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RESEARCH ARTICLE

OXIDATIVE STRESS AND GENETIC INSTABILITIES AMONG PATIENTS WITH CARDIOVASCULAR DISEASE

Jiju JS[1], Rekha MV[1], Viji Krishnan[2], Jeyapal V[3], Halah Nazar[4] and Dinesh Roy D[4].

1. Research Scholar, Meenakshi University, West KK Nagar, Chennai- 600 078. TN, India.
2. Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur, Kerala.
3. Consultant Cardiologist, Hridayalaya Institute for Preventive Cardiology, Thiruvananthapuram – 695024, India.
4. Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram-695 024, India.

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Abstract

Cardiovascular disease (CVD) is a leading cause of mortality and is responsible for one-third of all global death in India. CVD will be the largest cause of death and disability by 2020 in India. Oxidative stress (OS) has long been associated with CVD. An increase in reactive oxygen species (ROS) elicited oxidative damage to DNA and other biomolecules may impair normal functions of tissue cells which lead to human aging and CVD disease. Genomic instability at the cellular level can directly affect CVD. The goal of the present study was to evaluate the oxidative stress and genetic instabilities among patients with CVD. Sixty subjects with CVD were selected as study subjects and thirty 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 using Cytokinesis Block Micronuclei (CBMN) Assay. Detailed demographic, clinical and lifestyle characteristics were compared with study subjects. The MDA value and Micronuclei frequency was significantly elevated in the study subjects with that of the control subjects. Although CVD cannot be cured, treatment can help to manage the symptoms and reduce the risk of further problems. CVD can be managed effectively with a combination of lifestyle changes, medicine and, in some cases, surgery.

Introduction:-

Cardiovascular disease (CVD) had become the leading cause of death in the developing world, as it has been in the developed world since the mid1900s (Mather et al., 2001; WHO 2002a). Nearly 50 percent of all deaths in high-income countries and about 28 percent of deaths in low and middle income countries are the result of CVD (Mathers et al., 2001). In Western countries where CVD is considered to be a disease of the aged 23% of CVD deaths occur below 70 years of age while in India 52% of CVD deaths occur below 70 years of age (Huffman et al., 2012; Ghaffar et al., 2004). CVD will be the leading cause of death and disability worldwide by 2020 mainly because it will increase in low and middle income countries (Murray and Lopez, 1996).

Corresponding Author:- Dinesh Roy D.
Address:- Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram-695 024, India.

Annual incidence of CVD per 100,000 per year, using verifiable method from different parts of India is scarce (Feigin, 2005). CVD have been gaining importance in India recently because of increased incidence of the disease. In 2000, there were an estimated 29.8 million people with coronary heart disease (CHD) in India out of total estimated population of 1.03 billion, or a nearly 3% overall prevalence (Census of India, 2001). In 2005, 53% of the deaths were on account of chronic diseases and 29% were due to cardiovascular diseases alone (Reddy, 2004).

The etiology and pathophysiology of CVDs are complex, but the major risk factors include unhealthy lifestyle behaviors were coupled with multifactorial complex interaction between environment and genetic factors (WHO, 2004). The main causes of CVD are tobacco use, an unhealthy diet, physical inactivity and increased OS etc. High blood pressure has no symptoms, but can cause a sudden stroke or heart attack. Major clinical manifestations of CVD include myocardial infarction, coronary artery disease, stroke and peripheral artery disease (Rosamond et al., 2008).

Oxidative stress has been implicated in the formation of DNA damage in atherosclerosis and other cardiovascular diseases (Andreassi, 2003). At present, there is good evidence that DNA damage play a pivotal role in atherosclerotic mechanisms. Both DNA strand breaks and chromosomal damage are present in the circulating cells of patients with atherosclerosis, and they related to disease severity (Andreassi, 2008). At the present time, there is consistent evidence supporting the notion that oxidative stress-induced-genetic instability is a relevant contributor of atherosclerotic plaque development and its acute complications (Mercer et al., 2007; De flora and Izotti, 2007).

