Biochemical and Molecular Instabilities in Obesity and its Risk for Cardiovascular Disease

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Manuscript Info

Abstract

Obesity, characterized by an increase in body weight that results in excessive fat accumulation, represents a social problem worldwide and has been recognized as a major factor for the pathogenesis of several diseases. Obesity is one of the most important risk factor for CVD. The goal of the present study was to evaluate the biochemical and molecular instabilities in obesity and its risk for cardiovascular disease. Thirty one obese subjects were selected as study subjects and twenty five healthy subjects without any chronic illness were selected as control for the present study. The role of oxidative stress measured by the level of oxidative stress marker, Malondialdehyde (MDA) and the DNA damages were quantified by Cytokinesis-block Micronuclei (CBMN) assay. Detailed demographic, clinical and lifestyle characteristics were compared with subjects. The MDA value and the micronuclei frequency was significantly elevated in study subjects as compared with that of control subjects. Obese individuals experience substantially elevated morbidity and mortality from all forms of CVD.

Introduction:-

Obesity is a serious global epidemic and possesses a significant health threat to humans (Rayner et al., 2009). It is a chronic disease of multifactorial origin that develops from the interaction of social, behavioural, psychological, metabolic, cellular and molecular factors (Kaufer et al., 2001). It is the condition under which adipose tissue increased and can also be defined as an increase in body weight that results in excessive fat accumulation. Obesity is considered as the largest public health problem worldwide, especially in industrialized countries (Bravo et al., 2006). The World Health Organization (WHO) defines obesity as a body mass index (BMI) >30 Kg/m² and defines overweight as with a BMI of 25 Kg/m² (Sikaris, 2004). The obesity related diseases are mediated by different regional fat distributions, such as visceral and subcutaneous adiposity. Upper body fat includes the visceral and abdominal subcutaneous depots. Many studies have shown that visceral and subcutaneous adiposity play different roles in health outcomes (Lear et al., 2007; Britton et al., 2013).

Obesity, in particular abdominal adiposity is associated with increased risk of cardiovascular disease (CVD) and diabetes mellitus (DM) (Wang et al., 2005). Adipose tissue synthesizes and secretes biologically active molecules that may affect CVD risk factor. CVD is the main cause of death and it imposes a burden across the world (Lopez et al., 2006). The prevalence of obesity exceeds 30% in adults and is associated with increased risk of such serious
health problems such as CVD, type 2 diabetes mellitus and various types of cancer. These co-morbid conditions are associated with greater use of health care services among obese patients (American Academy of Family Physicians (AAFP), 2013).

Obesity with dyslipidemia has been shown to promote the onset of CVD (Yang et al., 2008). This link is strongly related to oxidative stress (OS). Low levels of circulating high-density lipoprotein (HDL), enhanced clearance of HDL particles, increased post-prandial triglycerides (TG) values, and elevated plasma very low density lipoprotein (VLDL) levels promote reactive oxygen species (ROS) generation in the endothelium (Ceriello et al., 2002). In addition to a pro-inflammatory process, ROS can also directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen activated protein kinases and redox sensitive transcription factors, causing changes in protein/lipid expression and, therefore, irreversible oxidative damage (Ceriello et al., 2002). Due to ROS-mediated changes in lipid expression, further oxidation-derived products including oxidative low-density lipoprotein (Ox-LDL) can play a further critical role in CVD.

Malondialdehyde (MDA) is a marker of oxidative stress. MDA is an endogenous genotoxic product of enzymatic and oxygen radical induced lipid peroxidation whose adducts is known to exist in DNA isolated from healthy human beings (Laura et al., 2003). MDA is an important marker of lipid peroxidation which showed progression of atherosclerosis is correlated with oxidative stress and can be followed by MDA measurements (Mogadam et al., 2008).

Several processes are involved in obesity associated OS, 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 OS (Avignon et al., 2012). Obesity screening and its cytogenetic, biochemical and molecular studies was not much carried out in recent studies. Hence the present study was undertaken to evaluate the biochemical and molecular abnormalities, if any, in obese subjects and its risk for cardiovascular disease.

