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RESEARCH ARTICLE

A STUDY OF OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES IN OBESITY

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ABSTRACT

Obesity is a growing social problem. Obesity can restrict the quality of life and make the person susceptible to a plethora of chronic systemic diseases like Coronary artery disease, Hypertension, Diabetes mellitus, metabolic syndrome and even cancers. Obesity leads to a low grade inflammatory state which creates an oxidative stress in the body. The oxidative stress measured in obese individuals versus normal controls show an increased oxidative stress in the former putting them at increased risk of complications. We found that with increasing Body mass index the oxidative stress increased as seen in the rise of Malondialdehyde (158%) and fall of Glutathione peroxidase (20.76%), Glutahione reductase (29.45%) and Vitamin C (21.7%) which when compared between the two groups is statistically very significant ($p<0.0001$).

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INTRODUCTION

Obesity - characterized by high body mass index - is a growing social problem compounded by many different lifestyle and genetic factors. Globally it is on the rise (Flegal *et al*., 2002). Obesity can limit movement and decrease the quality of life in general and increase the complications of chronic systemic diseases like liver and cardiovascular diseases, diabetes mellitus and metabolic syndrome and even cancers. Childhood obesity poses risk for adult obesity and the subsequent complications. As prevalence of obesity increases, it therefore increases the load of its co-morbidities (Dietz and Robinson, 2005). Obesity is associated with a low grade chronic inflammation which promotes pro-inflammatory and oxidative state leading to an acute response. Therefore it has been hypothesized that the inflammation of adipose tissue in obese patients will lead to obesity related complications like insulin resistance (Hotamisligil *et al*., 1993). Oxygen which sustains life is also capable of forming oxy free radicals which when produced in excess amounts increase the susceptibility of lipids to peroxidation. These oxy free radicals can directly or indirectly damage in various organs by increasing the oxidative stress. The body has mechanism to counter the oxyfree radicals by an integrated action of enzymes [viz. Glutathione peroxidase (GPx), Glutathione reductase (GR), Superoxide dismutase (SOD), Catalase (Cat)], nutrients (Vitamin E, C) and other reducing substances (Glutathione) which constitute the anti-oxidant system. GPx catalyses the reduction of variety of hydrogen peroxide (ROOH and H_2O_2) using glutathione as a substrate, thereby protecting mammalian cells against oxidative stress (Blum and Fridovich, 1985). Oxidative stress will increase levels of oxidants and products of oxidation like ROS and Malondialdehyde (MDA) (Keaney *et al*., 2003) while reducing the anti-oxidant machinery (Olusi, 2002). Asessing body fat requires extensive technologies, so WHO has recommended the simpler measurement of BMI which correlates well with the fat content (Lopez *et al*., 2006). Studies have shown increased increase un oxidative stress and inflammation in persons with obesity. This may be due to the various adipokines produced by the adipocytes like like IL-6 (Fried *et al*., 1998), TNF-a (Hotamisligil *et al*., 1993), Monocyte chemoattractant protein 1 (MCP-1) (Sartipy and Loskutoff, 2003) Leptin (Friedman and Halaas, 1998), Adiponectin (Yamauchi *et al*., 2001; Berg *et al*., 2001; Maeda *et al*., 2002; Okamoto *et al*., 2002; Matsuda *et al*., 2002; Arita *et al*., 1999). Adiponectin which has a role in insulin sensitisation (Berg *et al*., 2001; Maeda *et al*., 2002; Okamoto *et al*., 2002) and as an anti-atherogenic (Maeda *et al*., 2002; Okamoto *et al*., 2002) is decreased in the circulation in obesity (Fujita *et al*., 2006). Hence decreased Adiponectin may lead to insulin resistance in obese individuals. Serum Adiponectin of humans correlate inversely with systemic oxidative stress (Fujita *et al*., 2006). In adipose tissue, the oxidative stress also suppresses the secretion and mRNA expression of Adiponectin

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while increasing the same for pro-inflammatory adipocytokines such as IL-6 and MCP-1 (Furukawa *et al*., 2004). The present study was designed to study the effects of obesity on the oxidative stress status by measuring stress markers like MDA, GPx, GR, and Vitamin E and Vitamin C.