Genomic instability at the cellular level can directly affect vascular function and/or lead to cell cycle arrest, apoptosis and senescence (Andreassi et al., 2008). There is increasing evidence of DNA damage accumulation in atherosclerotic plaque either as genomic alterations or as DNA adducts in nuclear DNA (Andreassi et al., 2011). Cytogenetic studies in primary cultures of human atherosclerotic plaques have shown elevated levels of chromosomal alterations, especially aneuploidy (Matturri et al., 2001). The determination of molecular alteration in atherogenic process has been evaluated by biomarkers of carcinogenic exposure, like DNA adducts and cytogenetic aberrations were correlated with occurrence of atherogenic risk factors and coronary artery disease (CAD) (Botto et al., 2001).

Reducing premature mortality, tobacco control, obesity, reducing alcohol consumption, increased physical activity, avoid unhealthy diet etc helps to prevent CVD. Saturated fatty acids are reduced in the diet has been shown to lower low-density lipoprotein (LDL) cholesterol levels in the blood, which is thought to be important in preventing cardiovascular disease (Smith et al., 2011). The best recommended action is to increase the intake of natural dietary antioxidant vitamins are good for cardiovascular disease. So far, many studies have been conducted to evaluate the role of oxidative stress and genetic instabilities among patients with CVD. No systematic studies were conducted to evaluate the oxidative stress and genetic instabilities in CVD patients. Hence the present study was undertaken to evaluate oxidative stress and genetic instability in patients with CVD.

Materials and Methods:-

Sixty subjects suffering with cardiovascular disease 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. 30 subjects without any chronic illness were 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 subjects. CBMN Assay was performed by using cytochalasin B for quantitating the extent of somatic DNA damages and MDA test was conducted to evaluate 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:-

Table 1:- Distributions of mean CBMN frequency and MDA value according to Demographic Characteristics of the study subjects

Category	Variables	Total	Percentage (%)	Mean CBMN Frequency	Mean MDA Value
Age (years)	<40	6	10%	13.36	1.70
	40-60	36	60%	13.45	1.38
	>60	18	30%	13.49	1.75
Sex	Female	27	45%	13.58	1.59
	Male	33	55%	13.34	1.45
Birth order (n)	<6	51	85%	13.44	1.58
	≥6	9	15%	13.48	1.13
BMI (kg/m^2)	<25	33	55%	13.36	1.46
	≥25	27	45%	13.55	1.56
Residence	Coastal	10	16.66%	13.75	1.79
	Rural	24	40%	13.27	1.24
	Urban	26	43.33%	13.5	1.58
Socio economic status	High	7	11.66%	13.08	1.46
	Low	17	28.33%	13.58	1.92
	Medium	36	60%	13.46	1.47
Height (cm)	142-162	35	58.33%	13.36	1.49
	163-183	25	41.66%	13.58	1.53
Weight (kg)	40-60	15	25%	13.24	1.48
	61-81	40	66.66%	13.78	1.60
	82-102	4	6.66%	13.97	0.73
	103-123	1	1.66%	14.8	1.62

Distribution of mean CBMN frequency and MDA value according to the demographic characteristics of the study subjects were given in table 1. Among the sixty study subjects, 6 subjects (10%) were below the age of 40 years and showed mean CBMN frequency of 13.36 and the MDA value of 1.70. 36 subjects (60%) were belonged to the age between 40 to 60 years old showed a mean CBMN frequency of 13.45 and MDA value of 1.38. The mean CBMN frequency (13.49) and MDA value (1.75) was comparatively higher for the subjects with the age above 60 years. The mean CBMN frequency (13.58) and MDA value (1.59) of female subjects was comparatively higher than male subjects. According to birth order the mean CBMN frequency (13.48) and MDA value (1.13) was higher in subjects with birth order of ≥6. On the basis of BMI, the distribution of mean CBMN frequency and MDA value was analyzed <25 Kg/m^2 showed a mean CBMN frequency 13.36 and MDA value 1.46. Subjects with BMI ≥25 Kg/m^2 showed a high mean CBMN frequency of 13.55 and MDA value of 1.5. Based on residence, majority of the study subjects belonged to urban (n=26) followed by rural area (n=24) and coastal area (n=10). The highest mean CBMN frequency (13.75) and MDA value (1.78) was observed in coastal area. The study subjects were grouped according to their socio-economic status as high, medium and low. The highest mean CBMN frequency (13.58) and MDA (1.92) value was observed in subjects belonged to low socio-economic status. The mean CBMN frequency (13.58) and MDA value (1.53) was comparatively higher for the subjects with height range from 163-183 cm. Subjects with weight between 103-123 kg was showed highest mean CBMN frequency (14.8) and MDA value (1.62).

