ORIGINAL RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Hematological and Molecular Cytogenetic Studies in Hypertensive Subjects

KEYWORDS: Hypertension, Cardiovascular disease, Hematological indices and Cytokinesis-block micronuclei assay

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ABSTRACT *Hypertension is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease. The prevalence of hypertension worldwide is projected to increase from approximately 1.0 billion in 2000 to 1.5 billion by 2025. Elevated BP results from environmental factors (diet, physical inactivity, toxins and psychosocial factors) and genetic factors. Hypertensive subjects have an increase in blood plasma viscosity, and in membrane rigidity of red blood cells (RBCs), a reduction of their ability to pass through microcapillary, changes in erythrocyte aggregation and disaggregation, high hematocrit and increased amounts of fibrinogen and triglycerides. Hemorheologic action alters microcirculation and increase cardiovascular risk in hypertensive patients. The study consists of fifty four hypertensive individuals with varying risk factors were selected as study subjects and 30 healthy individuals without any chronic illness were selected as control for the study.The aim of the present study was to investigate the hematological and molecular cytogenetic studies in subjects with hypertension. Detailed demographic, clinical and hematological characteristics were recorded and compared. The present study demonstrated that micronuclei frequency was significantly elevated in the study subjects than control subjects. Increased levels of somatic DNA damages were observed in hypertensive subjects with more risk factors associated with genetically based conditions and demographic variables. Abnormal values of hematological and biochemical characters were compared to normotensive subjects. Lifestyle modifications by increasing physical exercise and dietary control will help to lower blood pressure.*

Introduction

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg (Chobanian et al., 2003). It is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease (Fields et al., 2004). A systematic review in 2004 showed that the prevalence of hypertension varies greatly around the world, lowest in rural India (6.8% in females and 3.4% in males) and highest in Poland (72.5% in females and 68.9% in males) (Kearney et al., 2004). The prevalence of hypertension worldwide is projected to increase from approximately 1.0 billion in 2000 to 1.5 billion by 2025 (Franco et al., 2005).

The Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (Chobanian et al., 2003) provides a classification of blood pressure for adults aged ≥18 years. Hypertension is defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Hypertension is divided into two stages.

• Stage 1 includes patients with systolic blood pressure 140–159 mmHg or diastolic blood pressure 90–99 mmHg.

• Stage 2 includes patients with systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg.

Elevated BP results from environmental factors (diet, physical inactivity, toxins and psychosocial factors) and genetic factors. Dietary factors have a prominent, and likely predominant, role in BP homeostasis. A substantial body of evidence strongly supports the concept that multiple dietary factors affect BP (Smith et al., 2005).

Hypertensive subjects have an increase in blood and plasma viscosity, and in membrane rigidity of red blood cells (RBCs), a reduction of their ability to pass through microcapillary, changes in erythrocyte aggregation and disaggregation, high hematocrit and increased amounts of fibrinogen and triglycerides (Lebensohn et al., 2008). All these changes have a clear hemorheologic action that alters the microcirculation and increase the cardiovascular risk in hypertensive patients (Ajmani, 1997).

Red cell distribution width (RDW) is elevated in hypertensive patients compared with normotensives (FiratOzcan et al., 2013). RDW is higher in prehypertensive and hypertensive patients compared with healthy independently of age, inflammatory status and anemia. Higher RDW values are strongly correlated with higher systolic and diastolic blood pressures (AsliTanindia et al., 2012). Recently, several studies showed that a high RDW index predicts severe morbidity and mortality in various cardiac conditions, such as acute and chronic congestive heart failure (Felker et al., 2007; Oh et al., 2009), pulmonary hypertension, (Hampole

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et al., 2009) and stroke (Ani et al., 2009). Recent reports suggest that people with hypertension have lower Mean corpuscular volume (MCVs) than do subjects with normal blood pressure (Bruschi et al., 1986; Postnov et al., 1988).

The hereditary nature of essential hypertension has been established in many family studies up to 30% of blood pressure variability is considered to be genetically determined and an individual's genetic predisposition to hypertensive disease ranges from 15 to 35% (Binder, 2007). When a person has a first-degree hypertensive relative, then the risk of developing hypertension is twice that of the general population. The risk quadruples when the number of first-degree relatives of hypertensive subjects rises (Izzo and Black, 2003; Fuentes et al., 2000).

