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Evaluation of Serum Antioxidants and Lipid Peroxidation Status in Bangladeshi Obese Patients

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Abstract: Obesity is emerging as one of the fastest growing pandemics in modern times and is associated with various health related problems. Its prevalence is gradually increased in Bangladesh especially in urban areas. As there is no available reported work and systematically documented data regarding the serum antioxidant and lipid peroxidation levels in Bangladeshi obese patients, so the aim of this study was to examine the relationship between serum antioxidant vitamin C and lipid peroxidation (MDA) levels and thereby, pathophysiological correlation was established. In this study, 100 obese patients and 100 non-obese subjects were recruited. Demographic, anthropometric and clinical data were collected at routine visits. Obesity was determined by the body mass index (BMI). Serum antioxidant vitamin (vitamin C) was determined by UV-spectrophotometric method. The study showed that the antioxidant vitamin (C) was comparatively lower ($p>0.05$) in the obese group than in control subjects and lipid peroxidation (MDA) was significantly higher ($p<0.05$) in obese group than in the control subjects. Pearson's correlation analysis was carried out to determine the relationship between variables. In the patient groups, there was a positive relationship between Age and BMI ($r = 0.010$, $p = 0.925$); Age and MDA ($r = 0.078$, $p = 0.440$) but an inverse relationship between Age and Vitamin C ($r = -0.052$, $p = 0.606$); BMI and MDA ($r = -0.029$, $p = 0.778$); BMI and Vitamin C ($r = -0.158$, $p = 0.117$). In the control group, there was a positive relationship between Age and MDA ($r = 0.123$, $p = 0.221$); BMI and MDA ($r = 0.007$, $p = 0.942$); BMI and Vitamin C ($r = 0.152$, $p = 0.132$) but inverse relationship between Age and BMI ($r = -0.278$, $p = 0.005$); Age and vitamin C ($r = -0.269$, $p = 0.007$). We found decreased serum antioxidant (vitamin C) and elevated lipid peroxidation (MDA) in obese individuals than the controls.

Key words: Antioxidant, Lipid Peroxidation, Obese Patients, Bangladesh

INTRODUCTION

Nowadays, Obesity is becoming a serious problem all over the world. This problem is especially more acute in developed countries than the others. Obesity is a medical condition in which excess fat is accumulated in the body showing an adverse effect on health (who, 2000 and Haslam DW, 2005). A person is considered obese when his body mass index (BMI), exceeds 30 kg/m^2 (WHO, 2000). BMI is obtained by dividing a person's weight in kilograms by the square of the person's height in meters (WHO, 2000). According to World Health Organization, overweight and obese is defined as 30>BMI≥ 25 (kg/m2) and BMI≥30 (kg/m2) respectively (WHO, 2000).

Obesity increases various diseases such as heart diseases, type 2 diabetes, sleep apnea, certain types of cancer, osteoarthritis, depression, etc. (Haslam DW, 2005). It is mainly caused by excessive intake of food, lack of physical activity, genetic susceptibility, endocrine disorders, medications or psychiatric illness. It is reported that fast food intake may increase the risk of obesity (Ebbeling CB et al., 2007), (French SA et al., 2007),

(Binkley JK et al., 2000), (Pereira MAet al., 2005). Some people eat little but gain weight due to slow metabolism (Kushner, 2007), (Adams PG et al., 2000).

In 2015, about 600 million adults (12%) and 100 million children were obese (New England Journal of Medicine., 2017). In 21st century obesity is considered as one of the most serious public health problems (Barness LA, 2007). The prevalence of obesity is much in the modern world particularly in the Western world (WHO, 2000), (Woodhouse, 2008). In 2013, Obesity was classified as a disease by the American Medical Association (Pollack, 2013), (Weinstock, 2013).

