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RESEARCH ARTICLE

Oxidative Stress and DNA Damages in Obesity

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Abstract

Obesity is a serious global epidemic and possesses a significant health threat to humans. Obesity rates have been steadily rising over the past 25 years. Obesity may induce oxidative stress, causing oxidative damage of DNA. The oxidative stress in terms of serum lipid peroxidase levels (MDA) are compared in obesity and age matched healthy controls. The aim of the present study was to determine the levels of oxidative burden that may be attributed to obesity and to determine the resulting DNA damage by CBMN assay. The study was performed in 62 obese individuals and 22 healthy control subjects. The study revealed that there is an increased level of LDL cholesterol as well as triglyceride in subjects with increased abdominal circumference. These subjects were observed with raised levels of MDA and increased frequency of micronuclei. Lifestyle modifications with proper diet and physical activities can reduce the risk factors that lead to obesity thereby reduces the resulting oxidative DNA damage. Good nutrition and regular physical activity should take place at all ages and stages of life.

INTRODUCTION

Obesity is a serious global epidemic and possesses a significant health threat to humans (Rayner, 2009). It is considered as the largest public health problem worldwide (Bravo et al, 2006). Obesity is characterized by an increase in body weight that results in excessive fat accumulation (Sikaris et al, 2004) and has been recognized as a major underlying factor in the pathogenesis of several diseases (Alberti and Zimmet, 1998). Obesity, defined as a body mass index (BMI) greater than 30 kg/m^2, is now recognized as a risk factor for cardiovascular disease. This relative risk ranges from 2 to 8 when the BMI is in the range 35 to 50 kg/ m^2 (Manu et al, 2007). The prevalence of obesity exceeds 30% in adults and is associated with increased risk of such serious health problems as cardiovascular disease, type 2 diabetes mellitus and various types of cancer. These comorbid conditions are associated with greater use of health care services among obese patients (AAFP, 2013).

Abdominal adiposity is an important independent risk factor for cardiovascular disease, type 2 diabetes, dyslipidemia, and hypertension. The NHLBI defines abdominal obesity as: (NIH, 1998)

- Waist circumference greater than 40 in (102 cm) in men
- Waist circumference greater than 35 in (88 cm) in women

Individuals with larger waist circumferences have more than a fivefold greater risk of multiple cardiometabolic risk factors, even after adjusting for BMI, compared with individuals with waist measurements in the normal range (Ghandehari et al, 2009).

Obesity leads to, or significantly increases the risk of co-morbidities involving various body systems including cardiovascular, neurological, respiratory, musculoskeletal, skin, gastrointestinal, genitourinary, psychological and endocrine system (Kushner et al, 2003). Obesity is also a major risk factor for Metabolic Syndrome or Syndrome X, a cluster of risk factors that, when occurring together, increase a patient's risk for cardiovascular disease and Type 2 Diabetes (T2DM) (Eckel et al, 2005). Female obesity and underweight are known to adversely affect fertility through alterations of hormone patterns and the menstrual cycle. Obesity affects 30–75% of women with polycystic ovarian syndrome (PCOS) (Ehrmann, 2005).

Obesity rates have been steadily rising over the past 25 years, with current reports classifying more than one-third of US adults as obese (Ogden et al, 2012). The prevalence of obesity is increasing not only in adults, but also among children and adolescents (Rayner, 2009). Poor diet and lack of exercise are implicated as the major contributors to the rising incidence of obesity (Schonfeld and Warden,1997) but there is also an increasing volume of research exploring possible genetic factors causing, or at least contributing to obesity susceptibility in adult and juvenile populations (Kimberly and Scott, 2014).