Table 2:- Distribution of mean CBMN frequency and MDA value according to the clinical characteristics of the study subjects

Category	Variables	Total	Percentage (%)	Mean CBMN frequency	Mean MDA Value
H/o Diabetes	Yes	33	55%	13.51	1.63
	No	27	45%	13.4	1.37
H/o Hypertension	Yes	32	53.33%	13.45	1.58
	No	28	46.66%	13.05	1.44
H/o Dyslipedemia	Yes	44	76.66%	13.76	1.63
	No	16	26.66%	13.34	1.18
Fasting Blood sugar (FBS) (mg/dL)	<100	14	23.33%	13.33	1.35
	100-200	9	15%	13.41	1.37
	>200	37	61.66%	14	1.69
Total Cholesterol (TC) (mg/dL)	<200	26	43.33%	13.25	1.36
	200-240	11	18.33%	13.55	1.39
	>240	23	38.33%	13.59	1.73
High density lipoprotein (HDL) (mg/dL)	<40	19	31.66%	13.59	1.62
	40-60	41	68.33%	13.16	1.47
Low density lipoprotein (LDL) (mg/dL)	100-160	32	53.33%	13.31	1.47
	>160	28	46.66%	13.57	1.55
Triglyceride (TG) (mg/dL)	<150	40	66.66%	13.37	1.26
	150-300	20	33.33%	13.49	1.64

Distribution of mean CBMN frequency and MDA value according to the clinical characteristics of the study subjects were given in the table 2. The mean CBMN frequency and MDA value based on the H/o diabetes were analyzed. Study subjects with H/o diabetes showed a high mean CBMN frequency of 13.51 and MDA value was 1.63. Among the sixty study subjects, 32 subjects had the H/o hypertension and the mean CBMN frequency (13.45) and MDA value was 1.58. The mean CBMN frequency and MDA value based on the H/o dyslipidemia was observed. Study subjects with H/o dyslipidemia showed high mean CBMN frequency of 13.76 and MDA value of 1.63. The study indicates that the mean CBMN frequency (14) and MDA value (1.69) was higher with those who had fasting blood sugar (FBS) in the level of >200 mg/dL. Subjects with total cholesterol >240 mg/dL were showed high mean CBMN frequency (13.59) and MDA value (1.73). The mean CBMN frequency and MDA value was studied according to HDL, LDL and TG level. The subjects with HDL level <40 mg/dL were showed highest mean CBMN frequency (13.59) and MDA value (1.62). The subjects with LDL level >160 mg/dL were showed highest mean CBMN frequency (13.57) and MDA value (1.55). Subjects with TG level between 150-300 mg/dL were showed highest mean CBMN frequency (13.49) and MDA value (1.64).

Table 3:- Distribution of mean CBMN frequency and MDA value according to Lifestyle characteristics of the study subjects

Category	Variables	Total	Percentage (%)	Mean CBMN frequency	Mean MDA Value
Smoking	Yes	7	11.66%	13.52	1.92
	No	53	88.33%	12.92	1.46
Alcoholism	Yes	11	18.33%	13.46	1.57
	No	49	81.66%	13.02	1.50
Chewing	Yes	4	6.66%	13.49	1.87
	No	56	93.33%	12.89	1.49
Physical activity	Average	42	70%	13.46	1.51
	Good	5	8.33%	13.01	1.43
	Poor	13	21.66%	13.57	1.53
Diet	Non veg	46	76.66%	13.75	1.73
	Veg	14	23.33%	13.36	1.45

Distribution of mean CBMN frequency and MDA value according to lifestyle characteristics of the study subjects were given in table 3.The study subjects with the habit of smoking have increased mean CBMN frequency (13.52) and MDA value (1.92) compared to nonsmokers. The subjects with alcoholism observed in 11 (18.33 %) subjects

with mean CBMN frequency (13.46) and MDA value (1.57). Subjects with chewing habit observed in 4 (6.66%) subjects with mean CBMN frequency (13.49) and MDA value (1.87). Majority of the study subjects were belonged to average physical activity (n= 42; 70%) and the subject with poor physical activity have high mean CBMN frequency (13.57) and MDA value (1.53). The mean CBMN frequency and MDA value of the study subject was studied according to their diet, non vegetarians subjects have high mean CBMN frequency (13.75) and MDA value (1.73) compared to vegetarian subjects.

