Association between Inflammatory Markers and Cardiovascular Risk Factors in Women from Kolkata, W.B, India

Debdutta Ganguli[1], Nilanjan Das[1], Indranil Saha[1,4], Krishna Rao Sanapala[2], Debnath Chaudhuri[3], Saurabh Ghosh[2], Sanjit Dey[1]

Department of Physiology, University College of Science and Technology, University of Calcutta, West Bengal, India[1]; Human Genetics Unit, Indian Statistical Institute, Kolkata, India[2]; Department of Biochemistry and Nutrition, All India Institute of Hygiene and Public Health, West Bengal, India[3]; Present Address: Department of Community Medicine, RG Kar Medical College and Hospital, Kolkata, India[4]

Abstract

Background: Recent research has focused on the use of inflammatory biomarkers in the prediction of cardiovascular risk. However, information is scant regarding the association between these inflammatory markers with other cardiovascular risk factors in Asian Indians, particularly in women.

Objective: To explore the association between inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) and white blood cell (WBC) count and cardiovascular risk factors such as overall and central adiposity, blood pressure, lipid and lipoprotein variables and fasting glucose.

Methods: We conducted a cross-sectional analysis on 100 women aged 35 to 80 years. Participants were selected following cluster sampling methodology from 12 different randomly selected urban wards of Kolkata Municipal Corporation.

Results: Hs-CRP has a significant association with body mass index (BMI) ($p < 0.001$) and waist circumference (WC) ($p = 0.002$). Significant inverse associations were observed between high-density lipoprotein cholesterol (HDL-C) and both inflammatory markers, hs-CRP ($p = 0.031$) and WBC count, ($p = 0.014$). Apolipoprotein A1 (Apo A1) was also negatively associated with hs-CRP. WBC count has significant correlation with fasting glucose and total cholesterol (TC) /HDL-C ratio. Using logistic regression, adjusting for age, BMI (odds ratio/OR, 1.186; confidence interval/CI, 1.046-1.345; p=0.008) and WC (OR, 1.045; CI, 1.005-1.087; p=0.027) were the covariates significantly associated with hs-CRP.

Conclusion: In the present study, risk factors like BMI, WC, and HDL-C and apo A1 show significant association with hs-CRP. WBC count was significantly correlated with HDL-C, fasting glucose, TC/HDL-C ratio in women. (Arq Bras Cardiol 2011; 96(1): 38-46)

Keywords: Inflammation; reactive protein C; risk factors; leukocyte count; adiposity; dyslipidemias; women: India.

Introduction

Recent epidemiological studies have reported a strong and consistent association between cardiovascular disease (CVD) risk factors and inflammation[1,2] and the latter has been identified as an independent risk factor for CVD[3,4]. Of the novel inflammatory markers currently under investigation, C-reactive protein (hs-CRP) and white blood cell (WBC) count are the promising ones. A number of prospective epidemiological studies consistently demonstrated that hs-CRP[5,6] as well as WBC count[7] can independently predict vascular risk both in apparently healthy men and women, asymptomatic for traditional risk factors of CVD. In addition, both markers[1,2] also add prognostic information in patients with clinical signs of CVD, beyond that available from standard lipid-screening. A substantial number of cross-sectional studies have already established the fact that hs-CRP and WBC count have been found to significantly correlate with components of metabolic syndrome[8-10].

In India, data are scarce regarding the association of these inflammatory biomarkers with other CVD risk factors in adults, particularly among women. Thus the objectives of the present study were to investigate the association between hs-CRP and WBC count with risk factors such as body mass index, waist circumference, waist-hip ratio, blood pressure, lipid and lipoprotein variables and fasting glucose.

