ORIGINAL ARTICLE

CIRCULATING LEVELS OF CELL ADHESION MOLECULES IN HYPERTENSION

Kavita K Shalia, Manoj R Mashru*, Jagdish B Vasvani*, Rajashree A Mokul, Shilpa M Mithbawkar and Priti K Thakur

Sir H. N. Medical Research Society, Sir H. N. Hospital and Research Centre, Raja Rammohan Roy Road, Mumbai 400 004

*Sir H. N. Hospital and Research Centre, Raja Rammohan Roy Road, Mumbai 400 004

ABSTRACT

Hypertension causes complications such as coronary atherosclerosis and thrombosis wherein inflammatory factors play significant role. In the present study inflammatory molecules such as cell adhesion molecules (CAMs); endothelial (E)-selectin, platelet (P)-selectin, intercellular CAM-1 (ICAM-1), vascular CAM-1 (VCAM-1) and platelet endothelial CAM-1 (PECAM-1) were analysed in subjects newly diagnosed with hypertension with no secondary cause against normotensive healthy individuals. In each group 57 subjects were recruited and soluble (s) levels of CAMs were analysed by ELISA. As compared to controls median of sE-selectin (49.2%, $P=0.001$), sP-selectin (54.3%, $P=0.001$), and sICAM-1 (18.9%, $P=0.012$) were significantly elevated in hypertensive subjects. Significant negative correlation was observed of sP-selectin (spearman rank correlation coefficient (r_s) = -0.345, $p=0.027$) and sPECAM-1 (r_s = -0.446, $p=0.003$) with age in hypertension group. Hypertension may increase expression of certain CAMs while younger hypertensives in addition are also at increased risk of atherothrombosis.

KEY WORDS

Hypertension, Cell adhesion molecules, Selectins, ICAM-1, VCAM-1, PECAM-1.

INTRODUCTION

Cardiovascular diseases (CVDs) have emerged as a global health problem. Essential hypertension (EH), increases pressure in arteries with no specific medical cause is one of the major risk factor for CVD. It synergistically with other risk factors such as diabetes mellitus (DM), hypercholesterolemia, smoking etc., increases the risk for CVDs especially in the elderly (1-3). The pathologic effects of increased blood pressure such as endothelial dysfunction and disturbed balance between vasoconstrictive and vasorelaxing factors result in changes in blood vessels including fibromuscular

hyperplasia of vessel walls and accelerated arteriosclerosis (4, 5) leading to coronary artery disease (CAD), congestive heart failure, stroke and renal failure.

Currently, it is well known that inflammatory factors play significant role in the mechanisms that trigger and enhance remodeling of the vascular wall. These factors are released from damaged cells of the vascular wall and from cells infiltrating this wall, namely monocytes, lymphocytes, and granulocytes (6). Among inflammatory molecules cell adhesion molecules (CAMs) play important role in accelerating the process of atherosclerosis. They are selectins, endothelial (E) - and platelet (P) - which mediate leukocyte rolling on vascular endothelial wall and platelet-leukocyte interaction respectively. Firm attachment of cells to the vascular endothelial wall is carried out by intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) (7). Subsequent migration through endothelium requires expression of VCAM-1 and platelet endothelial cell adhesion molecule -1 (PECAM-1) (8).

Address for Correspondence :

Dr. Kavita K. Shalia,

Sir H. N. Medical Research Society,
Sir H. N. Hospital and Research Centre,
Raja Rammohan Roy Road,
Mumbai 400 004.