According to nutritional guidelines [16], overeating and poor dietary choice may lead to obesity (Marantz PR, 2008). From 1971 to 2000, the obesity rates were increased from 14.5% to 30.9% in the United States (Flegal KM et al., 2000). During this period, the consumption of food especially sweetened beverages was increased among the people (Wright JD et al., 2004). Carbohydrates were the primary sources of sweetened beverages (Caballero B, 2007), (Mozaffarian D et al., 2011). It is believed that the obesity rate was increased by the consumption of sweetened drinks (Malik VS et al., 2006), (Olsen NJ et al., 2009). However, the purpose of this study was to determine serum antioxidant and lipid peroxidation levels of obese patients and to increase awareness among them.

MATERIALS AND METHODS

Study Design:

This study was carried out in Chowmuhani Classic Hospital, Noakhali, Bangladesh. Ethical permission was taken from ethical committee of the respective hospital. For the study purpose, 100 obese patients were recruited as cases and 100 healthy volunteers were also recruited as control subjects matching with age, sex with the cases.

Data Collection:

Detailed patients' history was taken with a well-designed questionnaire by regularly attending to Chowmuhani Classic Hospital, Noakhali. Following data were collected from patients with metabolic syndrome and healthy volunteers: age, sex, blood pressure, plasma glucose level, and body mass index (BMI).

Blood Sample Collection and Processing:

5 ml venous blood sample was drawn from each patient and control in a metal-free sterile tube. The blood samples were kept at room temperature for about 30 minutes to clot and centrifuged at 3000 rpm for 15 minutes to extract the serum. Then the serum was taken in eppendorf tube and was stored at -80°C until the study day. These samples were then used for determining the serum levels of antioxidant (vitamin C) and lipid peroxidation (MDA).

Determination of serum MDA:

Lipid peroxidation was assessed by measuring serum malondialdehyde (MDA) level according to the modified method described by Satoh (Satoh K , 1978).

Determination of serum vitamin C:

Serum Vitamin C was estimated by phenyl-hydrazine spectrophotometry method (Lowry H et al,. 1945).

Statistical analysis:

Independent sample t-test and Pearson's correlation test were performed for the statistical analysis using the statistical software package SPSS, version 16.0 (SPSS, Inc., Chicago, IL).

RESULTS

Determination of serum MDA:

The absorbance of the supernatant was measured spectrophotometrically (UV- 1800 Spectrophotometer, Shimadzu, Japan) at 530 nm using 1,1,3,3-tetraethoxy-propane as standard. Thiobarbituric acid reactive substances (TBARS) were expressed as nmol/mL or µmol/L.

Table No. 1: Concentrations of standard MDA with corresponding absorbance

SL. No.	Concentration (nmol/ml)	Absorbance
1	0.4	0.005
2	1	0.13
3	2	0.24
4	3	0.37
5	4	0.48