Materials and method

Study participants

We randomly selected a population of 100 women aged between 35 and 80 years from an existing epidemiological study on cardiovascular risk assessment involving 701 women residing at urban wards staying for an average 29.8 years in Kolkata. The study was carried out following the WHO prescribed cluster sampling methodology[11] from 12

Mailing address: Sanjit Dey •
Department of Physiology, University College of Science and Technology, University of Calcutta, 92, APC Road, Kolkata- 700 009, West Bengal - India
E-mail: sanjitdey2003@yahoo.com
Manuscript received February 11, 2010; revised manuscript received February 11, 2010; accepted May 28, 2010.

different wards out of 141 urban wards of Kolkata Municipal Corporation according to the most recent census data. The wards or clusters were included in the study by simple random sampling without replacement. According to the cluster sampling strategy, in each of the chosen ward or cluster, a location near the center of the ward was taken as the starting point and a random direction was selected. Then the households were chosen randomly along the direction after checking for the compliance with inclusion criteria such as- women aged 35 or above regardless of her marital status, but not pregnant ones. The exclusion criteria were acute illness or any treatment of inflammatory or chronic infectious disease either before or at the time of the investigation. Subjects unwilling to participate in the study were excluded during the survey. Individuals using aspirin as a chronic medication were also excluded. None of the subjects reported taking lipid-lowering medication. Among the eligible subjects, 57 individuals were reported as postmenopausal and the remaining 43 were premenopausal women.

Subjects submitted their written consent to participate in the study prior to the survey, which was approved by the Human Ethical Committee of Department of Human Physiology, University of Calcutta.

Questionnaire

A questionnaire-based interview was used to collect information on smoking habits, use of chewing tobacco, alcohol consumption, personal and family medical history of hypertension, diabetes and myocardial infarction. The participants were interviewed about their menopausal status and history of any surgical intervention, such as hysterectomy. Women were considered pre-menopausal if they had experienced 1 or more regular bleeding episodes in the past 12 months. Women were considered postmenopausal if their menses had ceased naturally or surgically (e.g. hysterectomy) for a period of at least 12 months. The participants were also interviewed about their history of dyslipidemia, current use of anti-hypertensive or hypoglycemic or lipid-lowering drugs and use of postmenopausal hormone therapy. The validity of responses to questions on drug use was confirmed by checking medical records.

Anthropometric and biochemical measurements

Height was measured to the nearest 0.5 cm without shoes using an anthropometer. Weight was recorded in light clothing after removal of shoes to the nearest 0.1 kg. WC was measured using a non-stretch measuring tape at the midpoint between the lowest rib and the iliac crest in the horizontal plane. Maximum hip circumference was measured horizontally at the level of the maximum extension of the buttocks. For each of waist and hip circumference, two measurements to the nearest 0.5 cm were recorded. The mean of the two closest measurements was calculated. Waist-hip ratio (WHR) was calculated by the standard equation: WHR = Waist circumference (cm) / Hip circumference (cm).

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Blood pressure was measured on the right arm of participants in a relaxed, sitting position with the arm supported at heart level, using a standard mercury sphygmomanometer. Systolic and diastolic blood pressures were recorded as the onset of the first and fifth Korotkoff phases, respectively. For each of the measurement, two readings 5 minutes apart were taken and the mean of the two readings was calculated to obtain the final blood pressure. Participants were advised to avoid cigarette smoking, caffeinated beverages and exercise for at least 30 minutes before the blood pressure measurement.

Participants were requested to fast at least 10 hours before the blood samples were collected on next morning. Venipuncture was performed by a trained physician with the participants in a sitting position. Fasting serum glucose was measured by glucose oxidase-peroxidase method[12], serum total cholesterol was determined by cholesterol oxidase-peroxidase-amidopyrine method[13] and serum triglycerides (TG) were measured by glycerol phosphate oxidase-peroxidase-amidopyrine method[14] using assay kits from Randox Laboratories Ltd (Crumlin, Co. Antrim, United Kingdom) on a spectrophotometer (Bio-rad, Hercules, California, USA). HDL-C was also determined by the same method after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) by the polyethylene glycol PEG 6000. LDL- cholesterol (LDL-C) was calculated using the formula: LDL-C = total cholesterol- HDL-C - (TG/5)[15]. Lipoprotein (a) (Lp(a)), apolipoproteins A1 and B were measured by an automated turbidimetric immunoassay[16,17] with reagent kits from Randox Laboratories Ltd on a Randox RX Daytona Autoanalyzer system (Crumlin, Co. Antrim, United Kingdom).

