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Original Article

Increased concentration of serum MDA, decreased antioxidants and altered trace elements and macro-minerals are linked to obesity among Bangladeshi population



Md. Tarek Adnan^a, Mohammad Nurul Amin^{a, b}, Md. Giash Uddin^{a, b},
Md. Saddam Hussain^a, Md. Shahid Sarwar^a, Md. Kamrul Hossain^c, S.M. Naim Uddin^c,
Mohammad Safiqul Islam^{a, *}

^a Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh

^b Department of Pharmacy, Atish Dipankar University of Science and Technology, Uttara, Dhaka, Bangladesh

^c Department of Pharmacy, University of Chittagong, Chittagong-4331, Bangladesh

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ABSTRACT

Background and objective: Obesity is an emerging public health concern in Bangladesh. This study aimed to find the levels of the serum malondialdehyde (MDA), non-enzymatic antioxidants (vitamin A, C and E), trace elements (zinc and iron) and macro-minerals (calcium, potassium and sodium) in obesity and its action in disease advancement.

Methods: Level of lipid peroxidation was estimated by measurement of the serum concentrations of malondialdehyde (MDA). Vitamin A and E concentration was found by RP-HPLC method and vitamin C was assessed for serum ascorbic acid by UV spectrophotometric method. Serum trace elements (Zn and Fe) and macro-minerals (Na, K and Ca) were estimated by Atomic Absorption Spectroscopy (AAS).

Results: Our study observed significantly elevated concentrations of MDA ($p < 0.001$) and depleted concentrations of antioxidants (vitamin A, E and C) ($p < 0.05$) in the patient than control group. Analysis of serum trace elements (Zn and Fe) and macro-minerals (Na, K and Ca) and found that the mean values of Zn, Fe, Na, K and Ca were 0.39 ± 0.02 and 0.43 ± 0.03 , 3284.81 ± 34.51 , 162.18 ± 3.72 , 44.62 ± 2.13 mg/L for the patient and 0.91 ± 0.13 , 0.88 ± 0.06 , 2562.74 ± 95.92 , 243.58 ± 8.97 , 87.66 ± 2.10 mg/L for the controls, consequently. There was a substantial difference in trace elements and macro-minerals between the patients and controls ($p < 0.001$).

Conclusion: Our study proposes that increased serum concentrations of MDA and decreased non-enzymatic antioxidant and altered trace elements and macro-minerals are powerfully related with obesity.

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1. Introduction

According to American Medical Association, obesity is a disease affecting one American people in three individuals. Though obesity as a disease was debated for a long time, in 2008, Obesity Society has confirmed their support obesity as a disease, officially. Again, experts also proposed that obesity is more likely a risk factor than disease and it is a public health problem worldwide [1]. It is a

grievous health hazard that leads to the increase morbidity. Over the last 20 years it is a major public health issue in the US and worldwide [2,3]. In developing countries this is arising as health crisis when body mass index (BMI) is above 30. This health burden has reached outbreak proportions globally and is a key factor of chronic diseases. It is a complex condition which affects all ages and socioeconomic group. It originates from continuous energy surplus and extra lipid storage which causes constant increase in energy intake and decrease output. It may be due to the abnormal metabolism and micronutrient deficiencies. Various socioeconomic factors like lack of physical activity, sedentary lifestyle and consumption of high fat diet are also major contributors to the progression of the obesity [3]. It has gained outbreak dimensions,

* Corresponding author. Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

E-mail address: research_safiq@yahoo.com (M.S. Islam).

containing overweight above 1 billion adults and 30% of them are clinically obese which leads to major threat of chronic diseases and impairment. Obesity and overweight have been linked to several complications such as type 2 diabetes, heart diseases, hypertension, stroke, and certain forms of cancer. Pulmonary disorders are more common in obese people which have seen in diagnosis in recent years [4]. Moreover, it causes heightened risk of untimely death to severe chronic disorder that affects quality of life.

Alteration of lipoprotein, metabolic complications and elevated blood pressure are linked to overweight and obesity which increases incidence of coronary heart diseases [4]. Apart from this, oxidative damage can lead to cardiac arrhythmias, infraction, poor contractibility rate and cardiac failure or sudden death [5].

