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ORIGINAL ARTICLE

hs-CRP : A POTENTIAL MARKER FOR HYPERTENSION IN KASHMIRI POPULATION

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ABSTRACT

Hypertension is the most important public health problem in developing countries and one of the major risk factors for cardiovascular diseases, and it has been reported that hypertension is in part an inflammatory disorder and several workers have reported elevated levels of CRP in hypertensive individuals. The main aim of the present study was to evaluate the association between blood pressure and serum CRP levels across the range of blood pressure categories including prehypertension. A total of 104 patients and 63 control subjects were included in the present study. The level of CRP in the serum samples was estimated by a high sensitivity immunoturbidometric assay. Standard unpaired student's 't' test was used for comparison of hs-CRP levels between hypertensive patients and normotensive control subjects and between patient groups with different grades of hypertension and different durations of hypertensive histories. The mean serum hs-CRP level in hypertensive patients was 3.26 mg/L compared with 1.36 mg/L among normotensive control subjects (P<0.001). On comparison with normotensive control subjects, the hs-CRP levels vary significantly both with grades and duration of hypertension, with most significant difference found in patients with prehypertension (P<0.001), followed by Stage-I (P=0.01) and Stage-II(P=0.02) hypertensives. Significant difference in hs-CRP levels was also found in patients with shorter duration of hypertensive history (≤ 1year) when compared with those with ≥ 5 years of hypertensive history (P<0.01). Our study reveals a graded association between blood pressure and CRP elevation in people with hypertension. Individuals with prehypertension or with shorter duration of hypertension (≤1 Year) had significantly a greater likelihood of CRP elevation in comparison to chronic stage-I or stage-II hypertensives.

KEY WORDS

Hypertension, Inflammatory disorder, hs-CRP, Stage I.

INTRODUCTION

Hypertension is a common, asymptomatic, readily detectable and usually easily treatable disease that leads to lethal complications if left untreated (1). An estimate suggests that world over approximately 1 billion adults have hypertension (333 million in economically developed and 639 million in economically developing countries) (2). Hypertension is probably the most important public health problem in developed countries with over 50 million (29%) adults having hypertension (defined BP=140/90) in United States alone (3). The prevalence of hypertension also shows approximately 20% of individuals in stage II hypertension and nearly 50% in stage I hypertension, while as even higher prevalence of hypertension is being reported in non white population (1, 4). Elevated blood pressure is an established independent risk factor for cardiovascular diseases. Even the individuals with BP in prehypertensive range are considered at increased cardiovascular risk (3). Hypertension is a multifactorial trait that results from the net effect of environmental and genetic factors. Factors that may contribute to hypertension include excess dietary salt or alcohol intake, stress, age, genetics,

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physical inactivity, diet rich in saturated fats and family history. 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 C-reactive protein (CRP) found in people with hypertension with no evidence of Cardiovascular disease(CVD) (6), predict increased cardiovascular risk and predisposing factors (7-13).

C-reactive proteins (CRP) is a plasma protein, present in trace amounts ($\leq$1mg/L) in healthy subjects whose concentration increases 100 fold in response to injury, infection or inflammation. 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 (14, 15). CRP is primarily synthesized by liver in response to interleukin-6 (IL-6) and interleukin-1β (IL-1 β). As a risk assessment tool, it has good points like it is stable, has a long half life of 19 hours and shows small variation in values between fresh and frozen forms that makes it an excellent diagnostic marker (16, 17).

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). The present study is an attempt to evaluate the relationship of serum CRP levels and blood pressure across the range of blood pressure (BP) categories including prehypertension in the Kashmiri people.

MATERIALS AND METHODS

Patients attending the cardiology clinic of Sher-I-Kashmir Institute of Medical Sciences (SKIMS) for hypertension management were recruited for this study, with prior informed consent. Total 104 patients and 63 healthy control subjects with descriptive features given in Table 1, were included in the present study. Patients with chronic inflammatory diseases like rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythromatosus (SLE), autoimmune diseases, tuberculosis, diabetes, stroke, any hepatic or renal diseases and malignancies were excluded from this study. The patients under study were grouped on the basis of systolic blood pressure (SBP) and diastolic blood pressure (DBP) as prehypertensive [130-139/85-89mmHg], Stage-I [140-159/90-99 mmHg] and Stage-II [>160/>100mmHg] and on the basis of duration of hypertension as those with $\leq$ 1 year, 1-5 years and $\geq$ 5 years history of hypertension.