Definitions and diagnosis criteria

Obesity was defined as BMI greater than 25 kg/m^2 [18]. According to the Seventh Report of the Joint National Committee (JNC) on Prevention, Detection, Evaluation and Treatment of High Blood Pressure[19], subjects were considered hypertensive if the systolic blood pressure was ≥140 mm of Hg, diastolic blood pressure was ≥ 90 mm of Hg or both or they were taking anti-hypertensive medication. Diabetes was defined as fasting serum glucose level of 7.0 mmol/l or more (≥ 126 mg/dl) or on medication for diabetes by the criteria laid down by the National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATP III)[20]. The following cut-off points were used to define dyslipidemias such as (i) Hypercholesterolemia: total cholesterol level of 5.18 mmol/l or more (≥ 200 mg/dl) (ii) Hypertriglyceridemia: triglyceride level of 1.69 mmol/l or more (≥150 mg/dl) (iii) Low levels of HDL-C: HDL-cholesterol level less than 1.03 mmol/l (<40 mg/dl) and (iv) High levels of LDL-C: LDL-cholesterol level of 3.36 mmol/l or more (≥ 130 mg/dl) according to the diagnostic criteria of the NCEP, ATP III guidelines.

Measurement of hs-CRP and WBC count

The plasma concentrations of hs-CRP were assayed by a latex-enhanced, immunoturbidimetric assay[4] using a kit from Spinreact, Santa Coloma, Spain. The assay was performed on an autoanalyzer system (Microlab-300, Merck, Germany). According to the manufacturer's instructions, the intra-assay and the inter-assay coefficients of variation (CV) for hs-CRP were 4.3% and 8.4% respectively and the lowest detection

limit was 0.05 mg/l. WBC counts were done manually with the help of Neubauer's hemocytometer under the microscope within 24h after venipuncture[21]. For the sake of uniformity in the counting procedure, the same trained person was deputed during the whole investigative process. The procedure was repeated for at least three times for each of the specimens.

Statistical analysis

All statistical analyses were conducted in parallel for hs-CRP and WBC counts which were divided into respective tertiles based on the distribution in 100 study participants. One-way ANOVA (with Tukey's pairwise comparisons) was used to compare group means for continuous variables across the tertiles, Wilcoxon's rank-sum test was used for comparison of medians and the χ^2 test was used to compare proportions. Before statistical testing, data were checked for normality. Because the distribution of hs-CRP and Lp(a) were skewed, the variables were natural-log-transformed for all analysis. Pearson's correlation analysis was carried out to determine the association of inflammatory markers with cardiovascular risk factors. A between-group comparison of pre- and postmenopausal women was performed with analysis of covariance (ANCOVA) after adjustment of other covariates. Serum hs-CRP levels were divided into two categories (below and above) based on the median value of hs-CRP considered as the cut-off point (1.31 mg/l). Logistic unconditioned regression models were used to calculate odds ratios (OR) to evaluate the association between hs-CRP and other variables. All analyses were performed using Windows-based SPSS statistical package (version 10.0, Chicago) and MedCalc statistical software (version 10.1.6) and p-values <0.05 were considered significant.

Results

The prevalence of some baseline characteristics is shown in Table 1. The mean age of the study subjects was 48.3±9.8 yrs. A total of 35% of the women chewed tobacco at a regular or weekly frequency. No individual was reported to smoke either cigarettes or beedies or consume alcohol. Prevalence of postmenopausal women in the population was 57.0%, but none of them was found to take any hormone replacement therapy. The overall prevalence of obesity was 51.5% in the study population determined by BMI alone. Prevalence of hypertension and positive family history for hypertension were 36.0% and 25.0%, respectively. Diabetes was observed in 12.0% of women and 16.0% of subjects were reported with positive family history for diabetes. Overall 3.1% women were found with a history of myocardial infarction. Hypercholesterolemia was observed in 32.0% subjects. However, hypertriglyceridemia was observed in 44.0% of subjects and an almost similar percentage (45.0%) was observed for low levels of HDL-C. Furthermore, 29.0% of the women showed high-levels of LDL-C.