MATERIALS AND METHODS

The present study was conducted in Department of Biochemistry, GCS Medical College, Ahmedabad on 30 male cases in the age group of 25 – 40 years with a BMI > 30. Equal number of healthy subjects in the same age group with BMI <25 served as control. Subjects who had complications like diabetes mellitus, hypertension, renal failure, past myocardial infarction, hypothyroidism were not included in the study. A written consent was taken and a questionnaire prepared to collect medical history along with anthropometric parameters like height and weight. The study was a case control cross sectional study approved by the Ethics committee of the institution. The cases and controls were tested for MDA, GR, GPx, Vit C, Vit E, and serum Lipid profile (Triglyceride (TG), Total Cholesterol (TC), HDL-C, LDL-C). All the statistical analysis were performed by using IBM SPSS 20.0 statistical package and Microsoft Excel 2007. Results are expressed as mean± S.D. Paired t-tests were used for comparisons of the variables between the obese and non-obese subjects. Correlations were determined by Pearson's correlation coefficient method. Linear regression model was used to check the correlation between BMI and other independent parameters (oxidative stress markers). P-values <0.05 were considered statistically significant. 5 ml of venous blood was collected by venupuncture in fasting condition in a heparinized vial (10U/ml blood). The samples were centrifuged for 10 minutes at 3000 rpm.

Plasma was separated from the top and analysed for fasting Glucose (GOD-POD), Creatinine (Jaffe's), Triglyceride (GPO), Total Cholesterol (CHOD-POD), HDL-C (PTA, CHOD-POD) (Barham and Trinder, 1972; Henry, 1963; Clinical Diagnosis and Management by Laboratory Method, 16th ed, 1974; Vasilades, 1976; Mcgowan *et al*., 1983). LDL-C and VLDL-C were calculated as per Friedwald formula (Richard et al., 2006). Vitamin C was estimated as dehydroascorbate in Plasma using phenylhydrazine (Natelson, 1971). Hemolysate was prepared for estimation of MDA (TBARS assay) (Beuge and Aust, 1978), GPx and GR (Beutler, 1986; Worthington and Rosemeyer, 1975; Paglia and Valentine, 1967).

RESULTS AND OBSERVATIONS

Table 1: The body weight, height, body weight, BMI, was found to be 105.3 ± 6.75 kg, 1.73 ± 0.053 m, 35 ± 1.89 kg/m^2 respectively in obese subjects in comparison to 70 ± 6.85 Kg, 1.70 ± 0.65 m, 23.39 ± 1.76 kg/m^2 respectively of healthy control subjects. There is a significant increase in the MDA levels between the two groups i.e 8.05 ± 6.97 mmol/L in cases and 3.465 ± 0.41mmol/L. The rise in Case group is statistically very significant (p<0.0001) (Table-1). The antioxidant enzymes show a decrease in cases. GR among cases is 31.8 ± 1.7 mg/G Hb and 45.08 ± 2.33 mg/G Hb in controls with a very significant change (p< 0.0000). GPx also shows a fall in cases 30.6 ± 0.9 while in controls it is 38.62 ± 2.19 mg/G Hb with a very significant change (p<0.0001) (Table-1). Vitamin C acts as an antioxidant and is used up to contain the oxyfree radicals as evident in their plasma levels. It is 0.65 ± 0.04 mg/dl in cases and 0.83 ± 0.06 mg/dl in controls. This difference among control group and case group is also very significant (p<0.0000) (Table-1). The Lipid profile i.e TC, HDL-C, LDL-C, TG in the case group was 190.4 ± 18.56, 40.35 ± 2.29, 122.6 ± 14.53 and 170.86 ± 15.07 mg/dl respectively while in the healthy control group it was 158.8 ± 10.07, 46.46 ± 5.94, 88.8 ± 7.65 and 138.5 ± 6.97 mg/dl respectively. There is a rise in the levels of TC, LDL-C and TG in the control group and a slight fall in the HDL-C levels when compared with the control group (Table-1). With regard to case group, after applying regression analysis, R^2 is 0.9788 and P value is <0.0001. It means that the regression model is highly significant or highly appropriate for the given parameters i.e BMI against Oxidative stress markers. With regards to Control group, as P value is equal to 0.001 i.e less than 0.05, and R square = 0.9604 we can say the regression model also fits good in the control group for BMI with MDA, GR, GPx , Vitamin C.