Sera was separated from the blood of patient and control subjects by centrifugation at 3000rpm, and stored frozen at -70^{O}C until further analysis. The level of CRP in the serum samples was estimated by a high sensitivity (with lowest detectable level =0.18mg/L) immunoturbidometric assay on Hitachi 912 clinical chemistry autoanalyzer, using hs-CRP reagent kit from Audit Diagnostic Cork, Ireland. The CRP test is based upon the reaction between C-reactive protein in the sample and latex covalently bound antibodies against human CRP. Values were determined turbidometrically at 570nm at 37^{O}C using fixed time measurement with sample blank correction. Standardization of the method was performed by using different dilution of the stock standard provided with the reagent kit, having a value of 144mg/L of CRP.

The total serum CRP levels were expressed as mean ±SD. Standard unpaired student's 't' test was used for comparison of hs-CRP levels between hypertensive patients and normotensive control subjects and between patient groups with different grades of hypertension and different durations of hypertensive histories.

RESULTS AND DISCUSSION

The estimation of hs-CRP level in 104 patients and 63 control subjects, revealed significantly high level of hs-CRP (=3.26 mg/L) compared to control subjects (hs-CRP =1.36 , P<0.001) (Table 1). Within the patient group (n=104), the male hypertensive subjects does not show any significant difference in hs-CRP levels as compared to female hypertensive subjects (P>0.1). Also the serum hs-CRP levels was higher, though not statistically significant, in prehypertensive subjects (CRP =3.71 mg/L) as compared to stage I (3.18mg/L) or Stage II (3.02mg/L) subjects (Table 2). When compared with the hs-CRP levels in control subjects, the serum hs-CRP levels vary significantly, with most significant difference found in patients with prehypertension (P<0.001) followed by Stage I (P=0.01) and Stage II (P=0.02) hypertensives (Table 1). In patient groups hs-CRP levels was also found to vary with duration of hypertensive history (Table 3). High level of hs-CRP was found in patients with $\leq$ 1 year duration of hypertensive history (hs-CRP= 4.14mg/L) compared to those with 1-5 years of hypertensive history (hs-CRP=3.20mg/L) and those with $\geq$5 years of hypertensive history (hs-CRP=2.78mg/L). However, difference in level was significant only in patient group with $\leq$ 1 year duration of hypertensive history when compared to those with $\geq$ 5 years of hypertensive history. In the present study serum hs-CRP levels were estimated in hypertensive and normotensive control subjects, to evaluate any significant relationship between elevated serum hs-CRP levels with hypertension of different grades in both men and women. The

Table 1: Serum CRP levels (mg/L) in hypertensive patients and in Controls

Parameter (Units)	Patients (n=104)	Controls (n=63)	P-Value
hs-CRP Level (mg/L±SD)	3.26±1.37	1.36±0.26	< 0.001
Hypertension stage with a given level of hs-CRP (mg/L) ± SD			
Pre-hypertension (n=25)	3.71±1.23	1.36±0.26	<0.002
Stage-I Hypertension (n=48)	3.18±1.53	1.36±0.26	<0.01
Stage-II Hypertension (n=31)	3.02±1.23	1.36±0.26	<0.02
Number of subjects with a given level of hs-CRP (mg/L)			
< 1mg/L	Nil	7	
1-3mg/L	47	56	
>3mg/L	57	Nil	

Values are expressed as mean ± 1SD or percent, unless otherwise specified. "n" represents the number of Subjects.

present study revealed an association although graded, between the blood pressure (BP) and serum hs-CRP levels. Of particular interest, individuals with prehypertension were more likely to have significantly elevated serum hs-CRP levels compared to normotensive control subjects. The difference in the elevation levels of hs-CRP was also found to be duration dependent. Patients with shorter duration of hypertensive history (≤1 year) were found to have significantly elevated levels of hs-CRP compared to those with longer duration of hypertensive history (≥5 years). Similar association between blood pressure (BP) and CRP levels were reported earlier by Blake et al and others (3, 18-20) except in a Spanish study (21). Although the Ethnic differences in CRP levels have been also reported by two separate studies (4, 22) but we did not note the ethnicity of the subjects under study.