Table 2 shows the relationship between hs-CRP levels and various risk variables among the study subjects. BMI and waist circumference were each significantly higher (p= 0.001 and p= 0.021 for BMI and WC, respectively) across increasing tertiles of serum hs-CRP levels. Women in higher tertiles of hs-CRP seemed to have a tendency toward higher incidence of diabetes as well as higher triglyceride and lower apolipoprotein A1 levels, although the differences were not statistically significant.

Table 1 - Prevalence (%) of some baseline characteristics in 100 women

Characteristics	
Age (yrs), mean ± SD	48.3±9.8
Smoking status (%)	Nil
Tobacco chewing (%)	35.0
Alcohol consumption (%)	Nil
Postmenopausal (%)	57.0
Hormone use (%)	
Current	Nil
Former	Nil
Obesity (%)	51.5
Hypertension (%)	36.0
Positive family history of hypertension (%)	25.0
History of myocardial infarction (%)	3.1
Diabetes (%)	12.0
Positive family history of diabetes (%)	16.0
Hypercholesterolemia (%)	32.0
Hypertriglyceridemia (%)	44.0
Low level of HDL-C (%)	45.0
High level of LDL-C (%)	29.0

We next examined (Table 3) the relationship between WBC count and other risk variables in the study population. TC/HDL-C ratio increased significantly (p= 0.007) across the WBC count tertiles, whereas other variables such as fasting glucose, triglycerides, HDL-cholesterol, apolipoprotein A1 were non-significantly higher among the higher tertiles. BMI and waist circumference were higher in the higher tertiles of WBC count, but the differences were not statistically significant. Both systolic and diastolic blood pressures were also non-significantly higher among the higher tertiles. Women in higher tertiles have higher incidence of diabetes. The mean value of hs-CRP was higher across the tertiles of WBC count but was not statistically significant.

High-sensitivity C-reactive protein (hs-CRP) was significantly associated with measures of obesity such as BMI (r=0.373, p<0.001) and WC (r=0.301, p=0.002) after adjustment for age (Table 4), whereas no significant correlation was found with WHR (r=0.004, p=0.966). However, WBC count was non-significantly associated with BMI, WC and WHR after adjustment for age. Analysis of partial correlation coefficient revealed a significant negative correlation between HDL-C and hs-CRP (r= -0.220, p=0.031) and between HDL-C and WBC count (r= -0.247, p=0.014). Apolipoprotein A1 was also inversely associated (r=-0.237, p=0.031) with hs-CRP in women. Associations with WBC count were significant for biochemical variables such as fasting glucose (r=0.253,

Table 2 - Distribution of various risk factors according to tertiles of high-sensitivity C - reactive protein

