Original Article

Evaluation of Oxidative Stress Markers' Status in Obese Females Using Hormonal Contraceptives

Fauzia Aitazaz, Muhammad Aurangzeb, Radhia Khan, Samiullah Khan, Zakia Khan

ABSTRACT

Objective: To evaluate the variation in oxidative stress markers including Superoxide Dismutase (SOD), catalase and Malondialdehyde (MDA) and to correlate it with obesity in females using contraceptives.

Methodology: Comparative cross sectional study was a project of University of Lahore (UOL) in collaboration with Basic Health Unit Mudkey (periphery of Kasur) from January to December 2013. The study population comprised of 51 married females of age ranged 25 to 40 years. The study was approved by the Research Ethical Committee of UOL. Thirty-one females either using Combined Oral Contraceptive (COC) pills or progestin injections were enrolled for a period of 9 months, while 20 females not using any type of contraceptives were recruited as a control group. Weight in Kg and height in meter were estimated by the standard protocol. Body mass index (BMI) was calculated by the formula; weight in Kg/ height in m^2. Subjects having BMI greater than 25 Kg/m^2 were categorized as obese. Stress markers were estimated from blood samples. SOD was assayed by Nitroblue Tetrazolium Method by Spectrophotometer. Catalase and Glutathione levels were also determined using spectrophotometer. MDA levels were measured by Thiobarbituric acid assay reaction with MDA. Statistical analysis was performed by SPSS 20. For continuous variables mean and SD were determined; while frequency and percentages were used for estimation of categorical variables. Beta coefficients for association between obesity and the stress markers were obtained by regression analysis. P-value ≤ 0.05 was taken as statistically significant.

Results: SOD and MDA levels were significantly higher in obese group as compared to non-obese group (P-values 0.02, 0.04). No significant differences were noted in obese and non-obese subjects with respect to catalase (P-value 0.35) and Glutathione (P-value 0.9) concentrations. Regression analysis shows significant positive association of BMI with SOD (P-value 0.006) and MDA (P-value 0.000); however, catalase (P-value 0.28) and Glutathione (P-value 0.85) were not significantly associated with BMI.

Conclusion: Oxidative stress in obese are due to higher MDA levels. SOD is positively associated with BMI; however catalase and glutathione are not affected by BMI.

KEYWORDS: Catalase, Malondialdehyde Oxidative Stress, Superoxide Dismutase

INTRODUCTION

Obesity is a chronic inflammatory condition-reaching pandemic in developed and developing countries. It is associated with metabolic derangement and oxidative stress.[1] Use of contraceptive pills and injections are responsible for obesity in females of reproductive age.[2]

Hyperlipidemia, hyperleptinemia and release of inflammatory cytokines from adipocytes are the contributing factors for over production of Reactive Oxygen Species (ROS) including Superoxide (O_2^-) and Hydrogen Peroxide (H_2O_2) that causes oxidative stress, damage to DNA and RNA leading to cell death.[3] Moreover, oxidative stress is best reflected by products of lipid peroxidation Malondialdehyde (MAD).[4]

In obese subjects accumulation of abdominal fat enhances lipid peroxidation and subsequent production of MDA due to excess release of pro-inflammatory cytokine interleukin-6 (IL6), Tumor Necrosis Factor-alpha (TNF-α) and C Reactive Proteins (CRP).[3] These factors are responsible for activating the apoptotic cascade causing cell death and oxidative stress in obese subjects.

An antioxidant system is required to prevent oxidetive stress. Superoxide dismutase is the key component

Dr. Fauzia Aitazaz, *MBBS, MPhil*
Asst. Prof. Physiology
Azad Jammu Kashmir Medical College, Muzaffarabad
Dr. Muhammad Aurangzeb, *MBBS, FCPS*
Pathologist
CMH, Rawlakot
Radhia Khan, *MSc, PhD*
Asst. Prof. Biochemistry
MTI Bannu Medical College, Bannu
Dr. Samiullah Khan, *MBBS, FCPS*
Senior registrar of medicine
MTI Khalifa Gulnawaz Teaching Hospital, Bannu
Dr. Zakia Khan, *MBBS, FCPS*
Asst. Prof. Gyneacology
MTI Bannu Medical College, Bannu

Correspondence:
Fauzia Aitzaz
Email: doctorfauzia@gmail.com

of antioxidative system and it proves to be first line of defense against oxidative cell damage induced by Reactive Oxygen Species (ROS). It helps in elimination of hydrogen peroxide (H_2O_2) from superoxide that can be later on decomposed into water and oxygen by the action of another important antioxidant enzyme catalase, thus protecting cell from oxidative damage.[5]

In previous studies, some have documented higher activity of SOD in response to oxidative stress in obese subjects, while others reported contradictory results suggesting lower activity of antioxidant system in obese individuals.[6] Oxidant-antioxidant status in females using contra-ceptive are still debatable. Limited data is available concerning association of oxidative stress and obesity in these high-risk females. Keeping in view marked altered oxidative stress and higher prevalence of obesity in females using contra-ceptive pill and injections, it is important to understand the link bet-ween the two. This study was aimed to evaluate the alteration in oxidative stress markers including SOD, Catalase and MAD and to correlate it with obesity in females using contraceptives.

