Correlation of Arterial Stiffness and the extent of Somatic DNA Damage in Cardiometabolic Syndrome

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Abstract - This study was conducted to evaluate the correlation of arterial stiffness and the extent of somatic DNA damage by Cytokinesis-Block Micronuclei Assay in patients with cardio-metabolic syndrome. Fifty two patients with cardiometabolic syndrome and fifty two normal, healthy controls were included in this study. The arterial stiffness was accessed by various physiologic and biochemical factors such as heart rate, pulse pressure, systole, diastole, augmentation index, HDL, LDL etc. Both male and female subjects those reported cardiometabolic syndrome had abnormal range of physiologic and biochemical characteristics along with increased DNA damage. Significantly elevated augmentation index (34.62±11.46) was observed among the study subjects than the control subjects (8.67±1.08). The mean CBMN frequency of the study subjects was found to be 14.12±0.53, which is higher than the control subjects (10.33±0.70). These findings clearly demonstrate a significant correlation between arterial stiffness/augmentation index and the extent of DNA damage in cardiometabolic syndrome subjects and hence lifestyle modification with proper food, exercise and medication will help to reduce the arterial stiffness/augmentation index and the risk for cardiometabolic syndrome.

Index Terms - Arterial stiffness, Augmentation index, Cardiometabolic Syndrome, CBMN frequency, DNA damage

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1 INTRODUCTION

According to the World Health Organization, by 2030 nearly 23.6 million people will die from cardiovascular disorders [1], [2] and during the last decade, cardiometabolic disorders has progressively become a major worldwide public health problem, because of its association with increased risk of type 2 diabetes mellitus, atherosclerotic cardiovascular disease and all-cause mortality [3]. More than 100 million individuals suffer from this syndrome in the world. Cardiometabolic syndrome increases cardiovascular morbidity, and also mortality, by three- to fourfold.

The term "Cardiometabolic Syndrome" is generally used to indicate a clinical entity of substantial heterogeneity, represented by the co-occurrence of hypertension, impaired glucose tolerance, atherogenic dyslipidemia, central fat accumulation, insulin resistance, as well as prothrombotic, inflammatory states and cardiovascular disorders [3].

Although there is controversy about cardiometabolic syndrome, most clinicians find it a useful designation for identifying patients at high risk of cardiovascular disease and type 2 diabetes [4] [5].

The pathogenesis of the cardiometabolic syndrome is complex and so far incompletely understood but the interaction of physiologic, biochemical and genetic factors are known to contribute to its development [6], [7], [8] and [9].

Arterial stiffness is a generic term for arterial compliance, distensibility and elasticity [10]. Increased arterial stiffness is proposed as a possible mechanism in the initiation and/or progression of atherosclerosis and hypertension. Dyslipidemia is associated with a number of cardiovascular risk factors, which was supported by a study done in 2006 by The National Cardiovascular Disease Database in which the prevalence of dyslipidemia among CVD patients was 55.9% [11]. Stiffening of the arterial tree increases the systolic blood pressure (BP), and simultaneously decreases the diastolic BP, resulting in a wide pulse pressure [12]. Many studies have shown that arterial stiffness is the most important cause of cardiovascular complications and a major contributor to atherosclerosis, and thus to stroke, myocardial infarction, and renal failure [13], [14] and [15]. Arterial stiffness can be accessed through the various factors such as heart rate, pulse pressure, systole, diastole, augmentation index, HDL, LDL, etc.

The typical lipid abnormalities defined in patients with metabolic syndrome consist of decreased high-density lipoprotein (HDL) cholesterol, and increased small, dense low-density lipoprotein (LDL) cholesterol. The presence of atherogenic dyslipidemia in addition to metabolic syndrome has increased the risk of cardiovascular disease by 19 folds [16]. There are only few studies carried out earlier to correlate

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arterial stiffness and somatic DNA damage. Hence the present study was undertaken to quantify the extent of somatic DNA damages by Cytokinesis-block Micronuclei (CBMN) assay and arterial stiffness by assessing various factors such as heart rate, pulse pressure, systole, diastole, augmentation index, HDL, LDL etc. in patients with cardio-metabolic syndrome.

2 Materials and Methods

The present study group comprised of 52 patients (19 men and 33 women; mean age= 49.6 years) and 52 subjects including both males and females were selected as control subjects. These subjects were recruited from Hridyalayam, Institute of Preventive Cardiology, Trivandrum-24. Informed consents were obtained from all the study subjects according to the norms laid down by the Institutional Ethics Committee. Various clinical characteristics were recorded using proforma.

