REFERENCE VALUE FOR HIGH-SENSITIVITY C-REACTIVE PROTEIN IN THE NORTHERN PAKISTANI POPULATION

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ABSTRACT

Objective: High- sensitivity C - reactive protein (hS-CRP) is an acute phase protein that is often used for the risk estimation of cardiovascular diseases. Objective was to find out the reference range of hS-CRP in the healthy adult northern Pakistani population and compare the distribution in relation with age, gender, body mass index (BMI), ethnicity and smoking.

Study Design: Cross-sectional study

Place and Duration of Study: Army Medical College Rawalpindi from Nov 2007 - Mar 2009

Subjects and Methods: In this cross-sectional study a total of 297 healthy adults (>18 years) comprising 207 males, 90 females, Punjabis (n=178) and Pathans (n=119) were randomly included from Rawalpindi, Lahore, Peshawar and Sawabi Districts. History and clinical examination were carried out. Serum hS-CRP was analysed in Clinical Pathology Laboratory, Army Medical College Rawalpindi by two site sequential chemiluminiscent immunometric assay kit on immulite 1000(USA). Data were analysed by SPSS-16.

Results: The reference values for hS-CRP are presented as median (2.5th-97.5th percentile). In the healthy adult population of Northern Pakistan it was 1.84 mg/L (0.37-4.81) mg/l; in Punjabis 1.75 (0.30-4.65) mg/l and in Pathans 1.93 (0.50-5.30) mg/l. Females had higher hS-CRP levels 2.05 (0.53-5.66) mg/l as compared to the males1.76 (0.31-5.06) mg/l. Smokers had relatively higher hS-CRP 1.98 mg/l (0.47-5.56) mg/l than non-smokers 1.68 mg/l (0.30-4.60). Our data also revealed that hS-CRP had significant positive correlation with advancing age ($r=0.20$; $p<0.05$) and increasing BMI ($r=0.29$; $p<0.01$).

Conclusion: The reference range for hS-CRP in the population of Northern Pakistan is 0.37-4.81 mg/l. Pathans have higher hS-CRP levels than Punjabis. Demographic factors including age and BMI were positively correlated to the distribution of hS-CRP.

Key words: hS-CRP; reference value; BMI; ethnicity; smoking; Pakistani population

INTRODUCTION

High-sensitivity C - reactive protein (hS-CRP) is an acute phase reactant and is a new tool in the risk estimation of cardiovascular diseases (CVD) [1]. Numerous epidemiologic studies have consistently shown that hS-CRP levels provide a strong and independent indication of risk of future heart attacks, ischemic stroke (due to obstruction, not bleeding), and peripheral arterial disease, even among individuals who are thought to be free of vascular disease [2]. There is a large intra individual variation in the hS-CRP levels and hence a need for regional studies related to it [3].

Sources of variability of hS-CRP values include gender, age [4], body weight [5], diet [6] and level of physical activity [7] Moreover, racial and demographic correlations have been studied as potential determinants of hS-CRP levels. Most studies that examined smoking in relation to hS-CRP found strong direct associations [8]. Determination of reference range and factors associated with hS-CRP levels could lead to strategies designed to estimate the CVD risk and long term consequences in our northern Pakistani population. Identification of these associated factors in the adults would be particularly useful for lifestyle modification to prevent the disease.

The reference value for hS-CRP is still not known in the Pakistani population. Our study aims to determine the reference range of hS-CRP in the healthy population of northern Pakistan and to study its relationship with age, gender, ethnicity, BMI and smoking.

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SUBJECTS AND METHODS

The study was carried out at the Clinical Pathology Laboratories (CPL), Army Medical College Rawalpindi after approval from the institutional review committee. The study was carried out from Nov 2007-March 2009.