Table 1. Anthropometric and Biochemical parameters

	Body wt.	height	BMI	MDA	GR	GPx	Vit C	TC	HDL-C	LDL-C	TG
	Kg	M	Kg/m^2	mmol/L	mg/G Hb	mg/G Hb	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
Case	105.3 ± 6.75	1.73 ± 0.053	35± 1.89	8.05± 0.46	31.8± 1.7	30.6 ± 0.9	0.65 ± 0.04	190.4 ± 18.56	40.35 ± 2.29	122.6 ± 14.53	170.86 ± 15.07
Control	70 ± 6.85	1.70 ±0.65	23.39 ± 1.76	3.465 ± 0.41	45.08 ± 2.33	38.62 ± 2.19	0.83 ± 0.06	158.8 ± 10.07	46.46 ± 5.94	88.8 ± 7.65	138.5 ± 6.97
P (T<=t) two-tail				0.0001	0.0001	0.0001	0.0001				

The comparision of BMI with other oxidative stress parameters i.e MDA, GR, GPx, Vitamin E and Vitamin C was done using linear regression analysis.

	R square	P value	Intercept	MDA mmol/L	GR mg/G Hb	GPx mg/G Hb	Vit C mg/dl
Control	0.960361	<0.00000001	2.0735	3.3313	0.1544	0.0332	1.6448
Case	0.978872	<0.00000001	66.5566	0.5548	-0.6287	-0.456	-3.2447

The formula applicable in regression analysis was:

$BMI_{control} = 2.0735 + 3.3313MDA_{control} + 0.15435GR_{control} + 0.033175GPx_{control} + 1.6679Vit\ C_{control}$

$BMI_{case} = 66.5565 + 0.5548MDA_{case} - 0.6287GR_{case} - 0.456GPx_{case} – 3.2446Vit\ C_{case}$

DISCUSSION

In our study we have reported a significant increase in MDA (p<0.0000) by 158% which correlates very well with increased lipid peroxidation in obesity. The case group consisted of obese individuals without any chronic systemic diseases which shows

that even in absence of such systemic diseases there is is increased lipid peroxidation. The peroxidation of erythrocyte membrane destroys the membrane resulting in hemolysis. Current literature reports have also cited increased MDA levels and our results are in agreement. (Cazzola *et al*., 2004; Madhikarmi *et al*., 2013; Amirkhizi *et al*., 2007; Kelishadi *et al*., 2012; Yesilbursa *et al*., 2005) Vitamin C in our study has shown a fall by 21.7%. The antioxidant vitamins help in breaking the generation of oxyfree radicals in the membranes by scavenging them. Vitamin C helps in regeneration and has a sparring action on Vitamin E. Fall in Vitamin C levels signify accumulation of free radicals and its exhaustion is due to its consumption to maintain redox balance (Madhikarmi *et al*., 2013). GPx breaks the peroxide radicals into H_2O thereby reducing oxidative stress. GPx activity was found to be decreased by 20.76% ($p<0.0000$) in obese individuals in comparison to control healthy individuals which correlates with other studies (Blum and Fridovich, 1985). GR activity also shows a decrease of 29.45% and ($p<0.001$). A factor that may lead to this is the decreased supply of NADPH (Yesilbursa *et al*., 2005). Increased fat accumulation therefore leads to statistically and biologically significant reduction in GPx and GR due to the oxidative stress and lipid peroxidation states which are in agreement with current literatures (Keaney *et al*., 2003; Olusi, 2002; Shigetada Furukawa *et al*., 2004). So our study corresponds with the increasing BMI and decreasing enzyme status. This fall in the anti-oxidant enzymes may render the cells more prone to oxidative stress resulting in more tissue damage (Raes *et al*., 1987; Lapennna *et al*., 1998; Zwirska *et al*., 2003).

