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Impact on Some Antioxidant Enzymes and Vitamins in Obese Adolescents of North West Indians

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Abstract

Obesity is one of the most common health problems in developed and developing nations such as India. In India, prevalence of obesity among adolescents is 6–24 %. In addition, obesity is considered as one of the risk factors for the initiation of many chronic diseases. Oxidative Stress (OS) is a well-established hallmark of diseases such as diabetes mellitus, cancer, and cardiovascular diseases. The aim of the study was to evaluate OS markers in obese subjects with respect to normal healthy subjects. The present case–control study was carried out on 25 male obese adolescent children with body mass index (BMI) ≥25 kg/m2 and on equal number of male normal healthy children with BMI<15 kg/m2 in the age range of 10–19 years. These subjects were recruited from rural and urban areas of Amritsar District, Punjab, India. The levels of malondialdehyde (MDA), antioxidant enzymes/molecules such as superoxide dismutase (SOD), reduced glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), antioxidant Vitamin E and Vitamin C, and β-carotene along with total antioxidant activity (TAA) were evaluated. Significant increase in the concentration of MDA by 114.81% was observed in obese adolescents in comparison to normal healthy adolescents while a significant fall was observed in SOD (47.153%), GSH (60.79%), GR (43.39%), GPx (29.92%), TAA (54.08%), Vitamin E (45.45%), Vitamin C (56.14%), and β-carotene (32.88%) levels in obese adolescents as compared to normal healthy control adolescents. Aforementioned observations suggested that OS induced in the obese adolescents even in the absence of any chronic diseases. Therefore, obesity should be treated at the early stages to prevent further complications.

Keywords: Antioxidants, Cardiovascular Disease, Malondialdehyde, Obesity, Oxidative Stress, Reduced Glutathione Superoxide Dismutase, , Vitamin E, Vitamin C

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1. Introduction

Punjab is an economically advanced and physically robust state of the country, and Amritsar is an industrial city has a large population of affluent families, who are exposed to a modern lifestyle. Obesity is a global nutritional concern. The prevalence of obesity is high in developed countries and similar trends are being observed in recent years among children from developing countries.[1-3] Prevalence of obesity in India among children and adolescents is 6–24 %.[3,4] Punjabi society has achieved a socio-economic status similar to that of the developed societies, especially with respect to living conditions and nutritional intake.[5] Recently, many studies reported the role of obesity for the initiation of diabetes mellitus, hyperlipidemia, colon cancer, sudden death, and other cardiovascular diseases (CVD).[6] Oxidative stress is a well-established hallmark of many diseases.[7-10]

Many cellular processes such as control of membrane permeability, secretion of extra cellular matrix proteins, cell proliferation, cell migration, and cell deaths are found to be promoted by oxygen free radicals that have contributed to the development of diabetes and cancer.[8,9] Free radicals may produce their deleterious effects by; (a) deoxidizing cell membrane lipids thus altering the structural integrity of the membrane; (b) peroxidizing

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the lipids in maintaining the micro environment of membrane proteins or reacting both with proteins and with membrane lipids separately or concurrently. In addition, oxygen radicals and products of the reaction may alter the structure of DNA and RNA. It is apparent therefore that an excessive stress imposed upon the cell could lead to the cellular modifications and thereby, essential chemical components of the cells leading to alter function.[11]

The cell and plasma contain a variety of antioxidants and free radical scavenging molecules and enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione-S-Transferase, antioxidant vitamins such as Vitamin E, Vitamin C, and β-carotene which under normal circumstances help the cell to maintain a reducing environment by preventing the potentially deleterious effects of free radical on cell membrane and organelles.[6,7] Any changes in the activity of these enzymes and antioxidants, increase oxidative stress and could lead to the development of various diseases such as CVD, arthritis, diabetes, and cancer.[8,9,12]

Although the exact biochemical mechanisms responsible for the association between obesity and the development of diseases such as atherosclerosis, arthritis, diabetes, and cancer have not been completely elucidated, and data on the change in antioxidant molecules/enzymes is scant in obese adolescents of Punjab state in Amritsar District. Therefore, an attempt has been made to evaluate the oxidative stress markers by analyzing malondialdehyde (MDA), SOD, GSH, GR, and GPx along with change in antioxidant vitamins such as Vitamin E, Vitamin C, and β-carotene levels in obese adolescents of Amritsar District of Punjab.

2. Materials and Methods

The present case-control study was carried out in the Department of Biochemistry, Government Medical College, Amritsar, Punjab, India, on 25 male obese adolescent children with body mass index (BMI) ≥25 kg/m^2 and on equal number (n=25) of male normal healthy children with BMI <15 kg/m^2 in the age range of 10–19 years. These subjects were recruited from rural and as well as from urban areas of Amritsar District of Punjab state.