Discussion:-

An improper diet and inadequate physical activity are considered to be major component of an unhealthy lifestyle that contributes significantly to the pathogenesis of cardiovascular disease (CVD) (Ramesh et al., 2005). In the present study the subjects with poor physical activity showed high mean CBMN frequency than subjects with good physical activity.

Particularly low levels of HDL-c, and high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and triglycerides are associated with an increased CV risk (Choi et al., 2005). In the present study, subjects with low HDL- cholesterol (<40 mg/dL) were showed highest mean CBMN frequency of 13.59. Subjects with high value of LDL- cholesterol (>160 mg/dL) were showed highest mean CBMN frequency of 1.55. Subjects with 150 to 300 mg/dL of TC showed mean CBMN frequency of 13.49.

Cigarette smoking and hypercholesterolaemia influence the renin-angiotensin system (RAS) functions, including increased RAS mediated vasoconstriction, mitogenic signaling, and angiotensin II type 1 receptor (AT1R) expression as per the studies by Zak and Wita who concluded that the 1166C allele increases the risk of CAD which was associated with the presence of cigarette smoking and hypercholesterolaemia (Iwona Zak and KrystianWita, 2008). In the present study nearly 11.66% of study subjects were smokers and 88.33% were non smokers.Their mean CBMN frequency of subjects without smoking habit was 12.92 and subjects with smoking habit showed a mean CBMN frequency of 13.52.

According to Donaldson, (2004) reported that people who drink heavily have a high mortality from all causes and cardiovascular disease, including sudden death and hemorrhagic stroke. In the present study alcoholics (18.33%) showed increased mean CBMN frequency of 13.46 and non alcoholics (81.66%) showed decreased mean CBMN frequency of 13.02. The prevalence of CVDs in alcoholics is higher than non alcoholics.

The socioeconomic disparities are striking in the case of heart diseases being substantially higher in lower status individual as defined by education, occupational position or income (Andrew Steptoe and Michael Marmot, 2005). In the present study, subjects were high socioeconomic status showed mean CBMN frequency of 13.08 and low socioeconomic status showed a mean CBMN frequency of 13.58. This indicates that study subjects with low socio economic status have increased prevalence of CVDs.

Fasting plasma or blood glucose value of 7.0 mmol/l (126 mg/dl) or higher have two fold increased risk of stroke and cause diabetes (Mendis et al., 2011). In the present study, 14 out of 60 subjects had low FBS (mg/dl) level and showed a mean CBMN frequency 13.3. 37 study subjects (61.66%) had high FBS (mg/dl) level and showed a high mean CBMN frequency of 14.

Pezeshkian et al., (2001) showed that, MDA levels increased significantly in heart diseases. In the present study, the lipid peroxidation product i.e. MDA levels have been increased significantly in plasma of the patients with cardiovascular disease as compared to the controls. This increase in MDA levels might be resulted from increase in lipid peroxidation which increased lipid peroxidation itself is resulted from an increase in oxidative stress levels.

Conclusion:-

In conclusion, the present study involves oxidative stress and genetic instabilities among patients with CVD. The present study suggests that cardiovascular diseases (CVD) are a major public health and socioeconomic problem, since they occupy a leading position in the structure of mortality and disability not only in developed but also in developing countries. The existing evidence support the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that the antioxidant therapy may prove beneficial in combating these problems. The demographic, clinical and lifestyle characteristics of the present study

revealed that CVD is correlated with higher level of CBMN frequency and MDA value among study subjects. The majority of cardiovascular disease (CVD) causes can be controlled, treated or modified, such as high blood pressure, cholesterol, overweight/obesity, tobacco use, lack of physical activity and diabetes.