Risk factors	Tertiles of serum hs-CRP			
	1st	2nd	3rd	p value*
Hs-CRP, median, mg/l	0.606	1.314	5.317	
Interquartile range	(0.21-0.70)	(1.00-1.86)	(3.06-6.02)	
Age, yrs	50.4±12.2	46.8±8.5	45.7±7.1	0.142
Postmenopausal, %	57.5	54.5	58.8	0.723
BMI, kg/m^2	23.1±3.3	25.3±3.3	26.8±4.5†	0.001
Obesity, %	30.3	53.1	73.5	0.001
Waist circumference, cm	88.6±9.1	92.2±11.1	96.2±12.5†	0.021
Waist-hip ratio	0.90±0.03	0.90±0.03	0.89±0.03	0.921
Systolic blood pressure, mmHg	124.0±19.0	131.9±20.4	124.3±20.6	0.198
Diastolic blood pressure, mmHg	75.9±7.1	81.0±9.5	77.8±8.3	0.050
History of hypertension, %	27.2	42.4	38.2	0.415
Fasting glucose, mmol/l (mg/dl)	5.52±2.28	5.11±1.51	5.90±2.24	0.290
History of diabetes, %	12.1	6.0	21.4	0.344
Total cholesterol, mmol/l (mg/dl)	4.63±1.24	4.86±1.27	4.57±0.97	0.570
H/O Hypercholesterolemia, %	36.6	36.6	23.5	0.427
Triglyceride, mmol/l (mg/dl)	1.58±0.72	1.68±0.71	1.78±0.79	0.555
H/O Hypertriglyceridemia, %	36.6	45.5	50.0	0.520
HDL-cholesterol, mmol/l (mg/dl)	1.17±0.34	1.00±0.34	1.02±0.24	0.072
Low level of HDL-C, %	36.5	57.5	41.1	0.191
LDL-cholesterol, mmol/l (mg/dl)	2.67±1.21	3.07±1.31	2.73±1.02	0.340
High level of LDL-C, %	24.2	39.3	23.5	0.274
Total / HDL-cholesterol ratio	4.30±1.78	5.32±2.16	4.76±1.72	0.098
Lipoprotein (a), µmol/l (mg/dl)**	0.31	0.36	0.34	0.899
Apo lipoprotein A1, g/l (mg/dl)	1.50±0.28	1.44±0.22	1.39±0.17	0.183
Apo lipoprotein B, g/l (mg/dl)	0.93±0.21	0.95±0.22	0.88±0.16	0.397

*All values are expressed as mean±SD except for the categorical and skewed variables. *For normally distributed variables, p-values were computed with one-way ANOVA; for skewed variables, p- values were computed with Wilcoxon rank-sum test for the difference in medians and for categorical variables p-values were computed with chi-square test. **Values are medians. †Significantly different from 1st tertile (p<0.05).*

p=0.011), TC/HDL-C ratio (r=0.284, p=0.004) and hs-CRP (r=0.252, p=0.012). As strong associations were observed between hs-CRP and measures of obesity like BMI and WC, we therefore adjusted these variables along with age for calculating partial correlation coefficients between hs-CRP and other variables in all the above models. Associations of WBC count with other variables were computed after adjustment for age.

A comparison of levels of inflammatory markers in premenopausal and menopausal women is shown in Table 5. Both hs-CRP and WBC count did not significantly differ between the premenopausal and postmenopausal group. Adjusted geometric means were 1.68 and 2.66 mg/l respectively for hs-CRP (1.58-fold increase with menopause). Because menopause may be associated with changes in measures of obesity and other biochemical profile, we adjusted the means for variables such as BMI, waist circumference and HDL-cholesterol in addition with age.

Logistic regression analysis was performed on variables that were significantly correlated with hs-CRP (Table 6). BMI (OR, 1.186; CI, 1.046-1.345; p=0.008) and WC (OR, 1.045; CI, 1.005-1.087; p=0.027) were the covariates significantly and positively associated with hs-CRP after adjustment for age. However, no significant independent association was observed between HDL-cholesterol and hs-CRP.

Discussion

There is an emerging consensus that CVD has a multifactorial etiology, including atherosclerotic, prothrombotic and inflammatory components. Therefore, in addition to the assessment of conventional risk factors, new markers have been explored in prospective observational studies with the hope that they might improve the ability to predict the risk of developing cardiovascular events.

