

# Association of stress derived risk factors, Chlamydia pneumoniae and high sensitive C-reactive protein in Coronary Artery Disease patients

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## Abstract

**Background:** Atherosclerosis is a chronic inflammatory disease and has a multifactorial etiology. There is growing evidence that Chlamydia pneumoniae (*C. pneumoniae*) may be involved in the pathogenesis of atherosclerosis. In India higher incidence of persistence chronic infection of *C. pneumoniae* has been reported among CAD patients. There are reports mentioning association of established risk factors in the progression of CAD however, many CAD patients lack these factors suggesting the need to look for independent stress derived risk factors including socioeconomic status (SES), migration and life style for the assessment of CAD progression. **Methods:** During the period from March 2005 to September 2006, 192 patients with incident or prevalent CAD and age matched controls attending Cardiology out patient department of Safdarjung Hospital, New Delhi, India were enrolled. A detailed questionnaire was prepared to gather necessary information at the time of sample collection from all patient and controls that included SES or source of income, migration, nature of work or life style. ELISA was performed for the estimation of *C. pneumoniae* IgA and inflammatory marker hsCRP in CAD patients and controls. **Results:** Positivity for *C. pneumoniae*-IgA was significantly high in CAD patients with medium socio economic status (MSES), migrated and sedentary life style (SLS) respectively compared to controls (89 Vs 36,  $p < .001$ ; 99 Vs 45,  $p < .001$  and 97 Vs 33,  $p < .001$ ). Moreover, levels of hsCRP were found comparatively high in CAD patients with MSES (7.5mg/L), migrated (5.9mg/L) and SLS (5.8mg/L) as compared to other CAD patients group and controls. **Conclusions:** Independent stress derived risk factors MSES, SLS and migrated population had high risk of *C. pneumoniae* IgA along with high levels of hsCRP conceivably more prone to CAD in India.

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## INTRODUCTION

Coronary Artery Disease (CAD) is a major cause of morbidity and mortality in humans and is predicted to be the leading cause of death in the world. <sup>1,2</sup> Established risk factors for CAD such as elevated serum lipids, smoking, hypertension, diabetes, age, gender, and family history are associated with less than half of prevalence and severity of CAD. <sup>3</sup> Many patients with CAD lack conventional risk factors, suggesting that there are additional unidentified factors that contribute to vascular injury. <sup>4</sup> Epidemiological studies indicate that infectious agents may predispose patients to atherosclerosis and its adverse clinical events. <sup>4</sup> Organism implicated is intracellular pathogen including Chlamydia pneumoniae (*C. pneumoniae*) specific IgA and CAD has been reported. <sup>5,6</sup> Liu et al has reported association

of hsCRP in heart patients. <sup>7</sup> Further stress has been associated with life style diseases in developing country like India including CAD. <sup>8</sup> Other important risk factors for CAD are change in living habits particularly sedentary life style (SLS) which contribute to increasing burden of heart diseases and CHD mortality. <sup>9</sup> Socioeconomic status (SES) has also been reported as one of the most important risk factors for CHD in men and women. <sup>10,11</sup> There are no studies documenting the combined role of CRP as the inflammatory marker (high sensitivity CRP or hsCRP), seropositivity for bacterial pathogen such as *C. pneumoniae* IgA and socioeconomic variation, which include SES, migration factor and sedentary lifestyle. Hence this study is aimed for evaluating synergistic association between *C. pneumoniae* IgA and hsCRP and with additional risk factors in CAD patients which may help in deliberated diagnosis.

## **MATERIALS AND METHODS**

A total of 192 CAD patients (148 males and 44 females) and 192 age matched controls with no evidence of CAD (142 males and 50 females) attending the Cardiology out patient department of Safdarjung hospital from March 2005 to September 2006 were enrolled after prior written consent. The study received clearance from Ethical committee, Safdarjung hospital. A detailed questionnaire was prepared to gather necessary information from each patient that included SES or source of income, migration from native place to work place, nature of work or life style.

**Inclusion criteria:** Evidence of CAD required at least one of the following: (1) significant stenosis (70% of luminal diameter) in at least one major coronary artery proved by angiography and had undertaken either percutaneous coronary intervention or coronary artery bypass graft (CABG); (2) positive stress myocardial perfusion imaging studies for ischemia.

**Exclusion criteria:** Patients were not included if any of the following were present myocardial infarction or CABG in the preceding 3 months, unstable angina, significant valvular heart disease, blood pressure 180/100 mm.

## **COLLECTION OF SAMPLES**

Venous blood (2 ml) was collected in non- heparinized tubes from CAD patients and controls. Serum was separated within 2 hrs of blood collection and kept at -80°C until used for detection of antibodies to *C. pneumoniae* IgA and levels of hsCRP.

