Original Article

Role Of Oxidative Stress In Obesity With An Insight Into Type II Diabetes Mellitus

Suman S Dambal, Indumati V And Suchetha Kumari

ABSTRACT

In the present study, the antioxidant status of obese and overweight individuals was analyzed with an insight into the role of diabetes mellitus towards increasing oxidative stress in the subjects. The assay of enzyme superoxide dismutase (SOD) in erythrocytes and that of antioxidant vitamin C in serum was carried out. The assay of vitamin C alone cannot completely focus on the antioxidant status of the system; the Total antioxidant capacity (TAC) of the serum was also assayed to give a summation of the antioxidants in the system.

The study was carried out on five groups of subjects in the age group of 40-60 years. Fifty non-diabetic and non-obese subjects formed the Group A, which were designated as the Controls. The study group was categorized into 4 groups, of which, 50 overweight patients were taken as Group B and Group C consisted of 50 overweight subjects who were diagnosed with diabetes mellitus. Fifty obese individuals were considered as Group D and 50 obese subjects with diabetes mellitus were taken to be Group E.

The levels of the enzyme, superoxide dismutase were significantly higher ($p<0.0001$) and the Total Antioxidant Capacity and vitamin C levels were significantly lower [($p<0.0001$) and ($p<0.0054$)] respectively in the study groups, as compared to those in normal individuals, as the disease conditions like obesity and diabetes mellitus in the study groups contributed to increasing the load of the reactive oxygen species in the system, thus utilizing and depleting the dietary antioxidants.

Key Words: Body Mass Index, Diabetes Mellitus, Obesity, SOD, Total Antioxidant Capacity, Vitamin C

INTRODUCTION

Obesity may be referred to as a disease condition, wherein excess body fat accumulates to such an extent, that it affects the health adversely [1]. Obesity occurs primarily due to the lack of physical exercise and to a lesser extent, due to a decrease in the basal metabolic rate [2]. BMI (Body mass index) is used as an index to measure body fatness and thus, the obesity status [3]. Obesity has been implicated in the pathogenesis of several diseases like diabetes mellitus, myocardial infarction, etc [4].

Diabetes mellitus is a condition which results from insulin resistance, which is characterised by elevated glucose levels in the blood. The conditions of obesity and diabetes mellitus contribute significantly towards the production of excess free radicals [5].

Free radicals are reactive oxygen species having an unpaired electron, which are generated under physiological conditions during aerobic metabolism. These free radicals have the potential to trigger chain reactions when they happen to react with proteins, lipids and other biological molecules, which are fatal to the cell[6]. Under normal conditions, the free radicals which are produced, are scavenged by a repertoire of enzymatic antioxidants like SOD, catalase, glutathione peroxidase, etc. and also by non- enzymatic antioxidants like ascorbic acid (vitamin C), αTocopherol , ceruloplasmin, glutathione, etc., thus preventing oxidative stress, which is a state which ensues due to the excess accumulation of free radicals in the system[7].

Super Oxide Dismutase (SOD) is an enzymatic antioxidant that selectively acts on the superoxide free radicals and dismutates them into lesser toxic O2 and H2O2. Further, there are non-enzymatic antioxidants like vitamin C, vitamin E, etc, which act as worthy reducing agents and oxidise the free radicals and reduce cellular toxicity [8].

In the present study, the antioxidant status of obese and overweight individuals were analysed with an insight into the role of diabetes mellitus towards increasing oxidative stress in the subjects. The assay of enzyme superoxide dismutase in erythrocytes and that of antioxidant vitamin C in serum was carried out. The assay of vitamin C alone cannot completely focus on the antioxidant status of the system; the Total Antioxidant Capacity of serum was also assayed to give a summation of the antioxidants in the system.

METHODS

The obesity status was categorized by analysing the BMI. The study was carried out on five groups of subjects in the age group of 40-60 years. Fifty non-diabetic and non-obese subjects (BMI 18.5-24.9 kg/m2) formed the Group A, which were designated as the Controls. The study group was categorised into 4 groups, of which, 50 overweight patients (BMI 25.0-29.9 kg/m2) were taken as Group B and Group C consisted of 50 overweight subjects who were diagnosed with diabetes mellitus. Fifty obese individuals (BMI 30kg/m2 and above) were considered as Group D and 50 obese subjects with diabetes mellitus were taken to be Group E.