E-mail: Kavita_shalia@hnhospital.com,
kavitashalia@hotmail.com

Increased blood pressure apart from various pathological changes (9) also increases production of free radicals by angiotensin II (10), latter being significantly correlated with inflammation (11). Incomplete oxidation and enhanced production of free radicals may be associated with medial thickening, which could prolong the diffusion of oxygen from the vascular lumen (12). This increased production of free radicals in endothelium may trigger a cascade activating the transcription factor nuclear factor kappa B (NFkB), which in turn regulates expression of molecules such as ICAM-1 (13). Thus expression of CAMs may be regulated at the transcription level (14) that may consequently influence serum concentration of soluble forms of CAMs. Experimental evidence came in when Buemi et al (15) demonstrated the relationship between increased blood pressure and increased levels of adhesion molecules in plasma. In their study, even a short-term increase in blood pressure, induced with cold pressor test (CPT) was accompanied by a significant increase in soluble (s) levels of ICAM-1.

Soluble levels of some of these molecules such as E-selectin, P-selectin, ICAM-1 and VCAM-1 have been explored in essential hypertension (16-26). The relationship between C-reactive protein (CRP) and hypertension has also been studied (27, 28) wherein prevalence of hypertension was demonstrated to be high in subjects with second, third and fourth quartile of high sensitivity (hs) CRP as compared to subjects in the first quartile. In India Hypertension has emerged as a major public health problem (29). The prevalence of hypertension (systolic blood pressure [SBP]/ diastolic blood pressure [DBP] $\geq 160/ \geq 95$ mmHg) in urban population has increased from 2–4% to 10–15% and in rural population from 1–2% to 4–8% from mid-1950s to at the end of 20th century (30). To evaluate the significance of above inflammatory markers in hypertension, soluble levels of CAMs which are index of their increased expression were analysed in subjects diagnosed with high blood pressure at the time of the recruitment of the study and confirmed with hypertension with no secondary cause and their levels were again evaluated at 3 months to know the influence of antihypertensive treatment.

MATERIALS AND METHODS

Subjects detected with elevated BP (SBP/DBP $> 135/85$ mmHg as per National Cholesterol Education [NCEP] ATP III Guideline) (31) at the time of the recruitment in the study and ruled out for any secondary causes were selected under hypertension group. They were all non-diabetic with fasting blood sugar below 110 mg/dl. They were then prescribed antihypertensive medications such as Angiotensin converting

enzyme (ACE) inhibitors, Angiotensin II type 1 receptor (AT1R) blockers, Beta-blockers, Calcium channel blockers and Diuretics. The controls were healthy individuals with SBP/DBP $\leq 135/85$ mm Hg or less, with no other risk factors for CVD or clinical symptoms of any other organic disease.

Blood sample was collected after over night twelve hour fast. Those subjects with fasting glucose levels > 110 mg/dl, serum transaminases, Blood Urea Nitrogen (BUN), and Creatinine levels beyond normal range or abnormal ECGs were excluded from the Control group. As per the selection criteria in each group, subjects were recruited with their informed consent. Information regarding their demographic status, clinical history, family history and medications were noted down in detail. The ethical committee of Sir H. N. Hospital and Research Centre approved the study protocol.

Estimation of Soluble levels of CAMs: An aliquot of serum was stored at -80°C for estimation of sE-selectin, sP-selectin, sICAM-1, sVCAM-1 and sPECAM-1 levels by commercially available enzyme-linked immunosorbent assay (ELISA) kits with monoclonal antibodies against each, according to the manufacturer's instructions. For determination of sICAM-1, sVCAM-1, sE-selectin, and sP-selectin, ELISA kits from R & D Systems were used. The minimum detectable levels of sICAM-1, sVCAM-1, sE-selectin, and sP-selectin were 0.35, 1.26, 0.1, and 0.5 ng/ml respectively. The intra assay coefficient of variations of sICAM-1, sVCAM-1, sE-selectin, and sP-selectin, were 4.8%, 3.5%, 5.1% and 6.5%, respectively and inter assay coefficient of variations were 10.1%, 7.7%, 7.4%, and 7.1% respectively. sPECAM-1 was analysed using ELISA kit from Bender MedSystem wherein minimum detectable level was 0.06 ng/ml, intra assay coefficient of variation was 2.5% and inter assay coefficient of variation was 7.4%. Serum hs CRP was analysed using enzyme immunoassay test kit from DSL (Webster, TX) with minimum detectable level of 1.6 ng/ml. The intra and inter assay coefficient of variations of hsCRP assay were 4.8% and 6.1% respectively.