The control of blood pressure (BP) is crucial in the prevention of adverse outcomes. However, hypertension can be asymptomatic. The detection and control of BP is, thus, a major public health challenge in the United States (Kwok et al., 2006). Lifestyle changes should have a central role in helping to manage hypertension in all patients with BP values >130/80 mmHg. These include weight loss, increase in physical exercise, reduction of alcohol intake, smoking cessation and perhaps most important, low sodium intake to levels <2.4 g⁄dL. Low salt intake should be encouraged through appropriate dietary counseling (George et al., 2008).

Recent analyses have shown that as of the year 2000, there were 972 million people living with hypertension worldwide, and it is predicted to be 1.56 billion worldwide by the year 2025 (Kearney et al., 2005). Over the past few years, genetic studies of hypertension have increased dramatically; nevertheless, their conclusions allowed only a partial understanding of the molecular mechanisms involved in its development. These studies mostly concern correlation analyses between hypertension development and specific gene loci, either by examining the entire genome or focusing on specific genes or polymorphisms. The present study was carried out to investigate the hematological and molecular cytogenetic studies in hypertensive subjects.

Materials and Methods

Fifty four individuals with hypertension were selected as study subjects and thirty normal healthy subjects without any chronic illness were selected as control for the present study.Detailed demographic and clinical characteristics were recorded using proforma. They were referred from Girija's lab, Thiruvananthapuram to Genetika, Centre for Advanced Genetic studies, Thiruvananthapuram, Kerala.

Five ml of venous blood was collected aseptically from all the subjects by venepuncture after overnight fasting. The 2ml blood was transferred into plain tube and allowed to clot, serum separated immediately, blood sugar and lipid profiles were estimated. The 1 ml of blood was used for hematology analysis by using 5 part hematology analyzer. The remaining 2ml blood was transferred into the vacutainer containing sodium heparin for quantifying the extent of somatic DNA damages by Cytokinesis-block micronuclei (CBMN) assay.

Result

The mean CBMN frequency of the study subjects was 12.87 and control subjects was 10.51. The mean CBMN frequency of the study subject was higher than the control subjects.

Distribution of mean CBMN frequency according to various demographic and anthropometric characteristics of the study subjects were given in table 1.

Table: 1

Variables	Category	Number (Percentage)	Mean CBMN Frequency
Age (Years)	29- 49	20 (37.03%)	12.82
	50- 69	34 (62.96%)	12.93
Birth order	<3	20 (37.37%)	12.40
	≥3	34 (62.97%)	13.03
Consanguinity	Yes	4 (7.40%)	13.52
	No	50 (92.52%)	12.71
Residence	Rural	33 (61.11%)	13
	Urban	17 (31.48%)	12.90
	Coastal	4 (7.40%)	11.35
Height (cm)	132-151	7 (12.96%)	12.93
	152-171	32 (59.24%)	13.28
	172-191	15 (27.77%)	13.28
Weight (kg)	43-62	17 (31.48%)	12.56
	63-82	28 (51.85%)	12.63
	83-103	9 (16.66%)	13.45
BMI (kg/m^2)	<25	29 (53.70%)	12.63
	≥25	25 (46.26%)	12.89

Distribution of mean CBMN frequency according to various clinical characteristics of the study subjects were given in table 2.

Table: 2

Variables	Category	Number (Percentage)	Mean CBMN Frequency
Family H/o Hypertension	Yes	15 (27.77%)	13.98
	No	39 (72.22%)	12.68
H/o Diabetes	Yes	25 (46.29%)	13.71
	No	29 (53.07%)	12.01
H/o Dyslipidemia	Yes	13 (24.07%)	13.63
	No	41 (75.92%)	12.53
H/o CAD	Yes	4 (7.40%)	13
	No	50 (92.59%)	12.78
H/o Chest pain	Yes	48 (88.88%)	12.85
	No	6 (11.11%)	12.53
Asthma	Yes	8 (14.81%)	13.1
	No	46 (85.18%)	12.59
Obesity	Yes	42 (77.77%)	12.84
	No	12 (22.22%)	12.65

Distribution of mean CBMN frequency according to biochemical characteristics of the study subjects were given in table 3.

