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Role of Inflammation in Developing Cardiovascular Risk in Pre-hypertensive & Hypertensive Patients

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Abstract

Background: *Hypertension is an important worldwide public-health challenge because of its high frequency and concomitant risks of cardiovascular and kidney disease. Due to its high mortality and morbidity early diagnosis and effective prevention is important. C-reactive protein (CRP), one of the hepatic acute phase reactants, has been associated with decreased endothelium-dependent relaxation, a potential risk factor for hypertension. However, the relationship between CRP and hypertension has not been well elucidated.*

Methodology & Patients: *The purpose of the study was to analyze the correlation between plasma hsCRP and lipid profile in pre-hypertensive as well as hypertensive in Indian patients, attending Diabetic clinic at Index Hospital, Indore and normal control subjects from within campus and surrounding areas. A total of 150 in which 50 newly diagnosed hypertensives (age and sex matched), 50 pre-hypertensives (age and sex matched) and 50 normo-tensive healthy subjects (age and sex matched) were selected for the study.*

Results: *On applying Pearson correlation no significant p value was seen on comparing SBP and DBP with hsCRP and lipid profile parameters in prehypertensive subjects. On comparing hsCRP with individual lipid profile parameters in pre-hypertensive subjects, significant correlation was found only between hsCRP and TG levels (P=0.020).*

Conclusion: *hsCRP values are highest among the pre-hypertensive subjects as compared to normal and hypertensive subjects. Thus it signifies its role as marker of chronic inflammatory process involved in evolution of hypertension. Hypertensive subjects have significantly higher hsCRP level as compared to normal healthy subjects. Thus high hsCRP levels support the hypothesis of chronic inflammation underlying the pathogenesis of hypertension. Our results suggest a correlation exists between hsCRP and hypertension more significantly with prehypertension. So estimation of serum hsCRP can be a good diagnostic as well as prognostic marker in diagnosing prehypertensives and prevent the occurance of hypertension and cardio vascular disorders thereby.*

Keywords: *Hypertension, Pre-hypertension, Inflmmation, hsCRP, Risk, Cardiovascular morbidity.*

Introduction

A Hypertension is a commonly occurring, readily detectable disease. It is most often is asymptomatic in nature that leads to lethal complications if left untreated. According to JNC 7 systolic blood pressure is more than 140mmhg and diastolic more than 90 mm hg is considered as stage I hypertension. In this regard the concept of pre-hypertension, defined as a systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80– 89 mmHg was introduced as the new guideline for the management of blood pressure.[1]

The concept of pre-hypertension, defined as a systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80– 89 mmHg was introduced as the new guideline for the management of blood pressure.[1] There are many established risk factors for development of hypertension which reiterates the importance of an early diagnosis preferably at the stage of pre-hypertension.

Many studies demonstrated that the pre-hypertensives had higher levels of blood glucose, insulin resistance, total cholesterol, low density lipoprotein cholesterol, and triglycerides, higher body mass index, abnormalities of glucose metabolism and lower levels of high-density lipoprotein cholesterol than the normotensive group.[2,3,4]

A growing body of evidences indicates that vascular inflammation may be involved in both the initiation and development of hypertension.[5] In healthy individuals C-reactive proteins (CRP) is present in plasma in minimal amounts but the concentration increases 100 fold in response to injury, infection or inflammation.[6, 7] The association of inflammatory markers with pre-hypertension and hypertension is not very clear. Few studies, however, have explored interrela-tions between levels of CRP and Hypertensive risk factors, and data from these reports have been inconsistent. [8-11]

Hence, we hypothesized that the pre-hypertensive and hypertensive condition is associated with a proinflammatory condition that can be linked to a significant increase in the levels of hsCRP in plasma. This is a simple, cross-sectional observational study of hypertensive patients. In this study patients will be evaluated for serum high sensitive CRP levels which will be correlated with degree of hypertension (HTN), lipid profile and will be compared with normal healthy subjects. The purpose of the study was to analyze the correlation between plasma hsCRP and lipid profile in pre-hypertensive as well as hypertensive in Indian patients, attending Diabetic clinic at Index Hospital, Indore and normal control subjects from within campus and surrounding areas.