Statistical analysis: Results are expressed as frequency and percentages, mean $\pm$ SD for parametric variables and median with inter quartile ranges for non-parametric variables. Significance of difference in the distribution of frequency was measured by chi square statistics. For parametric variables analysis of significance of difference of means between two groups was performed by student's unpaired t-test and of same group between two collection points by paired t-test. For non-parametric variables analysis of significance of difference of medians between two groups was carried out by Mann Whitney- U test and of same group between two collection

points by Wilcoxon signed-rank test. Correlations were evaluated by Spearman's rank correlation test. P-value <0.05 was considered statistically significant. Analyses were performed using statistical software SPSS (version 15.0, Chicago, IL).

RESULTS

Demographic data of control and hypertension groups is as demonstrated in Table 1. The two groups were matched for age and BMI while SBP and DBP were significantly elevated in hypertension group as compared to controls.

Table 1: Demographic data of Control and Hypertension groups

	Control	Hypertension	P value
No (M/F)	57 (27/30)	57 (33/24)	
Age (Years)	47.6± 9.69	48.9± 10.4	0.492
Age<40 /age≥40 Years	11/46	09/48	0.622
Pre/Post-menopausal women	10/20	10/14	0.528
Height (Meters)	1.63± 0.10	1.61± 0.09	0.391
Weight (Kg)	67.5± 14.3	67.6± 12.7	0.097
BMI (Kg/meter ²)	25.1± 4.43	25.7± 3.93	0.442
Waist(Cms)	86.5± 11	85.9±12.8	0.795
Hip (Cms)	97.8± 11.4	97.1 ±13.1	0.767
Waist to hip Ratio	0.88± 0.071	0.88± 0.07	0.767
SBP (mmHg)	115.6±11.1	156.3 ±17.1	0.001
DBP (mm Hg)	78.7 ±6.88	97.9± 15.1	0.001
Total Cholesterol (mmol/L)	5.65 ±3.92	5.44± 1.052	0.693
HDL-Cholesterol (mmol/L)	1.21± 0.234	1.25±0.249	0.395
Total cholesterol/HDL-C	4.7 ±0.23	4.47± 0.31	0.733
Triglyceride (mmol/L)	1.33± 0.74	1.48± 0.86	0.331
LDL cholesterol (mmol/L)	3.61± 2.32	3.6 ±1.1	0.999
WBC count/μl	6779 ±1518	7447 ±1588	0.005
Smoking	-	01 (1.7%)	
Consumption of Alcohol	-	7 (12.3%)	
Family History	24 (42.1%)	41 (69.5%)	0.0013
Hyperlipidemia	25 (43.85%)	39 (68.4%)	0.014

Analysis of soluble levels of CAMs between control and Hypertension group: Table 2 depicts median of soluble levels of CAMs of control and hypertension group. As compared to controls there was significant rise observed in sE-selectin of 49.2% (p=0.001), sP-selectin of 54.3% (p=0.001), and sICAM-1 of 18.9% (p=0.012) in hypertensive subjects. With respect to sVCAM-1, sPECAM-1 and hsCRP the differences in the medians between control and hypertension group were

Table 2: Soluble levels of cell adhesion molecules

CAMs	Control (n=57)	Hypertension (n=57)	P value
sE-selectin (ng/ml)	19.7 (12.9/31.7)	29.4 (20.2/41.3) (49.2%↑)	0.001
sP-selectin (ng/ml)	110 (80/150)	169.75 (134/201.5) (54.3%↑)	0.001
sICAM-1 (ng/ml)	264.7 (233/305)	314.6 (231/365) (18.9%↑)	0.012
sVCAM-1 (ng/ml)	497 (407/647)	553 (358/732) (11.26%↑)	0.524
sPECAM-1 (ng/ml)	51.3 (43.6/76)	62.3 (44.2/75.6) (21.4% ↑)	0.505
hsCRP (mg/L)	0.92 (0.27/2.23)	0.83 (0.29 / 1.64) (9.78% ↓)	0.902

statistically not significant. The analysis of CAMs according to gender, age groups, BMI groups, menopausal status, and family history with respective controls is depicted in Table 3, 4, 5, 6, and 7 respectively.

