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ROLE OF ADIPONECTIN, INTERLEUKIN-6 AND SOMATIC DNA DAMAGES FOR PREDICTING CARDIOVASCULAR DISEASES AMONG SUBJECTS WITH PREHYPERTENSION

Anaswara AA	Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram - 695024, Kerala
Sharmila K	Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur -680005
Aishwaria J	Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram - 695024, Kerala
Viji Krishnan	Department of Biochemistry, Jubilee Mission Medical College and Research Institute, Thrissur -680005
Dinesh Roy D*	Genetika, Centre for Advanced Genetic Studies, Pettah P O, Thiruvananthapuram - 695024, Kerala *Corresponding Author

ABSTRACT Prehypertension is one of the important potential candidates and independent predictor for both cardiovascular and cerebrovascular diseases. Newer biomarkers for the early diagnosis and prediction for cardiometabolic syndrome and CVD events among prehypertension is essential. The aim of the present study is to assess the role of Adiponectin and Interleukin-6 (IL-6) among prehypertensive subjects and to correlate with the various risk factor associate with Cardiometabolic Syndrome in prehypertensive subjects. A case-control study was conducted. The study group included 157 clinically diagnosed prehypertentsive subjects and 100 age and sex matched healthy control subjects. Adiponectin and Interleukin-6 (IL-6) had a significant role in association with somatic DNA damage in prehypertensive subjects and its earlier prediction of CMS. The increased mean CBMN frequency among the study subjects with 95% of confidence interval revealed its predictability for CMS. Adiponectin and interleukin-6 were also proved its strong predictability for CMS. Overall, this study recorded wider understanding of biochemical and molecular events in prehypertensive subjects which leads to high risk for CVD.

KEYWORDS : Prehypertension, cardiovascular diseases, Cardiometabolic Syndrome, CBMN Assay, Adiponectin and Interleukin-6

INTRODUCTION

Prehypertension is emerging as an important public health concern with a strong relation between prehypertension and CVD events. According to the "Seventh Joint National Committee Report on High Blood Pressure Prevention, Detection, Evaluation and Treatment (JNC-7)" "prehypertension can be referred as systolic blood pressure (SBP) of 120 - 139 mmHg or a diastolic blood pressure (DBP) of 80 - 89 mmHg in adults" (Chobanian et al 2003). Prehypertension becomes one of the important independent clinical conditions associated with metabolic syndrome, affecting both macro vascular and micro vascular pathology. According to Vasan et al (2001) "prehypertension often leads to clinical hypertension with an approximate rate of 20% over 4 years in accordance with the age". "Prehypertension may cause up to 66% increase in CVD mortality" Mainous et al (2004). Wu et al (2013) proved that, "prehypertension is one of the important potential candidates and independent predictor for both cardiovascular and cerebrovascular diseases".

According to Celik et al (2013), "prehypertensive patients have 31% higher risk of coronary heart disease (CHD), 49% greater risk of stroke and 44% higher risk of total cardiovascular events. Around 18 million people in world die due to diabetes and hypertension. Hence the clustering of these risk factors is a global concern. In short, CMS is an epidemic to the modern world and its subsequent downstream effects on the cardiovascular system and the cerebrovascular system causing a catastrophic attack to the human population. Subjects with Cardiometabolic Syndrome (CMS) are at two times greater risk to die from coronary heart disease (CHD) and have three fold greater risk to have a heart attack. According to Upadhyaya et al (2014) "chronic inflammation is associated with several metabolic disorders like hypertension, diabetes mellitus, obesity and cardiovascular disease". Bao et al (2015) reported that, "number of obesity- related peptide hormones has been identified for the contribution to the pathogenesis of Metabolic Syndrome (MetS). Adipose tissues are considered to be in the release of several inflammatory and immune mediators. Laboratory studies show that adiponectin suppresses several pathophysiological processes related to obesity, including insulin resistance, endothelial dysfunction, inflammation and atherosclerosis".