Materials and Methods

Study design- Cross sectional hospital based study.

Study site- The study was carried out at the Department of Biochemistry, Index Medical College, Indore, M.P

Duration of study- The duration of study was one year (01.12.2014 to 09.12.2015).

Selection of cases and controls- A total of 150 in which 50 newly diagnosed hypertensives (age and sex matched), 50 pre-hypertensives (age and sex matched) and 50 normo-tensive healthy subjects (age and sex matched) were selected for the study. Written informed consent was taken from them. Cases were selected from clinically newly diagnosed hypertensive and pre-hypertensive patients attending the medicine outdoor patient department of Index Hospital, Nemawar Road, Indore, M.P and age and sex matched healthy control subjects were also selected from the attendants of hypertensive patients and healthy subjects from the Hospital campus for the study.

Inclusion criteria

Subjects between the age group of 40 -60 years were selected. Samples from cases were collected before institution of anti-hypertensive treatment. The criterion for diagnosis of hypertension was systolic pressure of ≥140 mm of Hg and diastolic pressure of ≥90 mm of Hg; pre-hypertension was

systolic pressure of ≥120mmHg to ≤140mmHg and diastolic pressure of ≥80mmHg to ≤ 90mmHg. The criteria for the selection of controls were age and sex matched healthy normotensive individuals (systolic pressure ≤ 120mmHg and diastolic pressure ≤ 80mmHg) without any family history of hypertension.

Exclusion criteria

Hypertensive patients who were already on anti-hypertensive treatment were excluded from the study. Study subjects were examined systematically to exclude any disease (Secondary hypertension) or factors known to cause or those that were associated with hypertension. Subjects with any underlying condition or taking any drugs like steroids, oral contraceptive pills, and thyroxin were also excluded from the study. Similarly, subjects with any underlying condition or taking any drug known to alter serum lipid levels were excluded from the study. Subjects who were smokers, suffering from any inflammatory condition, malignancy, obese and pregnant women, were also excluded from the study.

Methods for Analysis of Test Parameters:

Blood pressure measurement[11]: In a quiet and comfortably seated study subject, two BP readings were taken five minutes apart, on both arms, with a mercury sphygmomanometer (cuff size, 12.5 x 40 cm). The SBP and DBP were read to the nearest 2 mm Hg. The first and fifth phases of Korotkoff's sounds were taken as the criteria for SBP and DBP respectively. The average of two consecutive readings was recorded.

Serum hsCRP[12]: For the estimation of serum hsCRP, 2 ml of fasting, venous, non haemolysed blood sample was withdrawn without the aid of a tourniquet, in a plain sterile bulb. The blood samples were analysed immediately. The estimation of serum hsCRP was done on XL-600 Automatic Analyzer with the kit (Erba Mannheim) based on the measurement of antigen-antibody reaction by the end-point Method.

Estimation of Serum hs C - reactive protein [13-15]

This was done by using Latex –immunoturbidi-metric high sensitivity method.

Principle Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

Reference Range Serum hs-CRP in adults – 2.68 to 8.5 mg/L

Determination of HDL-Cholesterol in Serum or Plasma Method:[16]

Precipitation and enzymatic determination by AutoZyme Cholesterol Reagent Kit: Accurex biomedical Principle: Phosphotungstate /Mg2+precipitates chylomicrons, LDL and VLDL fraction. High Density Lipoprotein (HDL) fraction remains uneffected in supernatant. Cholesterol content of HDL fraction is assayed using AutoZyme Cholesterol.