## **SEROLOGY AND ANTIBODY LEVEL OF ATHEROSCLEROTIC MARKER**

Detection of antibodies for *C. pneumoniae* specific IgA was performed using commercially available ELISA kit (R-Biopharm AG, Germany) as per manufacturer's instructions. Index number was calculated by dividing the absorbance for the sample by the calculated average cut off control value. An Index value >1.1 is predicted as *C. pneumoniae* positive; 0.9–1.1 as equivocal and <0.9 as *C. pneumoniae* negative. All the calculations were done consider in only true positive *C. pneumoniae* cases. For detection of antibodies to hsCRP, ELISA was performed using kits (Calibiotec Inc., USA) as per manufacturer's instructions. More than 3 mg/L of hsCRP in serum was considered as hsCRP positive in a dichotomized result.

## **HSCRIP LEVEL AND CONVENTIONAL RISK FACTORS**

CAD patients and controls that were positive for hsCRP were further divided into 14 groups on the basis of additional risk factors as follows (i) Group1- low SES (LSES) in patients, (ii) Group 2- LSES in controls, (iii) Group 3- medium SES (MSES) in patients, (iv) Group 4- MSES in controls, (v) Group 5- high SES (HSES) in patients, (vi) Group 6- HSES in controls, (vii) Group 7- non-migrant patients, (viii) Group 8- non-migrant controls, (ix) Group 9- immigrant CAD patients, (x) Group10- immigrant controls, (xi) Group 11- patients leading normal lifestyle (NLS), (xii) Group12- controls leading NLS, (xiii) Group13- patients leading sedentary lifestyle (SLS), (xiv) Group 14- controls leading SLS.

## **STATISTICAL ANALYSIS**

SPSS version 12.0 for Windows (SPSS Inc., Chicago, USA) was used for statistical testing. All serological results were dichotomized as positive or negative. For comparing diagnostic assays, the chi – square test, fisher-exact statistic for binary related variables was used. Simultaneously, an alpha level of 0.05 was set as the level of significance.

## **RESULTS**

### **ASSOCIATION OF IGA SEROLOGY WITH INDEPENDENT RISK FACTORS IN CAD PATIENTS**

In CAD patients with MSES and HSES significantly high seropositivity for

*C. pneumoniae*-IgA was detected as compared to controls ( $p<0.001$ ,  $p=0.017$ ).

**Figure 1**

Table 1: Association of IgA with stress derived independent risk factors in CAD patients and controls

Independent risk factors		Positive no. of Patients	Positive no. of Control	Odds Ratio	P Value
Socio Economical Status (SES)	Low SES (Pt=57) (Control=29)	42 (73.68)	15 (51.72)	2.61	0.055
	Medium SES (Pt=103) (Control=101)	89 (86.40)	36 (35.64)	12.70	<0.001*
	High SES (Pt=32) (Control=62)	22 (68.75)	25 (40.32)	3.28	0.017*
Migration	Non-Migrated (Pt=84) (Control=74)	55 (65.47)	31 (41.89)	2.62	0.004*
	Migrated (Pt=108) (Control=118)	99 (91.66)	45 (38.13)	18.03	<0.001*
Life Style	Normal (Pt=75) (Control=95)	57 (76.00)	43 (45.26)	3.85	<0.001*
	Sedentary (Pt=117) (Control=97)	97 (82.90)	33 (34.02)	14.26	<0.001*

\*Statistically significant, Pt=Patients, percentage in parenthesis.

Positivity for C. pneumoniae-IgA was significantly high in migrated and non-migrated CAD patients (p<0.001, p=0.004) compared to controls (Table1).

**LEVELS OF HSCRP IN INDEPENDENT RISK FACTORS**

Socio-economic status (SES) was divided into three groups; LSES, MSES, and HSES. It was classified as (i) high for employer/owners with >five employees/ professionals of university level; owning a four-wheeler and earning >30,000 INR/ month (ii) medium for salaried employees with working skills and intermediate or graduate level of education; owning a two-wheeler and earning >10,000 INR/ month and (ii) low for under-employed or small property owners, generally with no permanent employment, with no working skills and with a monthly income of <10,000 INR. Median levels of hsCRP (mg/L) were measured in additional risk factors of CAD patients and controls. Levels of hsCRP was higher in CAD patients with LSES, MSES and HSES (mg/L 4.42, 7.48, and 3.61) compared to controls (mg/L 2.6, 1.43 and 1.82) respectively.