"Elevated IL-6 levels are associated with mortality among subjects with obesity, inflammation, stress and coronary heart disease (Shireen Hashmat et al 2016). IL-6 in MetS appeared to act on several key factors, which contributed to insulin resistance, elevated glucose production in liver, together with inhibition of the insulin mediated glucose uptake in skeletal muscle and the facilitation of hypertension". Andreassi et al (2009) observed that, "DNA damages are the emerging risk factor which plays a vital role in atherosclerosis and coronary artery disease. Since, it is due to the effect of multiple endogenous and exogenous factors such as oxidative stress and hypertension". Moreover, the interplay between genetic determinants and environmental factors are still unknown. Management of hypertension through the prevention and early detection is very important for the public health. The genetic basis of the prehypertension and cardiometabolic syndrome, has not yet been thoroughly investigated. Moreover, newer biomarkers for the early diagnosis and prediction for Cardiometabolic syndrome and CVD events among prehypertension is essential. Hence the present study was undertaken to identify the various biochemical and molecular biomarkers for the early detection of Cardio Metabolic Syndrome among subjects with prehypertension. The precise objectives are to evaluate the biochemical changes and to evaluate the role of Adiponectin and Interleukin-6 (IL-6) concentration in prehypertensive subjects and also to quantify and correlate extent of DNA damage among subjects with prehypertension.

METHODS

A case-control study was conducted to assess the various molecular and biochemical risk factors and to correlate the extent of somatic DNA damages by CBMN Assay along with the emerging risk markers in cardiometabolic syndrome. 157 clinically diagnosed pre-hypertentsive subjects and 100 age and sex matched healthy control subjects were selected in order to identify the CVD risks in prehypertensive subjects. After 12 to 14 hours of fasting, eight ml (8 mL) of venous blood was collected and 2 mL of blood was transferred aseptically to a sodium heparinized vacuutainer for evaluating mean CBMN frequency (Fenech, 1993) for somatic DNA Damage study and serum was separated into a labeled container and the following investigations were performed; Fasting blood sugar, Lipid profile, High sensitivity C reactive proteins (hsCRP), Fibrinogen, Malondialdehyde (MDA). ELISA method was done for estimating Adiponectin (Bobbert et al 2008) and Interleukin-6 (Yamagishi et al

1992) using ELISA Kit from CusoBio.

RESULTS AND OBSERVATIONS

Clinical parameters like systolic pressure, diastolic pressure, heart rate, pulse pressure and augmentation index were recorded and compared among study and control subjects. All the parameters except pulse pressure values were higher among study subjects than the control subjects. Moreover, these differences have statistical significance (Table: 1). Biochemical parameters like FBS, total cholesterol, HDL-C, LDL-C and triglycerides were analyzed and compared among study and control subjects. The study demonstrated that, FBS, total cholesterol, LDL-C and triglycerides were showed a statistically significant increased difference among the study subjects than the control subjects. However, the study subject showed a decreased level of HDL-C compared to control subjects (Table: 2).

In order to predict the cardiovascular risk among the subjects, new biomarkers such as, Adiponectin and IL-6 were performed. The study subjects showed a statistically significant decreased value of Adiponectin compared to control subjects (t= -2.139; p= 0.034). However, the level of IL-6 was higher among study subjects than the control subjects (t= 2.96; p= 0.004). A statistically significant increase of CBMN frequency (t= 11.341; p <0.001) was also observed among study subjects (Table: 3). Inflammatory markers such as, hsCRP and fibrinogen were evaluated and compared among study and control subjects. Study demonstrated that, hsCRP was significantly higher among study subjects (7.42±2.52) than control subjects (0.70±0.19) (t=26.614; p=<0.001). The level of fibrinogen was also higher among study subjects (400.70±86.34) than the control subjects (299.24±90.78) (t=9.002; p=<0.001) (Table: 4).

The distribution of emerging biochemical and molecular marker among the study and control group was analyzed in the t test which expressed a statistically significant result with p<0.05 and hence univariate analysis was performed to calculate the odds ratio. The odds ratio of the corresponding parameters such as adiponectin, interleukin-6, fibrinogen, malondialdehyde and mean CBMN frequency were found to be 2.129, 8.46, 10.853, 12.562 and 29.053 respectively with a 95% confidence interval (Table: 5). Adiponectin showed a negative correlation with interleukin-6 and fibrinogen. Parameters like IL-6, MDA and mean CBMN frequency showed a strong positive correlation with fibrinogen. Adiponectin had a negative correlation and interleukin-6 had a strong positive correlation with mean CBMN frequency. While considering emerging risk markers, IL-6 showed a positive correlation whereas, adiponection showed a negative correlation with mean CBMN frequency.