Measurement of LDL Cholesterol in Serum or Plasma[17]**:**

Method: Indirect using Friedewald equation LDL Cholesterol was measured by indirect method using the Friedewald equation. The Friedewald equation: In the most widely used indirect method, cholesterol, triglyceride, and HDL cholesterol are measured and LDL cholesterol is calculated from the primary measurements using the empirical equation of Friedewald and colleagues. [LDL chol] = [Total chol] – [HDL chol] – [Triglyceride / 5] where all concentrations are given in milligrams per deciliter. The factor [Triglyceride / 5] is an estimate of VLDL cholesterol concentration, and is based on the average ratio of triglyceride to cholesterol in VLDL.

Results

A cross sectional hospital based study was designed to analyze the relation between plasma hsCRP and lipid profile in pre-hypertensive as well as hypertensive subjects over a period of one year. Cases were selected from clinically newly

diagnosed hypertensive and pre-hypertensive patients attending the Medicine OPD of Index Hospital, Indore, Madhya Pradesh and age and sex matched healthy control subjects were also selected from the attendants of hypertensive patients and healthy subjects from the hospital campus for the study.

A total of 150 cases in which 50 newly diagnosed hypertensives (age and sex matched) were selected for the study. Subjects between the age group of 40-60 years were selected. The criteria for diagnosis of hypertension were systolic pressure of ≥140 mm of Hg and diastolic pressure of ≥90 mmHg. Pre-hypertension were systolic pressure of 120 to 140 mmHg and diastolic pressure of ≥80 mmHg to ≤90 mmHg. The criteria for the controls were age and sex matched healthy normotensive individuals (systolic pressure ≤120 mmHg and diastolic pressure ≤80 mmHg) without any family history of hypertension.

Hypertensive patients who were already on anti-hypertensive treatment were excluded from the study. Study subjects were examined systematically to exclude any disease (secondary hypertension) or factors known to cause or associated with hypertension.

Demographic details were noted in all subjects along with recording of systolic and diastolic blood pressure. Blood samples were selected in plain vials and serum was separated for biochemical analysis.

The estimation of serum hsCRP was done on XL-600 automatic analyzer with the kit (Erba Mannheim) based on the measurement of antigen antibody reaction by the end-point method. All the lipid profile parameters were measured on semiautomated biochemistry analyzer using manual kits. LDL cholesterol was measured by indirect method using the Friedewald equation.

Table 1: Distribution of study groups with different grades of hypertension and Controls as per the categorization of serum CRP levels

	Prehypertensive		Hypertensive		Control	
	No	%	No	%	No	%
<1 mg/litre	20	40%	38	76%	47	94%
1-3mg/litre	21	42%	11	22%	3	6%
>3mg/litre	9	**18%**	1	**2%**	0	**0%**
Total	50		50		50	

Table 1 show 18% of pre-hypertensive was having > 3mg/litre serum CRP levels. In hypertensive groups majority of study participants 76% were having <1mg/litre serum CRP levels.

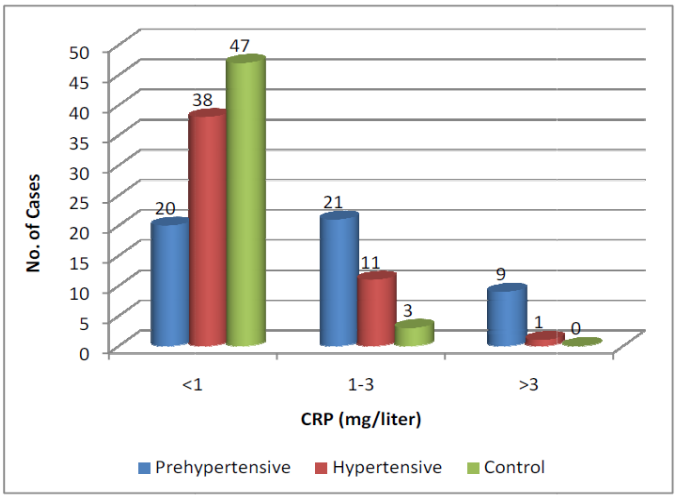

Figure 1: Distribution of study groups with different grades of hypertension and Controls as per the categorization of serum CRP levels

