C-reactive Protein: Adjunct to Cardiovascular Risk Assessment

S Bahadursingh[1], K Beharry[1], K Maharaj[1], C Mootoo[1], P Sharma[1], J Singh[1], K Teelucksingh[1], R Tilluckdharry[2]

ABSTRACT

Objectives: *The study aimed to investigate whether elevated plasma high sensitivity C-reactive protein (hs-CRP) levels are independently associated with an increased risk of cardiovascular disease (CVD) and to then assess the effectiveness of the addition of hs-CRP testing to cardiovascular risk assessment by standard lipid screening.*

Methods: *A retrospective hospital-based case-control study was designed. All patients attending Cross Crossing Medical Centre (CCMC) for routine cardiovascular assessment or emergency treatment were included. Cases were defined as patients with a cardiovascular event and controls as those without an event. Data collected included blood measurements of hs-CRP and cholesterol, demographic data, drug and risk factor history.*

Results: *Odds ratio of 1.84 (95% CI 1.00, 3.38) indicated that a patient with elevated hs-CRP is 1.84 times more at risk of CVD than one with normal hs-CRP. Additionally, the association between hs-CRP and CVD was found to be independent of the other risk factors (p = 0.058). Hs-CRP ranked fourth as an indicator of risk above smoking and diabetes, and patients with both high hs-CRP and high cholesterol (OR = 9.5) were 3.5 times more at risk of CVD than someone with high cholesterol alone (OR = 6.0).*

Conclusions: *Hs-CRP testing enhanced the clinical identification of patients at risk of cardiovascular events. It can therefore contribute to timely implementation of effective lifestyle modification and pharmaceutical intervention.*

Proteína C reactiva: Complemento para la Evaluación del Riesgo Cardiovascular

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RESUMEN

Objetivos: *El estudio apuntó a investigar si los niveles de proteína C reactiva (PCR-hs) de alta sensibilidad de plasma elevado están independientemente asociados con el aumento del riesgo de la enfermedad cardiovascular (ECV), y luego evaluar la efectividad de añadir la prueba PCR-hs para la evaluación del riesgo cardiovascular mediante detección (screening) estándar de lípidos.*

Métodos: *Se diseñó un estudio caso-control retrospectivo con sede en el hospital. En el mismo fueron incluidos todos los pacientes que asistían al Centro Médico Cross Crossing (CCMC) para una evaluación cardiovascular de rutina o para tratamiento de urgencia. Los casos fueron definidos como pacientes con controles y eventos cardiovasculares, o sin un evento. Los datos recogidos incluyeron mediciones sanguíneas de PRC-hs y colesterol, datos demográficos, historia de factores de riesgo y de drogas.*

Resultados: *El odds ratio de 1.84 (95% CI 1.00, 3.38) indicó que un paciente con PCR-hs elevado tiene un riesgo de ECV 1.84 veces mayor que uno con PCR-hs normal. Además, se halló que la asociación entre el PCR-hs y la ECV era independiente de los otros factores de riesgo (*p = *0.058). La PCR-hs alcanzó el cuarto lugar como indicador de riesgo, por encima del hábito de fumar y la diabetes, y los pacientes con alto PCR-hs y alto colesterol (OR = 9.5) presentaban riesgo de ECV 3.5 veces mayor que aquellos que sólo tenían colesterol alto (OR = 6.0).*

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Conclusiones: *Las pruebas de PCR-hs mejoraron la identificación clínica de pacientes con riesgo de eventos cardiovasculares. Por lo tanto, estas pruebas pueden contribuir a implementar oportunamente una modificación para un estilo de vida y una intervención farmacéutica que sean efectivos.*

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INTRODUCTION

Many studies have demonstrated the effectiveness of lifestyle change, optimal risk factor management and pharmaceutical intervention in both primary and secondary prevention of cardiovascular events (1, 2). Despite this, cardiovascular disease (CVD) remains the leading cause of mortality in Trinidad and Tobago and much of the western world (1, 3). A major contributor to this problem is the lack of timely detection of persons who should be targeted for intervention.

This predicament stems partly from a lack of screening in the community. However, considerable blame can be attributed to the current methods of cardiovascular risk assessment since as many as half of those with coronary heart disease have none of the established risk factors (1). The challenge remains, therefore, to enhance the efficiency and effectiveness of cardiovascular risk assessment.

