



EVALUATION OF OXIDATIVE STRESS, ANTIOXIDANT STATUS AND TOTAL LIPID PROFILE IN STUDENTS WITH EATING DISTRESS

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ABSTRACT

Background: Eating disorders cause a manifold of medical complications to its victims. While numerous studies on this context have been conducted worldwide, very limited reports are available with reference to the Indian population. Therefore, we made an attempt to examine the manifestation of oxidative and nitrosative stress among students with eating distress in Mysore. In conjunction to the above, we evaluated the total antioxidant capacity of the participants. An analysis of the total lipid profile was also undertaken. **Methods:** A case-control comparison study was made using serum samples from students with and without eating distress. A series of biochemical tests were conducted and subsequently analysed using statistical software SPSS version₁₄. Analysis of variance (ANOVA) was utilised to uncover any significance difference existing between the groups. **Results:** Variations in superoxide dismutase, catalase and NOS scavenging activities as well as differences in lipid peroxidation by-products confirmed the existence of both oxidative and nitrosative stress in students prone to eating disorders. On the other hand, the mean total antioxidant capacity appeared to be normal in both control and subject groups. An increase in the mean values of triglycerides, high density lipoprotein and very low density lipoprotein were observed in the subject groups. Although mean total cholesterol content did not deviate significantly from the standard values, hypercholesterolemia was observed in some individuals of the subject groups. **Conclusions:** Eating distress is not merely a psychological manifestation; it affects the human system in many ways leading to an impaired defence system against oxidative stress and disruption of the normal lipid level rendering them prone to the risks of cardiovascular and related diseases.

KEYWORDS: Eating disorders, Oxidative stress, Total antioxidant capacity, Lipid profile.

INTRODUCTION

Erratic and aberrant eating habits as an effect of extreme obsession with one's body image constitute eating disorders. A position paper from the Academy of Eating Disorders (AED) asserts that Anorexia Nervosa (AN), Bulimia Nervosa (BN) and their variants are characterized under Biologically Based Mental Illness (BBMI) and demand identical health care as that of other diseases under BBMI like depression, schizophrenia and bipolar disorder.^[1] Sharp and Freeman declare that the medical complications of Anorexia Nervosa range from premature advanced osteoporosis to life endangering cardiovascular problems.^[2] This manifold infestation of health issues may also hold good for Bulimia Nervosa as well as Binge Eating Disorder (BED).

Oxidative stress is the imbalance caused when the body's ability to disable Reactive Oxygen Species (ROS) is impaired. Finkel and Holbrook state that the degree of oxidative stress is determined by the balance between ROS production and antioxidant defences.^[3] The body is armed with a defence system to counteract ROS which

includes enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase.^[3] The estimation of these enzymes and lipid peroxidation (LPO) by-products gives us an insight on ROS and degree of oxidative stress present in an individual.

The effects of oxidative and nitrosative stress are seen in various diseases and play a vital role in their progression. These topics of vast discussion and on-going research are however, studied sparsely in case of eating disorders. The presence oxidative stress is already confirmed in case of anorexia patients.^[4] Revelations of contrasting SOD levels,^[5-6] variations in catalase activity^[6] and reports of presence of lipid peroxidation products^[7] implies an altered antioxidant enzyme system thus proving the association of oxidative stress in the Anorexia Nervosa patients. Similarly, reduced nitric oxide synthase (NOS) scavenging activity^[5] and marked production of NOS isoform in anorexia patients^[8] suggest the involvement of nitrosative stress in eating disorders.

Apart from the oxidative damage repairing enzymes, a variety of non-enzymatic molecules are also involved in scavenging ROS.^[3] These low molecular mass molecules, which are introduced into our systems through daily diet include, ascorbates, pyruvates, flavonoids and so on. The quantification of these molecules in the serum is arrived at by estimating the total antioxidant capacity (TAC). A significant reduction of TAC in anorexia patients has already been demonstrated earlier.^[9] Unfortunately, such studies as well as research on oxidative and nitrosative stress were not found to be available in case of bulimics and binge eaters.