Correlation Data: Table 8 depicts correlation data. Within control group among all CAMs only sPECAM-1 demonstrated significant correlation with hsCRP. Within hypertension group significant negative correlation was observed of sP-selectin and sPECAM-1 with age.

Effect of antihypertensive treatment on soluble levels of cell adhesion molecules: Thirty seven out of 57 subjects detected with elevated blood pressure at the time of recruitment in the study and then started with antihypertensive treatment were followed up at three months. Eight of them were each on combination of calcium channel blocker plus beta blocker and on AT1R blocker while seven each were on calcium channel blocker, beta blocker and ACE inhibitors. Therefore analysis of soluble levels of CAMs was carried out on combined data of all the patients on different antihypertensives. Demographic data of these subjects is depicted in Table 9. There was significant decrease in both SBP and DBP from basal level to follow-up at 3 months. Figure 1 (sE-selectin and sP-selectin), Figure 2 (sICAM-1 and sVCAM-1) and Figure 3 (sPECAM-1 and hsCRP) demonstrate soluble levels of CAMs before starting the medication and at follow up of 3 months. Among CAMs there was significant fall only in sP-selectin (20.5%, p=0.006) (Figure 1) and non-

Fig 1: Soluble levels of E-selectin and P-selectin on follow-up of antihypertensive treatment

Fig 2: Soluble levels of ICAM-1 and VCAM-1 on follow-up of antihypertensive treatment

Fig 3: Soluble levels of PECAM-1 and hsCRP on follow-up of antihypertensive treatment

significant fall sICAM-1 of 19.9% (Figure 2) while there was no significant change in any other soluble levels of CAMs. Follow up data of CAMs demonstrated strong correlation of sVCAM-1 with DBP ($r=0.449$, $p=0.047$) and sP-selectin with SBP ($r=0.749$, $p=0.005$).

DISCUSSION

In the present study the status of soluble levels of CAMs that may aggravate the risk of atherosclerosis and thrombosis was determined in freshly diagnosed hypertensives.

We observed significant increased levels of sE-selectin, sP-selectin and sICAM-1 as well as increasing trend of sVCAM-1 in newly diagnosed hypertensives not yet treated with antihypertensives as compared to controls. Similar to our findings literature reports elevated levels of sE-selectin (16-18), with exceptions by DeSouza et al (19) and Ferri et al (20); s-P-selectin (21-24), sICAM-1 (11, 17, 19, 25, 26) and sVCAM-1 (17-19) in hypertension. In the present study, as compared to controls increasing trend of sPECAM-1 in hypertension group was also observed.

E-selectin is a marker of endothelial activation. It demonstrated strong correlation with DBP within hypertension group. Blann et al (16) and Ferri et al (20) have reported similar findings. In the present study within both controls as well as hypertension group there was no significant difference in sE-selectin levels between subjects of BMI groups of 18-25kg/m² and >25-32 kg/m². However median value of sE-selectin of obese subjects of BMI group >32kg/m² as compared to 18-25kg/m² and >25-32 kg/m² BMI groups was elevated in control group by 94.5% and 63.0% (NS) and in hypertension group by 89.2% and 76% (NS) respectively. Ferri et al (32) have analysed the data of hypertension against control with obesity as additional risk factor and have reported that obesity causes elevation of sE-selectin only. In their study plasma E-selectin correlated with BMI. However our data suggests that obesity in addition to blood pressure increases sE-selectin. In the present study sE-selectin was elevated by two fold in pre-menopausal hypertensive women as compared to healthy pre-menopausal women. Similar finding has been reported by Miller et al (33). However Lamon-fava et al (34) have reported that oestrogens can decrease the expression of CAMs. Increasing blood pressure in pre-menopausal women observed in our study and as suggested by Miller et al (33) may abolish the protective effect of oestrogen concentrations. Significant rise in sE-selectin in pre-menopausal freshly diagnosed hypertensive women demonstrates susceptibility of these women towards atherosclerosis in spite of pre-menopausal status protection.