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Table No: 3

Variables	Category	Number (Percentage)	Mean CBMN Frequency
Glucose (mg/dL)	70-130	40 (75.92%)	12.15
	>130	14 (24.07%)	13.10
TG (mg/dL)	<60	4 (7.40%)	12.80
	60-165	38 (70.37%)	12.88
	>165	12 (22.22%)	12.92
TC (mg/dL)	<200	28 (51.85%)	12.51
	200-240	17 (31.48%)	12.55
	≥240	9 (16.66%)	13.18
HDL (mg/dL)	<40	21 (38.88%)	13.24
	40-60	30 (55.55%)	12.65
	>60	3 (5.55%)	12.53
LDL (mg/dL)	<100	6 (11.11%)	13.05
	100-160	33 (60.11%)	12.66
	>160	15 (27.77%)	13.6
VLDL (mg/dL)	<30	33 (60.11%)	12.63
	≥30	21 (38.88%)	13.25
Blood urea (mg/dL)	15-50	52(96.22%)	12.84
	>50	2 (3.70%)	13.65
Serum creatinine (mg/dL)	<1.5	48 (88.88%)	12.74
	≥1.5	6 (11.11%)	13.23
Serum uric acid (mg/dL)	<5	38 (70.37%)	12.78
	≥5	16 (29.62%)	12.84

Distribution of mean CBMN frequency according to Hematological characteristics of the study subjects

Table: 4

Variables	Category	Number (Percentage)	Mean CBMN Frequency
WBC (*10^3/uL)	4 to 11	47 (87.03%)	12.78
	>11	7 (12.96%)	13.5
RBC (*10^6/uL)	<4	7 (12.96%)	12.44
	4 to 5.50	45 (83.33%)	12.35
	>5.50	2 (3.70%)	12.96
HGB (g/dL)	<12	8 (14.81%)	13.58
	12	8 (14.81%)	12.98
	>12	38 (70.37%)	13.72
MCV (fL)	<80	3 (5.55%)	12.3
	80 to 100	51 (94.44%)	12.90
RDW-CV (%)	11 to 16	51 (94.44%)	12.87
	>16	3 (5.55%)	12.96
RDW-SD (fL)	35 to 56	53 (98.14%)	12.2
	>56	1 (1.85%)	12.88
Platelet (*10^3u/L)	<150	8 (14.81%)	12.92
	150 to 400	45 (83.33%)	12.3
	> 400	1 (1.85%)	13.25
ESR (mm/h)	0 to 22	40 (74.07%)	12.78
	>22	14 (25.92%)	13.12

The hematological characteristic observations were recorded (table 4). The hematological parameters included in the study were WBC (white blood cell), RBC (Red blood cells), HGB (Hemoglobin), MCV (Mean corpuscular volume), RDW-CV (Red cell distribution width- coefficient of variation), RDW-SD (Red cell distribution width - standard deviation), Platelet and ESR (Erythrocyte sedimentation rate). Subjects with WBC count >11x103/uL showed the highest mean CBMN frequency (13.5). Subjects with RBC count >5.50x106/uL were considered to be abnormal with a mean CBMN frequency of 12.96. Subjects with hemoglobin count >12 g/dL were showed a high mean CBMN frequency of 13.72. On the basis of MCV, majority of the subjects belonged to the normal range and mean CBMN frequency was 12.90. Subjects with RDW-CV value >16 showed the highest mean CBMN frequency of 12.96. Based on the RDW-SD range, subjects with >56 fL showed the highest mean CBMN frequency of 12.88. In the case of ESR, subjects with >22 mm/h of ESR showed highest mean CBMN frequency of 13.12. Based on platelet count, majority of the subjects belonged to the normal range and showed a mean CBMN frequency of 12.3.

Discussion

Most of the earlier studies on elevated blood pressure (BP) have concentrated only on clinical hypertension, which is defined by the JNC 7 guidelines as systolic blood pressure(SBP) ≥ 140 mmHg and/or diastolic blood pressure(DBP) ≥ 90 mmHg (Chobanian et al., 2003). The present study consist of 45 hypertensive subjects with a broad age range, which allowed a detailed analysis of the association between mean CBMN frequency and blood pressure.

According to Ramaswamy et al., (2015) when age increased, there was a decline innormal BP, and at the same time an increase in prehypertension. Consistent with several other studies, a significant increase in BP with age was observed (Anand et al., 2000). Among the present study, hypertension peaked at 50–69 years and showed mean CBMN frequency of 12.93.

The present study showed that rural area had highest mean CBMN frequency of 13. Hypertension is responsible for 57% of stroke deaths and 24% of coronary heart disease deaths in India (Gupta et al., 2004). The present study also showed that subjects with H/o CAD showed highest mean CBMN frequency of 13.