METHODOLOGY

This comparative cross sectional study was the project of the University of Lahore (UOL), in collaboration with Basic Health Unit (BHU) Mudkey (periphery of Kasur) from January to December 2013. The study population comprised of 51 married females of age ranged 25 to 40 years. Research Ethical Committee of UOL approved the study. Healthy married females with reproductive age were included in the study. Unmarried females, lactating, menopausal and females with any pelvic pathology were excluded. Subjects with history of neurological disorders, metabolic diseases including hepatic disorders, diabetes mellitus and cancer that could influence their oxidative markers were also excluded from the study.

Thirty-one females either using Combined Oral Contraceptive (COC) pills or progestin injections for a period of 9 months were enrolled, while 20 females of the same age group not using any type of contraceptives were recruited as control group. All participants were enrolled by purposive sampling technique from BHU, Mudkey. Informed consent was taken from each participant. Obesity status was evaluated and compare among the females not using contraceptives (control group) and females using contraceptive. Subjects were further subdivided into obese and non-obese groups based on BMI for comparison of stress markers and to find out the association between obesity and oxidative stress markers.

All relevant information was recorded on predesign questionnaires. Height in meters and weight in Kg were estimated by Stadiometer and weighing scale (ZT - 160 NSL) BMI was calculated by formula weight in Kg/ height in m^2. Subjects having BMI > 25 Kg/m^2 were considered obese as per WHO guidelines for Asians.[7] Blood samples (5ml) were drawn for estimation of stress markers including SOD, Catalase, Glutathione and MDA. Serum was separated from blood samples by centrifugation within one hour of collection and stored at -70° C until assayed. SOD was assayed by Nitroblue Tetrazolium (NBT) method by spectrophotometer. Catalase and Glutathione levels were also determined by spectrophotometer procedure. The MDA levels were measured by Thiobarbituric Acid (TBA) assay reaction with MDA.

***Statistical Analysis*:** SPSS version 20 was used to analyze the data. Continuous variables were expressed as mean ± SD (standard deviation), while categorical variables (obesity) were presented as frequency and percentages. Normality of data was checked by Shaprio Wilk Test. Independent t-test was used to compare stress markers among obese and non-obese females. Regression analysis was applied to show the association between independent variable (obesity) and dependent variables (oxidative stress markers). Results of regression analysis were expressed as beta co-efficient and standard error. P-value $\leq$ 0.05 was considered statistically significant.

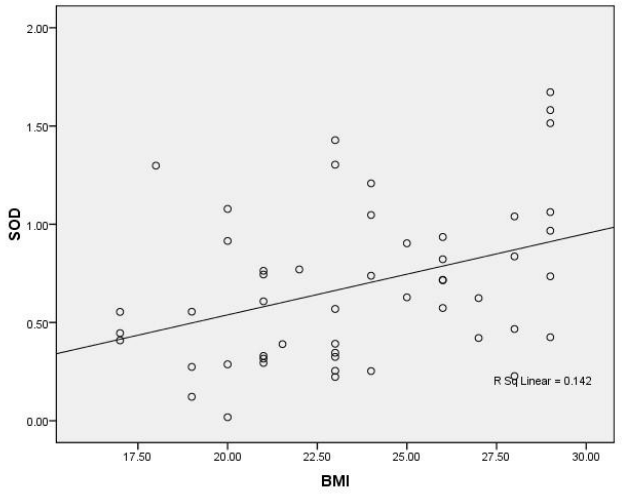
RESULTS

This study was comprised of 51 females of mean aged 30.8±5.4 year. Out of total 51 participants, 24 (47%) were obese having BMI > 25 Kg/m^2 and 27 (53%) were non-obese. Of total population, 21 females were using progestin injections, while 10 were on low dose COC pills. Twenty females were not using any type of contraceptive.

In the females using contraceptives 16 (51.6%) were obese as compared to 8 (40%) of obese females who were not using contraceptives. Current results reveal that SOD levels were higher in obese group as compared to non-obese group, the difference was statistically significant (P-value 0.000). No significant differences were noted in obese and non-obese subjects with respect to catalase (P-value 0.35) and glutathione (P-value 0.9) concentrations. Statistically significant difference was observed in mean MDA concentration among the obese and non-obese females (P-value 0.04). MDA was higher in obese subjects than in non-obese subjects (Table1). Scattered plot graph confirms the

significant positive association between SOD and obesity (Figure 1).