Tissue antioxidants provide a protective action against reactive oxygen species (ROS) and radicals. This protective action contains enzymatic and non-enzymatic antioxidants. Vitamins A, E and C are crucial antioxidants [6]. Hydrophobic vitamin E ceases lipid peroxidation and changes to O_2^- and $\cdot OH$ to lesser extent of reactive forms. Like vitamin E lipophilic vitamin A is a good free radical hunter. It provides defense against lipid peroxidation and from oxidation of DNA. Hydrophilic vitamin C can instantly remove ROS and hydroperoxides [4].

Trace elements and minerals are important in regulating of usual body metabolism. The trace elements like zinc, iron are needed for energy metabolism, protein formation, play antioxidants role etc. The major minerals like sodium, potassium, calcium, etc. act a crucial part in nerve transmission, muscle contraction, immune system of health and acid-base balance. Zinc is an essential trace element in synthesis of DNA, gene expression and several enzymatic reactions. It is an essential element for the growth and development of human. Again accumulation of zinc in adipose tissue results in production of adipokines in obese people [7]. Iron is an essential cofactor important for energy production, metabolism and synthesis of DNA. Again, it can also be harmful due to free radical production. Obesity may contribute to decreased iron absorption from duodenum. The peptide hormone hepcidin derived from liver is the key regulator of iron homeostasis. Pro-inflammatory cytokine IL-1, IL-6 released from adipose tissue in obese individuals also induce hepcidin release [8]. Sodium is a mineral required for the regulation of blood and body fluids, nerve signal transmission and heart activity. Potassium is also a mineral required for normal cellular function. It is a cationic intracellular electrolyte that conducts electricity in the body along with sodium and calcium. It plays crucial role in heart function, skeletal and smooth muscle contraction [9]. In addition, calcium is an important mineral provide defense against obesity by the inhibition of 1 α and 25-dihydroxycholecalciferol synthesis utilizing dietary calcium. Thus it results decreased in vivo oxidative stress and inflammation, and give relaxing result on smooth muscle [10]. Considering the health burden of obesity and the role of lipid peroxidation, antioxidants, trace elements and macro-minerals, we designed current research projects to investigate the mentioned parameters in Bangladeshi obese population which is yet to be reported.

2. Material and methods

2.1. Study design and blood sample collection

The Ethics Committee of the Dhaka Medical College and Hospital, Dhaka, granted the research protocol. This case-control study recruited 100 obese individuals and 100 healthy volunteers as control groups. Each obese individuals and control group was informed regarding the function of the study and written permission was received from them. The principles of Declaration of

Helsinki were followed throughout the all investigations. The study subjects had not taken any antioxidants which would alter the level of antioxidants, trace elements and macro-minerals. With a pre-designed questionnaire socio-demographic data were collected. Different biographical characteristics (age, height, weight) and BMI were collected for both obese individuals and control groups.

Overnight fasting blood was taken from each obese individuals and control group. The blood samples are allowed to clot for 1 h at normal temperature and after centrifugation at 3000 rpm for 15 min the extracted serum from the collected blood samples were kept into micro tubes and stored at $-80^\circ C$ until analysis. Samples were then employed for determining the serum level of triglyceride (TG), total cholesterol (TC), HDL, LDL, antioxidant vitamin A, C and E, lipid peroxide (MDA) as well as trace elements and macro-minerals.

2.2. Chemicals and reagents

All the chemical and reagents utilized in the research study were analytical grades from renowned commercial company. Standards of zinc, iron, sodium, potassium and calcium were obtained from Buck Scientific, USA. Used standards of retinol, α -tocopherol and α -tocopheryl acetate collected from Sigma Chemical Co., USA. HPLC grade chemicals and reagents were obtained from Active Fine Chemicals Limited, Dhaka, Bangladesh. Nitric acid, hydrochloric acid (37%) and nitric acid were obtained from Merck, Germany. Other ancillary and required chemicals of indicated grade were provided by Department of Pharmacy, Noakhali Science and Technology University, Bangladesh.

2.3. Quantification of MDA level

Serum MDA level was evaluated in accordance with our earlier published method [11,12] applying thiobarbituric acid (TBA) reagent. The supernatant absorbance was assessed spectrophotometrically at absorbance of 530 nm and MDA level was showed as nmol/mL.

2.4. Determination of vitamins A, E and C

Vitamin A and vitamin E was determined by the RP-HPLC according to procedure of our previously published paper [13]. In the liquid-liquid extraction process, reconstitution of dried extract into a mobile phase was followed. Subsequently, level of vitamin A and E were determined at 291 nm absorbance. To determine the vitamin C concentration, phenyl-hydrazine based spectrophotometric method was employed [11,12].