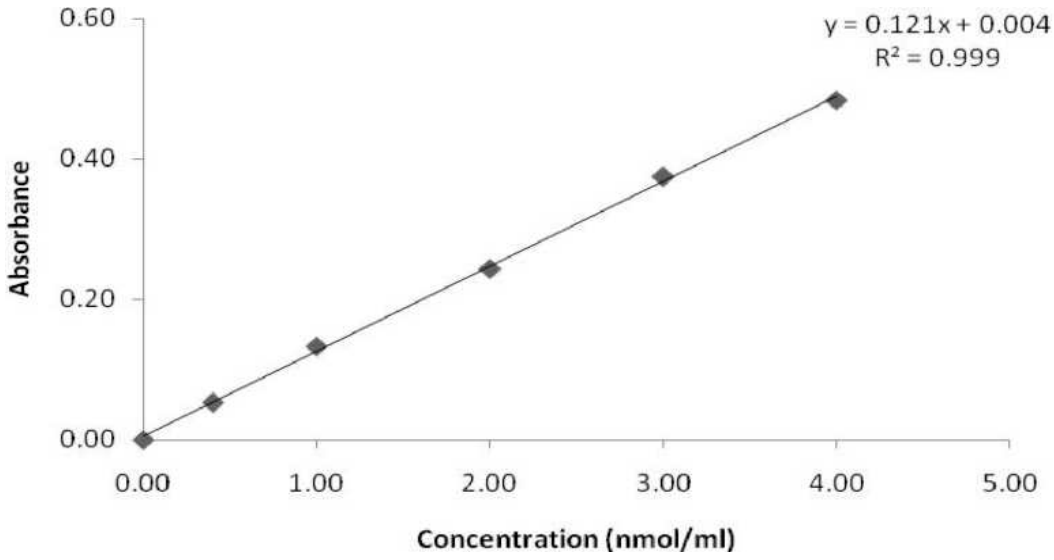

Figure 1. Standard curve of MDA

Anthropometric and demographic profile:

This study comprised of 100 obese patients as cases and 100 normal healthy adults as controls. All data are expressed as mean±standard error of mean (mean±SEM). Anthropometric and demographic profile of the patients and controls are represented in table 2. It was observed that mean age of the obese patients and controls were 48.93±1.25 and 52.38±1.66 years respectively. Statistically significant difference was not found ($p=0.098$) for age between patients and control groups.

According to World Health Organization (WHO), obesity is more common in women than men (WHO, 2016). But in this study, it was observed that men were more prevalent for obesity than women. The relative percentage of the obese men and women was 55% and 45% v_s 59% and 41% in patient and control groups respectively. Statistical analysis showed that the obese subjects had significantly higher level of BMI in comparison to control subjects ($p<0.05$). It was observed that mean value of BMI of the obese patients and controls were 28.43±0.27 and 21.68±0.20 respectively.

Table No. 2: Anthropometric and demographic profile of the study population

Variables	Patient group	Control group	p value
	Value ± SEM		
Age (years)	48.93±1.25	52.38±1.66	0.098
	Value, n (%)		
Sex			
Male	55 (55%)	59 (59%)	
Female	45 (45%)	41 (41%)	
	Value ± SEM		
BMI (kg/m^2)	30.43±0.27	23.68±0.20	0.000**

**p<0.05 (Significant difference between patient and control groups at 95% confidence interval)

Table No. 3: Serum level of MDA & vitamin C in the study population

Parameters	Values (Mean±SEM)		p value
	Patient group	Control group	
BMI (kg/m^2)	30.43±0.27	23.68±0.20	0.000**
MDA (µmol/L)	3.6424±.07251	0.1889±.00678	0.000**
Vitamin C(µmol/L)	20.5889±2.01869	28.2930±1.57242	.132

**p<0.05 (Significant difference between patient and control groups at 95% confidence interval)

From the table 3, it is said that Patient groups have an average BMI value 28.43±0.27 (kg/m^2), MDA value 0.1889±0.00678 µmol/L and Vitamin c value 20.5889±2.01869 µmol/L. On the other hand, control groups have an average BMI value 21.68±0.20 kg/m^2, MDA value 3.6424±.07251 µmol/L and vitamin C value 28.2930±1.57242 µmol/L.

Correlation analysis of the variables:

Pearson's correlation analysis was carried out to determine the relationship between variables (Table 4). In the patient group, there was a positive relationship between Age and BMI (r = 0.010, p= 0.925); Age and MDA (r = 0.078, p = 0.440) but an inverse relationship between Age and Vitamin C (r = -0.052, p = 0.606); BMI and MDA (r = -0.029, p = 0.778); BMI and Vitamin C (r = -0.158, p = 0.117). In the control group, there was a positive relationship between Age and MDA (r = 0.123, p = 0.221); BMI and MDA (r = 0.007, p = 0.942); BMI and Vitamin C (r = 0.152, p = 0.132) but inverse relationship between Age and BMI (r = -0.278, p = 0.005); Age and vitamin C (r = -0.269, p = 0.007).