2.1 Inclusion Criteria

All subjects recruited for the study were vegetarian, and there was no positive family history of diabetes, CVD in these subjects. These subjects were interviewed by questionnaire regarding the detailed information on their lifestyle, medical history, diet etc. and after obtaining the written consent, these were considered for the study after taking approval from the Ethical Committee of the Institution.

2.2 Exclusion Criteria

The obese adolescents, who were suffered with complication such as diabetes mellitus, hypertension, hypothyroidism, renal failure, hepatic diseases, acute illnesses, recurrent myocardial infarction, unstable angina and those not on any weight lose treatment and inherited obese individuals were excluded from the study.

2.3 Measurements of Anthropometric Parameters

The examination of body weight was done by taking weight in kilogram (kg) and height was measured in centimeters. The BMI was calculated from the formula: BMI = weight in kg/(height in meters).^{2} A complete lipid profile, fasting and postprandial glucose levels, and Blood pressure (systolic and diasystolic) were carried out in adolescents, who entered the study as per a predesigned proforma for assessing the signs of chronic heart failure, diabetes and also the presence of any exclusion criteria.

2.4 Collection of Blood

Blood samples were collected in EDTA and plain vacutainer from both the groups (control as well as obese subjects).

2.5 Preparation of Serum

Plain vacutainer was kept at 37°C for 20 min and then centrifuged at 3000rpm for 15 min. A clear supernatant (serum) was used for the estimation of Vitamin E, Vitamin C, and β-carotene.

2.6 Preparation of Plasma and Red Blood Cell Hemolysate

EDTA containing vacutainer were immediately centrifuged at 4000 rpm at 4°Cand a clear supernatant (plasma) was used for the estimation of MDA and total

antioxidant activity (TAA). The 100µl of red blood cell (RBC) were taken and lysed with 2.0ml ice cold water and the clear lysate obtained after spinning down the cell debris at 8500g for 10 min at 4°Cwas used for the analysis of SOD, reduced GSH, GPx, and GR.

- Estimation of MDA: MDA level in serum was assessed by the new colorimetric method of Satoh, 1978.[13] The MDA(representing lipid peroxidation) values expressed in terms of nmol/ml.
- Estimation of SOD: SOD levels were measured in erythrocyte by using Jaiswal *et al.,* 2013[14] method in RBC hemolysate.
- Estimation of reduced GSH: The levels of GSH from whole blood was estimated by applying the method of Giustarini *et al*., 2013.[15]
- Estimation of GPx (EC 1.11.1.9): The activity of GPx in erythrocytes was estimated spectrophotometrically by applying the method of Paglia and Valentine, 1967.[16] The decrease in absorbance at 340 nm was measured and expressed in terms of GPx (U/g of Hb).
- Estimation of GR (1.6.4.2): Erythrocyte GR activity was determined by the method described by Worthington and Rosemeyer, 1975.[17]
- Estimation of Vitamin E (α-Tocopherol): Vitamin E was estimated in plasma according to the method of Baker and Frank, 1968.[18]
- Estimation of β-carotene: The levels of β-carotene was done by Quaife *et al*. method, 1968.[19]
- Estimation of Vitamin C: Plasma Vitamin C was estimated by Natelson, 1971.[20]
- Estimation of TAA: Plasma total antioxidant capacity was estimated by the ferric reducing ability of Plasma assay by applying the method of Benzie and Strain, 1996.[21]

2.7 Statistical Analysis

The data was expressed as mean ± standard deviation and analyzed with the SPSS 16.0.7(SPSS, Chicago, IL, United States) for Microsoft Windows XP (Redmond, WA, United States) statistical software package. Differences between the obese and control subjects were evaluated using the Student's independent samples *t*-test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Anthropometric Measurements

The anthropometric measurements of both obese and normal healthy control subjects are summarized in the Table1. The body weight, height, age, and BMI was 55.15 ± 5.35kg, 144.92 ± 7.64cm, 14.30 ± 6.50years, and 26.25± 3.84 kg/m,^{2} respectively, in obese subjects corresponding to body weight, height, age, and BMI 28.67 ± 4.40kg, 143.10 ± 5.23cm, 14.20 ± 5.45years, and 14.02 ± 2.27 kg/m^2, respectively of healthy control subjects.

3.2 Malondialdehyde

The concentration of MDA observed in obese adolescents was 0.58 ± 0.05 nmol/ml and in normal healthy adolescents was 0.27 ± 0.07 nmol/ml. A significant increase by 114.81% ($P<0.001$) was recorded in obese subjects with respect to normal healthy subjects [Table 2].