Patients with Anorexia Nervosa are known to suffer from cardiovascular diseases leading to their premature deaths. Hence many studies have been conducted on the lipid profile of anorexia patients. Reports from studies across the globe show varied results with respect to the total lipid profile parameters. In case of AN, while one study presented with increased total plasma cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) but normal low density lipoprotein (LDL)^[10], another depicted the same outcome except for an increase in LDL too.^[11] Many such studies, report high values in at least one such parameter in anorexia nervosa patients.^[12-14]

Patients with both Bulimia Nervosa and Binge Eating Disorder are known to consume high quantities of food or in other words go on bingeing episodes. Thus high dietary intake of lipid may cause them to have high cholesterol. Earlier it was predicted in a report that serum cholesterol levels higher than the recommended range may be seen in patients with Bulimia Nervosa^[15] which was later clinically proven in another analysis.^[16] Enhanced TC^[17] and along with it higher TG^[18] have also been reported in bulimia nervosa patients. Detailed analysis of lipid profile on binge eaters have not yet been conducted extensively therefore research articles on lipid profiling of BED are unavailable.

Studies on eating disorders pertaining to oxidative and nitrosative stress, total anti-oxidant capacity and lipid profile have not been conducted in India. In view of this, we conducted this study wherein we analysed the aforementioned parameters among a non-clinical sample of students with eating distress extracted from the student population of Mysore city, Karnataka. This study, to the best of our knowledge is the first of its sorts to be conducted in India.

METHODS

On receiving ethical clearance from the Institutional Human Ethical Clearance Committee of University of Mysore, we surveyed 1600 students, aged 15-25 years, studying at Mysore using two self-report questionnaires, EAT-26 (Eating Attitudes Test - 26)^[19] and BES (Binge Eating Scale)^[20]. Among the 417 students who scored high in either of the questionnaires 35 students (13 male

and 22 females) were interviewed in person. Their high scores in the questionnaires, altered body mass index (BMI) along with erratic eating practices were indicative of eating distress. This aided us in selecting these students as subjects in our study. Similarly 35 students scoring low in both the questionnaires with normal BMI and eating habits were taken as controls. Among the 35 subjects, 18 scored higher in EAT-26 and 17 scored higher in BES. Thus, two different groups were identified within the subjects according to their type of eating distress. Henceforth, in this article, these two groups are referred to as the high scorers of EAT-26 and the high scorers of BES. All these high scorers were not clinically diagnosed with a particular eating disorder due to lack of facilities and expertise in the field of eating disorders in the Indian context. However, high scores in the universally accepted standard questionnaires and other factors such as varied BMI, extensive starvation or binge eating clearly implied that the subjects in our study were susceptible to having an eating disorder.

Blood samples were collected from subjects and controls on receiving their consent in a written format. The samples were centrifuged at 4000 rpm for 10 minutes to obtain blood serum. The serum samples were then subjected to analysis of SOD, Catalase, Lipid Peroxidation, NOS scavenging activity and Total antioxidant capacity.

SOD analysis was conducted according to the method specified by Kazari Das.^[21] In this method the superoxide radicals produced by the reduction of riboflavin are scavenged by SOD. The leftover superoxide radicals react with hydroxylamine hydrochloride to produce nitrites which in turn react with Griess reagent to produce a pink azo compound whose absorbance was determined at 543 nm. Aebi method^[22] was used for the determination of Catalase activity where the decomposition of hydrogen peroxide by catalase was noted by observing the decrease in absorbance read at 240nm. The variation in absorbance per unit time was used as a measure for catalase activity. The method by Buege and Aust^[23] was utilized to measure reaction of malondialdehyde (MDA) with thiobarbituric acid - trichloroacetic acid - hydrochloric acid assay (TBA-TCA-HCl assay) to assess the extent of lipid peroxidation. The pink coloured MDA-TBA product was read at 535nm. NOS Scavenging Activity was analysed using the procedure described by Green *et al.*,^[24]. The reaction of reactive nitrogen species produced by sodium nitroprusside at physiological pH with Griess reagent yields a pink colour compound which was read at 546nm. Finally, Total Antioxidant Capacity was measured using the method by Prieto P *et al.*,^[25] where the conversion of Molybdenum (Mo VI) to molybdenum (Mo V), by antioxidants and its further reaction with phosphate under acidic pH resulted in the formation of a green coloured complex. This was read spectrophotometrically at 695nm. Here we used vitamin

C as standard. Lowry's method ^[26] was used for protein estimation.

The serum samples isolated were also used for lipid profile comprising of chemical parameters such as total cholesterol (TC), HDL- cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol. ERBA kits were used for the estimations. Cholesterol, HDL-cholesterol and Triglyceride estimations were carried out in the laboratory. LDL- cholesterol and VLDL- cholesterol were calculated utilizing the results from the estimations conducted.