It was a cross-sectional study conducted on a total of 297 subjects. They were healthy adults aged between 18 and 73 years comprising of males (n=207) and females (n=90). Punjabis (n=178) and Pathans (n=119) were randomly selected from Rawalpindi, Lahore, Peshawar and Sawabi districts. The data were collected from the council office of these districts after which random selection was done. History and physical examination were carried out by a doctor and laboratory screening was performed. Only healthy subjects were recruited. Smoking history was taken and coded into non-smokers and current smokers. BMI was used as a measure of relative body weight. Individuals who were obese (BMI > 30), hypertensive or on drugs and pregnant women were excluded from the study.

Blood sample (5ml) was obtained by venepuncture at Clinical Pathology Laboratories (CPL), Army Medical College Rawalpindi. Serum was separated by centrifugation at 1500×g for 15 minutes. Blood samples were stored in Eppendroff tubes at -80 0Ċ until hS-CRP analysis was performed.

Estimation of Serum hS- CRP

Serum hS-CRP was analysed by two-site sequential chemiluminescent immunometric assay kit with a Lot No. LK CRP 10161 on Immulite 1000 (Siemens, LA, California, USA) according to valid published method (9) by following the manufacture instructions. The analytical sensitivity was 0.1 mg/L. Control materials were run with every batch. Coefficient of variation of the method was 4.8 percent.

Statistical Analysis

The data were analyzed using standard SPSS software version-16. Descriptive studies were done according to which data were found to be non-Guassian. The range was calculated using the 2.5th and 97.5th percentiles to for reference interval. Furthermore variations in the reference value of hS-CRP with respect to age, gender, BMI and smoking were studied. The correlation between hS-CRP and different factors was determined by Spearman test. A p value < 0.05 was considered significant.

RESULTS

In this cross-sectional study a total of 297 subjects of both sexes were recruited, aged between 18-74 years. The baseline characteristics of the participants are given in table 1. Median hS-CRP level (95% CI) in the total population was 1.84 mg/l with a range of 0.37-4.81 mg/l as is evident from the histogram given in figure 1. Females had higher hS-CRP levels as compared to the males with median (range) 2.05 mg/l (0.53-5.66 mg/l) and 1.76 mg/l (0.31-5.06 mg/l) respectively. The box plot of distribution of hS-CRP according to gender is illustrated in Figure 2. hS-CRP distribution in northern Pakistani population particularly in Pathans 1.93 mg/l (0.50-5.30) mg/l and Punjabis 1.75 mg/l (0.30-4.65) mg/l is exhibited in Fig -1.

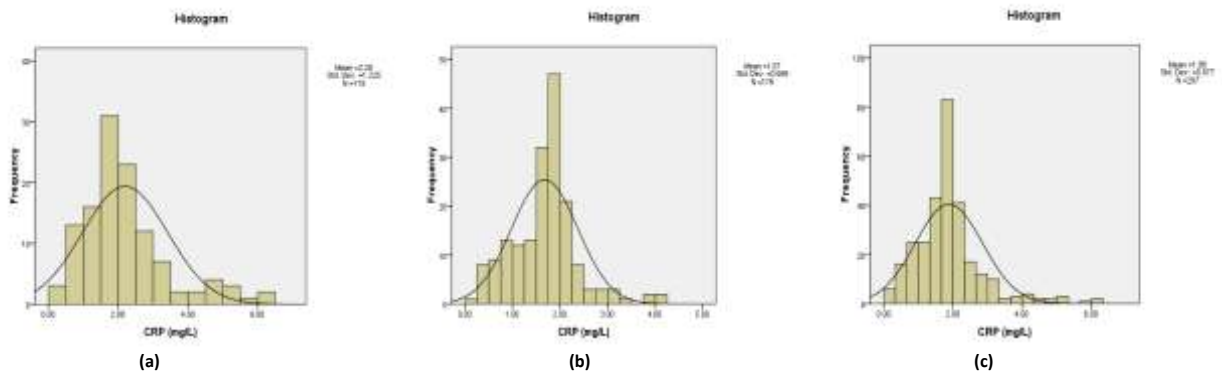

Figure 1: Distribution of CRP in the (a) Punjabis (b) Pathans (c) Total population

Our data also showed that hS-CRP level rose with both, advancing age and increasing BMI (Table-2). Smokers had relatively higher hS-CRP values with the median level being 1.95 mg/l in smokers and 1.76 mg/l in non-smokers. Correlation study revealed positive correlation between hS-CRP level and age (r=0.20; p <0.05) and BMI (r=0.29 and p<0.01).