Figure 1: Scattered Plot Between Mass Index (BMI) and Superoxide Dismutase (SOD) (n=51)



BMI = Body Mass Index, SOD = Superoxide dismutase

Table 1: Comparison of Mean Scores Among Study Groups (n=51)

Stress Markers	Obese Females n(%) 24(47)	Non-obese Females n(%) 27(53)	P value
	Mean ± SD	Mean ± SD	
Body Mass Index (BMI) Kg/m^2	26.95 ± 1.50	20.85 ± 2.21	0.000*
Superoxide Dismutase (SOD) U/ml	0.84 ± 0.38	0.58 ± 0.39	0.02*
Catalase (CAT) U/ml	2.75 ± 0.07	2.77 ± 0.09	0.35
Glutathione (GSH) U/ml	0.42 ± 0.58	0.41 ± 0.07	0.9
Malondialdehyde (MDA) µmol/L	0.73 ± 0.47	0.55 ± 0.313	0.04*

Kilogram/meter^2 (kg/m^2), Units/Milliliter (U/mL), micromole/Litter (µmol/L), P-value ≤ 0.05 was considered significant.

Regression analysis shows significant positive association of BMI with SOD (p value 0.006) and MDA (p value 0.000). However, catalase (p value 0.28) and Glutathione (p value 0.85) were not significantly associated with BMI (Table2).

DISCUSSION

Current study was aimed to evaluate the oxidative stress by estimating stress markers and status of obesity in the females using contraceptive pills and injections. Association between obesity and oxidant-antioxidant status in these females was also explored. SOD, catalase and glutathione are crucial parameters of antioxidants defense system. Glutathione is a potent antioxidant that is involved in protection of proteins required for nucleic acid synthesis and helps in repairing of DNA.[8]

Table 2: Regression Analysis between BMI and Oxidative Stress Markers (n=51)

Oxidative Stress Markers	Beta coefficient (β)	Standard Error	P value
Superoxide Dismutase (SOD)	0.041	0.015	0.006*
Catalases (CAT)	- 0.003	0.007	0.284
Glutathione (GSH)	0.001	0.003	0.85
Malondialdehyde (MDA)	1.661	1.297	0.000*

Independent variable is Body Mass Index (BMI), Dependent variables are Oxidative Stress Markers, P-value ≤0.05 was considered significant.

Generation of ROS and resultant high levels of end product of lipid peroxidation MDA with insufficient antioxidant enzymes reflect the oxidative stress.[9] Intense oxidative stress has harmful effects on cellular functions, intracellular signaling, gene regulation, apoptosis and results in cellular death.[10]

Strong evidences are available showing that the females using oral as well as injectable contraceptives have positive effect on their BMI causing obesity.[2] Growing evidences also indicate that the white adipose tissues in obese subjects are associated with excess generation of ROS including superoxide (O_2^-) and H_2O_2, accompanied by augmented expression of NADPH oxidase and decline in anti-oxidative enzymes that are required to resist ROS mediated cellular damage.[3] If oxidation by ROS exceeds the antioxidants defense system, oxidative stress is generated.[10]

We found significant higher levels of MDA in obese subjects and it was significantly associated with BMI, suggesting the presence of oxidative stress in these subjects. Current result concerning positive association of MDA with BMI is supported by study conducted in China.[6] Higher MDA levels in obese subjects are also reported by researches conducted in various other regions of world including Jordan and Saudi Arabia that justified our results.[3,4]

Present study also found higher levels of anti-oxidant SOD in obese subjects than non-obese subjects and was positively related with BMI. SOD is believed to be the first line of antioxidant defense system. This finding is confirmed by the past study from Jordan that documented similar relation.[3] However, inconsistent results are documented by other studies conducted in

Saudi Arabia, and documenting decrease activity of SOD in obese subjects.[4]

Current study did not find any influence of obesity on catalase and glutathione. Present results are in line with Al-Dalaeen et al who reported no impact of BMI on catalase and glutathione.[3] While on the other hand, Albuali et al reported decrease in glutathione levels in obese subjects.[4]

Increase in MDA but not increase in above mentioned antioxidant results in imbalance between oxidant-antioxidant systems causing oxidative stress in obese subjects. Increase in the first line of antioxidant defense SOD in obese subjects of current study might be in the response to generation of oxidative stress induced by over production of ROS and higher lipid peroxidation reflected by higher MAD levels. Activation of SOD counteracts the effect of ROS and protects cells from damaging effects of oxidants.

Current findings are justified by An H et al who reported the overproduction of ROS and activation of the antioxidant defense system in obese subjects.[6] Contradictory to our results, Das et al documented decrease activity of SOD, glutathione and catalase along with higher MDA levels and subsequent oxidative stress in obese population.[9]

***Limitations*:** The causality between BMI and oxidative stress could not be established due to cross sectional study design. Due to Small sample size the result cannot be generalized to whole Female population.

CONCLUSION

Oxidative stress in obese is due to higher MDA levels. SOD is positively associated with BMI; however, catalase and glutathione are not affected by BMI.

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Conflicts of Interest: None.

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Author's Contribution:

Dr. Fauzia Aitazaz	Study design, data collection, manuscript writing, revising all intellectual contents and accountable for al research work.
Radhia Khan	Manuscript writing, revising all intellectual contents and accountable for al research work.
Dr. Samiullah Khan	Statistical analysis, interpretation of results, manuscript writing, revising the final version and accountable for al research work.
Dr. Zakia Khan	Statistical analysis, interpretation of results, manuscript writing, revising the final version and accountable for al research work.

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