2.5. Determination of trace elements and macro-minerals

Serum concentrations of trace elements and macro-minerals were evaluated by both flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) adopting the method reported in our earlier published paper [14,15]. Serum sample dilution was done with deionized water 1:10 and several concentrations of minerals (0.5, 1.0, 2.0, 5.0 and 10.0 mg/L) were applied to develop calibration curve. Then the concentrations of trace elements and macro-minerals were assessed by taking the absorbance's at 213.9, 248.3, 589, 766.5, 422.7 nm for zinc, iron, sodium, potassium and calcium in the order given.

2.6. Statistical data analysis

All the values of MDA, antioxidant vitamins, trace elements and macro-minerals were showed as the mean \pm standard error mean

(mean \pm SEM). The resultant values were equated between obese individuals and the control group with independent sample t-tests. Pearson's correlation was employed to correlate among different study parameters and $p < 0.05$ was believed to be statistically important. The statistical calculation of data was executed by employing SPSS statistical software, version 19.0 (Armonk, NY: IBM Corp.)

3. Results

This study made up of obesity patients as cases and healthy individuals as controls. Our findings on the assessed parameters in obese individuals and control groups are shown in Table 1 and Table 2.

3.1. Anthropometric and demographic profile of the study population

Anthropometric and demographic feature of the patients and controls are depicted in Table 1. In our study, female contained the most prominent percentage for patient and control group than male subjects. The percentage of female individuals in the patient and control group was 60% and 63%, respectively. The average age of the patients and control group were 48.93 ± 1.25 and 52.38 ± 1.66 years respectively. There were substantial dissimilarities in the mean value of BMI between the patients ($33.88 \pm 0.27 \text{ kg/m}^2$) and the controls ($22.98 \pm 0.17 \text{ kg/m}^2$).

3.2. MDA and antioxidants (vitamin A, E, C) level

Serum MDA and antioxidants level for both obese individuals and control groups are depicted in Table 2. At 5% level of significance, only obese group presented significantly higher level of MDA ($2.21 \pm 0.03 \text{ nmol/mL}$, $p < 0.001$) than the controls. Again, this study found significantly lower concentrations of vitamin A ($0.32 \pm 0.027 \text{ }\mu\text{g/mL}$, $p < 0.05$), vitamin E ($4.09 \pm 0.25 \text{ }\mu\text{g/mL}$, $p < 0.05$) and vitamin C ($5.72 \pm 0.12 \text{ }\mu\text{g/mL}$, $p < 0.001$) for obese group than control group.

3.3. Status of trace elements and macro-minerals

The mean concentrations of trace element and macromolecules for obese and control group given in Table 3, where control group showed maximum value either than obese group that was 0.91 ± 0.13 , 0.88 ± 0.06 , 243.58 ± 8.97 and 87.66 ± 2.10 , mg/L for Zn, Fe, K and Ca, respectively. The difference between the values obtained for these two groups was found statistically significant ($p < 0.001$). But mean concentrations of Na levels were significantly higher in obese group (3284.81 ± 34.51 , $p < 0.001$) than control group.

Table 1
Characteristics of study population.

Parameter	Obese group	Control group	p value
No. of subjects	100	100	
Male: Female	40/60	37/63	
Age (Years)	48.93 ± 1.25	52.38 ± 1.66	0.098 ^{NS}
BMI (kg/m^2)	33.88 ± 0.27	22.98 ± 0.17	0.000**
Total Cholesterol (mg/dl)	219.66 ± 6.06	192.12 ± 2.84	0.000**
Triglyceride (mg/dl)	224.43 ± 7.13	155.16 ± 5.04	0.000**
HDL (mg/dl)	43.62 ± 1.09	45.4 ± 1.27	0.145 ^{NS}
LDL (mg/dl)	128.48 ± 3.35	112.33 ± 3.10	0.000**

Values are expressed as Mean \pm SEM, NS: Not significant, BMI: Body mass index, ** $p < 0.05$ (Significant difference between obese group and control groups at 95% confidence interval).

Table 2
Serum vitamin A, C, E and MDA level of study population.