Table 3: Soluble levels of cell adhesion molecules with respect to gender

CAMs	Men		P value	Women		P value
	Control (n=27)	Hypertension (n=33)		Control (n=30)	Hypertension (n=24)	
sE-selectin (ng/ml)	26.4 (15.5/35.2)	29.3 (20.8/35.4)	0.188	15.9 (8.25/25.2)	30 (18.2/47.2)	0.002
sP-selectin (ng/ml)	108 (78/120)	170 (136.3/201)	0.01	115 (85/130)	148.5 (116/211)	0.01
sICAM-1 (ng/ml)	264.7 (237/320)	287 (222/345)	0.729	263 (216.4/299)	339.7 (299.5/386)	0.001
sVCAM-1 (ng/ml)	472.5 (435/640)	513 (344/682.5)	0.784	517 (397.5/663)	675 (438/809)	0.130
sPECAM-1 (ng/ml)	59.9 (40.2/67)	62.6 (44.2/82.9)	0.197	49.8 (44.1/78.6)	53.1 (43.2/69.7)	0.614
hsCRP (mg/L)	0.52 (0.15/1.39)	0.89 (0.38/1.65)	0.117	1.28 (0.37/2.82)	0.76 (0.1/2.26)	0.195

Elevated sP-selectin is a marker of platelet activation and thus its increasing levels may impart thrombogenic status on these hypertensive subjects. In the present study healthy but obese individuals (BMI group $>32\text{Kg/m}^2$) only demonstrated marginal but significant rise in sP-selectin of 18.2% ($p=0.026$) and 20.37% ($p=0.042$) as compared to BMI groups of 18-25kg/ m^2 and $>25\text{-}32\text{ kg/m}^2$ respectively. It also demonstrated strong positive correlation with total cholesterol. Similar to this finding Kvasnicka et al (35) have reported elevated levels of sP-Selectin in overweight hyperlipidemics without clinical evidence of cardiovascular disease, diabetes or hypertension. Within hypertensive group sP-selectin demonstrated increasing trend with respect to BMI as well. Hypertensives with BMI $>32\text{kg/m}^2$ demonstrated remarkable rise in sP-selectin of 84.4% ($p=0.047$) and 42.7% (NS) as compared to BMI groups 18-25kg/ m^2 and $>25\text{-}32\text{ kg/m}^2$ respectively. Among CAMs hypertensive men demonstrated prominent rise only in sP-

selectin as compared to control men. Within hypertension group significant negative correlation was observed of sP-selectin with age. Supporting the above data, hypertensives of age group <40 years demonstrated although non significant but increase in sP-selectin of 37.24% as compared to hypertensives of age group ≥ 40 years.

ICAM-1 is constitutively expressed at low levels and is also influenced by cytokines. In the present study there was marginal but significant association of sICAM-1 with hypertension. sVCAM-1 also demonstrated non-significant trend towards hypertensives. Pre-menopausal hypertensive women demonstrated notable increase of sICAM-1 while post-menopausal hypertensive women demonstrated although non-significant similar rise in sVCAM-1 as compared to respective healthy women.