In light of the recognition of CVD as an inflammatory disease (4), there has been much interest in the use of markers of inflammation in risk assessment. One such marker, that has shown great promise, is C-reactive protein [CRP] (5).

C-reactive protein is produced in the liver (6) as well as macrophage-derived foam cells (6) and is elevated in both acute and chronic inflammatory states (6). Additionally, it has been implicated in several aspects of the pathogenesis of CVD, including monocyte chemotaxis, endothelial activation, tissue factor expression and stimulation of pro-inflammatory cytokine release by macrophages and foam cells (6). Elevated levels have also been implicated in the injury of arterial endothelium, thus promoting the formation of atherosclerotic lesions (6).

The objective of this study therefore was to investigate whether elevated plasma high sensitivity C-reactive protein (hs-CRP) levels are associated with an increased risk of cardiovascular disease in the local population. It also aimed to investigate the value of hs-CRP as an adjunct to cardiovascular risk assessment by examining the benefits of adding hs-CRP testing to standard lipid screening.

SUBJECTS AND METHODS

A retrospective hospital-based case-control study was designed. All patients attending the Cross Crossing Medical Centre from January 2003 to March 2005 for either a routine cardiovascular examination or for emergency cardiovascular treatment were included in the study. This yielded a total of 300 study participants.

From this sample, cases were designated as persons with an incident or prevalent cardiovascular event (including angina pectoris, myocardial infarction, angioplasty, aorto-coronary bypass, cerebrovascular event, transient ischaemic attack and/or peripheral artery disease). Controls were designated as persons free of a cardiovascular event up to March 2005. Persons with conditions known to cause independent increases in plasma CRP levels were excluded from the study. These conditions included recent and/or active bacterial and/or viral infection, malignancy, connective tissue disorders (systemic lupus erythematosus), rheumatoid arthritis, pregnancy or hormone replacement therapy use.

The baseline data collected from patients' records at the end of the study period included:

* Blood measurements (plasma hs-CRP and cholesterol)
* Demographic data (age, gender and ethnicity)
* History of risk factors for cardiovascular disease (diabetes mellitus, hypertension and smoking)
* Drug history (aspirin and/or statin use)

Plasma hs-CRP levels were determined using the Solid Phase Chemiluminescent Immunometric Assay with the IMMULITE® 2000 Analyzer.

The protocol for this study was approved by the Ethics Committee of the Faculty of Medical Sciences, The University of the West Indies, St Augustine Campus.

Statistical analysis

The risk of CVD associated with an elevated hs-CRP (> 0.8 mg/L) was generated through the calculation of odds ratios. The significance of the association between plasma hs-CRP levels and CVD was then investigated using the non-parametric Mann Whitney U test which compared hs-CRP levels in the cases and controls.

The significance of the association between CVD and established risk factors was also investigated for the study population. The Student's *t*-test was used to compare the difference in mean age between the cases and the controls. The chi-square test was used to compare the significance of the difference in the proportion of those with hypertension, diabetes mellitus, smoking history and hypercholesterolaemia (as indicated by statin use) between the cases and the controls. Odds Ratio for these risk factors were also generated.

Logistic regression was performed with CVD as the dependent variable and the other established risk factors for CVD (dichotomous variables) along with CRP as covariates. Adjusted odds ratios (with 95% confidence interval) as well as adjusted *p* values were generated for CRP and the other risk factors. This allowed for the comparison of risk associated with CRP to that for the other risk factors.

The individual relationship of plasma hs-CRP levels

with established risk factors was then investigated, followed by linear regression to generate adjusted *p* values for these associations. These tests assisted in determining the existence of independence or association between plasma hs-CRP and each of the individual risk factors.

To investigate the benefit of adding CRP to standard lipid screening, patients were categorised into the following 4 groups:

* Patients with normal hs-CRP plasma levels and normal cholesterol
* Patients with normal hs-CRP plasma levels and high cholesterol
* Patients with high hs-CRP plasma levels and normal cholesterol
* Patients with high hs-CRP plasma levels and high cholesterol

Odds Ratio was generated for each category to compare the relative strength of the combinations of plasma markers as indicators of cardiovascular risk.