6 ml of venous blood was collected in sodium heparinised vaccutainers under strict sterile conditions from all subjects and 2-3ml of the blood was used to quantify the extent of DNA damages by Cytokinesis-block Micronuclei Assay [17]. The frequency of micronuclei among 1000 binucleated cells were counted and analyzed. The remaining blood samples were used to carry out other biochemical tests. The collected data were subjected to statistical analysis using statistical package for social survey (SPSS).

5 to 6 drops of whole blood samples was transferred to a vial containing 10 ml of RPMI 1640 medium supplemented with 15% foetal bovine serum to carry out CBMN assay. To that 10µgm/ml of phytohaemagglutinin (PHA) was added and incubated at 37^0C for 72 hours. After 44 hours of PHA stimulation, cytochalasin B was added to the cultures to give a final concentration of 4.5µg/ml. After 28 hours of addition of cytochalasin B, the whole contents were transferred into a sterile centrifuge tube and centrifuged for 10 minutes at1000 rpm, removed the supernatant, shaken the pellet in a cyclomixer. Added 10 mL of 0.075M KCl solution to the cell button and kept at 37^0C for 10 minutes. After this added 2 drops of freshly prepared fixative (methanol: acetic acid in the ratio 3:1). Again centrifuged at 1000 rpm for 10 minutes and removed the supernatant, mixed the cell button in a cyclomixer and added 10 ml of freshly prepared fixative and centrifuged at 1000 rpm for 10 minutes. Repeated this process until the supernatant becomes clear and cell button becomes white. From the cell button, prepared the cell suspension and 7-8 drops of cell suspension was dropped on pre cleaned, labeled and chilled slides from a particular height. The slides were flamed gently on spirit lamp, blown gently on the material and air dried. Stained the slides with 10-20% Giemsa stain solution and allowed to remain for 10 minutes. After 10 minutes the excess stain was washed off with running water and slides were air dried. The slides were examined at 100X magnification. The number of micronuclei in not less than 1000 binucleated cells was scored and the distribution of micronuclei among binucleated cells was recorded.

FBS and PPBS were estimated by enzymatic end-point method using glucose oxidase (GOD)-peroxidase (POD) reaction [18]. Total cholesterol was estimated by enzymatic end point method using cholesterol oxidase - peroxidase (CHOD- PAP) method [19], Triglyceride by glycerol phosphate oxidase – peroxidase (GPO-PAP) [20], HDL cholesterol by homogenous enzymatic colorimetric test [21] and LDL cholesterol by homogenous enzymatic colorimetric test [22].

3 Results

Demographic characteristics: The present study comprised of 104 subjects (52 study subjects & 52 control subjects); of them 39.4% were males and 60.6% were females. The age of the study subjects ranged from 25 to 78 years with a mean age of 49.61. Regarding the age of the control subjects, it ranged from 33 to 62 years with a mean age of 53.03.

Majority of the study subjects belong to urban area (51.9%) followed by rural area (48.1%). Regarding the educational qualification of the study subjects, majority attained higher secondary education (46.2%). On the basis of the occupational type, 46.2% of the study subjects were manual labourers, 46.2% were house wives and 7.7% had sedentary type of occupation. Moreover, 69.2% of the study subjects belong to average socioeconomic status followed by high (17.3%) and low (13.5%) economic status. Based on the dietary habits, majority (96.2%) of the study subjects have mixed type of diet, whereas only 3.8% are vegetarians.

Biochemical characteristics: The present study evaluated various biochemical investigations viz: FBS, PPBS, Total Cholesterol, HDL Cholesterol, LDL Cholesterol and Triglycerides and compared with that of control subjects. Moreover the study frankly demonstrated a significant difference ($p<0.05$) between the study subjects and the control subjects (Table no: 1)

Physiological characteristics: In the present study, various physiological parameters like systole, diastole, heart rate, pulse pressure, augmentation index and the body mass index of the study subjects and the control subjects were observed which showed a statistically significant difference. The augmentation index of the study subjects showed a mean value of 34.62 ± 11.46 and the control subjects showed a mean value of 8.67 ± 1.08 ($t=16.247$; $p=<0.001$).