DISCUSSION

This is first study in Pakistan and we have found that the reference range of hS-CRP (95% CI) in the healthy population of

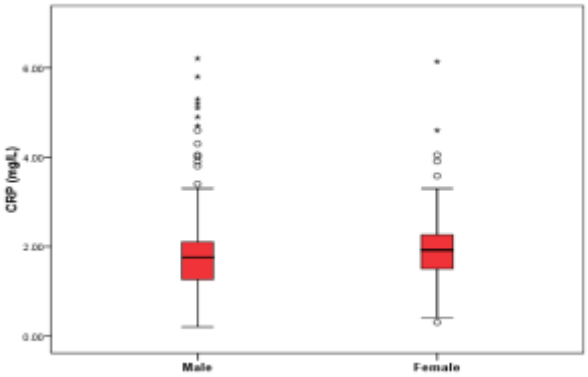

Figure 2: Distribution pattern of CRP in healthy Pakistani adults according to gender (Male n=207; Female n=90)

Table-1: Baseline characteristics of the participants

Parameters	Total population n=297 Median (range)	Punjabis n=178 Median (range)	Pathans n=119 Median(range)
Age (years)	39 (18-73)	35 (19 – 73)	38 (18-72)
BMI (kg/m2)	24.6(17.36 - 30.07)	24.4 (17.36-28.64)	25 (18 -30.01)
Smokers (%)	98 (32.9)	62 (34.8)	35(29.4)
Non-smokers (%)	199 (67)	137(76.96)	63(52.9)
Males (%)	207 (69.7)	123 (69.1)	84 (70.6)
Females (%)	90 (30.3)	55 (30.9)	35 (29.4)

Table-2: Distributions and associations of hS-CRP with age, BMI, gender, ethnicity and smoking.

Independent variable	% in group	CRP Median (95% CI)	p-Value
Age (years)			0.000
<45 n=236	79.5	1.76 (0.30-4.32)	
≥45 n=61	20.5	2.12 (0.48-5.95)	
Body Mass Index(kg/m2)			0.000
<25 n=155	52.2	1.75 (0.30-4.72)	
≥25 n=145	48.8	1.94 (0.42-5.24)	
Gender			0.029
Males n=207	69.7	1.76(0.31-5.06)	
Females n=90	30.3	2.05 (0.53-5.66)	
Ethnicity			0.013
Punjabis n=178	59.9	1.75 (0.30-4.65)	
Pathans n=119	40.1	1.93 (0.50-5.30)	
Smoking status			0.001
Non-smokers n=199	67.1	1.68 (0.30-4.60)	
Smokers n=98	32.9	1.98 (0.47-5.56)	

Northern Pakistan is 1.84 mg/l (0.37-4.81 mg/l) which is lower than the Aboriginal Australians, Hungarian population, Latin Americans and Hispanics, while it is comparable to the levels in the Thai and Brazilian population and higher than the Chinese population. hS-CRP levels determined by ultra sensitive method in a healthy Hungarian population revealed mean hS-CRP level of 3.57 mg/L [10]. Another study on the population distribution of hS-CRP values in Aboriginal Australians shows mean value of 7.3 mg/L in females and 4.1 mg/l in males [11]. A study titled 'CRP levels Among Women of Various Ethnic Groups Living in USA' shows median hS-CRP levels in black, white, Hispanic and Asian women were 2.96 mg/L, 2.02 mg/L, 2.06 mg/L and 1.12 mg/L respectively [12]. Another study from Canada on hS-CRP as a screening test for cardiovascular disease in the healthy multiethnic population revealed the mean

values of 3.74 mg/l, 2.59 mg/L, 2.06 mg/L and 1.18 mg/L in Aboriginals, Asians, Europeans and Chinese respectively [13]. A population-based study in Brazil showed mean level of 0.89 mg/L in males and 1.96 mg/L in females.