Table 1. Anthropometric analysis of normal healthy control and obese adolescents

Anthropometric assays	Group 1 (Normal Healthy Control Subjects)	Group 2 (Obese Subjects)
Subject number	25	25
Sex	Male	Male
Height (cm)	143.10±5.23[a]	144.9±7.64 (+1.27)[NS]
Weight (kg)	28.67±4.40	55.15±5.35 (+92.36)[b,***]
Age (years)	14.20±5.45	14.30±6.50 (+0.70)[NS]
BMI (kg/m^2)	14.02±2.27	26.25±3.84 (+87.23)***

[a]Values are expressed as mean ± SD of 25 observations, bValues in parentheses represent percentage changes in obese adolescents as compared to normal healthy adolescents, ***$P<0.001$, [NS]Not significant. BMI=Body mass index, SD=Standard deviation

Table 2. Changes in malondialdehyde and superoxide dismutase, reduced glutathione, glutathione peroxidase, and total antioxidant activity in normal healthy and obese adolescents

Biochemical assays	Group1 (Normal Healthy Control Subjects)	Group2 (Obese Subjects)
Malondialdehyde (nmol/ml)	0.27±0.07[a]	0.58±0.05 (+114.81)[b,***]
Superoxide dismutase (U/mgHb)	1.08±0.31	0.55±0.08 (−47.15)**
Reduced glutathione (mmol/L)	2.78±0.33	1.09±0.28 (−60.79)***
Glutathione peroxidase (U/g of Hb)	61.43±9.58	43.05±7.13 (−29.92)*
Glutathione reductase (U/g of Hb)	14.15±2.57	8.01±1.71 (−43.39)**
Total antioxidant activity(μmol/L)	325.51±11.14	149.47±9.62 (−54.08)***

[a]Values are expressed as mean ± SD of 25 observations, [b]Values in parentheses represent percentage changes in obese adolescents as compared to normal healthy adolescents, *P<0.05, **P<0.01, ***P<0.001. SD=Standard deviation

3.3 Antioxidants Enzymes and Total Antioxidant Activity

The results of antioxidant enzymes such as SOD, GSH, GR, GPx, and TAA are summarized in Table2. A significant decrease by 47.15% (from 1.08 ± 0.31 to 0.55 ± 0.08 U/mgHb in SOD, by 60.79% (from 2.78 ± 0.33 to 1.09 ± 0.28 mmol/L) in GSH, by 29.92% (from 61.43 ± 9.58 to 43.05 ± 7.13 U/g of Hb) in GPx, by 43.39% (from 14.15 ± 2.57 to 8.01 ± 1.71 U/g of Hb) in GR, and by 54.08 (325.51 ± 11.14 to 149.47 ± 9.62 μ mol/L) in TAA was observed in obese subjects with respect to normal healthy subjects. The P values observed for SOD, GSH, GPx, GR, and TAA are P< 0.01, P< 0.001, P< 0.05, P< 0.01, and P< 0.001, respectively.

3.4 Antioxidant Vitamins

The summary of results of Vitamin E, Vitamin C, β-carotene, and Vitamin D represented in Table3. The levels of Vitamin E, Vitamin C, β-carotene, and Vitamin D observed in obese adolescents was 0.66± 0.14 mg/dl and in normal healthy adolescents was 1.21 ± 0.94mg/dl, 1.25 ± 0.14 mg/dl, 22.90 ± 6.26mg/dl and 16.95 ± 14.21 mmol/L, respectively, in obese adolescents in comparison to the levels of 1.21 ± 0.94mg/dl, of Vitamin E, 2.85 ± 0.43mg/dl, of Vitamin C, 34.12 ± 5.63mg/dl of β-carotene, and 24.50 ± 15.60 mmol/L of Vitamin D in normal healthy adolescents. The alteration in Vitamin E, Vitamin C, β-carotene, and Vitamin D were found to statistically significantly decreased by 45.45% (P< 0.01), 56.14 (P< 0.01), 32.88 (P < 0.01), and 30.81 (P<0.05), respectively.