In the present study, we found significant associations

Table 3 - Distribution of various risk factors according to tertiles of white blood cell count

Risk factors	Tertiles of white blood cell count			
	1st	2nd	3rd	p value*
WBC Count, [x 10^9/l]	4.7±0.4	6.3±0.5	8.6±0.7	
Age, yrs	48.5±10.0	48.0±8.8	46.4±10.1	0.663
Postmenopausal, %	57.5	54.5	58.8	0.723
BMI, kg/m^2	24.3±3.8	24.7±3.7	26.2±4.4	0.147
Obesity, %	43.7	60.6	52.9	0.395
Waist circumference, cm	91.0±11.1	92.0±10.4	94.0±12.4	0.539
Waist-hip ratio	0.89±0.03	0.90±0.03	0.90±0.03	0.355
Systolic blood pressure, mmHg	124.1±18.3	127.9±21.1	128.1±21.2	0.673
Diastolic blood pressure, mmHg	77.1±8.1	78.6±10.1	79.0±7.3	0.642
History of hypertension, %	36.3	30.3	41.1	0.649
Fasting glucose, mmol/l	4.91±1.07	5.63±2.22	5.98±2.46	0.091
History of diabetes, %	6.0	9.0	20.5	0.154
Total cholesterol, mmol/l	4.34±1.13	4.98±1.25	4.74±1.05	0.077
H/O Hypercholesterolemia, %	21.1	45.5	29.4	0.099
Triglyceride, mmol/l	1.63±0.69	1.68±0.78	1.74±0.76	0.555
H/O Hypertriglyceridemia, %	39.9	48.4	44.1	0.758
HDL-cholesterol, mmol/l	1.15±0.28	1.06±0.30	0.99±0.34	0.096
Low level of HDL-C, %	33.3	45.4	55.8	0.178
LDL-cholesterol, mmol/l	2.45±1.20	3.09±1.25	2.94±1.05	0.069
High level of LDL-C, %	18.1	33.3	35.2	0.242
Total / HDL-cholesterol ratio	3.94±1.34	5.12±1.96†	5.31±2.13†	0.007
Lipoprotein (a), µmol/l**	0.34	0.40	0.22	0.085
Apo lipoprotein A1, g/l	1.46±0.23	1.45±0.23	1.43±0.22	0.183
Apo lipoprotein B, g/l	0.91±0.21	0.95±0.22	0.88±0.15	0.350
Hs-CRP, median, mg/l**	0.94	1.81	1.87	0.064

*All values are expressed as mean±SD except for the categorical and skewed variables. *For normally distributed variables, p-values were computed with one-way ANOVA; for skewed variables, p- values were computed with Wilcoxon rank-sum test; for the difference in medians and for categorical variables p-values were computed with chi-square test. **Values are medians. †Significantly different from 1st tertile (p<0.05).*

between hs-CRP levels and adiposity measures such as BMI and waist circumference. These results are consistent with the experimental findings suggesting that adipose tissue is a major source of cytokines, including IL-6, which is an important determinant of hepatic CRP synthesis[8,22-24]. However, in adults, stronger associations between body fat and CRP values have been reported for women compared with men[25]. The Third National Health and Nutrition Examination Survey (NHANES III) in United States including both men and women aged ≥20 years, revealed that higher BMI is associated with higher CRP concentrations, even in younger adults[26]. A significant association of CRP levels with BMI was also reported among elderly men and women in the Cardiovascular Health Study[22] as well as by Mendall et al[23]. Lemieux et al[27] observed that despite the fact that the amount of total body fat (measured as BMI) was the best correlate of CRP levels, the highest plasma CRP concentrations were observed among individuals who had concurrent elevations in visceral adiposity (measured as waist circumference) and in total body fatness. Additional support for this observation came from the study by Hak et al[24] where they reported that CRP was strongly correlated with waist circumference even after BMI adjustment. The relative contributions of intra-abdominal and subcutaneous adipose tissue in the generation of CRP remain unclear. However, in the present study, waist circumference showed significant association with hs-CRP, although the association disappeared after controlling for BMI (data not shown). This phenomenon could be explained by the strong collinearity of waist circumference and BMI in the study subjects.