The study was approved by the Ethical and the Research Committees of the Institute. After obtaining informed consent from the subjects, 5 mL of venous blood was drawn, of which 1mL was transferred into a fluoride bottle for the estimation of SOD, Haemoglobin and Random Blood sugar (RBS). Haemoglobin estimation was carried by the Cyanmethaemoglobin method. The whole blood was then centrifuged and plasma was used for the assay of RBS by the GOD-POD method. The suspended erythrocytes were washed thrice with normal saline and were lysed with cold distilled water to attain a dilution of 1:20, which was used for SOD estimation. The remaining 4mL was transferred into a plain bottle and was centrifuged and the

separated serum was used for the estimation of vitamin C and Total Antioxidant Capacity.

SOD activity:

The principle of the SOD activity assay was based on the inhibition of nitro blue tetrazolium (NBT) reduction. The illumination of riboflavin in the presence of O2 and electron donor like methionine generates superoxide anions, which in turn react with NBT to form a blue coloured complex. In the test, as the enzyme extract is added, the superoxide radicals which are generated are dismutised, thus reducing the amount of superoxide radicals, which explains the reduction in the formation of formazan. This reduction is proportional to the amount of superoxide dismutase in the sample.

One unit of SOD activity is defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions.

The procedure which was adopted was that of Beauchamp and Fridovich [9]. The reaction mixture contained 2.5ml of methionine phosphate buffer [pH 7.8], 1x 10-2 M methionine, 16.8x10-5 M NBT and 1.17x10-6 M riboflavin, with suitably diluted erythrocyte haemolysates in a total volume of 3ml. The illumination of the solution which was taken in 10ml beaker, was carried out in an aluminium foil lined box, with a 15 W fluorescent lamp, for 10 minutes. Controls without the enzyme source were always included. The absorbance was measured at 560 nm. The values were expressed in Units/mg Hb [10].

Vitamin C estimation: Vitamin C, which is a major soluble antioxidant in the body fluid, is estimated by the Dinitrophenyl hydrazine (DNPH) method [11]. It works on the principle that a good reducing agent like vitamin C can undergo a reversible conversion to its oxidised form, dehydroascorbic acid. Both these forms couple with 2, 4-dinitrophenyl hydrazine to yield an osazone, which gives a red colour with sulphuric acid. The copper in the dinitrophenyl hydrazine reagent acts as a catalyst, and the intensity of the colour is read at 520nm.

The serum which is taken for the analysis is first deproteinised with 5% TCA and is incubated with the DTC reagent which contains 0.1M DNPH, 0.027M of copper sulphate and 0.66 M of thiourea for 60 minutes at 60oC for an hour, followed by the addition of 4.5M sulphuric acid (H2SO4). The intensity of the colour which is formed is read at 520 nm against a blank which is treated similarly as the test, with distilled water instead of serum.

Total Antioxidant Capacity:

The total antioxidant capacity of the serum was analysed by the phosphomolybdenum method [12]. This quantitative assay is based on the conversion of Molybdenum (Mo VI) by reducing agents like antioxidants to molybdenum (Mo V), which further reacts with phosphate under acidic pH, resulting in the formation of a green coloured complex, the intensity of which is read spectrophotometrically at 695nm.

The serum to be analysed, is treated with 5% TCA to precipitate the proteins. The deproteinised sample is then treated with the total antioxidant capacity (TAC) reagent, which contains 28mM sodium dihydrogen phosphate and 4mM ammonium heptamolybdate, in a 0.6M of Conc. H2SO4, at 90oC for ,90 minutes in a water bath. Following incubation, the mixture is cooled and the optical density of the green complex which is formed is read at 695nm.

Statistical Analysis:

The results were expressed as Mean ± Standard Deviation (S.D). The statistical significance was determined by one-way analysis of variance (ANOVA). The p-value < 0.05 was considered as a significant value. All statistical analyses were carried out by using the instant statistical package (Graph pad prism version 3.0).