Statistical calculations were executed with Statistical Package for Social Sciences (SPSS) version 12.0 for Windows®.

RESULTS

In the sample of 300 study participants, 224 were male and 76 female, with 67% of participants between the ages of 41 to 60 years (Fig. 1). Eighty-six per cent of participants were of East Indian extraction, 9% of African descent, 3% Caucasian and 2% of other ethnicities. There were 89 persons (29.7%) with CVD of which 33% were found to have elevated plasma hs-CRP levels (> 0.8 mg/L). Two hundred and eleven persons (70.3%) were free of cardiovascular events of which 18% had elevated plasma hs-CRP levels.

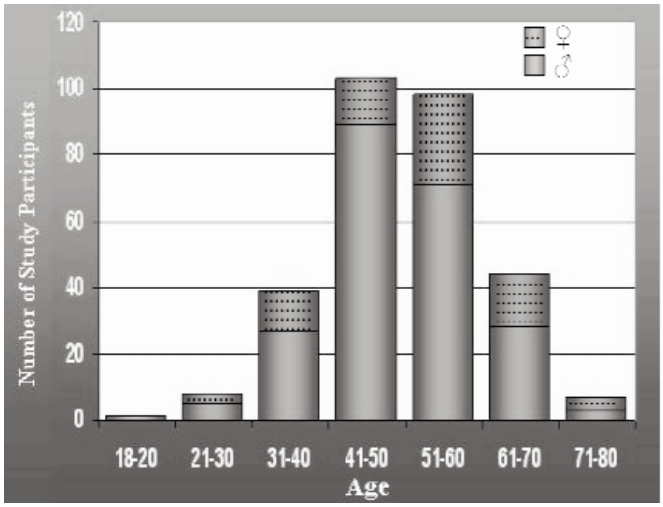


Fig. 1: Distribution of ages of study participants showing the proportion of males and females in each age group

The distribution of CRP was found to be rightward skewed for the study population. Thus box plots were constructed to compare the distribution of CRP in the cases and controls (Fig. 2). Median CRP was found to be 0.08 mg/L higher in cases than in controls, with a 0.25 mg/L difference in interquartile range between cases and controls (Fig. 2).

An odds ratio of 1.84 (95% CI 1.00 , 3.38) indicated that a patient with elevated hs-CRP (> 0.8 mg/L) is 1.84 times more at risk of CVD than one with normal hs-CRP. The Mann Whitney *U* test generated a *p* value of 0.055, indicating that the association between CRP and CVD approached significance.

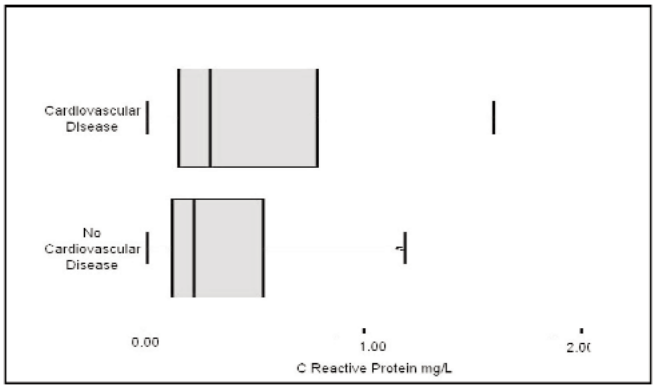


Fig. 2: Box plots comparatively illustrating the distributions of plasma hs-CRP levels in two groups of the study population: those with cardiovascular disease (n = 211) and those without (n = 89).

Logistic regression generated an adjusted odds ratio of 1.97 (95% CI 0.98, 3.96) substantiating the previous finding that elevated hs-CRP increases risk of CVD. The adjusted *p* value of 0.058 confirmed the possible association between CRP and CVD and showed it to be independent of other risk factors.