Moreover, we observed that Pathans have higher levels of hS-CRP than Punjabis which shows that hS-CRP in healthy adults is affected by demographic and behavioral factors. Both baseline hS-CRP concentrations and drug-induced hS-CRP changes are highly variable and potentially subject to genetic regulation [14]. Moreover, genetic variants on the CRP locus and dietary and lifestyle factors are responsible for the interindividual variability of plasma hS-CRP concentrations [14]. This may be one of the causes of the difference in the hS-CRP value between Pathans and Punjabis. It is also a common observation that use of diesel-run cars vehicles is more in Peshawar and its adjacent areas; some studies have shown that air pollution was associated with activation of inflammatory/oxidative stress pathways [15]. Hence, increased hS-CRP in Pathans may be because of greater particulate matter especially sulphate primarily formed by oxidation of SO2 from tail pipe emissions of diesel cars and trucks [16]. Results of twin and family studies have led to estimations that genetic factors could contribute up to 35-50% of the phenotypic variation of plasma hS-CRP concentrations [17]. This may be one of the contributing factors which results in different baseline levels of hS-CRP among various populations of the world and a relatively constant level in a certain ethnic group. All these data supports the necessity of establishing regional hS-CRP values.

According to our study females tended to have higher reference levels for hS-CRP than males which is comparable to some earlier studies stating that females tended to have higher levels than males [18]. A probable reason for this may be more adipose tissue in women which is consistent with the notion that excess adipose tissue contributes to a chronic inflammatory state through a range of metabolic pathways [19]. Another study shows that leptin is present in higher quantities in women and the leptin receptor resembles the cytokine receptor which includes the interleukin- 6 receptor. Thus it regulates the synthesis of CRP and is involved in the inflammatory mechanisms [20].

BMI also showed correlation with hS-CRP levels in our study. Leptin has again been shown to be high in the obese and as mentioned earlier it has a potential role in the inflammatory mechanisms. The identification of factors which are amenable to intervention, like smoking and BMI, reinforces the need for population- based preventive strategies.

Age was also strongly associated with the hS-CRP levels in our study comparable to others which found direct associations between them [21]. It is a common observation that as age advances the level of psychological stress also increases and so does the physical activity. Both these factors have shown to chronically activate the innate immune response, driving an inflammatory stimulus [22]. Hence, it emphasizes the role of psychosocial risk factors on inflammatory mechanisms and chronic states with advancing age.

Our study showed higher levels of hS-CRP among current smokers as compared to non-smokers which agrees with most studies that examined smoking in relation to hS-CRP levels and found direct associations [9]. Most probably excessive smoking results in increased oxidative stress thus contributing to the underlying inflammatory mechanism. Recent study has shown that there is a positive dose dependent relationship between current cigarette smoking and increased hS-CRP levels and the most possible reason for this could be the induction or exacerbation of inflammation [23].

Most studies conducted largely involve high income countries and ours is one of the few important ones involving the population from a low-to-middle income country .By far our study is the first of its kind to determine the hS-CRP levels in the healthy population of

northern Pakistan. This study was unique because it investigated a novel biomarker in a representative sample of the adult Pakistani population.

One of the limitations of our study was that the study population was selected from the northern part of Pakistan and involved just two provinces so the findings are not representative of the other two provinces of Pakistan. For future researchers it has opened venues for comparison and further study on the total population of Pakistan.

In conclusion, the reference range for hS-CRP in the population of Northern Pakistan is 0.37-4.81 mg/l. We have demonstrated that hS-CRP levels in adults are affected by anthropometric, ethnic and sex-specific factors. Females have higher hS-CRP levels than males while Pathans have higher values than Punjabis. Age and BMI are positively correlated with the reference level of hS-CRP.

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