Conclusion

Obese individuals are prone to oxidative stress reflected through the alterations in MDA, Vitamin C, Glutathione peroxidase and Glutathione reductase levels. This oxidative stress is responsible for the plethora of chronic systemic including cancers and ageing affecting the quality of life. So obesity should be taken seriously and managed promptly. Subsequent studies should focus on effect of hypolipidemic therapy, diet, vitamin and mineral supplements, exercise and their role in decreasing oxidative stress in obese individuals.

REFERENCES

Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. 2007. Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women?. *ARYA Atherosclerosis Journal*, 2(4): 189-92.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, *et al*. 1999. Paradoxical decrease of an adipose-specificprotein, adiponectin, in obesity. *Biochem Biophys ResCommun*., 257:79—83

Barham D. and Trinder P. 1972. An improved colour reagent for the determination of blood glucose by the Oxidase system; Analyst 1972 Feb : 97(151):142-5

Berg AH, Combs TP, Du X, Brownlee M. and Scherer PE. 2001. The adipocyte-secreted protein Acrp30 enhances hepaticinsulin action. *Nat Med*., 7:947—53.

Beuge, J.A. and Aust, S.D. 1978. The thiobarbituric acid assay. *Meth. Enzymol*., 52,306-307.

Beutler E. 1986. Red cell metabolism- A manual of biochemical methods, Vol.16. London: Edinburgh Churchill Livingstone Publication.

Blum J. and Fridovich I. 1985. nactivation of glutathione peroxidase by superoxide radical. *Arch Biochem Biophys*., 240(2): 500-8.

Cazzola R, Rondanelli M, Russo-Volpe S, Ferrari E, Castaro B. 2004. Decreased membrane fluidity and altered susceptibility to peroxidation and lipid composition in overweight and obese female erythrocytes. *J Lipid Res*., 45(10): 1846-51.

Dietz WH. and Robinson TN. 2005. Clinical practice. Overweight children and adolescents. *N Engl J Med*., 352: 2100.

Flegal KM, Carroll MD, Ogden CL, Johnson CL. 2002. Prevalence and trends in obesity among US adults, 1999–2000. *JAMA*, 288 (14):1723–7.

Fried SK, Bunkin DA. and Greenberg AS. 1998. Omental and sub-cutaneous adipose tissues of obese subjects releaseinterleukin-6: depot difference and regulation by gluco-corticoid. *J Clin Endocrinol Metab*., 83:847—50.

Friedman JM. and Halaas JL. 1998. Leptin and the regulation of bodyweight in mammals. *Nature*, 395:763—70.

Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. 2006. Systemic oxidative stress is associatedwith visceral fat accumulation and the metabolic syn-drome. *Circ J*., 70:1437—42.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, YamadaY, Nakajima Y, *et al*. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*, 114: 1752—61.

Gul M, Kutay FZ, Temocin S, Hanninen O. 2000. Cellular and clinical implications of glutathione. *Ind J Exp Biol*., 38(7): 625-34.

Henry R.J. 1963. Standard methods of clinical chemistry, Vol. 4.

Henry J.B.1974. Diagnosis and management by Laboratory methods, W.B. Saunders Co. Ltd.; 16th edition.

Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adiposeexpression of tumor necrosis factor-alpha: directrole in obesity-linked insulin resistance. *Science*, 259:87—91.