Table 1: Comparison of Clinical Parameters among study and control subjects

Variable	Study		Control		t	p
	Mean	Sd	Mean	Sd		
Systolic pressure (mmHg)	132.73	5.26	119.43	1.17	24.861	<0.001
Diastolic pressure (mmHg)	93.88	5.47	79.43	1.17	26.016	<0.001
Heart Rate	88.12	12.04	71.96	0.35	13.405	<0.001
Pulse Pressure	38.94	1.97	40	0	-5.35	<0.001
Augmentation index	20.17	4.2	12.99	0.46	17.021	<0.001

Table 2: Comparison of Biochemical Parameters among study and control subjects

Category	Study		Control		t	p
	mean	sd	mean	sd		
FBS	120.68	26.73	96.63	18.37	7.887	<0.001
Total Cholesterol	203.59	49.07	161.37	29.38	7.760	<0.001
HDL	45.92	8.72	58.06	4.23	-12.971	<0.001
LDL	139.24	35.34	112.57	13.73	7.202	<0.001
Triglycerides	168.68	46.23	119.15	21.17	10.058	<0.001

Table 3: Comparison of Emerging Risk Markers among study and control subjects

Category	Study		Control		t	p
	mean	sd	mean	sd		
Adiponectin (µg/mL)	8.08	6.54	10.73	6.95	-2.139	0.034
IL-6 (pg/mL)	11.14	5.78	7.98	5.69	2.96	0.004
CBMN frequency	12.73	1.30	10.79	1.41	11.341	<0.001

Table 4: Comparison of Inflammatory Markers among test and control subjects

Category	Test group		Control group		t	p
	mean	sd	mean	sd		
hsCRP	7.42	2.52	0.70	0.19	26.614	<0.001
Fibrinogen	400.70	86.34	299.24	90.78	9.002	<0.001

Table 5: Distribution of odds ratio in emerging biochemical and molecular markers among study and control group

Parameters	Conc. / cutoff	Study		Control		95% CI for OR		
		N	%	N	%	OR	L	U
Adiponectin	<12.07	51	67.1	23	48.9	2.129	1.01	4.487
	>12.07	25	32.9	24	51.1			
IL-6	>5.6	67	88.2	22	46.8	8.46	3.435	20.835
	<5.6	9	11.8	25	53.2			
Fibrinogen	>352	123	78.3	25	25	10.853	6.011	19.595
	<352	34	21.7	75	75			
Malondialdehyde	>2.01	128	81.5	26	26	12.562	6.882	22.931
	<2.01	29	18.5	74	74			
Mean CBMN frequency	>11.6	138	87.9	20	20	29.053	14.636	57.671
	<11.6	19	12.1	80	80			

DISCUSSION

"Prehypertension", the term introduced by "The Joint National Committee (JNC) VII" and is defined as "blood pressure (BP) levels of 120–139 mmHg for systolic and 80–89 mmHg for diastolic BP, respectively". Grossman et al (2006) reported that, "prehypertension" is common, even in young "so called" healthy subjects" and often "it is associated with metabolic syndrome and other cardiovascular (CV) risk factors" (Grotto et al 2006). The present study was undertaken to evaluate the various risk factors associated with prehypertensive subjects and their risk for cardiometabolic syndrome by assessing the various biochemical, inflammatory and molecular biomarkers. An attempt was also made to assess the association between prehypertension and some known risk factors for CVD, specifically prediabetes, overweight or obesity, waist circumference, physical activity, smoking, vegetables and fruit intake. Greenlund et al (2004) reported that, "persons with pre-hypertension had a higher prevalence of risk factors for CVD morbidity than those with a normal BP; including dyslipidemia, overweight and obesity, and diabetes mellitus". According to Grossman et al (1996) "coexistence of hypertension and type 2 diabetes may cause adverse effects for CVD". Chuang et al (2013) reported that, "CRP has been independently associated with increases in BP". Sesso et al in 2003 observed "a positive association between higher concentration of hsCRP and prehypertension". A study done by Sinha et al (2017) also reported "the association between diastolic blood pressure and hsCRP level in prehypertensive group". The present study also observed that hsCRP was higher among prehypertensive subjects than normotensive. LI Jian-jun (2006) point out that, "the clinical utility and importance of elevated levels of inflammatory markers for predicting cardiovascular risk is gaining much recognition, so that respect CRP has been the most intensively investigated in clinical studies". According to Shafi et al 2010 and Dawri et al 2014, "inflammation is one of the major pathological factors for the development prehypertension and hence, hsCRP was found as an independent risk factor for development of hypertension". In the present study "t" test was performed and observed that there was a statistically significant elevation in hsCRP concentration among the study subjects than the controls (t= 26.614; p= 0.001). However, it was observed that, hsCRP was not found as an independent predictor for CMS. The present study also observed that hsCRP had a positive correlation with fibrinogen, MDA, IL-6 and mean CBMN frequency.