Materials and Methods:-
Thirty one obese patients were selected for this study. The samples were referred from Hridayalaya, Institute for Preventive Cardiology, Thiruvananthapuram to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. Twenty five healthy subjects without any chronic illness were also selected as control for this study. Detailed demographic, clinical and lifestyle characteristics were recorded using proforma. In this study, Cytokinesis Block Micronuclei (CBMN) assay and Malondialdehyde test (MDA) was carried out in each subject. CBMN assay was performed by using Cytochalasin B for quantitating the extent of somatic DNA damages and MDA test was conducted to evaluate the oxidative stress.

Seven ml of blood sample was collected by venepuncture and transferred two ml of blood into sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by Cytokinesis-Block Micronuclei (CBMN) assay. The remaining five ml of blood was transferred into a plain tube. Blood was allowed to clot, serum separated immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer. The level of the serum lipid peroxide marker, MDA was determined using thiobarbituric acid as main reagent and measuring these values on photoelectric colorimeter at 540nm.

Two ml blood was added to a culture tube containing 10 mL RPMI 1640 supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 100µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44th hours of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M KCl) for 1 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

Result:--
Thirty one obese subjects and twenty five normal healthy individuals were selected and analyzed for the present study. The mean CBMN frequency of study subjects was 14.20 and that of the control subjects was 10.40 which showed a statistically significant difference (p<0.001). The MDA value of the study subjects was 1.90 and that of
the control subjects was 1.20. The study frankly demonstrated that the level of MDA was increased among the study subjects than the control subjects.

Table 1 - Distribution of mean CBMN frequency and mean MDA value according to the demographic characteristics of the study subjects.

| Category      | Variables   | Total | Percentage (%) | Mean CBMN Frequency | Mean MDA Value |
|---------------|-------------|-------|----------------|---------------------|----------------|
| Age (Years)   | <55         | 20    | 64.5           | 14.23               | 1.81           |
|               | >55         | 11    | 35.4           | 14.26               | 2.08           |
| Sex           | Female      | 19    | 61.2           | 13.90               | 2.05           |
|               | Male        | 12    | 38.7           | 14.80               | 1.89           |
| Occupation    | Non-sedentary | 15   | 48.3           | 14.20               | 1.38           |
|               | Sedentary   | 16    | 51.6           | 14.29               | 2.55           |
| BMI (Kg/m²)   | <30         | 27    | 87.09          | 14.12               | 1.95           |
|               | >30         | 4     | 12.9           | 15.07               | 2.24           |
| Residence     | Rural       | 21    | 67.7           | 14.09               | 2.04           |
|               | Coastal     | 3     | 9.6            | 14.33               | 2.16           |
|               | Urban       | 7     | 22.5           | 14.7                | 1.74           |
| Religion      | Hindu       | 12    | 38.7           | 14                  | 1.39           |
|               | Christian   | 13    | 41.9           | 14.3                | 2.42           |
|               | Muslim      | 6     | 19.3           | 14.5                | 2.24           |
| Social Status | Low         | 17    | 54.8           | 13.96               | 2.01           |
|               | Medium      | 4     | 12.9           | 14.52               | 1.41           |
|               | High        | 10    | 32.2           | 14.63               | 3.32           |