In the present study, the mean levels of hemoglobin and erythrocyte count were found to be significantly higher in the hypertensive group. The mean levels of Hemoglobin and Erythrocyte count in hypertensive were 17.97, 6.1. From these findings it can be concluded that in primary hypertension, the mean hemoglobin and erythrocyte count are increased significantly. These findings are similar to the earlier findings by Giacomoet al., (1986), Massimo et al., (1992), Dan et al., (1996) and Al-Muhanaet al., (2006). Other results also show positive correlation between blood hemoglobin and blood pressure in hypertensives by Giacomoet al., (1986), Dan et al., (1996) and Al-Muhanaet al., (2006).

In the present study, the mean levels of thrombocyte count were found to be significantly higher in the hypertensive group. The mean levels of thrombocyte count in hypertensive were 460.24. The above findings show that there is increased platelet count in hypertensive subjevts. These results are significantly consistent with the studies reported by Giacomoet al., (1986), Khandekaret al., (2006), Paul et al., (2007) and Al-Muhanaet al., (2006).

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The risk of familial heritability of essential hypertension is positively correlated with the number of hypertensive relatives. When a person has a first-degree hypertensive relative, then the risk of developing hypertension is twice that of the general population. The risk quadruples when the number of first-degree relatives of hypertensive subjects rises (Izzo and Black, 2003; Fuentes et al., 2000). The current study also showed subjects with family H/o hypertension had mean CBMN frequency of 13.98.

There is increasing evidence that complex interactions between genes and environment play an important role in determining the risk of various common diseases such as hypertension (Giacomo et al., 1986). It is now accepted that certain environmental factors act as regulators of gene expression, promoting the development of a disease and speeding or slowing its course in persons with a clear genetic predisposition (Massimo et al., 1992; Dan et al., 1996). The present study illustrated that DNA of hypertensive subjects were showed significant damage, by increased mean CBMN frequency in lymphocytes.

Body size is an important determinant of blood pressure, tall adolescents normally have higher blood pressures than those who are short (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The present study also showed that increased mean CBMN frequency shown by tall subjects (13.28).

The prevalence of essential hypertension is highest in obese adolescents (Bartosh and Aronson, 1999). The present study showed a significant relationship between obesity and hypertension. Study subjects had a significant relationship between the mean CBMN frequency (12.84) and obesity of hypertensive subjects.

The increases in total cholesterol or triglyceride level with blood pressure were greater in overweight than in lean subjects. This suggests that body mass in itself or factors associated with body mass are related to concomitant elevations of blood pressure and blood lipids (Julius et al., 1990). Other research suggests that the lowering of LDL cholesterol level is associated with lowered incidence of hypertension (Ekelund et al., 1988). But the present study showed as the level of LDL increases micronuclei were also increases, which showed that hypertensive subjects had high LDL cholesteroland DNA damage.

RDW is higher in prehypertensive and hypertensive patients compared with healthy independently of age, inflammatory status and anemia. Higher RDW values are strongly correlated with higher systolic and diastolic blood pressures (AsliTanindia et al., 2012). The present study showed that the high range for RDW showed highest mean CBMN frequency when compared to reference range for RDW (11.8% to 14.8%).

According to Al-Muhanaet al.,(2006) the mean levels of Mean Corpuscular Volume (MCV) were found to be significantly higher in the hypertensive group. The mean levels of MCV in hypertensive were 110.81 and in controls were 87.56. The MCV appears to be inversely related to systolic and diastolic blood pressures. The findings of the present study showed that there is increase in mean corpuscular volume (MCV) in hypertensive subjects.

Conclusion

In conclusion, the present study involves Hematological and Molecular Cytogenetic Studies in Hypertensive Subjects. Hypertension and dyslipidemia are widely recognized risk factors for cardiovascular disease. From the present study it can be concluded that in patients with hypertension, significant changes were seen in hematocrit, hemoglobin, RBC count, WBC count and platelet count which can be used for early detection of hypertensive prone individual. The present study observed a clear relationship between increased micronuclei frequencies and the severity of hypertension; however, more direct experimental data are needed at this point to evidence DNA damage accumulation in hypertension. Rapid urbanization, lifestyle changes, dietary changes and increased life expectancy are factors attributable to this rising trend.

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