Results:

The levels of the enzyme, superoxide dismutase were significantly higher (p<0.0001) and the levels of Total Antioxidant Capacity and vitamin C were significantly lower (p<0.0001) and (p<0.0054) respectively in the study groups, as compared to the controls [Table/Fig 1]; [Table/Fig 2-4].

Subjects	SOD units/mg Hb Mean±s.D	VitC ug/ml Mean±S.D	TAC ug/ml Mean±S.D
Group A(n=50)	2.26±0.40	0.23±0.15	199.5±28.85
Group B(n=50)	2.63±0.45**	0.18±0.08*	130.4±17.07**
groupC(n=50)	2.80±0.35**	0.14±0.04*	125.7±12.89**
GroupD(n=50)	3.22±0.38**	0.12±0.06*	124.3±10.69**
GroupE(n=50)	3.70±0.48**	0.10±0.04*	115.5±8.623**

[Table/Fig. 1]: Super Oxide Dismutase, Vitamin C and Total Antioxidant Capacity Levels in Controls and Study Groups B-E

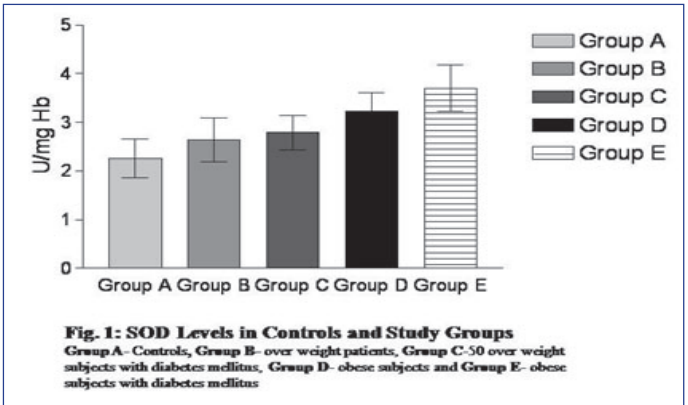

Fig. 1: SOD Levels in Controls and Study Groups
Group A- Controls, Group B- over weight patients, Group C-50 over weight subjects with diabetes mellitus, Group D- obese subjects and Group E- obese subjects with diabetes mellitus

[Table/Fig: 2]: SOD Levels in Controls and Study Groups

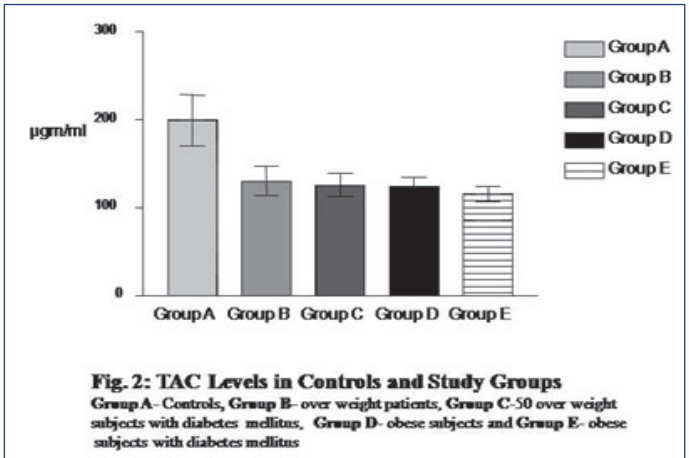

Fig. 2: TAC Levels in Controls and Study Groups
Group A- Controls, Group B- over weight patients, Group C-50 over weight subjects with diabetes mellitus, Group D- obese subjects and Group E- obese subjects with diabetes mellitus

[Table/Fig: 3]: TAC Levels in Controls and Study Groups

DISCUSSION:

The results indicate that the antioxidant status of the study groups was impaired, as compared to the controls. Obesity is a pathological condition which is spawned by excess adiposity [13]. Obesity is closely