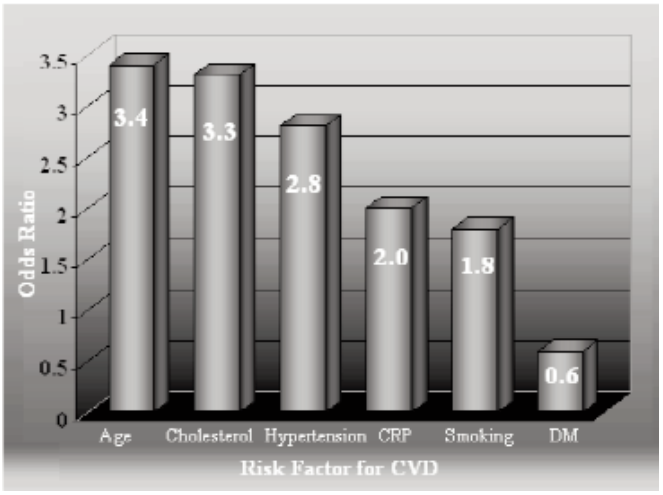
Univariate and adjusted *p* values and risk estimates for established risk factors (age, hypercholesterolaemia, diabetes mellitus, hypertension and smoking) are given in Table 1. Fig. 3 illustrates that when compared to other risk factors for CVD, CRP ranks fourth as an indicator of risk above smoking and diabetes.

Investigation of the individual relationship between hs-CRP and the established risk factors for CVD showed the adjusted *p* values to be all statistically insignificant, indicating no association between hs-CRP and the other risk factors (Table 2). This confirmed the role of CRP as an independent risk factor for CVD.

The high *p* value (0.824) for the association between cholesterol and hs-CRP strongly indicated their independence and therefore supported the use of hs-CRP as an adjunct to lipid screening. Odds ratio calculations with combinations of these two plasma markers, as shown in Fig. 4, indicated that an individual with both high hs-CRP and high cholesterol was 3.5 times more at risk of CVD than another individual with high cholesterol alone and 7.4 times more at

Table 1: Summary of baseline clinical characteristics of 300 study participants, including *p* values and odds ratios generated in both univariate and multivariate analyses on these characteristics (risk factors)

			Univariate analysis		Multivariate analysis	
Baseline Characteristic	**Persons with CVD (cases) n = 89**	**Persons without CVD (controls) n = 211**	***p* value**	**Odds Ratio (95% CI)**	***p* value**	**Odds Ratio (95% CI)**
High sensitivity C-reactive protein mg/L			0.055	1.84 (1.00 – 3.38)	0.058	1.97 (0.98 – 3.96)
Median	0.298	0.218				
Interquartile Range	0.69	0.44				
Statin Use %	77.53	39.81	< 0.001	5.22 (2.95 – 9.21)	< 0.001	3.32 (1.77 – 6.23)
Mean age (yr)	55.02	47.33	< 0.001	4.52 (2.47 – 8.27)	< 0.001	3.38 (1.72 – 6.64)
Male (n = 224)	53.94 ± 8.89 (n = 68)	46.73 ± 8.85 (n = 156)	< 0.001	5.07		
Female (n = 76)	58.52 ± 9.89 (n = 21)	49.04 ± 11.61 (n = 55)	0.001	4.11		
History of smoking %	26.97	16.11	0.030	1.92 (1.06 – 3.48)	0.084	1.81 (0.92 – 3.56)
Diabetes mellitus %	31.46	23.70	0.161	1.48 (0.85 – 2.56)	0.175	0.64 (0.34 – 1.22)
Hypertension %	53.93	25.59	< 0.001	3.40 (2.03 – 5.72)	0.001	2.78 (1.54 – 5.03)



Age: >55 in women, >45 in men; Cholesterol: elevated – as indicated by statin use; CRP: C – reactive protein; DM: Diabetes mellitus

Fig. 3: Risk of cardiovascular disease (adjusted odds ratios) associated with established risk factors and plasma high sensitivity C-reactive protein

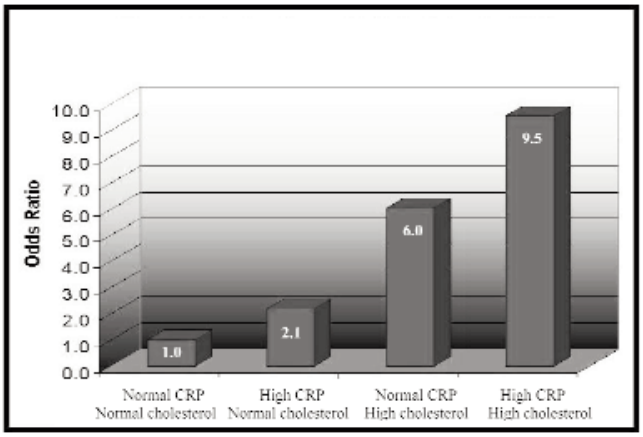


Fig. 4: Histogram illustrating the use of both cholesterol and hs-CRP testing as an indicator of risk of cardiovascular disease as compared to either hs-CRP or cholesterol testing alone.

