSION

Antioxidant is defined depending on the circumstances, generally they are defined as substances that oppose or prevent the action of oxidants. However this definition is appropriate for an *in vitro* reaction of an antioxidant. But in a biological or *in vivo* situation this definition is modified as a substance that opposes the damaging effect of an oxidant[8]. There are many physiologically relevant mechanisms that generate oxidants and free radicals. For example, mitochondria produce free radicals and superoxide[9]. During the flow of electrons in the respiratory chain a small percent of the electrons (1-3%) "Leak" to oxygen forming superoxide[10]. Peroxisomes consume a large portion of the oxygen of the cell for metabolic reactions involving oxygen. For their normal metabolism, they produce

H_2O_2. However peroxisomes also have catalase and hence it does not allow the accumulation of H_2O_2. Phagocytic cells like macrophages produce superoxide through their respiratory burst oxidase to destroy bacterial infection [11]. Myeloperoxidase is another enzyme of the phagocytic cells used to kill pathogens. Myeloperoxidase produces Hypochlorous acid which is a potent pro-oxidant[12]. The endoplasmic reticulum also produces reactive oxygen species because it has NADPH- cytP450 reductase enzyme used in metabolism of Xenobiotic[13]. The plasma membrane contains enzymes like lipoxygenases and cyclooxygenases which generate biologically active oxidized lipids during the biosynthesis of prostaglandins thromboxanes, Leukotrienes and HETEs. In addition to these, diet, air, atmosphere and environment can also generate reactive oxygen species and free radicals. Free radical generation and scavenging as well as production of oxidation and their neutralization by antioxidants are regulated processes useful for the organism to survive. However during some pathological circumstances the pro-oxidants exceed the antioxidant defense system. This is the underlying cause of many disorders like diabetes cardiovascular diseases, cancer and obesity. The antioxidant capacity of the plasma or serum represents the total antioxidant molecules that can produce antioxidant defense to the organism. In our study, we found that the total antioxidant capacity of serum of diabetics was significantly lower than that of the obese subjects (P<00005). This suggests that diabetics have a higher oxidative stress when compared with obese subjects. DPPH is a stable Nitrogen centered free radical which can be scavenged by antioxidant present in the serum. In our study the DPPH radical scavenging activity was lower in diabetic serum compared with serum from obese subjects. These results are in agreement with the reduced antioxidant capacity in diabetics compared with obese subjects. Lipoprotein oxidation is the key step in the initiation of atherosclerosis. Lipoproteins like LDL and HDL are both subject to oxidative modification. When HDL is oxidized, it loses its atheroprotective function and becomes a proatherogenic molecule. The serum of diabetics as well as obese subjects appeared to have been oxidized since the 234 nm absorbing substances were higher than the control subjects. The lipoprotein oxidation profile of control serum shows these distinct phases of oxidation, namely the lag phase, log phase and the stationary phase. Whereas, the serum of diabetics as well as obese subjects showed only the decline phase of oxidation, in this phase, the oxidation products of lipids breakdown and react with the protein part of the lipoprotein. In both diabetes and obesity these appears to be oxidative stress resulting in the higher oxidation of lipoprotein. In our study we found all the obese subjects having moderate to high CRP levels suggesting that the obese subjects were in a chronic pro-inflammatory state.

CONCLUSION

Our study showed that the diabetics had lower antioxidant capacity and DPPH radical scavenging activity than obese subjects. The lipoprotein oxidation of both diabetic serum and obese serum was comparable. The obese subjects had moderate to high levels of C - reactive protein, an acute phase protein in this serum.

Conflict of interest

There is no conflict of interest

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