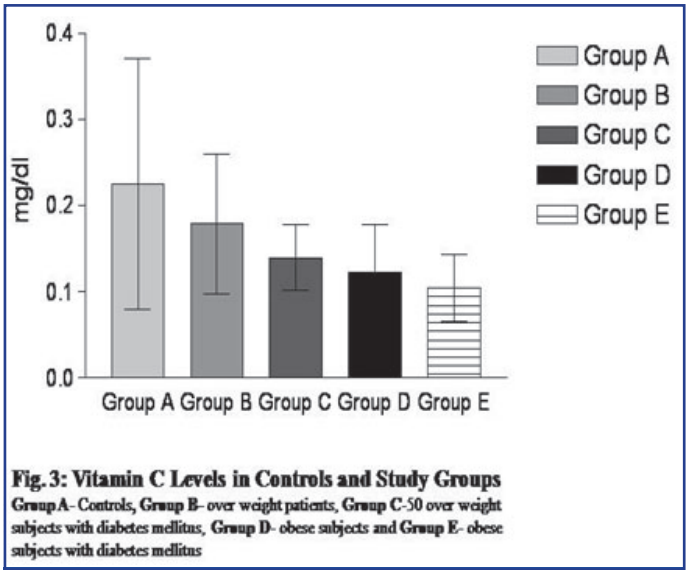

Fig. 3: Vitamin C Levels in Controls and Study Groups
Group A- Controls, Group B- over weight patients, Group C-50 over weight subjects with diabetes mellitus, Group D- obese subjects and Group E- obese subjects with diabetes mellitus

[Table/Fig: 3]: TAC Levels in Controls and Study Groups

associated with oxidative stress. The possible mechanism of obesity related oxidative stress includes increased oxygen consumption and subsequent free radical production via mitochondrial respiration, diminished antioxidant capacity, increased fat deposition and cell injury, thus causing increased rates of free radical formation [14]. It is observed that the incidence of obesity in turn, induces an increase in the probability of the development of several anomalous health disorders like diabetes mellitus, myocardial infarction, etc., as obesity alters not only the physical state of the body, but also the physiological and the metabolic state. One probable reason for the pathogenesis of obesity leading to diabetes mellitus is oxidative stress, which could be induced by several mechanisms, for eg, the increased fat content in the body can stimulate a condition of adipose tissue oxidant stress [15]. This in turn leads to a condition of endoplasmic reticulum stress, which triggers aberrant glucose production in the liver by the excess accumulation of free radicals. Further, this leads to the suppression of a lipoprotein, adipocyte ApoE. This suppression activates gluconeogenesis inappropriately. Exposure to hyperglycaemia leads to a functional limitation of the hexose monophosphate shunt, which increases free radical production and thus contributes towards increased oxidative stress.

This state leads to the increased production of the endogenous enzyme, SOD, by the cells. Hence, it could be hypothesized that increased levels of SOD activity, as seen in the obese subjects. was because of the adaptive response of the living system towards fixing oxidative stress. Further, an increase in the SOD levels of the obese subjects who had diabetes as compared to the normal obese subjects, could be due to additional oxidativestress in those subjects, which was probably contributed by the hyperglycaemic conditions. Though the increased activity of the enzyme, SOD decreases the amount of superoxide free radicals; its activity leads to the outcome of other free radicals like hydrogen peroxide and hydroxyl radicals, which have to be in turn scavenged by other antioxidants [16].

Vitamin C, a dietary antioxidant is utilised in scavenging the free radicals. Since the oxidative stress in the study groups was higher as compared to the controls, the dietary vitamin C was exhausted.

The total antioxidant capacity is an assay, which summarises the levels of various dietary antioxidants in the body. As observed, there was an overall decrease in the total antioxidant capacity of serum in the study groups as compared to the normal individuals, as the disease conditions like obesity and diabetes mellitus in the study groups contributed to increase the load of the reactive oxygen species in the system, thus utilising and depleting the dietary antioxidants.

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AUTHORS:
1. Dr. Suman S Dambal: Associate Professor, KIMS, Hubli, Karnataka
2. Dr. Indumati V: Associate Professor, VIMS, Bellary, Karnataka
3. Dr. Suchetha Kumari: Department of Biochemistry, KSHEMA, Nitte University, Mangalore, Karnataka

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Suman.S.Dambal, Associate Professor, Dept. of Biochemistry, Karnataka Institute of Medical Sciences, Hubli-580029, Karnataka, INDIA, E-mail: sumandambal25@gmail.com, Cell Phone: 9449027755

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