According to Szmitko et al (2007), adiponectin, due to its antiinflammatory and anti-atherogenic properties, appears to have important protective effects at cardiovascular level. Ouchi et al (2006) reported that, a progressive increase in the knowledge of the biological actions of adiponectin has been observed, which in turn, led to recognize the importance of its functions and its cardio protective role. Hence, in the present study, an attempt was made to assess and evaluate the role of adiponectin as diagnostic and prognostic biomarker for cardiometabolic syndrome among subjects with prehypertension. In the current study, subjects with prehypertension showed a statistically decreased level of adiponectin than the control subjects (t= -2.139; p= 0.034). Moreover, the concentration of serum IL-6 was statistically higher among study subjects (t= 2.96; p= 0.004).

Sung et al (2009) observed that, hypoadiponectinemia is involved in the pathophysiology of atherosclerosis. According to Calle et al (2012), adiponectin is a collagen-like plasma protein secreted exclusively by adipocytes. This protein has anti-inflammatory, anti-atherogenic, and potent insulin sensitizing effects, which may be partially mediated by suppression of TNF-α and IL-6. Moreover, Calle et al (2012) add on that, decreased concentration of adiponectin is associated with metabolic syndrome, insulin resistance and diabetes mellitus. Jain et al (2017) observed an inverse association of adiponectin with waist circumference, indicating that the association between abdominal obesity and insulin resistance may be mediated by lowered adiponectin. Similarly, Chang et al (2015) observed an inverse association of adiponectin with obesity among Taiwanese and Japanese children. In the present study a positive correlation of IL-6 with hsCRP, fibrinogen, mean CBMN frequency and MDA were observed among prehypertensive subjects.

According to Francisqueti et al (2017), "the oxidative stress will increase due to the fat accumulation among subjects with metabolic syndrome. Components of MS, like dyslipidemia and insulin resistance (IR) are responsible for the increased production of reactive oxygen species and subsequently raise the oxidation of lipid products, proteins and DNA, which causes the endothelial dysfunction". In the current study also the MDA (OS marker) was observed to be statistically significant with an odds ratio of 12.562, which clearly indicates its associated risk for cardiometabolic syndrome. The present study observed a positive correlation between the concentration of MDA with fibrinogen, IL-6, mean CBMN frequency, systolic BP, diastolic BP, heart rate, augmentation index, FBS, TC, LDL-C, TG, urea, uric acid, hsCRP and creatinine. Abdominal circumference showed weak positive correlation with the concentration of MDA. A negative correlation was also observed between HDL-C and MDA.

The present study can be summarized as the emerging risk markers (Adiponectin and Interleukin-6) had a significant role in association with somatic DNA damage in prehypertensive subjects and its earlier prediction of CMS. The increased mean CBMN frequency among the study subjects with 95% of confidence interval also revealed its predictability for CMS. Overall, this study recorded wider understanding of biochemical and molecular events in prehypertensive subjects which leads to high risk for CMS. In short, the current study demonstrated that, prehypertension and oxidative stress together play an important role for DNA damage which leads to cardiometabolic syndrome.

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