Table 2: Distribution of study groups with different grades of hypertension and controls as per the categorization of serum total cholesterol levels

	Prehypertensive		Hypertensive		Control	
	No	%	No	%	No	%
<200 mg/dl	25		24		36	
200-239 mg/dl	17		19		8	
≥240 mg/dl	8		7		6	
Total	50		50		50	

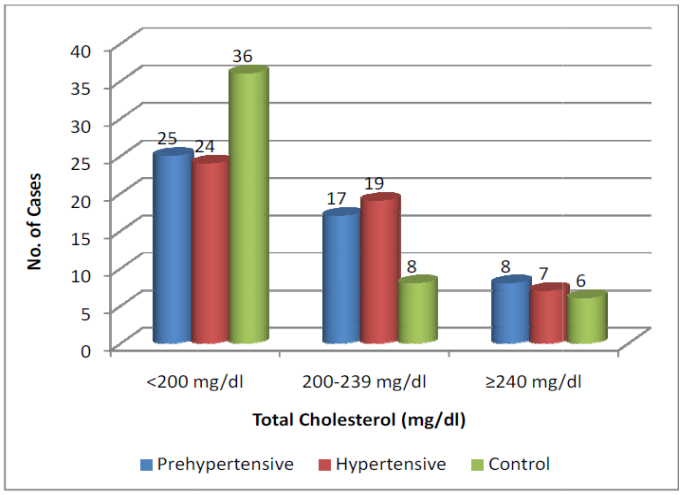

Figure 2: Distribution of study groups with different grades of hypertension and controls as per the categorization of serum total cholesterol levels

Table 3: Comparison of mean values of various parameters in study groups with different grades of hypertension and control group

	Control		Prehypertensive		Hypertensive		ANOVA
	Mean	±SD	Mean	±SD	Mean	±SD	p value
Age	37.98	6.717	40.46	5.939	40.86	7.039	.063
SBP	110.60	4.92	130.44	5.13	148.28	7.84	000
DBP	73.20	3.23	85.08	2.22	92.32	3.88	000.
CRP	0.36	0.31	1.77	1.35	0.82	0.72	.000
CHL	180.04	43.60	197.86	51.51	198.72	35.39	.060
LDL	112.75	46.24	130.58	48.88	119.51	36.63	.130
HDL	40.08	3.22	36.86	6.16	41.08	6.02	.000
TG	136.06	30.42	152.10	41.91	190.66	85.66	.000
Non HDL cholesterol	139.96	43.99	161.00	48.13	157.64	35.68	.034

All the readings were noted for respective group of subjects' i.e. normal healthy group, pre-hypertensive and hypertensive group. Mean and SD values were calculated using statistics. Unpaired student t test was applied group wise to compare various parameters with systolic and diastolic blood pressures. HsCRP was correlated with various lipid profile parameters in respective study groups. The various parameters were compared in all three groups using ANOVA test. The mean hsCRP value of pre-hypertensive group was higher than the control group and hypertensive group. The mean hsCRP value of pre-hypertensive group was also significantly higher than the hypertensive group.

Table 4: Correlation between BP and CRP in normal healthy subjects

	r value	p value
SBP and CRP	.004	.979
DBP and CRP	.172	.231

Table 5: Correlation between BP and lipid profile in normal healthy subjects

	r value	p value
SBP and CHL	.169	.241
DBP and CHL	-.037	.798
SBP and LDL	.182	.206
DBP and LDL	-.017	.909
SBP and HDL	-.093	.519
DBP and HDL	.038	.795
SBP and TG	-.123	.396
DBP and TG	-.160	.268
SBP and Non HDLCHL	.174	.226
DBP and NON HDL CHL	-.040	.785