Parameter	Obese group	Control group	p value
MDA (nmol/mL)	2.21 ± 0.03	1.08 ± 0.03	0.000**
Vitamin A ($\mu\text{g/mL}$)	0.32 ± 0.027	0.52 ± 0.078	0.017*
Vitamin E ($\mu\text{g/mL}$)	4.09 ± 0.25	5.28 ± 0.41	0.014*
Vitamin C ($\mu\text{g/mL}$)	5.72 ± 0.12	8.14 ± 0.16	0.000**

Values are expressed as Mean \pm SEM, MDA: Malodialdehyde, ** $p < 0.05$ (Significant difference between obese group and control groups at 95% confidence interval).

Values are expressed as Mean \pm SEM, ** $p < 0.05$ (Significant difference between obese and control groups at 95% confidence interval).

3.4. Correlation study

Pearson's correlation is employed to demonstrate inter-element correlations between the patient and the control groups for the reported elements. Tables 4 and 5 shows the correlation coefficient and the statistical significance value. Among all demonstrated relationship between antioxidant vitamins, trace elements and macro minerals for obese group and control group, only control group exhibited statistically significant ($p < 0.05$) positive correlation between MDA and Fe ($r = 0.280$, $p = 0.024$), and negative correlation between Zn and Na ($r = -0.288$, $p = 0.022$) but all other positive and negative correlation between vitamin and other trace elements and macro minerals were not found statistically significant for both of the experimental groups.

4. Discussion

The prevalence of obesity in Bangladesh is endlessly increasing and represents a major clinical threat. In our study, we found increased serum MDA and decreased concentration of serum antioxidant vitamin A, E and C in obese patient with respect to healthy controls. This study also found that low serum concentrations of Zn, Fe, Ca and K and increased Na level strongly associated with obese individuals.

ROS are potentially harmful, though sometimes beneficial for biological process. Lipid peroxidation take place by chain reaction of free radicals while oxygen acts as major factor in the production of lipid peroxides which is not beneficial for health. In a good biological system, a balance is required between formation and elimination of ROS. Antioxidants play a vital role to maintain this biological balance [14]. MDA is the last product of lipid peroxidation which is the significant biomarker of oxidative stress. Oxidative stress causes cellular damage and release pro inflammatory cytokines [16]. Previous study found that obesity is related with the heightened chance of atherosclerosis while oxidative change of lipoproteins may bring an important function in pathogenesis of atherosclerosis [17]. Peroxidation of lipids also linked to the oxidative modification of lower concentration of lipoproteins and causes formation of atherosclerotic lesions [18]. Increased lipid peroxidation in obese cases also involved in hypertension,

Table 3
Serum level of Zn, Fe, Na, K and Ca in the study population.

Parameter	Obese group	Control group	p value
Zn (mg/L)	0.39 ± 0.02	0.91 ± 0.13	0.000**
Fe (mg/L)	0.43 ± 0.03	0.88 ± 0.06	0.000**
Na (mg/L)	3284.81 ± 34.51	2562.74 ± 95.92	0.000**
K (mg/L)	162.18 ± 3.72	243.58 ± 8.97	0.000**
Ca (mg/L)	44.62 ± 2.13	87.66 ± 2.10	0.000**

Table 4
Correlation study among various study parameters in patient and control group.

Correlation Parameters	Obese group		Control group	
	r	p	r	p
Vitamin A and Vitamin E	-0.038	0.707	0.135	0.181
Vitamin A and Vitamin C	0.052	0.604	-0.024	0.812
Vitamin A and MDA	-0.009	0.929	-0.099	0.327
Vitamin A and Zn	0.091	0.370	0.025	0.844
Vitamin A and Fe	0.097	0.339	-0.022	0.863
Vitamin A and Ca	0.111	0.270	0.094	0.458
Vitamin A and K	0.112	0.375	0.159	0.115
Vitamin A and Na	0.049	0.697	-0.051	0.617
Vitamin C and MDA	0.027	0.793	0.034	0.737
Vitamin C and Zn	0.013	0.900	0.109	0.396
Vitamin C and Fe	-0.040	0.694	0.049	0.701
Vitamin C and Ca	-0.032	0.750	-0.017	0.895
Vitamin C and K	-0.210	0.093	-0.159	0.114
Vitamin C and Na	0.013	0.916	-0.040	0.693
Vitamin E and Zn	0.039	0.696	0.009	0.942
Vitamin E and Fe	-0.150	0.136	0.090	0.475
Vitamin E and Ca	-0.066	0.517	0.021	0.870
Vitamin E and K	0.141	0.261	0.103	0.309
Vitamin E and Na	-0.136	0.280	-0.048	0.632
MDA and Zn	0.007	0.946	0.074	0.563
MDA and Fe	-0.058	0.565	0.280	0.024*
MDA and Ca	0.004	0.965	-0.018	0.889
MDA and K	-0.183	0.145	0.066	0.516
MDA and Na	0.145	0.250	0.035	0.733