Table 4: Soluble levels of cell adhesion molecules with respect to age groups

CAMs	Age group <40 years		P value	Age group ≥ 40 years		P value
	Control (n=11)	Hypertension (n=9)		Control (n=46)	Hypertension (n=48)	
sE-selectin (ng/ml)	19.4 (12.6/26.9)	30.3 (21.9/34.8)	0.044	20.8 (12.9/33.2)	28.7 (18.6/42.6)	0.003
sP-selectin (ng/ml)	108 (78/150)	199 (169/280)	0.019	115 (85/125)	145 (132.1/195)	0.034
sICAM-1 (ng/ml)	237 (179/274)	308 (199/368)	0.133	266.7 (241.5/320)	317 (239/369)	0.037
sVCAM-1 (ng/ml)	451 (401/611)	629.5 (450/1249)	0.134	511 (416/663)	513 (341/703)	0.854
sPECAM-1 (ng/ml)	47.0 (40.2/83.2)	66.6 (61.6/91)	0.088	59.7 (44.1/76)	53.1 (41.7/69.7)	0.750
hsCRP (mg/L)	0.61 (0.13/1.6)	0.98 (0.7/1.62)	0.652	0.96 (0.32/2.34)	0.76 (0.25/1.89)	0.674

Table 5: Soluble levels of cell adhesion molecules with respect to BMI

CAMs	BMI 18-25 Kg/m ²		BMI>25-32 Kg/m ²		BMI >32 Kg/m ²	
	Control (n=30)	Hypertension (n=31)	Control (n=21)	Hypertension (n=20)	Control (n=6)	Hypertension (n=6)
sE-selectin (ng/ml)	16.5 (8.53/27)	27.9 (20.2/41.3) (p=0.001)	19.7 (15.3/35)	30 (19.5/33.7) (p=0.363)	32.1 (15.3/44)	52.8 (26.4/61.2) (p=0.114)
sP-selectin (ng/ml)	110 (98/120)	137 (133/194) (p=0.03)	108 (100/123)	177 (138/224) (p=0.05)	130 (105/140)	252.6 (219/286) (p=0.243)
sICAM-1 (ng/ml)	250 (216/288)	287 (225/340) (p=0.082)	274 (255/322)	320 (259/385) (p=0.078)	313.7 (236/353)	359 (388/403) (p=0.071)
sVCAM-1 (ng/ml)	504 (443/643)	547 (411/732.7) (p=0.577)	511 (396/755)	611 (335/868) (p=0.76)	377.5 (294/699)	458 (299/519) (p=1.00)
sPECAM-1 (ng/ml)	47.9 (39.3/69)	60.4 (42.93/83.3) (p=0.301)	60.7 (44.1/69)	62.4 (43.3/68) (p=1.00)	84.2 (45/100)	75.9 (69/82.9) (p=0.857)
hsCRP (mg/L)	0.49 (0.12/1.4)	0.98 (0.15/1.76) (p=0.582)	1.24 (0.44/2.2)	0.77 (0.46/1.6) (p=0.706)	2.3 (1.0/2.8)	3.23 (0.89/5.6) (p=0.1)

Another molecule that was high-lighted in the present study was sPECAM-1. It is with wide variety of function in platelet activation, inflammation, cell survival and immune response and has role in atherosclerotic plaque as well as thrombus formation. Within control group it demonstrated significant correlation with hsCRP. Elevated sPECAM-1 has been reported in pregnancy induced hypertension by Zeister et al (36) and in women with preclampsia by Krauss et al (37, 38).

Ours is the first study to report strong negative correlation of sPECAM-1 with age in hypertensive subjects with no secondary cause with corresponding rise in hypertensives of age group < 40 years (25.4% [p=0.037]) as compared to age group ≥ 40 years and in pre-menopausal hypertensive women (56.1% [p=0.02]) as compared to post-menopausal hypertensive women.