Statistical Analysis

The obtained data was tabulated and analysed by using statistical software SPSS version_{14.01}. Mean and standard error were used for comparison. Analysis of variance (ANOVA) was employed to find out any significance difference existing between the different groups. $p < 0.05$ was considered to be significant.

RESULTS

The results from the estimation of SOD & catalase activity revealed that both EAT -26 and BES high

scorers displayed increased SOD activity but decreased Catalase activity when compared to the controls. The lipid peroxidation results implied the presence of MDA in all groups i.e. controls (0.1098 ± 0.01 n moles of MDA/mg of protein), EAT-26 and BES high scorers, with the highest MDA present in case of the high scorers of BES (0.3026 ± 0.05 n moles of MDA/mg of protein) followed by the EAT high scorers (0.2894 ± 0.05 n moles of MDA/mg of protein). This data proved to be statistically significant with $p < 0.05$ (Table 1).

We found an upsurge in the NOS scavenging activity in high scorers of both EAT-26 and BES than in controls, i.e. $31.24 \pm 2.09\%$ in controls, $50.41 \pm 4.85\%$ in EAT-26 high scorers and $47.39 \pm 5.27\%$ in BES high scorers showing a significant variation ($p < 0.05$) among the groups. Thus, implying the presence of nitrosative stress. The most astonishing revelation in this study was the escalated TAC in the high scorers of both EAT-26 (122.49 ± 33.94 $\mu\text{g/ml}$) and BES (159.97 ± 37.97 $\mu\text{g/ml}$) than in controls (61.87 ± 15.88 $\mu\text{g/ml}$). Significant differences ($p < 0.05$) were also observed between the groups in case of total anti-oxidant capacity (Table 1).

Table 1: Comparison of mean values of SOD, LPO, CAT, NOS scavenging activity and TAC between controls and high scoring subjects of EAT-26 and BES.

Biomarkers	Type	Number	Mean $\pm$ SE	df	F	p
SOD (Units of SOD/ mg of protein)	Control	35	0.0440 ± 0.005	2	2.118	0.128
	EAT-26	18	0.0668 ± 0.01			
	BES	17	0.0646 ± 0.01			
LPO (n moles of MDA/ mg of protein)	Control	35	0.1098 ± 0.01	2	9.357	0.000*
	EAT-26	18	0.2894 ± 0.05			
	BES	17	0.3026 ± 0.05			
CAT (k/ml)	Control	35	0.0370 ± 0.01	2	0.987	0.378
	EAT-26	18	0.0129 ± 0.004			
	BES	17	0.0182 ± 0.01			
NOS (%)	Control	35	31.24 ± 2.09	2	9.302	0.000*
	EAT-26	18	50.41 ± 4.85			
	BES	17	47.39 ± 5.27			
TAC ($\mu\text{g/ml}$)	Control	35	61.87 ± 15.88	2	3.878	0.025*
	EAT-26	18	122.49 ± 33.94			
	BES	17	159.97 ± 37.97			

* indicates significant value ($p < 0.05$)

Results from the total lipid profile, displayed that although the subject groups exhibited lesser mean values of TC than the controls (Figure 1), 11.1% of EAT-26 high scorers were slightly hypercholesterolemic. The HDL values were almost similar in case of the controls and the high scorers of EAT-26 but BES high scorers presented with a higher mean HDL. Mean TG levels in both the subject groups were found to be elevated when compared to the controls i.e. 74.87 ± 4.74 mg/dl, 101.52 ± 5.94 mg/dl and 98.99 ± 6.96 mg/dl for controls, high scorers of EAT-26 and high scorers of BES respectively. Likewise the highest VLDL values were seen in the high scorers of EAT-26 (20.30 ± 1.18 mg/dl) closely followed by the BES high scorers (19.79 ± 1.39 mg/dl) when

compared with the controls (14.97 ± 0.94 mg/dl). Both mean TG and VLDL values showed statistical significance ($p < 0.05$) between the groups. LDL values of the subject groups were lower than the controls with the least in case of BES high scorers.