Reference:-

1. Andreassi, M.G., (2003): Coronary atherosclerosis and somatic mutations: an overview of the contributive factors for oxidative DNA damage. Mutat. Res; 543:67-86.
2. Andreassi, M.G., (2008): DNA damage, vascular senescense and atherosclerosis.J. Mol. Med. (Berl), 86, 1033-1043.
3. Andreassi, M.G., Barale, R., Iozzo, P., Picano, E., (2011): The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. Mutagenesis, 26, 77–83.
4. Andrew Steptoe., and Michael Marmot., (2005): Socioeconomic Status and Coronary Heart Disease: A Psychobiological Perspective. Socioeconomic status and coronary heart disease: a psychobiological perspective. In 'Aging, Health, and Public Policy' edited by L. J. White. Population Council, New York.
5. BottoN,Rizza A., Colombo, M.G., Mazzone, A.M., Manfredi, S., Massetti, S., et al., (2001): Evidence for DNA damage in patients with coronary artery disease Mutatte Res, 493; 23-30.
6. Census of India (2001): Population Projection for India and States 2001-2026. Report of the Technical Group on Population Projections Constituted by the National Commission on Population, Office of Registrar General and census Commissioner, India. 2006.
7. Choi, H.K., Seeger, J.D., (2005): Lipid profiles among US elderly with untreated rheumatoid arthritis—the Third National Health and Nutrition Examination Survey. J Rheumatol;32:2311–6.
8. De Flora, S., Izzotti, A., (2007): Mutagenesis and cardiovascular diseases Molecular mechanisms, risk factors, and protective factors. Mutat. Res; 621:5-17.
9. Donaldson, I.M., (2004): Bon santé: is wine good for your health? Int Med J.;34(5): 221–223.
10. Feigin, V.L., (2005): Stroke epidemiology in the developing countries. Lancet; 365:2160-61.
11. Ghaffar, A., Reddy, K.S., Singhi, M., (2004): Burden of noncommunicable diseases in SouthAsia. British Medical Journal; 328:807-10.
12. Huffman, M.D., and Engelgau, M.M., (2012): Economic impact of non communicable diseases in India. India Health Beat; Vol 6(2).
13. Integrated Management of Cardiovascular Risk. Geneva (2008): 2002a WHO CVD Program.Iwona Zak and KrystianWita. The risk of coronary artery disease associated with cigarette smoking and hypercholesterolemia is additionally increased by the presence of the AT 1 R Gene 1166C Allele. Biochemical Genetics;469(11-12);799-809.
14. Mercer, J., Mahmoudi M., (2007): Bennett DNA damage, p53, apoptosis and vascular disease, Mutat. Res. 621 75–86.
15. Mathers, C.D., A. Lopez., C. Stein, D., Ma Fat., C. Rao, M., (2001): Inoue, and others. "Deaths and Disease Burden by Cause: Global Burden of Disease Estimates for 2001 by World Bank Country Groups." In Disease Control Priorities Project Working Paper 18. Bethesda, MD.
16. Matturri, L., Cazzullo, A., Turconi. P., Lavezzi, A.M., Vandone, P.L., Gabrielli, L., Fernandez Alonso, G., Grana, D., Milei, J., (2001): Chromosomal alterations in atherosclerotic plaques. Atherosclerosis;154:755-761.
17. Mendis, S., Puska, P., Norrving, B., (2011): Global Atlas on Cardiovascular Disease Prevention and Control.. World Health Organization (i collaboration with the World Heart Federation and World Stroke Organization), Geneva.
18. Murray and Lopez., (1996): Global Burden of Disease and Injury Series, Vols. I and II, Global Health Statistics. Boston: Harvard School of Public Health.
19. Pezeshkian, M., Nouri, M., Zahraei, M., Afrasiabi, A., Abadi, N.A., (2001): Study of MDA, antioxidant vitamins,lipoproteins serum levels and anthropometry parameters in coronary artery disease patients. Medical Journal of Islamic Academy of Sciences;14:5–8.
20. Ramesh, L., Bijlani Rama, P., Vempati Raj, K., (2005): Yadav, RoomaBasu Ray, Vani Gupta, Ratna Sharma, Nalin Mehta, and Sushil C. Mahapatra. The Journal of Alternative and Complementary Medicine.11(2): 267-274.
21. Reddy, K.S., (2004): Cardiovascular disease in non-western countries.NEngl J Med; 350: 2438-40.
22. Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S.M., Ho, M., Howard, V., Kissela, B., et al., (2008): Heart disease and stroke statistics—2008 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation, 117, e25–e146.
23. Smith, G.N., Luo, Z.C., Fraser, W.D., Julien, P., Deal, C.L., Audibert, F., Xiong, X., Walker, M., (2011): Tracing the origins of "fetal origins" of adult diseases:programming by oxidative stress? Med Hypothesis 66:38-44.
24. World Health Organization. (2004): Cardiovascular Disease: Prevention and control, WHO Global Strategy on diet, physical activity and health. WHO, Geneva.