The subjects were grouped based on their demographic characteristics such as age, sex, occupation, BMI, residence, religion and social status (Table 1). Among the 31 study subjects, 20 subjects (64.5%) were belonged to below the age of 55 years and showed a mean CBMN frequency of 14.23. The mean CBMN frequency of 14.26 were shown by 11 subjects (35.4%) with the age of >55 years. The highest mean MDA value (2.05) was showed >55 years of age group. The mean CBMN frequency (14.80) of male subjects was comparatively higher than female subjects (13.90) but highest mean MDA value (2.05) was showed in female subjects. On the basis of BMI, <30 Kg/m² showed mean CBMN frequency of 14.12 and >30 Kg/m² showed mean CBMN frequency of 15.07. Highest mean CBMN frequency (15.07) and mean MDA value (2.24) was showed by subjects with BMI of >30 Kg/m². The mean MDA value of subjects belonged to sedentary and non-sedentary lifestyle characters were 2.55 and 1.38 respectively. The highest mean CBMN frequency (14.29) and MDA value (2.55) was observed in subjects having sedentary lifestyle. Majority of the study subjects were belonged to rural (67.70%) followed by urban area (22.50%) and coastal area (9.60%). The highest mean CBMN frequency was observed in urban area (14.70). Subjects belonged to coastal area showed a highest mean MDA value of 2.16. According to data the highest mean CBMN frequency was showed in the Muslim religion (14.5) and highest mean MDA value was showed in Christian religion (2.42). The mean CBMN frequency was studied according to the social status. The mean CBMN frequency (13.96) and mean MDA value (2.01) of 17 (54.8%) subjects were belonged to low social status. From the study subjects, 4 (12.9%) were showed a high social status with a mean CBMN frequency of 14.52 and mean MDA value of 1.41. 10 subjects (32.2%) were belonged to middle social status and showed a high mean CBMN frequency (14.63) and mean MDA value (3.32).
Table 2 - Distribution of mean CBMN frequency and mean MDA value according to the clinical and lifestyle characteristics of the study subjects

| Category        | Variables | Total | Percentage (%) | Mean CBMN frequency | Mean MDA Value |
|-----------------|-----------|-------|----------------|---------------------|----------------|
| H/o Diabetes    | No        | 18    | 58.06          | 14.01               | 1.98           |
|                 | Yes       | 13    | 41.9           | 14.57               | 2.003          |
| H/o Hypertension| No        | 10    | 32.2           | 14.05               | 1.8            |
|                 | Yes       | 21    | 67.7           | 14.34               | 2.3            |
| H/o CAD         | No        | 16    | 51.6           | 14.15               | 1.48           |
|                 | Yes       | 15    | 48.3           | 14.35               | 2.46           |
| H/o Cancer      | No        | 28    | 90.3           | 14.16               | 1.84           |
|                 | Yes       | 3     | 9.6            | 15.03               | 2.006          |
| FBS (mg/dL)     | <126      | 13    | 41.9           | 14.13               | 1.6            |
|                 | ≥126      | 18    | 58.06          | 14.3                | 2.2            |
| Total Cholesterol (mg/dL) | ≤200 | 10    | 32.2           | 14.1                | 1.9            |
|                 | ≥200      | 21    | 67.7           | 14.3                | 2.02           |
| HDL (mg/dL)     | <40       | 17    | 54.8           | 14.2                | 1.5            |
|                 | ≥40       | 14    | 45.1           | 14.27               | 2.5            |
| LDL (mg/dL)     | ≤100      | 12    | 38.7           | 14.1                | 1.4            |
|                 | ≥100      | 19    | 61.2           | 14.3                | 2.7            |
| Smoking         | No        | 9     | 29.03          | 13.9                | 1.32           |
|                 | Yes       | 22    | 70.9           | 14.3                | 2.26           |
| Alcoholism      | No        | 11    | 35.4           | 13.9                | 1.66           |
|                 | Yes       | 20    | 64.5           | 14.4                | 2.17           |

The subjects were grouped based on their clinical and lifestyle characteristics (Table 2). The mean CBMN frequency based on H/o diabetes were analyzed among the study subjects. Study subjects with H/o diabetes were showed a mean CBMN frequency of 14.57 and the subjects without H/o diabetes was showed a mean CBMN frequency of 14.01. Study subjects with H/o diabetes were showed a mean MDA value of 2.003 and the subjects without H/o diabetes was showed a mean MDA value of 1.98. This indicates that H/o diabetes have a correlation with mean CBMN frequency and mean MDA value. Among the 31 study subjects, 21 (67.7 %) subjects had H/o hypertension and the mean CBMN frequency was 14.34. The remaining subjects showed no H/o hypertension and mean CBMN frequency of 14.05. Subjects were belonged to H/o hypertension showed a mean MDA value of 2.3. The study evaluate that the mean CBMN frequency level and mean MDA level were higher with those who have H/o CAD. The mean CBMN frequency was studied according to the H/o cancer. 28 (90.3%) subjects showed no H/o cancer disease and the mean CBMN frequency was 14.16. The remaining subjects showed H/o cancer disease and mean CBMN frequency was 15.03. Highest mean MDA value (2.006) was observed in subjects belonged to H/o cancer.