Keaney, J.F., Jr., *et al*. 2003. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler. Thromb. Vasc. Biol.,* 23:434–439.

Kelishadi R, Hashemipour M, Sheikh HA, Samani SG. 2012. Changes in serum lipid profile of obese or overweight children and adolescents following a lifestyle modification course. *ARYA Atheroscler*., 8(3): 143-8.

Lapennna D, Gioia S, Ciotani G, Mezzetti A, Ucchino S. 1998. Glutathione related antioxidant defenses in human atherosclerotic plaques. *Circulation*, 97(19): 1930-4.

Lopez AD, Mathers CD, Ezzati M, Jamison DT. and Murray CJL. 2006. Global Burden of Disease and risk factors. The World Bank and Oxford University Press.

Madhikarmi NL, Murthy KRS, Rajagopal V, Singh PP. 2013. Lipid peroxidation and antioxidant status in patients with type 2 diabetes in relation to obesity in Pokhara–Nepal. *J Diabetol*., 1(3): 3-8.

Maeda N, Shimomura I, Kishida K, Nishizawa H, MatsudaM, Nagaretani H, *et al*. 2002. Diet-induced insulin resis-tance in mice lacking adiponectin/ACRP30. *Nat Med*., 8:731—7.

Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, *et al*. 2002. Role of adiponectin in preventing vascular steno-sis. The missing link of adipo-vascular axis. *J Biol Chem*., 277:37487—91.

Mcgowan, M. W. Artiss, J. D. Stranberg, D. R. Zak, B. A. 1983. Peroxidase coupled method for the colorimetric determination of serum Triglycerides, *Clin.Chem*., 29, 538-542.

Natelson S. 1971. In Techniques in Clinical Biochemistry. CC Thomas, USA, 162: 288.

Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y,Kumada M, *et al*. 2002. Adiponectin reduces atheroscle-rosis in apolipoprotein E-deficient mice. *Circulation*, 106:2767—70.

Olusi, S.O. 2002. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans.*Int. J. Obes. Relat. Metab. Disord.,* 26:1159–1164.

Paglia DE. And Valentine WN. 1967. Studies on the quantitative andqualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*., 70(1): 158-69

Raes M, Mihiels MC. and Remacle J. 1987. Comparative study of the enzymatic defense systems against oxygen derived free radicals: the key role of glutathione peroxidase. *Free Radic Biol Med*., 3(1): 3-7.

Richard A. McPherson, Matthew R. Pincus, John Bernard Henry, 2006. Henry's Clinical diagnosis and management by Laboratory investigations 21st Ed

Sartipy P. and Loskutoff DJ. 2003. Monocyte chemoattractant pro-tein 1 in obesity and insulin resistance. *Proc Natl Acad SciU S A*, 100:7265—70.

Shigetada Furukawa, Takuya Fujita *et al*. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation*, Volume 114 Number 12 December, 1752-1761

Tietz, N.W. 1976. Foundamentals of Clinical Chemistry, W.B. Saunders, R.S. Phila, p 1211.

Vasiliades, J. 1976. Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono-creatinine picric acid complex; *Clin Chem*., Oct;22(10);1664-71

Worthington DJ. and Rosemeyer MA. 1975. Glutathione reductase from human erythrocytes. Molecular weight, subunit composition and aggregation properties. *Eur J Biochem*., 60(4): 459-66.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, HaraK, *et al*. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy andobesity. *Nat Med*., 7:941—6.

Yesilbursa, D., Z Serdar, A Serdar, M Sarac, S Coskun and C Jale. 2005. Lipid peroxides in obese patients and effects of weight loss with orlistat on lipid peroxides levels. *International Journal of Obesity*, 29, 142–145

Zwirska KK, Jochem J, Rybus KB, Polaniak R, Birkner E. 2003. Assessment of blood superoxide dismutase, glutathione peroxidase activity and malondialdehyde concentration as oxidation status parameters in obese women. *Pol Arch Med Wewn*., 110(1): 725-31.

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