Discussion

Hypertension is an important worldwide public-health challenge because of its high frequency and concomitant risks of cardiovascular and kidney disease.[18] Industrialization, urbanization, and associated lifestyle changes have led to an epidemiologic transition.[19] Due to its high mortality and morbidity early diagnosis and effective prevention is important. In this regard the concept of pre-hypertension, defined as a systolic blood pressure of 120–139 mmHg and/or a diastolic blood pressure of 80– 89 mmHg was introduced as the new guideline for the management of blood pressure by the seventh

report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7).[20]

A statistical analysis conducted by National Health and Nutrition Examination Survey from 1999 to 2006 found the prevalence of pre-hypertension in disease free adults is 36.3% in all over World.[19] Many studies demonstrated that the pre-hypertensives had higher levels of blood glucose, insulin resistance, total cholesterol, low density lipoprotein cholesterol, and triglycerides, higher body mass index, abnormalities of glucose metabolism and lower levels of high-density lipoprotein cholesterol than the normotensive group.[2-5] There are many established risk factors for development of hypertension which reiterates the importance of an early diagnosis preferably at the stage of pre-hypertension. A growing body of evidences indicates that vascular inflammation may be involved in both the initiation and development of hypertension.[5] This is evident from the elevated levels of inflammatory markers like Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) and Creactive protein (CRP) found in people with hypertension. In healthy individuals C-reactive proteins (CRP) is present in plasma in minimal amounts but the concentration increases 100 fold in response to injury, infection or inflammation.[21]

CRP is named so for its ability to precipitate the somatic C-polysaccharides of Streptococcus pneumoniae and is the first acute phase protein to be described.[6, 7] CRP is primarily synthesized by liver in response to interleukin-6 (IL-6) and interleukin-1ß (IL-1 ß). As a good biological marker it is stable, half life of 19 hours and small variation in values between fresh and frozen forms.[22,23] The relevance of elevated levels of inflammatory markers in predicting cardiovascular risk is gaining increasing recognition and in that respect CRP has been the most intensively investigated in clinical studies.[5] In the year 2001, a cross-sectional study conducted by Bautista et al, for the first time measured CRP in hypertension and found CRP to be an independent risk factor for the development of hypertension. Although, CRP is an efficient marker for inflammation, it is not detectable at a very low level (i.e < 3 mg/l) by routine lab methods. Specialized techniques can detect hsCRP at a level lower than 3 mg/l and so are more important for detection of the pro-inflammatory state at the earliest.[23]

The association of inflammatory markers with pre-hypertension and hypertension is not very clear. Few studies, however, have explored interrelations between levels of CRP and hypertensive risk factors and data from these reports have been inconsistent.[8-11] Hence, we hypothesized that the pre-hypertensive condition is associated with a pro-inflammatory condition that can be linked to a significant increase in the levels of hsCRP in plasma.

The prevalence of hypertension in the late nineties and early twentieth century varied among different studies in India, ranging from 2-15% in Urban India and 2-8% in Rural India. Review of epidemiological studies suggests that the prevalence of hypertension has increased in both urban and rural subjects and presently is 25% in urban adults and 10-15% among rural adults.[22]

Hypertension is an important cause of cardiovascular and kidney disease.[1, 2] Due to its high mortality and morbidity early diagnosis and effective prevention is important. It is an established risk factor for development of atherosclerosis and various cardiovascular diseases (CVDs) like coronary heart disease (CHD), renal failure, congestive heart failure (CHF), ischemic and haemorrhagic stroke and peripheral vascular disease. [1, 2]

In this regard the concept of pre-hypertension defined as a systolic blood pressure of 120-139 mmHg and/or a diastolic blood pressure of 8—89 mmHg was introduced as the new guideline for the management of blood pressure.[3] There are many established risk factors for development of hypertension which reiterates the importance of an early diagnosis preferably at the stage of pre-hypertension.