This study indicates that, the subjects with smoking habits have increased mean CBMN frequency and mean MDA values were compared to nonsmokers. The alcoholism was observed in 20 (64.5%) subjects with a mean CBMN frequency was 14.4. The study subjects without alcoholism (35.4 %) showed a mean CBMN frequency of 13.9. Highest mean MDA value (2.17) was observed in subjects had alcoholism.

**Discussion:-**

According to Christopher, (2007) body weight tends to increase with age. In the present study it is correlated that when age increases body weight also increases. BMI greater than or equal to 25 is overweight and BMI greater than or equal to 30 is obesity (WHO, 2015). The subjects with <30kg/m2 of BMI were showed a mean CBMN frequency of 14.12 and the subjects with >30 Kg/m2 of BMI were showed a mean CBMN frequency of 15.07. So the present study also revealed that obese individuals have BMI greater than 30 had a higher mean CBMN frequency.

Mahadik et al., (2007) observed that the prevalence of abdominal obesity and hypertriglyceridemia was significantly higher in the urban population compared to the rural population. The mean CBMN frequency was higher in subjects belonged to urban area (14.7) followed by coastal (14.33) and rural (14.09) area. So from the present study it is suggested that urbanization and change in lifestyle can mediate the progress of obesity and CVD.
National institute of health, (1998) reported that obese individuals are at increased risk of diabetes mellitus, cardiovascular disease, hypertension, and certain cancers, and other pathologic conditions. Present study also revealed that subjects with various risk factors like history of cancer, history of diabetes, history of hypertension, history of coronary artery disease, etc showed an increased mean CBMN frequency (Table 2).

According to Zimlichman et al., (2005) in young adults found higher rates of smoking among obese individuals relative to their overweight and normal weight counterparts, and obese smokers smoked more cigarettes per day than overweight or normal weight smokers. The present study also proved that the subjects with smoking habit have an increased mean CBMN frequency.

Sayon et al., (2011) found that severe drinkers were associated with weight gain while light to moderate alcohol drinkers, especially wine drinkers, was not associated with obesity. In the present study the CBMN frequency (14.4) was increased in alcoholic subjects.

Ghosh et al., (2006) reported that lipid profile i.e. high HDL cholesterol, high LDL cholesterol, high total cholesterol, high triglycerides plays an important role in cardiometabolic syndrome. The evidence found from the present study indicates that the mean CBMN frequency of fasting blood sugar and HDL cholesterol was high among the study subjects. From the present study the elevated level of LDL cholesterol was observed in the study subjects.

The MDA assay, a widely use method for measuring lipid peroxidation in determining oxidative stress (Nair et al., 2008). This suggests that MDA value plays an important role in measuring the level of oxidative stress. In the present study showed that the mean MDA values were higher in the study subjects (1.90) than control subjects (1.20). The increased MDA values were observed among subjects belonged to advanced age, history of hypertension, history of diabetes, history of CAD and history of cancer. Subjects who had lifestyle characters such as habit of smoking, alcohol consumption demonstrated increased MDA value.

Conclusion:
In conclusion, the present study involves biochemical and molecular instabilities in obesity and its risk for cardiovascular disease. The present study suggests that the long-term implications of the obesity epidemic and its consequences are extremely serious. Obesity has a great influence on its risk factors for CVD and the development of atherosclerosis. The demographic, clinical and lifestyle characteristics of the present study subjects revealed that obesity is correlated with higher level of mean CBMN frequency and MDA value among study subjects. The findings imply that there is a strong evidence for increased risk of developing CVD in subjects suffering from obesity. Increase antioxidant defenses in obese subjects could be useful to prevent and treat obesity co-morbidities. Weight loss associated with physical activity has been found to be the most efficacious approach to prevent CVD and specific diet administered to induce weight loss.

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