In the present study it has been observed that hs-CRP has a significant inverse association with HDL-C and apolipoprotein A-1. In addition, there was an inverse association between WBC count and HDL-C. HDL cholesterol has consistently shown an inverse association with systemic markers of inflammation in many prospective studies[5,24,28]. A recent study by Birjmohun et al[29] have demonstrated that apparently

Table 4 - Correlation coefficients for hs-CRP and white blood cell count with anthropometric and biochemical variables in 100 women

	ln hs-CRP		WBC count	
	Pearson, r	p-value	Pearson, r	p-value
BMI (kg/m²) †	0.373	<0.001	0.197	0.051
Waist circumference (cm) †	0.301	0.002	0.128	0.208
Waist-hip ratio†	0.004	0.966	0.120	0.236
Systolic blood pressure (mmHg)‡	0.043	0.672	0.080	0.431
Diastolic blood pressure (mmHg)‡	0.068	0.510	0.109	0.279
Fasting plasma glucose (mmol/l)‡	0.087	0.398	0.253	0.011
Total cholesterol (mmol/l)‡	0.023	0.817	0.127	0.207
Triglyceride (mmol/l)‡	0.152	0.139	0.072	0.476
HDL-cholesterol (mmol/l)‡	-0.220	0.031	-0.247	0.014
LDL-cholesterol (mmol/l)‡	0.053	0.607	0.152	0.133
TC/HDL-C ratio‡	0.130	0.206	0.284	0.004
Lipoprotein (a) (µmol/l)‡	-0.025	0.824	-0.203	0.062
Apolipoprotein A1 (g/l)‡	-0.237	0.031	-0.190	0.153
Apolipoprotein B (g/l)‡	-0.063	0.569	-0.064	0.631
High-sensitivity CRP (mg/l)‡	-	-	0.252	0.012

†Partial correlation coefficients controlled for age. ‡Partial correlation coefficients controlled for age, BMI and waist circumference.

healthy individuals with genetically determined isolated low HDL-C levels are more susceptible to low-dose endotoxin challenge in comparison with subjects with normal or high HDL-cholesterol levels. The study revealed a strong inverse association between HDL-C and apo A-1 levels *versus* leukocyte response and plasma CRP levels, supporting an anti-inflammatory effect of HDL-C. In our present investigation, the association between plasma CRP level and low-HDL-C values has been shown to persist even after adjustments for BMI and waist circumference, suggesting an independent association between these variables among the individuals.

Table 5 - Comparison of inflammatory biomarkers in premenopausal and menopausal women

	Premenopausal (n=43)		Postmenopausal (n=57)	
	Mean	SE	Mean	SE
Age, years	39.6	3.37	53.7	8.28
hs-CRP, mg/l*†	1.68	0.88 to 2.47	2.66	2.00 to 3.32
WBC Count [x 10^9/l]*	6.43	0.32	6.74	0.27

**Adjusted for age, BMI, Waist circumference and HDL-cholesterol. †Geometric mean (95% CI) are shown for hs-CRP because its distribution is skewed.*

Recent evidence supports a wide array of antiatherogenic effects by HDL-C, including anti-inflammatory effects[30].

In the present investigation, WBC count showed a significant positive correlation with plasma hs-CRP levels and variables such as fasting blood sugar and TC/HDL-C ratio. The ARIC cross-sectional study of young and middle-aged people[31] revealed that WBC count was associated with fasting insulin and blood glucose in an analysis not stratified by gender. A recent study in the Asian Indian population reported by Gokulakrishan et al[32] has shown that leukocyte count was positively associated with fasting plasma glucose and insulin resistance. It is therefore possible that inflammation and endothelial function are among several common antecedents for both diabetes and coronary heart disease[33]. Association between plasma CRP concentrations and WBC count have also been consistently reported in many clinical findings[2,34].

We did not find a clear influence of menopausal status on the level of inflammatory biomarkers. Similar observations were reported by Hak and her group[24] where both age and age-and-BMI-adjusted levels of CRP were found slightly higher in postmenopausal than in premenopausal women, but the difference was not statistically significant. In a comparative study of postmenopausal vs. premenopausal women by Sites et al[35], increased levels of CRP were found to be related to increased body fat, but not to menopausal status. However, numerous clinical trials have demonstrated that exogenous hormone replacement therapies, as well as endogenous hormone levels were associated with increased CRP levels along with other markers of inflammation such as fibrinogen and WBC count in postmenopausal women[36,37]. Further studies are needed to address the association between inflammation and menopause.