Table No. 4: Correlation analysis of the variables

Correlation Parameters	Patient group		Control group	
	R	P	R	P
Age and BMI	0.010	0.925	-0.278	0.005**
Age and MDA	0.078	0.440	0.123	0.221
Age and Vitamin C	-0.052	0.606	-0.269	0.007
BMI and MDA	-0.029	0.778	0.007	0.942
BMI and Vitamin C	-0.158	0.117	0.152	0.132

DISCUSSION

Obesity has been considered as a general health problem around the world. It has been associated with a markedly increased oxidative stress. Antioxidant vitamins play an outstanding role in the protection against oxidative stress. The antioxidant status has been estimated by determining the levels of vitamin C. Obesity is reported to be a risk factor for Diabetes Mellitus (DM). DM is associated with increased lipid peroxidation and decreased antioxidant status.

Considering all of these facts an attempt has been made to investigate the serum levels of trace antioxidant (vitamin C) and lipid peroxidation (MDA) of obese patients and attempt has also been taken to find out the correlation between serum levels of these components in obese patients.

This study was not only to determine several biochemical parameters which may contribute to the pathogenesis of obesity, but we also analyzed the anthropo-demographic features of the patients and controls and found out the relationship of these parameters with the disease process. All these data could help us find out the reasons of morbidity and mortality rate of obese patients.

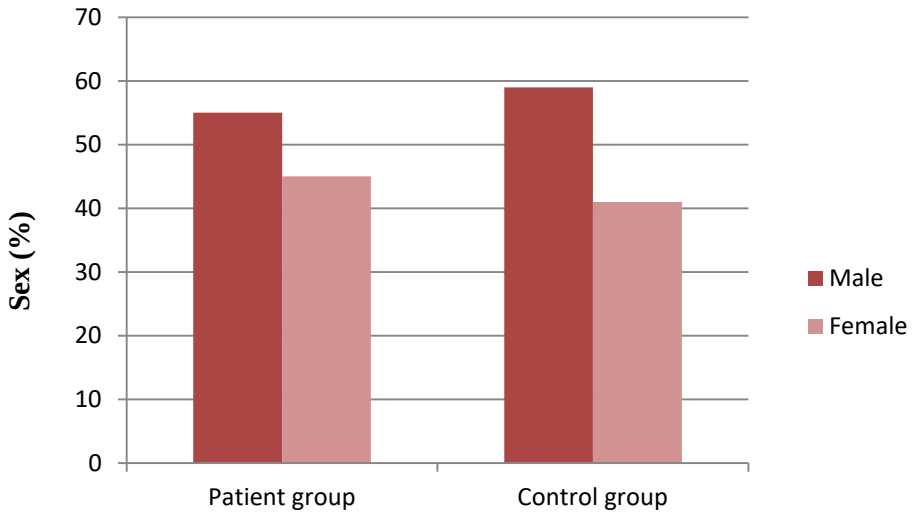

Figure 2. Comparison of sex distribution between patient and control groups

In this study, we have shown in figure 2 that we have observed obesity in 55% male individuals than 45% female individuals. On the other hand, we have collected 59% male and 41% female individuals as control to compare with obese patients.

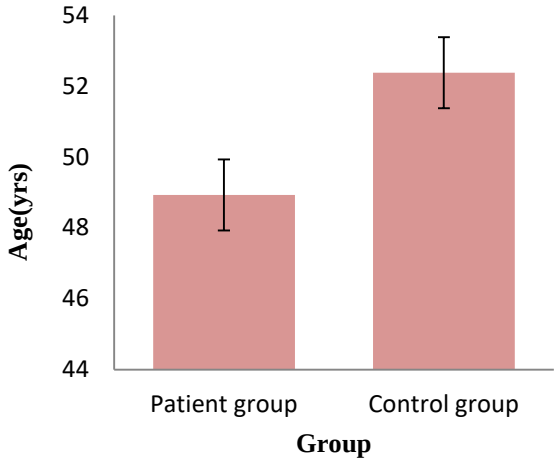

Figure 3. Comparison of age between patient and control groups

From figure 3, it reveals that obesity is more prevalent at 48.93±1.25 years. We have collected male and female individuals with average age 52.38±1.66 years as control to compare with obese patients.