r = Correlation co-efficient; p = Significance value; Negative values specify opposite correlation. *Correlation is significant at 0.05 levels (two-tailed).

hypercholesterolemia, diabetes or coronary heart disease [19]. In our study we observed that significantly higher level of MDA in obese individuals than control group ($p < 0.001$).

Vitamins A, E and C are major antioxidants which provide defense against ROS and radicals. Vitamin A is an excellent free radical trapper found in the cellular membranes of tissues [6]. It protects against peroxidation of lipids. Previous study found that supplementation of antioxidant vitamin decrease the lipid peroxidation [20]. In many enzymatic reactions vitamin E and C act as co-factor. Vitamin E is a lipid soluble antioxidant [21] which acts as a peroxyl scavenger and prevents lipid peroxidation [22]. Obese individuals have lower concentrations of vitamin E, reported by earlier study [23]. Vitamin C is a water soluble antioxidant vitamin which leads to rapid depletion with limited intake of fruits and vegetables [24]. It directly scavenges ROS and lipid hydroperoxides. Previous study found that supplementation of vitamin C is associated with prevention of obesity [25]. In our study, we observed that significantly decreased level of vitamin A, E and C in obese individuals than control group ($p < 0.005$).

Table 5
Comparison of inter-element relationships between the obese and control groups.

Correlation Parameters	Obese group		Control group	
	r	p	r	p
Zn and Fe	0.165	0.100	0.068	0.594
Zn and Ca	0.117	0.245	0.071	0.579
Zn and K	0.073	0.563	-0.021	0.872
Zn and Na	0.158	0.209	-0.288	0.022*
Fe and Ca	0.057	0.575	0.015	0.905
Fe and K	0.022	0.860	0.040	0.754
Fe and Na	-0.097	0.441	0.045	0.723
Ca and K	-0.016	0.902	0.145	0.248
Ca and Na	-0.179	0.154	-0.082	0.517
K and Na	-0.113	0.368	-0.020	0.847

r = Correlation co-efficient; p = Significance; Negative values specify opposite correlation. *Correlation is significant at 0.05 levels (two-tailed).

Increased level of MDA and reduced level of antioxidant vitamins suggest some degree of tissue destruction in obese individuals due to oxidative stress. Oxidative stress is related to increase free radical and can interact with crucial cellular components such as membrane lipids, DNA and proteins which involved in the modification of normal role of these components and causes cell damage [14]. So, our study indicates that an increase in MDA with reduced antioxidants level may be a potential causative factor for the pathogenesis of obesity.

Minerals acts major role in proper working of immune system of human and changes in the level of these minerals may give negative impact on biological processes and leads to various undesired effects [13]. Micronutrients may influence various physiological body functioning, affects immune system activity and heighten the comorbidity disorder [26]. It is reported that there are strong association between micronutrient deficiency and obesity. Evidence suggests that such inadequacy or deficiency can affect metabolism of leptin and insulin. There are various pathways by which deficiency of micronutrient leads to impair regulation of appetite and metabolism of energy. Micronutrients deficiency in obesity may also occur due to altered metabolic activity and excretion [27].

Zinc (Zn) is an important trace element and adequate amount of Zn is needed for optimal synthesis of nucleic acid, metabolism of protein, cells proper functioning and regulation [28]. In human body, Zn plays role in adipose tissue metabolism, leptin secretion regulation and informs brain for fat storage. It also increases free fatty acid release and glucose reuptake and also involves in the glucose regulation [29]. These observations are similar with our findings that presented significantly decrease level of Zn in obese individuals ($p < 0.001$).