The CBMN assay revealed that the study subjects showed a mean CBMN frequency of 14.12 ± 0.53 and the control subjects showed a mean CBMN frequency of 10.33 ± 0.70. Here also, a statistically significant difference was observed between study subjects and the control subjects (t=31.99; p=<0.001).

Thus in this study, an increased CBMN frequency was observed among the study subjects with abnormal physiological characteristics like systole, diastole, heart rate, pulse pressure, augmentation index and body mass index (Table no: 2). Moreover, an increased CBMN frequency was also observed among the study subjects with an abnormal range of biochemical characteristics (Table no: 3).

Contribution of genetic, physiological and biochemical parameters for augmentation index in cardiometabolic syndrome patients are given in Table 4 & 5. It clearly demonstrates that the major parameters like CBMN frequency, systole, diastole, heart rate, pulse pressure, BMI, FBS, PPBS, total cholesterol, triglycerides, HDL-C and LDL-C had a significant role to influence an increased in augmentation index among the cardiometabolic patients. Thus this study clearly indicates a positive correlation between arterial stiffness and the extent of somatic DNA damage in cardiometabolic syndrome.

Table 1: Physiological characteristics

Physiological Characteristics	Category	N	Mean	sd	t	p
Age	Case	52	49.62	13.52	-.865	.389
	Control	52	51.50	8.01		
BMI	Case	52	31.08	5.70	7.800	<0.001
	Control	52	24.16	2.91		
Systole	Case	52	136.42	21.27	5.827	<0.001
	Control	52	119.06	3.04		
Diastole	Case	52	91.25	15.05	5.653	<0.001
	Control	52	79.27	2.64		
Heart rate	Case	52	80.40	12.96	4.833	<0.001
	Control	52	71.63	1.78		
Pulse pressure	Case	52	45.42	14.75	2.617	.010
	Control	52	40.06	1.04		
Augmentation index	Case	52	34.62	11.46	16.247	<0.001
	Control	52	8.67	1.08		
BMI	Case	52	31.14	5.68	10.071	<0.001
	Control	52	22.87	1.69		
FBS	Case	52	100.52	14.47	7.672	<0.001
	Control	52	83.52	6.77		
PPBS	Case	52	133.08	43.08	2.725	.008
	Control	52	115.90	14.46		
TC	Case	52	224.40	29.79	9.820	<0.001
	Control	52	180.91	11.52		
HDL-C	Case	52	46.32	5.64	-3.669	<0.001
	Control	52	50.13	4.93		
LDL-C	Case	52	150.56	20.21	12.427	<0.001
	Control	52	108.37	13.81		
TG	Case	52	173.37	25.30	13.271	<0.001
	Control	52	121.61	12.29		
CBMN frequency	Case	52	14.12	.53	31.119	<0.001
	Control	52	10.33	.70		

Table 2: Distribution of Physiological characteristics of Cardiometabolic patients

Distribution of Physiological characteristics of Cardiometabolic patients				
	Variable	**Number**	**Percentage (%)**	**CBMN Frequency**
Systole	Normal range	14	26.92	13.15
	Abnormal range	38	73.07	14.78
Diastole	Normal range	14	26.92	14.12
	Abnormal range	38	73.07	14.90
Heart rate	Normal range	21	40.38	14
	Abnormal range	31	59.61	14.98
Pulse pressure	Normal range	19	36.53	13.47
	Abnormal range	33	63.46	14.89
Augmentation index	Normal range	2	3.84	13.13
	Abnormal range	50	96.15	15
BMI	Normal range	22	42.30	14.26
	Abnormal range	30	57.69	15.10

Table 3: Distribution of Biochemical characteristics of Cardiometabolic patients

Distribution of Biochemical characteristics of Cardiometabolic patients				
	Variable	**Number**	**Percentage (%)**	**CBMN Frequency**
FBS	Normal range	43	82.69	13.13
	Abnormal range	9	17.30	14.91
PPBS	Normal range	42	80.76	13.08
	Abnormal range	10	19.23	14.74
TC	Normal range	19	36.53	14.18
	Abnormal range	33	63.46	15.07
HDL-C	Normal range	40	76.92	13
	Abnormal range	12	23.07	14.76
LDL-C	Normal range	11	21.15	13.37
	Abnormal range	41	78.84	15.04
TG	Normal range	31	59.61	13.77
	Abnormal range	21	40.38	14.95