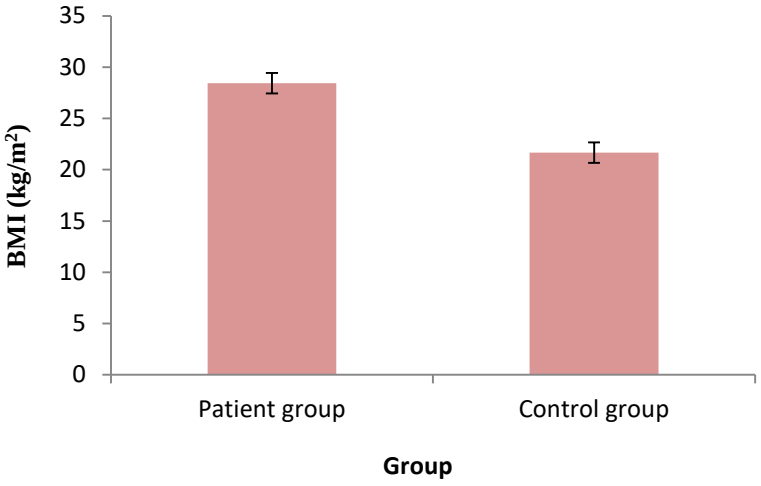

Figure 4. Comparison of BMI between patient and control groups

From figure 4, we observe that obesity in male and female individuals has an average BMI of 28.43±0.27 kg/m^2 and we have collected male and female individuals with average BMI 21.68±0.20 kg/m^2 as control to compare with obese patients.

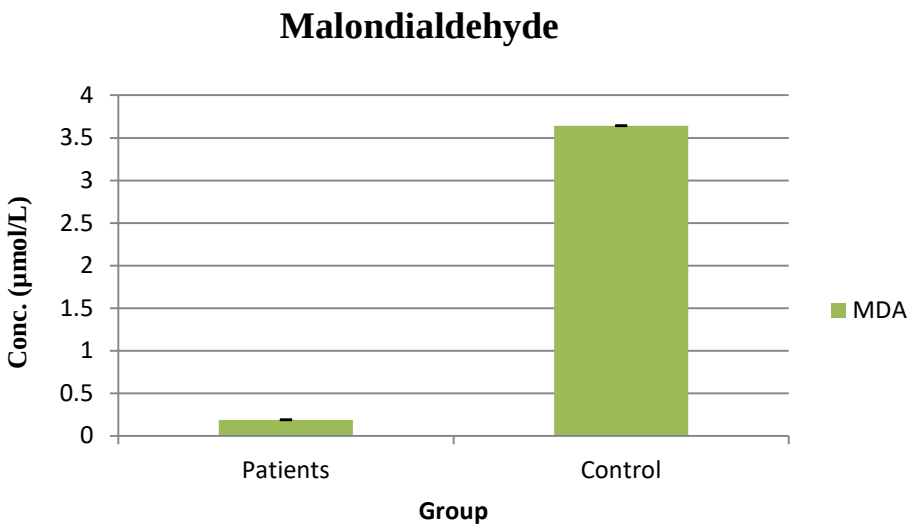

Figure 5. Comparison of MDA between patient and control groups

From above figure it indicates that in patients group male and female individuals have an average MDA of 0.1889±.00678 µmol/L. At the same time, in control group male and female individuals have an average MDA of 3.6424±.07251 µmol/L.