Oxidative stress (OS) and obesity are closely related (Atabek et al, 2004). Oxidative stress occurs when the generation of reactive oxygen species (ROS) and other radical species exceeds the scavenging capacity by antioxidants due to excessive production of ROS and/or inadequate intake or increased utilization of antioxidants (Sharma et al, 1996). In obese individuals, elevated levels of fatty acids increase the level of oxidative stress via activation of NADPH oxidase and increased production of reactive oxygen species (ROS) (Furukawa et al, 2004). The increase in obesity associated OS is probably due to the presence of excessive adipose tissue itself, because adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines, including TNF-α, IL-1, and IL-6 thus, obesity is considered a state of chronic inflammation. These cytokines are potent stimulators for the production of reactive oxygen and nitrogen by macrophages and monocytes therefore, a rise in the concentration of cytokines could be responsible for increased OS (Fonseca et al, 2007). Adipose tissue also has the secretory capacity of angiotensin II, which stimulates Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase comprises the major route for ROS production in adipocytes (Morrow, 2003). Excessive fat accumulation can cause cellular damage due to pressure effect from fat cells. Cellular damage in turn leads to high production of cytokines such as TNF-α, which generates ROS in the tissues, increasing the lipid peroxidation rate which could contribute to the development of atherosclerosis (Khan et al, 2006).

Obesity is a chronic disease of multifactorial origin. It has been reported that obesity may induce systemic OS (Fernandez et al, 2011). Several processes are involved in obesity associated oxidative stress, caused by an overload of nutrients and in particular high fat and high carbohydrate meals. An increment of fat levels corresponds to increased energy storage, mitochondrial oxidation of nutrients, and oxidative stress (Avignon et al, 2012). So far, many studies have been conducted to evaluate the role of somatic DNA damage in obesity. No systematic studies were conducted to evaluate the extent of somatic DNA damage that cause obesity and to correlate it with oxidative stress. Hence the present study was undertaken to aware the people about the biochemical, molecular cytogenetics of oxidative stress and DNA damages in obesity.

Materials and Methods

Sixty two individuals with obesity were selected for this study. The samples were referred from various centres of Kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. Twenty two subjects were also selected as control for this study. Detailed, social and clinical characteristics were recorded using proforma. In this study Cytokinesis Block Micronuclei (CBMN) Assay was performed on each sample by using cytochalasin – B for quantitating the extent of somatic DNA damages and (Malondialdehyde) MDA test was performed for detecting the oxidative stress.

Collected eight ml of blood sample by venepuncture and transferred 3 ml of blood to sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by cytokinesis-block micronuclei (CBMN) assay. Lymphocyte cultures for each subject were prepared in sterile bottles by adding five to six drops of blood sample to 10 ml RPMI 1640 supplemented with 100 units/ ml penicillin, 100 units/ ml streptomycin, 15% fetal bovine serum and 1% phytohemagglutinin. At 44^{th} hr after initiation, cells were blocked in cytokinesis by adding cytochalasin B (Sigma, final concentration, 4.5µg/ml). The total incubation time for all cultures was 72 hr. After incubation, the

cells were fixed in 3:1 methanol/glacial acetic acid, dropped onto clean microscopic slides, air dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored at 100X magnification. The number of micronuclei per 1,000 binucleated cells was recorded.

The remaining five ml of blood was transferred into a plain tube. Blood was allowed to clot and separated the serum immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer. The level of the serum biomarker for oxidative stress, malondialdehyde was determined using thiobarbituric acid as main reagent and the values are measured on a semi-autoanalyser at 540 nm.

Results

Sixty two individuals with obesity under the age range 31 to 70 years were selected for the study and 22 age matched healthy individuals were selected as control for the study. Various demographic, clinical, lifestyle and physiological condition were recorded and correlated with the extent of DNA damages and oxidative stress. The Cytokinesis block micronuclei assay revealed that the mean CBMN frequency was statistically higher among the study subjects (13.47) than the healthy control subjects (10.54). The MDA value for study subjects was 2.01 whereas in control subjects it was 1.12. Thus the MDA test in the present study showed a statistically significant difference (Table: 1).

Table 1: Comparison of Mean CBMN frequency and MDA value among the study and control subjects

Subjects	Number	Mean CBMN Frequency	Mean MDA Value
Study Subjects	62	13.47	2.01
Control Subjects	22	10.54	1.12

The age of the study subjects ranged from 31 to 70 years with a mean age of 33.84 years. The highest mean CBMN frequency observed among the subjects belonging to 61 to 70 years. The mean CBMN frequency of males and females were 13.37 and 13.68 respectively, which showed a statistically significant difference. Based on the body weight, subjects with more than 70 kg body weight showed the highest mean CBMN frequency.