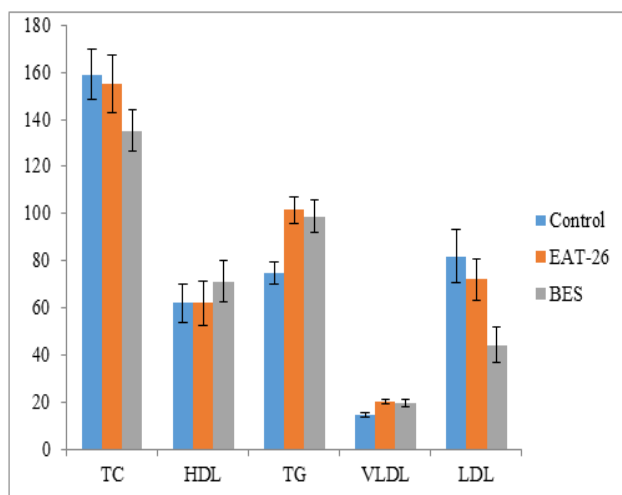


Figure 1: Comparison of mean values of lipid profile between controls and high scoring subjects of EAT-26 and BES (TG & VLDL displayed $p < 0.05$ indicating significant value).

As males and females have different normal range values for the various parameters of total lipid profile, we further made comparisons between females of the different groups and also within the males of the different groups. We observed that the female high scorers of EAT-26 had higher TC, HDL and lower LDL when compared to the controls (Table 2). However, the high scoring EAT-26 males in our study displayed higher TC and VLDL than the controls (Table 3). The high scorers of BES show an increase in TG, HDL and VLDL levels when compared to the controls (Figure 1). Both males and females of BES high scorers showed these differences in the same parameters when compared to their counterparts in the control group (Tables 2 & 3) with the exception of HDL in case of male high scorers of BES. The mean TG and VLDL values were statistically significant ($p < 0.05$) between the only female experimental groups. In general, meagre variations from normal ranges of different lipid content were noted in the subjects.

Table 2: Comparison of mean values of lipid profile between female controls and female high scoring subjects of EAT-26 and BES.

Bio markers	Type	Number	Mean $\pm$ SE	df	F	p
TC	Control	21	155.20 $\pm$ 15.08	2	0.895	0.417
	EAT	12	166.46 $\pm$ 16.48			
	Binge	10	132.86 $\pm$ 10.51			
HDL	Control	21	51.81 $\pm$ 8.28	2	1.977	0.152
	EAT	12	65.35 $\pm$ 12.94			
	Binge	10	81.26 $\pm$ 10.49			
TG	Control	21	73.32 $\pm$ 5.91	2	5.039	0.011*
	EAT	12	101.64 $\pm$ 7.8			
	Binge	10	96.96 $\pm$ 8.76			
VLDL	Control	21	14.66 $\pm$ 1.18	2	5.039	0.011*
	EAT	12	20.32 $\pm$ 1.57			
	Binge	10	19.39 $\pm$ 1.75			
LDL	Control	21	88.72 $\pm$ 14.48	2	4.212	0.022*
	EAT	12	80.77 $\pm$ 11.21			
	Binge	10	32.21 $\pm$ 5.03			

* $p < 0.05$ indicates significant value.

Table 3: Comparison of mean values of lipid profile between male controls and male high scoring subjects of EAT-26 and BES.

Biomarkers	Type	Number	Mean $\pm$ SE	df	F	p
TC	Control	14	164.94 $\pm$ 16.14	2	1.150	0.333
	EAT	6	131.83 $\pm$ 11.52			
	Binge	7	138.83 $\pm$ 15.98			
HDL	Control	14	77.81 $\pm$ 15.83	2	0.623	0.545
	EAT	6	56.08 $\pm$ 12.17			
	Binge	7	56.94 $\pm$ 14.36			
TG	Control	14	77.21 $\pm$ 8.12	2	2.312	0.121
	EAT	6	101.28 $\pm$ 9.39			
	Binge	7	101.90 $\pm$ 12.12			
VLDL	Control	14	15.44 $\pm$ 1.62	2	2.312	0.121
	EAT	6	20.25 $\pm$ 1.87			
	Binge	7	20.38 $\pm$ 2.42			
LDL	Control	14	71.67 $\pm$ 18.26	2	0.200	0.820
	EAT	6	55.49 $\pm$ 12.58			
	Binge	7	61.51 $\pm$ 14.88			

DISCUSSION

Eating disorders pose great medical threats to their bearers. These threats need to be frequently assessed by means of specific biomarkers for a better insight into these disorders and how they manifest. Considering this, we conducted the current study to explore the possibilities of existence of oxidative and nitrosative stress, and an altered lipid profile among students with eating distress.