Many studies demonstrated that the pre-hypertensive had higher levels of blood glucose, insulin resistance, total cholesterol, low density lipoprotein cholesterol and triglycerides, higher body mass index, abnormalities of glucose metabolism and lower levels of high-density lipoprotein cholesterol than the normotensive group.[4,5,6]

The association of inflammatory markers with pre-hypertension and hypertension is not very clear. Few studies however, have explored interrelations between levels of CRP and hypertensive risk factors and data from these reports have been inconsistent.[10-13]

The mean hsCRP value of pre-hypertensive group was higher than the control group and hypertensive group. The mean hsCRP value of pre-hypertensive group was also significantly higher than the hypertensive group.

On comparing the parameters of lipid profile among the three study groups, mean cholesterol levels was highest in hypertensive group followed by pre-hypertensive and lowest in control group. These differences however were not significant statistically. Similarly the differences in mean values of LDL were also statistically non significant. Mean HDL level was highest in hypertensive group followed by normal group and lowest in pre-hypertensive group. The difference in mean HDL levels on comparing control group with pre-hypertensive groups and pre-hypertensive group with hypertensive group was highly significant ($p<0.001$). But there was no significant difference in mean HDL values of control and hypertensive groups. Thus HDL was lowest in pre-hypertensive group.

No correlation was established between systolic or diastolic blood pressure values and hsCRP or lipid profile parameters in normal healthy subjects. No association was found values of hsCRP and lipid profile parameters in normal healthy subjects.

On applying Pearson correlation no significant p value was seen on comparing SBP and DBP with hsCRP and lipid profile parameters in prehypertensive subjects. On comparing hsCRP with individual lipid profile parameters in pre-hypertensive subjects, significant correlation was found only between hsCRP and TG levels ($P=0.020$)

Similar comparison of hsCRP with lipid profile parameters in hypertensive subjects showed positive correlation with total cholesterol and LDL, while no significant correlation was established between hsCRP and HDL, TG or non HDL cholesterol.

CRP increases the blood pressure may be by several mechanisms. It decreases the production of nitric oxide by endothelial cells, so indirectly inhibits vasodilatation. On the other hand, it increases leukocyte adhesion, platelet activation, oxidation and thrombosis. CRP upregulates the angiotensin type-1 receptor so mediates the angiotensin-II mediated increase in blood pressure.[25, 26] Pulse Wave Velocity and augmentation index, a measure of arterial stiffening was associated with many circulating inflammatory markers in recent studies suggesting that inflammation may play a role in arterial stiffness. All these facts indicate CPR has a role in development of hypertension. Ki Chul Sung and workers found hsCRP to be an independent risk factor for development of hypertension in Korean population.[27] In study by Sinha S et al (2014) also we get higher level of hsCRP in prehypertensive and hypertensive group than control.[28]

Conclusion

Among the lipid profile parameters cholesterol and LDL values show no significant difference among normal healthy subjects, pre-hypertensive and hypertensive subjects. HDL levels were significantly lower in pre-hypertensive subjects as compared to normal healthy subjects. The triglycerides were deranged with higher values in hypertensive subjects as compared to the normal and pre-hypertensive subjects. Non HDL cholesterol levels were significantly higher in pre-hypertensive and hypertensive subjects as compared to normal healthy subjects.

hsCRP values are highest among the pre-hypertensive subjects as compared to normal and hypertensive subjects. Thus it signifies its role as marker of chronic inflammatory process involved in evolution of hypertension.

Hypertensive subjects have significantly higher hsCRP level as compared to normal healthy subjects. Thus high hsCRP levels support the hypothesis of chronic inflammation underlying the pathogenesis of hypertension. hsCRP levels showed positive correlation with TG levels in pre-hypertensive patients. hsCRP levels showed positive correlation with total cholesterol and LDL levels in hypertensive subjects.

Thus dyslipidemia raised hsCRP levels are seen pre-hypertensive and hypertensive subjects. These parameters may be used to catch early stages of pre-hypertensive cases developing into hypertensive over a span of time. It can guide for timely intervention to avoid and delay long term effects and morbidity associated with hypertension.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee according to Helsinki declaration

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