Table 6: Soluble levels of cell adhesion molecules with respect to menopausal status

CAMs	Pre menopausal			Post menopausal		
	Control (n=10)	Hypertension (n=10)	P value	Control (n=20)	Hypertension (n=14)	P value
sE-selectin (ng/ml)	16.8 (11.5/29.3)	36.3 (13.9/49.7)	0.01	16.5 (7.64/22.7)	26.0 (18.2/41.3)	0.002
sP-selectin (ng/ml)	115 (95/130)	186 (153/250)	0.049	108 (80/125)	156.8 (98/185)	0.02
sICAM-1 (ng/ml)	0.02 (207/298)	374 (342/406)	0.01	261 (236/290)	308.5 (287/369)	0.032
sVCAM-1 (ng/ml)	527 (409/724)	575 (458/732)	0.929	451 (359/558)	691 (370/896)	0.067
sPECAM-1 (ng/ml)	68.9 (45.3/85.8)	70.4 (59.7/85.2)	0.82	45.3 (35.6/64.1)	45.1 (39.8/55.4)	0.684
hsCRP (mg/L)	1.32 (0.276/3.2)	0.11 (0.02/2.18)	0.087	1.14 (0.32/2.42)	1.00 (0.44/3.17)	0.756

Table 7: Soluble levels of cell adhesion molecules with respect to family history of cardiovascular disease

CAMs	Absence of family history of cardiovascular disease		P value	Presence of family history of cardiovascular disease		P value
	Control (n=33)	Hypertension (n=16)		Control (n=24)	Hypertension (n=41)	
sE-selectin (ng/ml)	23.3 (12.2/35.5)	24.5 (18.1/38.9)	0.271	18.2 (13.7/27.3)	31.74 (23.8/41.4)	0.001
sP-selectin (ng/ml)	110 (98/120)	137.6 (133/188)	0.617	130 (111/150)	178.9 (134/224)	0.04
sICAM-1 (ng/ml)	250 (220/292.7)	228 (221/315)	0.988	270 (254/328)	327 (281.5/386)	0.020
sVCAM-1 (ng/ml)	475.9 (418.5/625)	494 (369/807)	0.722	511 (396/729)	567 (350/730)	0.833
sPECAM-1 (ng/ml)	50.9 (40.9/77.8)	60.4 (43.8/75.6)	0.818	59.4 (44.1/67.2)	63.6 (43.6/76.3)	0.302
hsCRP (mg/L)	0.56 (0.17/2.38)	0.32 (0.13/1.62)	0.634	0.98 (0.44/1.77)	0.89 (0.46/1.98)	0.895

Table 8: Bivariate correlation within control and hypertension group

Risk factors		r _s	P value
sCAMs in Controls			
sE-selectin	Weight	0.319	0.016
	Hip	0.291	0.030
sP-selectin	Total Cholesterol	0.277	0.037
sICAM-1	BMI	0.329	0.013
	Weight	0.303	0.022
	Hip	0.345	0.009
	SBP	0.308	0.020
	DBP	0.291	0.028
	Total cholesterol	0.434	0.001
	Total Cholesterol/HDL	0.265	0.046
	Triglyceride	0.282	0.033
hsCRP	LDL-cholesterol	0.366	0.005
	BMI	0.333	0.012
	Waist	0.398	0.003
	sPECAM-1	0.469	0.001
sCAMs in Hypertension			
sE-selectin	DBP	0.271	0.046
sP-selectin	Age	-0.345	0.027
	Weight	0.338	0.031
sICAM-1	Triglyceride	0.345	0.027
sPECAM-1	Age	-0.446	0.003
hsCRP	Hip	0.390	0.033