A recent study focused on oxidative stress in patients with AN demonstrated an increase in SOD in case of Anorexics.^[5] Similar results were observed in our study among the EAT-26 high scorers. In addition to this, the decreased catalase activity that we observed paralleled with the results from a previous study conducted on patients with AN.^[6] They also observed an increase in SOD activity and decrease in catalase activity. The occurrence of MDA in the high scorers of EAT-26 concurred with the results from another analysis where they too expressed the presence of LPO by-products in AN.^[7] From the above outcomes we can be ascertained that presence of oxidative stress was apparent in high scorers of EAT-26. Moser *et al.*, stated that AN appeared to cause an impairment of enzymatic and non-enzymatic oxidative systems due to constant decrease in food intake thus generating more ROS.^[27] This may very well be the justification for our findings.

Although not heavily explored, nitrosative stress through nitric oxide (NO) too forms a common denominator for stress-related diseases.^[28] Earlier experimentations have revealed that NOS activity was found to be reduced in patients with AN, whereas the expression on NOS iso-forms was unaffected.^[5] In contradiction, a different study declared a substantial increase in NO production and a prominent manifestation of NOS iso-forms.^[8] We noticed elevated NOS scavenging activity in high scorers of EAT-26 confirming the existence of nitrosative stress. As observed in the high scorers of EAT-26, BES high scorers also exhibited equivalent outcomes. However no reports thus far have been made in regard with correlations between BED and oxidative and nitrosative stress. Further in-depth analysis of BED needs to be undertaken.

In addition to the oxidative damage repairing enzymes, diverse non-enzymatic molecules as well assist in scavenging ROS. The efficacies of these anti-oxidants are gauged as a measure of TAC. A study to determine the serum anti-oxidant capacity in AN patients demonstrated a significant decrease in TAC.^[9] However, quite surprisingly, our investigation revealed elevations in TAC in both the subject groups than in controls. This could possibly be an adaptation to a heightened level of oxidative stress at an initial phase.^[29-30]

Lipid profiling is used to screen abnormalities in lipid contents. As mentioned earlier patients with Anorexia Nervosa have known to suffer from cardiovascular

diseases. Therefore many studies on the lipid profile of anorexia patients have been made. Although the subjects from our study didn't display higher TC, hypercholesterolemia was observed in a significant fraction of EAT-26 high scorers corresponding to the fact that AN patients very often displayed hypercholesterolemia.^[12,14] While one investigation revealed that women with Anorexia presented with an increased TC, TG & HDL,^[10] another portrayed the same results of higher serum levels of TC, HDL with an addition of LDL.^[11] Similar to these earlier researches we found elevations in different parameters of lipid profile among the subject groups. In case of BES high scorers, these differences may be attributed to the disturbed eating habits including consumption of high fat food. Many arguments for the causes of hypercholesterolemia in AN have been proposed, one of which is the augmentation in the process of resorption in case of exogenic cholesterols.^[31] This could explain the hypercholesterolemic condition seen in EAT-26 high scorers.

CONCLUSIONS

Incidences of eating disorders are now common in India; however studies on their diagnosis are relatively uncommon. Rarer still, are in-depth studies on the effects of oxidative and nitrosative stress on these disorders. Advanced studies on the consequences of inconsistencies in the parameters of total lipid profile also need to be conducted to understand these disorders better so that we, as a society can be well equipped in dealing with eating disorders. Our investigation was limited due to the dearth of an appropriate diagnostic tool to clinically prove the presence of eating disorders in our subjects. However we may be the first ones in India to evaluate the presence of oxidative stress, nitrosative stress and to conduct total lipid profile on a non-clinical sample of students with eating distress.

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ABBREVIATIONS

AED: Academy of eating disorders
 AN: Anorexia nervosa
 ANOVA: Analysis of variance
 BBMI: Biologically based mental illness
 BED: Binge eating disorder
 BES: Binge eating scale
 BMI: Body mass index
 BN: Bulimia nervosa
 CAT: Catalase
 EAT-26: Eating attitudes test-26
 HDL: High density lipoprotein cholesterol
 LDL: Low density lipoprotein cholesterol
 LPO: Lipid peroxidation
 MDA: Malondialdehyde
 NOS: Nitric oxide synthase
 ROS: Reactive Oxygen Species

SOD: Superoxide dismutase

TAC: Total antioxidant capacity

TBA: Thiobarbituric acid

TC: Total cholesterol

TCA: Trichloroacetic acid

TG: Triglycerides

VLDL: Very low density lipoprotein cholesterol