Table 2: The association between plasma hs-CRP levels and risk factors for cardiovascular disease generated by both univariate and multivariate analysis.

Risk factor for cardiovascular disease	**Univariate Analysis: *p* value for association between plasma hs-CRP and named risk factor**	**Multivariate analysis Adjusted *p* Value**
Hypercholesterolaemia	0.990	0.824
Age	0.365	0.769
Hypertension	0.054	0.104
Smoking	0.187	0.579
Diabetes	0.318	0.706

risk than one with high hs-CRP alone.

DISCUSSION

The results of this retrospective case-control study showed plasma hs-CRP levels to be not only independently associated with CVD but they clearly demonstrated substantial improvement of cardiovascular risk assessment by standard lipid-screening alone. These findings have several important implications.

The addition of hs-CRP testing to routine cardiovascular risk assessment may allow for the enhanced clinical identification of susceptible patients who have none of the established risk factors for CVD. It will also allow for the reclassification of low risk patients to higher risk, based on recognition of an additional risk factor. This improved clinical identification can therefore motivate appropriate primary and secondary prevention measures in terms of lifestyle changes (diet modification, exercise, weight loss, smoking cessation) as well as pharmacological intervention (aspirin and/or statin use).

It is noteworthy that at least three separate studies (7, 8, 9) showed that statins reduce the risk of a cardiovascular event independent of their effect on LDL levels, suggesting that their benefits may be due in part to their effects on inflammation. Therefore, the recommendation of statins to persons with normal cholesterol but high CRP may be of therapeutic benefit and merits a clinical trial for substantiation. Additionally, the future holds potential for drugs aimed directly at lowering CRP levels themselves, since CRP is indicated not only as a marker of the cardiovascular risk but also as a contributor to the pathological process itself.

The use of hs-CRP testing in risk assessment has several unique advantages. High sensitivity C-reactive protein is a continuous variable which, when measured, gives a more precise indication of risk than the categorical variables. Additionally, the test is inexpensive, standardized and widely available (10), and levels can be measured in the fasting or non-fasting state, since they are minimally affected by diet intake and have no circadian variation (5).

Another promising concept arises from the fact that CRP levels are stable over many months to years in persons with atherosclerotic disease. It may be possible therefore, for doctors to warn patients of a potential problem up to 40 years in advance (11). Since the commercial assay is economical and can be used with standard hospital and outpatient laboratory equipment, screening using this predictor of cardiovascular risk would be practical in many clinical settings (4). Consequently, its use as a routine test would be feasible.

Notwithstanding these promising results, there were some unavoidable limitations to this study. Data on Body Mass Index (BMI), level of activity and alcohol use were unavailable, making it impossible to adjust for these established risk factors in the regression model. Also, the study was done in a short study period with a small sample size. High sensitivity C-reactive protein was tested only once, and some patients were on aspirin and statin that may have altered plasma hs-CRP (12). Additionally, statin use would have lowered baseline cholesterol levels, making it more feasible to employ statin use as an accurate indicator of an elevated cholesterol level.

It is recommended, therefore, that a large-scale cohort study be conducted to confirm the role of CRP in the prediction of CVD, inclusive of risk factors not considered in this study.

A larger sample would also allow for investigation and definition of additional ranges of hs-CRP as indices of risk. In addition, investigation of the relationship between CRP and individual events would be worthwhile to establish whether CRP elevation varies with different cardiovascular events.

In summary, this study shows potential benefit to the addition of hs-CRP to standard lipid screening. In light of the tremendous impact of CVD on mortality, morbidity, productivity and the economy, hs-CRP testing should be given strong consideration as a viable adjunct to cardiovascular risk assessment.

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