Majority of the study subjects (51.61%) belonged to rural area followed by urban (32.25%) and coastal area (16.12%). Majority of the subjects belonged to Hindu (n=33, 53.22%) followed by Christian (n=21, 33.87%) and Muslim (n=8, 12.90%) religions and the highest mean CBMN frequency was recorded in Christians (13.52). The study subjects were grouped according to abdominal circumference. The mean CBMN frequency (13.60) was found to be higher in subjects with >100 cms abdominal circumference. This study clearly indicates a significant relationship between mean CBMN frequency and the abdominal circumference.

The mean CBMN frequency and MDA value according to the clinical and lifestyle characteristics were also studied. The major risk factors like diabetes, hypertension, dyslipidemia, cancer, smoking and alcoholism showed significant contribution for the increase in CBMN frequency in obese subjects. Random blood sugar (RBS), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) levels were significantly elevated in the study subjects compared to the control group. Other life style factors such as diet, socioeconomic status and area of residence were found to influence the CBMN frequency among obese ones. The MDA value was also found to be raised in all these subjects with various risk factors. This study reveals that there is an increased level of somatic damages as well increased level of oxidative stress in obese subjects.

Discussion

According to WHO, BMI greater than or equal to 25 is overweight and BMI greater than or equal to 30 is obesity. The present study also revealed that obese individuals with BMI greater than or equal to 30 had a higher mean CBMN frequency. Jay Schwartz (2014) reported that female obesity rates have risen faster than male obesity rates. In 2007 and 2008, 35.5 percent of women and 32.2 percent of men who were at least 20 years old were obese, according to Centers for Disease Control and Prevention (CDC) survey. The present study also suggests that the incidence of obesity is higher in females than males. Cynthia et al (2010) reported that among men, there is no significant trend between education level and obesity prevalence. Among women, obesity prevalence increases as

education decreases. The current study revealed that there is no significant relationship between education and obesity in both males and females.

National institute of health (1998) reported that obese individuals are at increased risk of diabetes mellitus, cardiovascular disease, hypertension, and certain cancers, among other conditions. High levels of random blood sugar, total cholesterol, LDL, triglyceride etc in the present study also suggests that obese individuals are at increased risk of cancer, diabetes, hypertension and coronary artery disease.

Renehan et al (2008) revealed an association between obesity and cancer has been reported across populations worldwide. A meta analysis has shown that increased BMI was associated with higher risk of both common and less common cancers. The evidence of increased DNA damage among the obese subjects with the H/O cancer in the present study also suggests that these subjects are at increased risk of cancer.

Park et al (2003) reported that prevalence of metabolic syndrome increases dramatically from 5%–6% in normal-weight (body mass index (BMI) < 25 kg/m^2) men and women to 22%–28% in overweight (BMI > 25 kg/m^2) adults and 50%–60% in obese (BMI ≥ 30 kg/m^2) individuals. The evidence from the present study indicates that the metabolic syndrome increases with increase in BMI.

Christopher (2007) reported that body weight tends to increase with age. This can be correlated with the present study that body weight increases with increasing age. Davis (2009) reported numerous factors influencing obesity, overconsumption of caloric dense foods is one major culprit. The present study revealed that in non vegetarians the incidence of obesity is higher.

Conclusion

The distribution of MDA value and mean CBMN frequency according to various demographic, clinical and lifestyle characteristics of the study subjects with obesity was analysed. The individuals who had reported for obesity showed a higher CBMN frequency and the factors supporting these include increased age, increased body weight, an increase in BMI, an increase in abdominal circumference and individuals with history of diseases. BMI should be calculated and plotted annually in children to aid early recognition of inappropriate weight gain. Discussions about good nutrition and regular physical activity can and should take place at all ages and stages of life. Physical exercise and activity are particularly important for maintaining weight loss over the long term.

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