Table 4

Correlation between CBMN frequency	Pearson Correlation- r	p
Age	-.121	.223
BMI	.547**	.000
Systole	.481**	.000
Diastole	.432**	.000
Heart rate	.355**	.000
Pulse pressure	.285**	.003
FBS	.554**	.000
PPBS	.247*	.012
TC	.650**	.000
HDL-C	-.302**	.002
LDL-C	.710**	.000
TG	.742**	.000
Augmentation index	.822**	.000

Table 5

Correlation between Augmentation index	Pearson Correlation- r	p
CBMN frequency	.822**	.000
Age	.082	.406
BMI	.479**	.000
Systole	.655**	.000
Diastole	.467**	.000
Heart rate	.396**	.000
Pulse pressure	.533**	.000
FBS	.561**	.000
PPBS	.188	.056
TC	.600**	.000
HDL-C	-.302**	.002
LDL-C	.663**	.000
TG	.711**	.000

4 Discussions

Associations between increased arterial stiffness and a number of cardiovascular diseases such as hypertension, atherosclerosis, and coronary heart disease are reported [23]. In addition to its potential etiologic role in cardiovascular disease, elevated arterial stiffness may serve as an early marker for the detection of asymptomatic atherosclerotic lesions and/or structural arterial changes resulting from hypertension [24] and [25]. Given the insidious nature of the atherosclerotic and/or hypertensive processes, early recognition of arterial changes may identify individuals at risk for clinical complications of atherosclerosis or hypertension; this identification may provide for early modification of risk factors and delay or reverse the disease process.

Gosh et al [26] reported that lipid profile i.e. low HDL cholesterol, high LDL cholesterol, high total cholesterol, high triglycerides plays an important role in cardiometabolic syndrome. Lahdenpera et al [27] observed that hypertriglyceridemia is an independent risk for coronary artery disease. In the present study, elevated LDL cholesterol and triglycerides and decreased HDL cholesterol was observed among cardiometabolic patients.

Hypertension, diabetes, and dyslipidemia are labelled as conventional risk factors for their strength of evidence supporting role in the pathogenesis of CAD. It has been indicated that about 80–90% of CAD patients have atleast one of the conventional risk factors [28]. In the present study, the data provided more convincing information that the DNA damages were found higher in those subjects with abnormal range of systole, diastole, FBS and PPBS.

Wilson et al [29] confirmed that the cardiometabolic syndrome is a powerful predictor for T2DM. In addition, an elevated blood glucose level (100 mg/dL) has been associated with the highest risk for development of diabetes. The combination of obesity, elevated blood glucose level, and low HDL-cholesterol level is associated with a 12-fold risk for the development of cardiovascular disease which may lead to genetic disorders. In the present study, the extent of DNA damage was found in those subjects with abnormal range of Body Mass Index.

As atherosclerosis progresses the tunica media thickens and tunica intima becomes rigid, thus reducing the arterial elasticity [30]. Reduced arterial distensibility has been shown to be associated with atherosclerotic events [31]. Hypertension has also been well known as another cardiovascular risk factor, which might influence the arterial stiffness. Diabetes has been reported to accelerate arterial stiffness whereas the role of dyslipidemia is unclear. Stefanadis et al [32] found that coronary ischemic disease was substantially associated with increased aortic stiffness. When arteries are stiffer and the pulse pressure higher, the reflected wave arrive earlier and augment the central systolic blood pressure, rather than the diastolic blood pressure, which increases the left ventricular workload and compromises the coronary blood flow [33]. In this study it is clearly indicated that subjects with abnormal range of heart rate, pulse pressure and augmentation index have high mean CBMN frequency with increased DNA damage. Thus the present study clearly demonstrated that there is a positive correlation between arterial stiffness and the extent of DNA damage in cardiometabolic syndrome.

5 Conclusions

Arterial stiffness may be important in the etiology and natural history of several cardiovascular outcomes such as hypertension, atherosclerosis and coronary heart disease. This study clearly indicates that there is an increase in DNA damage in cardiometabolic syndrome which leads to coronary artery disease. In this study both male and female subjects those reported cardiometabolic syndrome had abnormal range of physiologic and biochemical characteristics with increased DNA damage. Overall study indicates that there is a positive correlation between arterial stiffness and the extent of DNA damage in cardiometabolic syndrome. This is mainly due to the lifestyle and dietary habits and sometimes due to hereditary problems. Lifestyle modification with proper food, exercise and medication will reduce the arterial stiffness and the risk for cardiometabolic syndrome.

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