Table 6 - Multiple logistic regression analysis with hs-CRP‡ as the dependent variable and other risk factors as independent variables in 100 women

	Adjusted for age			Adjusted for age + BMI + WC		
	β*	OR(95%CI)†	p-value	β*	OR(95%CI)†	p-value
BMI (kg/m²)	0.171	1.186(1.046, 1.345)	0.008		-	
Waist circumference (cm)	0.044	1.045(1.005, 1.087)	0.027		-	
HDL-cholesterol (mmol/l)		-		-0.023	0.977(0.943, 1.012)	0.202

*‡A dichotomous datum high-sensitivity C-reactive protein (hs-CRP ≤ 1.31/hs-CRP > 1.31) was the dependent variable. *β indicates regression coefficient. †OR, odds ratio; CI, confidence interval. Here, odds ratio or e^{β} is the increase in odds associated with a unit increase in the independent variable.*

Inflammation plays a fundamental role in atherothrombosis[38]. Hs-CRP, a measure of inflammation, is a mediator as well as marker of atherothrombosis. Although a number of other inflammatory markers have been investigated, the 'hs-CRP' level has the most stability, assay precision, accuracy and availability. C-reactive protein has gained official recognition as a cardiac test by the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA)[4]. The utility of CRP testing in patients with myocardial infarction, stable or unstable angina has been well established[39]. Elevated hs-CRP levels in these clinical settings identify patients with higher inflammatory burdens who are at higher risk of future ischemic events. In addition, an elevated CRP level provides additional prognostic value to traditional cardiac risk factors. Therefore, in a high-risk individual, an elevated hs-CRP level should even further alert both the physician and the individual to the need for aggressive risk-lowering strategies.

To the best of our knowledge, the present study is the first to examine the association between systemic inflammatory markers such as hs-CRP and WBC count and other cardiovascular risk factors in a general population-based sample from India and it focuses particularly on women. An additional strength of this study was the quality of sample collection and the precision in the measurement of CRP and other associated biomarkers. However, some issues of our study need to be addressed. This study was conducted in an apparently healthy population with a low or without current exposure to factors like clinically established CVD, such as-myocardial infarction and smoking, respectively. The above factors are strong determinants of CRP levels and therefore the choice of our population facilitates the investigation of confounders associated with CRP. So, additional studies are needed to verify the findings in both epidemiological and clinical settings in this population. Secondly, this study includes the use of a single measurement of inflammatory markers which may not accurately reflect long-term inflammation status. Multiple measurements over time and changes in those measurements may provide a more accurate mechanism for predicting risk in individuals.

Conclusion

Our results indicate that in an apparently healthy population of women with a low burden of smoking and clinically established cardiovascular disease, BMI and waist circumference were associated with levels of systemic biomarkers such as- hs-CRP and WBC count. Because inflammatory mediators are directly involved in atherogenesis, these results suggest an important mechanism through which obesity might affect the risk of cardiovascular disease. We also observed a significant association between inflammatory markers and lipoprotein variables such as HDL-C and apo-A1, which was independent from adiposity measures. These findings add to a growing body of evidence that HDL-C and apo-A1 protect against cardiovascular disease by mechanisms that extend well beyond their involvement in cholesterol transport. The present findings thus reinforce the importance of these inflammatory biomarkers for cardiovascular risk prediction algorithms along with the standard screening tools, particularly in women.

Acknowledgements

This work was supported by the Department of Science and Technology, Government of India, [Grant Number SSD/WS/059/2004].

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by Department of Science and Technology Government of India.

Study Association

This article is part of the thesis of doctoral submitted by Debdutta Ganguli, from University of Calcuta.

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