4. Discussion

Free radicals are continuously produced during aerobic metabolism. Oxidative damage caused by free radicals is counteracted by a number of enzymes and vitamins. The imbalance between the rate of free radical production and the effect of protective antioxidants leads to oxidative damage, which is also known as oxidative stress.[20,21] SOD, the first line of defense against the deleterious effects of reactive oxygen species (ROS) in the cells by catalyzing the dismutation of O_2^- radicals to H_2O_2 and O_2.[9,22] In human three isoenzymic forms such as CuZn-SOD (cytosol), Mn-SOD (mitochondria), and extra cellular-SOD (plasma) exists. The presence of SOD in various compartments of our body immediately dismutates O_2^- radicals and protects the cells from oxidative damage. In the present study, a fall of SOD activity by 25.63% (P< 0.05) in obese adolescents was recorded, might results in an increased flux of O_2^- radicals and hence reflects the tissue damage/injury.

At the second step of the antioxidant defense system is GPx and CAT independently degrade H_2O_2 to water. Reduced GSH is required as a substrate by GPx to degrade H_2O_2. A significant reduction in the levels of GSH by 60.79% (P<0.001) was observed in obese subjects w.r.t. normal healthy control subjects [Table2]. GSH is a potent endogenous antioxidant that helps to protect cells from a number of noxious stimuli including ROS.[23,24] GSH is maintained in reduced state by an efficient GPx/GR system. Reduction in the levels of GSH confirms an increased susceptibility to oxidative damage in obese adolescents. Many studies[25,26] reported that inverse relationship exists between MDA and GSH status. GSH depletion of 20% to 30% can impair the cell defense against the toxic action of xenobiotic and may lead to cell injury/death.

The activity of GPx, selenium-containing enzyme catalyses the reduction of variety of hydrogen peroxide

Table 3. Changes in Vitamin E, Vitamin C, β-carotene and Vitamin D levels in normal healthy and obese adolescents

Biochemical assays	Group1 (Normal Healthy Control Subjects)	Group2 (Obese Subjects)
Vitamin E (mg/dl)	1.21±0.94[a]	0.66±0.14 (−45.45)[b,**]
Vitamin C (mg/dl)	2.85±0.43	1.25±0.14 (−56.14)**
β-carotene (mg/dl)	34.12±5.63	22.90±6.26 (−32.88)*

[a]Values are expressed as mean±SD of 25 observations, [b]Values in parentheses represent percentage changes in obese adolescents as compared to normal healthy adolescents, *P<0.05, **P<0.01. SD=Standard deviation

(ROOH and H_2O_2) using GSH as a substrate, thereby protecting mammalian cells against oxidative stress.[27] It is well reported that low activity of this enzyme may render the tissue more susceptible to LPO damage. Accordingly, in the present work, we observed a significant decrease by 29.92% (P<0.05) in GPx activity upon increase in LPO level by 114.81% (P< 0.001). The significant reduction in GR activity (P<0.001, 43.39%) in obese adolescents attributed to increased oxidation or decreased synthesis of GSH.

These finding indicate that H_2O_2 cannot be neutralized efficiently in obese subjects. A significant inhibition of GPx, GR, and GSH causes the accumulation of H_2O_2 and lipid peroxides. If the amount of free radicals that cannot be efficiently neutralized then lipid peroxidation occurs subsequently. MDA is one of the most commonly used indicators of lipid peroxidation. In the present case–control study, a significant increase in MDA level by 114.81% (P< 0.001) was observed in obese adolescent's to corresponding control groups. Thus, elevation of LPO levels in obese adolescents confirms activation of pro-oxidative processes. Once LPO started, it spreads rapidly and affects a great number of lipid molecules generating excess free radicals and lipid hydroperoxides that can seriously alter the cell membranes, membrane fluidity, and permeability.

The levels of non-enzymatic antioxidants such as Vitamin C, Vitamin E, and β-carotene are significantly lower (P< 0.01) in obese subjects as compared to control group along with increase in MDA[Tables2 and 3]. These antioxidants are acting as LPO chain breaking molecules and scavengers to reduce the oxidative stress. It might be possible that there is a depletion of these vitamins due to reduced dietary intake. It is possible to increase the ascorbate alpha tocopherol and β-carotene concentration in the epithelial lining fluid by increasing the ascorbate intake through the children's diet. Wood *et al.*[28] observed dietary supplementation with vitamins (ascorbic acid, alpha tocopherol, and β-carotene) has some positive effect. However, in our study, no data was collected about the dietary intake of these vitamins.

Aforementioned observations strongly suggest increased oxidative stress in obese adolescents of Amritsar District of Punjab state even in the absence of disease such as diabetes mellitus, hypertension, hyperlipidemia, renal or liver disease, and therefore, obesity should be treated with the same attention such as other chronic diseases. Further studies using antioxidant vitamin supplementations alone and in combinations, lifestyle and dietary modification might be beneficial to reduce oxidative stress.

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4.2 Conflict of Interest: There is no conflict of interest.

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