r_s=Spearman's rank correlation coefficient

Several studies have been documented wherein protective effect of antihypertensives against vascular damage has been analysed. The studies have been carried out using ACE inhibitors (39, 40), AT1R blockers (39, 41-43), beta blockers (44) and calcium channel blockers (45-47). In the present study we could follow-up 37 of 57 hypertensive subjects after they had started with antihypertensive treatment. These patients were prescribed any one particular antihypertensive drugs discussed above. As there were only eight patients each on combination of calcium channel blocker plus beta blocker and on AT1R blockers and seven each on calcium channel blocker, beta blocker and ACE inhibitors, their data was combined. On follow-up there was significant fall only in sP-selectin and non-significant fall in sICAM-1 levels showing beneficial effect in addition to decrease in blood pressure while there was no significant change in sVCAM-1, sPECAM-1 or sE-selectin levels. Thus, sPECAM-1 may add risk of atherothrombosis to hypertensives even after control of blood pressure. Decrease in sP-selectin has been reported by ACE inhibitors (39), AT1R blockers (42), beta blocker (44) and calcium channel blocker (45-47).

In the present study median of hsCRP did not increase significantly in the hypertensives as compared to controls as documented in the literature (27, 28). Healthy Asian Indians are reported to have both greater insulin resistance and higher hs-CRP levels than do European Whites (48). In another study from United States, similar relationship was reported between Asians Indians and Caucasians (49) suggesting that many Asian Indians have an underlying proinflammatory state. Some of the reasons for elevated basal hsCRP levels; a marker of low-grade inflammation, have been highlighted in many studies

Table 9: Follow up at 3 months of hypertensive subjects on treatment

	Hypertension		P value
	At Basal level	on follow up	
No (M/F)	37 (27/10)		
Age (Years)	51.91± 9.63		
Ht (Meters)	1.62± 0.10	1.59± 0.09	0.375
Weight (Kg)	68.21±10.52	67.26± 9.64	0.174
BMI (Kg/meters ²)	26.53± 4.14	26.37± 4.57	0.581
Waist (Cms)	89.53± 10.59	90.35±9.77	0.376
Hip (Cms)	101.3± 10.05	101.06 ±10.45	0.912
Waist to hip Ratio	0.88± 0.073	0.89± 0.068	0.525
SBP (mmHg)	157.9±17.6	106.1 ±14.65	0.001
DBP (mmHg)	126.5 ±21.51	87.45± 9.88	0.001
Total Cholesterol (mmol/L)	5.64±36.2	5.23± 1.01	0.111
HDL-C (mmol/L)	1.31± 0.262	1.32 ±0.263	0.842
Total cholesterol/HDL-C	4.3 ±0.84	3.96± 0.82	0.117
Triglyceride (mmol/L)	1.34± 0.68	1.38± 0.68	0.062
LDL Cholesterol (mmol/L)	3.52± 0.99	3.24± 0.79	0.778
WBC count/μl	7495 ±1177	7029 ±1443	0.097
Ca-channel plus Beta blocker	8		
Calcium channel blocker	7		
Beta blocker	7		
ACE inhibitor	7		
AT1R blocker	8		

(50-51) and therefore larger population study with extensive analysis between plasma hsCRP and various parameters of demographic data, body composition, fat distribution and insulin sensitivity etc. will be required to determine its exact status in hypertension.

Overall small sample size is a major limitation of the study. Subsequently, although 37 of freshly diagnosed hypertensives who started on medications came for the follow-up study; there were only few subjects under each antihypertensive treatment. Therefore the effect of one particular drug on soluble levels of CAMs could not be studied. Follow up of longer duration is essential so as to analyse the beneficiary effect of these drugs in addition of maintaining normal blood pressure. Other aspects that could not be analysed in our study were the status of soluble levels of CAMs with respect to smoking habit and consumption of alcohol, as there was only one hypertensive subject with smoking habit and seven with occasional alcohol

consumption.

Novel finding of our study is strong negative correlation of sPECAM-1 and sP-selectin with age in hypertension group. Clinical and laboratory evidence suggests that hypertension may confer a prothrombotic or hypercoaguable state with abnormalities of coagulation, platelets and the endothelium-in fulfillment of Virchow's triad for thrombogenesis (52). Our results suggest that these makers may be useful and specific for pressure-induced endothelial and platelet activation with crucial role in increased atherothrombotic tendency seen in hypertension.

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