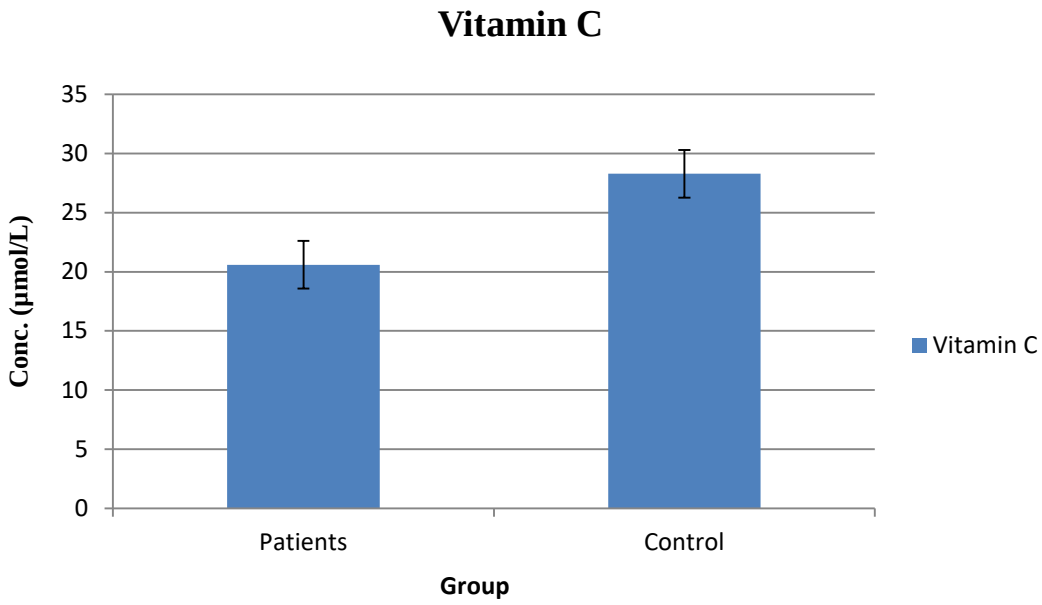

Figure 6. Comparison of Vitamin C between patient and control groups

From figure 6, we notice that in patient's group, male and female individuals have an average vitamin C of 20.5889±2.01869 µmol/L. On the other hand, in control group male and female individuals have an average MDA of 28.2930±1.57242 µmol/L.

CONCLUSION

The incidence of obesity has significantly increased worldwide during recent decades. Besides, obesity and obesity-related disorders constitute a serious threat to the health of all populations on earth. Obesity represents a risk factor for diseases including cancers, cardiovascular diseases, atherosclerosis, diabetes, etc., in which inflammation plays a crucial role in the pathogenesis. The findings of this study revealed that anthropo-demographic factors have considerable effect on the pathogenesis of obesity possibly due to their life style and dietary habit. Vitamins play an important role in the body and include antioxidant functions, immunomodulatory functions, etc. The present study found that depleted level of vitamin C in obese patients than the healthy subjects which supports the hypothesis that oxidation is a causative factor in the pathogenesis of obesity. Antioxidant supplementation may be recommended to scavenge the free radical action which may be useful as secondary therapy to prevent the oxidative damage in the tissue of obese patients. This study also explored that obese patients have higher serum concentration of MDA than the normal individuals which suggests the involvement of depleted serum lipid peroxidation (MDA) in the pathogenesis of obesity. Thus, dietary supplementation may be recommended to reduce the risk of obesity which may require further study to find out the role dietary components on the pathophysiology of obesity. Moreover, lifestyle changes and therapeutics that may reduce adiposity could offer the benefit of preventing obesity-related morbidity and mortality.

LIMITATIONS OF THE STUDY

Bangladesh is a developing country in South Asia. Most of the people in our country is illiterate and lives below the poverty line. May be due to lack of literacy, many obese patients and the normal objects were not interested to give 5 ml blood for research purpose. We faced many problems to convince them. Otherwise laboratory facilities were not available to conduct this study.

LIST OF ABBREVIATIONS:

MDA: Malondialdehyde, BMI: Body Mass Index.

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