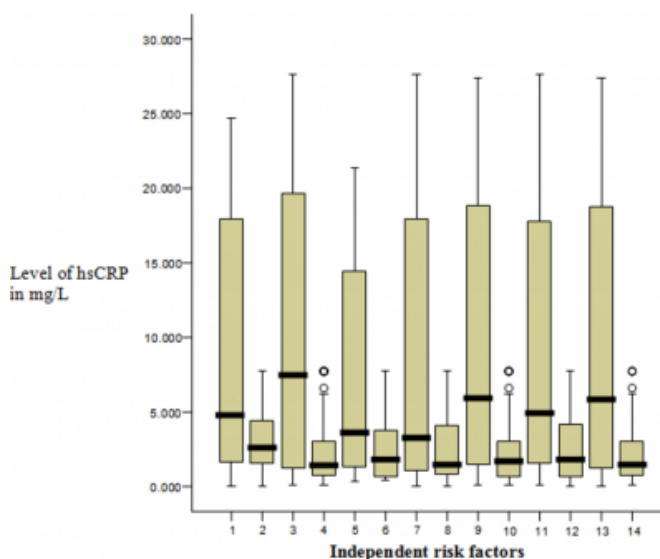
Migration status of CAD patients and controls were divided into two groups; non-migrants and migrants. Non-migrated and migrated CAD patients also showed higher levels of hsCRP (mg/L 3.27 and 5.8) compared to controls (mg/L 5.92 and 1.7).

Lifestyles led were classified using basic metabolic rate

(BMR) as a cut-off. They were classified as (i) sedentary <1.6 BMR (which included chair-bound or bed-bound or seated work with no option of moving around and little or no strenuous leisure activity) (ii) normal as 1.6 to 1.9 (which included seated work with discretion and requirement to move around but little or no strenuous leisure activity or standing work; eg housewife, shop assistant) and (iii) strenuous > 1.9 (which included significant amounts of sport or strenuous leisure activity (30-60 min 4-5 times per week) or strenuous work or highly active leisure). All participants were classified in the first two groups; that is, sedentary (SLS) and normal lifestyles (NLS) as the patients and controls were well matched. CAD patients with normal and SLS showed higher hsCRP levels (mg/L 4.92 and 5.84) compared to controls (mg/L 1.82 and 1.47) (Fig 1).

**Figure 2**

Fig 1: Levels of high sensitive C – reactive Protein in coronary artery disease patients with stress derived Independent risk factors.



Legends:

On X axis - Additional risk factors for CAD.

Y axis- Levels of hsCRP (mg/L in serum).

1= Low SES in CAD patients, 2= Low SES in controls, 3= Medium SES in CAD patients, 4= Medium SES in controls, 5= High SES in CAD patients, 6= High SES in controls, 7= Non-migrated in CAD patients, 8= Non-migrated in controls, 9= Migrated in CAD patients, 10= Migrated in controls, 11= Normal LS in CAD patients, 12= Normal LS in controls, 13= Sedentary LS in CAD patients, 14= Sedentary LS in controls.

## DISCUSSION

The increase in CVD risk factor profile worldwide has been influenced by a cluster of lifestyle related variables, of which perceived stress is one of the most important factor.<sup>12</sup> Our study for the first time revealed that impact of socioeconomic status was very high as CAD migrated patients had highest risk (18 times) followed by SLS (14 times) and MSES (12 times) with *C. pneumoniae* infection. It has also been shown earlier that SES is related to divergences in both the physical and social environment and in the capacity to respond to stressful life experience.<sup>13</sup> Moreover, higher hsCRP levels were detected in MSES (7.5mg/L), migrated population (5.9mg/L) and SLS group (5.8mg/L) in CAD patients showing thereby that they are at higher risk for CAD. Previous reports also suggested that CRP >3mg/L represent high risk, CRP >10mg/L may be attributed to acute infection and CRP >20mg/L predicts cardiovascular events.<sup>14</sup> Moreover we detected significantly higher levels of hsCRP in MSES group in contrary to earlier report where LSES individuals were found to be more susceptible to infections and have a greater risk of developing chronic disease.<sup>15</sup> It may be the outcome of urbanization in Indian metro city life as well as increasing stressful or competitive environment at work place. Chandola et al, demonstrated that stress at work can lead to CHD through direct activation of neuroendocrine stress pathways and indirectly through health behaviors.<sup>8</sup> Our study divulges that migration from rural to urban has been emerging a major risk factor for CAD in Indian population. Present scenario all over the world ie, SLS is one of the major independent risk factor for CAD<sup>16</sup> and in our study we also found significant higher levels of hsCRP in this group of CAD patients. Several biological mechanisms could explain the beneficial effects of physical activity on CVD risk, including lowering of blood pressure, elevation in high density lipoprotein levels, increased insulin sensitivity, improved endothelial function, and reduced atherogenic cytokine production.<sup>17,18</sup> Thus all these independent risk factors which are directly or indirectly derived by stress have synergistic association of *C. pneumoniae* in CAD patients. MSES, SLS and migrated population have higher risk for CAD and significantly associated with seropositivity for *C. pneumoniae* IgA along with high levels of hsCRP in CAD patients. However, further longitudinal studies are required to know the mechanistic progression for these stress emerging additional risk factors.

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