Iron (Fe) is also an important trace element that acts as a co-factor, present in important macromolecules which are related to the production of energy, metabolic activity and synthesis of DNA. Fe deficiency is associated with obesity. Hcpidin is a peptide hormone comes from liver produced which is play role in iron hemostasis. It is an inhibitor of intestinal iron absorption and macrophage iron release. It provides its action by combining to iron exporter ferroportin (FPN) contributing FPN phosphorylation, degradation and reduced iron absorption in intestine. In obese subjects pro-inflammatory cytokines released from adipose tissue also accelerates hepcidin release. Again, lipocalin 2 produced by adipose tissue also an iron binding protein makes the Fe unavailable for hemoglobin formation resulting deficiency of iron and anemia which may impair homeostasis of mitochondrial and cellular energy and further increase fatigue of obese individuals. Fe is also associated with oxidative stress [8]. These observations are similar with our findings that presented significant reduced level of Fe in obese individuals ($p < 0.001$).

Sodium (Na) and potassium (K) are important minerals for the proper function of all cells, tissues and other organs of human body. They act as electrolyte which helps in conduction of electricity in human body. K plays a major function in skeletal and smooth muscle contraction [9]. Previous study explicated that decrease in K level associated with glucose intolerance and causes impaired insulin secretion [30]. As a result depletion of K is involved in impaired glucose metabolism and may be associated with obesity. This effect can be reversed by K supplementation [31]. Previous observational studies showed the associations between potassium and obesity are considered controversial [32].

Again it is considered that $[Na^+]/[K^+]$ is linked to obesity. The sugar-sweetened soft drink ingestion is also linked with salt intake [33] and it is associated with the incident of weight gain [34,35]. Usually, a heavy fat diet is high in Na^+ and low in K^+ [36] and salt sensitivity is concerned with insulin sensitivity [37] through the interaction of an atypical renin-angiotensin system. Most studies

suggested a relationship between insulin resistance and salt sensitivity [38,39]. Thus it is considered that low intake of K and high intake of Na may be associated to obesity. Above considerations are consistent with our findings. In this study, we observed significantly lower levels of K and higher levels of Na in the obese group than the control group ($p < 0.001$).

Calcium (Ca) can help in the energy metabolism. Ca supplementation in obese subjects assists in weight loss and fat absorption [9,40]. Previous studies mentioned that active form of vitamin D, 1 α and 25-dihydroxycholecalciferol cause adipocyte differentiation. It also induces the adipocyte lipogenesis, resists lipolysis, and increase triglycerides storage [41,42]. Ca gives a protective effect in case of obesity by the inhibition of 1 α and 25-dihydroxycholecalciferol. Inhibition of 1 α and 25-dihydroxycholecalciferol by Ca also attenuates the oxidative and inflammatory stress and provides a calming effect on smooth muscle [10]. So, decrease level of Ca play an important role in obesity. In this study, we observed significantly lower levels of Ca in the obese individuals than control group ($p < 0.001$).

According to the evidenced presented here, obesity is associated with lipid peroxidation and imbalance of intake of vitamins and minerals during the diet in all the patients included in our study. It is clear that antioxidant acts in slowing down the inflammation in obesity. It would be ideal increasing the consumption of food rich in antioxidants. In this context, we need to pay more attention to both food quantity and quality during a nutritional intervention along with proper physical exercise for weight control in obese individuals.

Despite of the importance of the research, we should report some limitations of this study. Firstly, we did not include the impact of dietary supplementation in our study parameters; therefore, further study may be needed to determine whether nutritional intervention can improve the condition of obese patients or not. Secondly, the study was carried out on a limited number of subjects. A large-scale study with higher number of samples from different regions of Bangladesh may represent the actual scenario in this population. Having these limitations, we hope our study will play an important role to create a new pathological tool for obese patients in Bangladesh.

5. Conclusion

The present study illustrated that Bangladeshi obese individuals have increased serum concentrations of MDA and altered concentrations of serum trace elements and macro-minerals compared with control groups. Serum concentrations of antioxidant vitamins, such as vitamin A, E and C were also found lower in obese individuals than control groups. This study found decreased level of serum Zn, Fe, K and Ca but increased level of serum Na in obese patients than control groups. Thus, increased serum MDA, altered serum antioxidants, trace elements and macro-minerals, may have an impact on the development of obesity in Bangladeshi obese individuals. So, proper nutritional intervention with vitamins, trace elements and macro-minerals may promote the obesity management and reduce its complications in Bangladeshi obese individuals.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dsx.2018.12.022>.

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