There are several potential mechanisms that may account for the observed relationship between blood pressure and CRP levels. Increased blood pressure may promote vascular inflammation by modulation of mechanical stimuli from pulsatile blood flow. Cyclic strain has been shown to increase the expression of soluble intercellular adhesion molecule-1(sICAM-1) and vascular cell adhesion molecule-1(VCAM-1) by endothelial cells (23) and also upregulate the secretion of monocyte chemoattractant protein-1 (MCP-1) (24, 25) that promote monocyte adhesion to endothelium. Furthermore, elevated blood pressure is also known to promote generation of reactive oxygen species (ROS) (26) as evident from a study where a significant correlation was observed between levels of CRP and mononuclear oxidative stress (27).

Table 2: Serum CRP levels in mg/L in patients with different grades of Hypertension

Hypertension Stage		P Value
Prehypertension (n=25) CRP=3.71±1.23	Stage I (n=48) = 3.18±1.53	>0.1
Prehypertension CRP=3.71±1.23	Stage II (n=31) = 3.02±1.23	>0.1
Stage I CRP =3.18±1.53	Stage II = 3.02±1.23	>0.5

Values are expressed as mean ± SD; Unless otherwise specified; Prehypertensive subjects (BP= 130-139/85-89) have CRP levels higher than the subjects with Stage-I (BP=140-159/90-99mmHg) Or Stage-II (BP=160-179/100-109mmHg) hypertension, but the difference is not statistically significant (P>0.1/0.5).

In the light of present findings and from several other studies we hypothesize hypertension per se may lead to multiple inflammatory stimuli at the vessel wall which in turn promote the production of a number of pro inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and CRP as a defense against injurious factors. Inflammation, common in hypertensives, decreases endothelium dependent relaxation, possibly by decreased capacity of the endothelium to generate vasodilatory factors, particularly nitric oxide (NO) which inturn raises blood pressure. This is substantiated by several studies which have shown inflammatory markers such as CRP as an independent determinant of endothelium dependent vascular function among patient with coronary heart disease (CHD) and this situation may also exist in patients with hypertension (28). CRP inhibits formation of nitric oxide by endothelial cells which in turn promote vasoconstriction, leukocyte adhesion, platelet activation, oxidation and thrombosis. Moreover, high levels of CRP may upregulate angiotensin receptors and enhance expression of plasminogen activator inhibitor-1 by endothelial cells (5). Both these changes could raise blood pressure and promote atherogenesis.

Table 3: Variation of hs-CRP levels with duration of hypertension

Duration of hypertension in years with serum CRP levels in mg/L±SD		P value
≤ 1 year, CRP = 4.14±1.34 (n=20)	>1-5 years, CRP = 3.17±1.40 (n=52)	> 0.1
≤ 1 year, CRP = 4.14±1.34	>5years , CRP = 2.95±1.46	<0.01
>1-5 years, CRP = 3.17±1.40	>5 years, CRP = 2.95±1.46 (n=32)	>0.5

CRP values in mg/L are expressed as Mean±SD; Duration of hypertension expressed in years & Subjects are divided in three groups depending on the duration of hypertensive history. Patients with a hypertensive history of ≤1 Year have significantly higher CRP levels (P< 0.01).

Our findings are in agreement to that one reported by Sesso et al, who also have shown a link between elevated CRP and increased risk of developing hypertension in a cohort study, including people with baseline blood pressure in prehypertensive range. Possible mechanisms for this association being oxidative stress and interaction with adhesion molecules, plasminogen activator inhibitor-1 and low density lipoprotein cholesterol (LDL-C) uptake (3). The findings of the present study, therefore suggests estimation of CRP levels as an essential / potential tool for early identification of individuals at risk for development of hypertension and eventually cardiovascular diseases. Since both elevated CRP and hypertension are independent determinants of cardiovascular risk, the finding of this study may provide a rationale for pharmacotherapy, in a broader subset of people with hypertension. Since the people with prehypertension may be at a greater CV risk than previously appreciated due to elevated CRP levels, hence strategies targeted to lower CRP levels may potentially provide increased clinical benefits.

In conclusion, our results suggest that increased serum CRP levels are associated with hypertension, more significantly with prehypertension and in new onset patients with hypertension, in Kashmiri people. Thus serum CRP estimation can be a potential tool for early identification of individuals at the risk for development of hypertension and eventually CVDs. However, because of the cross-sectional nature of our study these findings should be confirmed in prospective cohort studies, aimed at elucidating the role of CRP in the prediction, diagnosis and management of hypertension.

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