ORIGINAL ARTICLE
H-SENSITIVITY C REACTIVE PROTEIN LEVELS AS A MARKER OF CORONARY HEART DISEASE IN MIDDLE AGED INDIVIDUALS

Nadia Haleem, Zahid Irfan Marwat, Shazia Tauqeer*, Sarwat Abbasi
Department of Biochemistry, *Department of Physiology Ayub Medical College Abbottabad-Pakistan

Background: coronary heart disease is multifactorial inflammatory process which involves the accumulation of lipid macrophages and intimal plaques in smooth muscle cell in large and medium sized arteries. C reactive protein (CRP) which is an inflammatory marker is considered as global risk assessment for coronary heart disease. The objective of study is to determine the CRP level as risk marker in coronary heart disease in middle aged individuals. **Methods:** This cross sectional study was conducted in Hayatabad medical complex Peshawar and Rehman Medical Institute Peshawar. On the basis of predesigned questionnaire, 100 middle aged individuals of age 40-60 years and 50 normal subjects of same age were questioned by taking consent. Data was collected and analysed by SPSS-15. **Results:** It was founded that 74% of patients have higher values of CRP and 4% have high values of CRP in controls. The *t*-test applied at 95% confidence interval with mean difference of 22.096+2.36 of CHD individuals and 1.288±1.70 of control group. *P*-value was 0.001 which is found to be significant. **Conclusion:** It was observed that CRP has higher association with CHD.
Keywords: Coronary Heart Disease, C. Reactive Protein, Inflammatory Marker

INTRODUCTION

Coronary heart disease (CHD) is the clinical multifactorial inflammatory process which involves the fibro fatty accumulation of smooth muscle cell and lipid macrophages in intimal plaques in medium and large sized coronary arteries".[1] The most common leading cause of the coronary heart disease is the atherosclerosis.[2] Atherosclerosis is mainly due to imbalance between the oxygen supply to the heart muscle and myocardial demand.[3] Incidence of coronary heart disease in under developed country like Pakistan is about 6% in males and 4% in females, but active ischemia is two times greater in women. So it is basically suggested that in urban areas of the Pakistan one in five adults have coronary heart disease.[4] Coronary artery disease is responsible for about 500,000 deaths per year in United States, i.e., 25–30% of all deaths and is the most leading cause of the death in developed countries.[5] C Reactive protein is an "Acute Phase protein synthesized in the hepatocytes of liver which is responsible to promote inflammation." It is suggested to be the golden and significant marker of inflammation regulated by cytokines (tumour necrotic factor, interleukin 1 and interleukin 6).[6] Of all the markers of CHD, high sensitive C Reactive protein (CRP) is the important and influential biomarker that may tend to fulfil the required criteria for a novel marker of cardiovascular disease risk. National guidelines issued by American Heart Association basically given to measure the CRP in the prevention of coronary heart disease.[7] CRP binds with phosphochole (natural ligand) having highest affinity and this ligand has key role acting as ubiquitous at the polar head of phophatidylcholine in cell membrane and plasma lipoproteins. The binding of CRP to damaged or dead cells present significant quantity of lysophophotidyl choline but not showing on healthy living cells. CRP may attach to apoptotic cells but it is controversial. It may suggested that CRP only binds to platelet aggravated factor, oxidized phospholipids, normal concentrated VLDL, LDL, and small nuclear ribonucleoprotein particles (having no phosphocholine).[8] C Reactive protein resembles LDL-C is able to distinguish the individuals who may be undergoing coronary events only modestly and if established and known factors have been evaluated for estimation of absolute risk, then CRP appears to be very modest factor to provide the risk information.[9]

It does not break down and destroyed during sample collection and lab processing. C Reactive protein is present in the blood only when produced de novo in the liver when stimulus is present. So this gives us the careful evaluation of systemic inflammatory process in apparently healthy individuals as well those individuals having established coronary heart disease.[10] The aim of our current study was basically to determine the levels of serum CRP among middle aged coronary heart disease individuals and control individuals of same age group and to establish correlation of CRP levels with serum lipids and to find out the validity of the test performed through sensitivity and specificity.

MATERIAL AND METHODS

This is the cross sectional study in which two groups were studied. In first group A 100 coronary heart disease patients were taken and in second group B, 50 control subjects were taken who are free of CHD. These subjects were collected randomly from Hayatabad medical complex Peshawar and Rehman Medical Institute Peshawar. The approval of the current study was taken from ethical review committee. Aims and objectives of study were briefly explained to the patients and their consent was taken properly. All the required and relevant information was recorded on the well-designed questionnaire. History from subjects was taken in detail. Past history was also in record to find out the exact cause of risk factor involved in causation of coronary heart disease.

About 5 ml of blood was taken into the vacutainer tube without any coagulant. After coagulation of blood, these samples are centrifuged for at least 10 minutes at 3000 rpm in centrifuge machine to obtain the clear serum. Labelling of the samples were done properly. Serum CRP analysis was done. These investigations are performed in research laboratory of institute of basic sciences KMU Peshawar on daily basis. The quantitative C reactive protein is determined by lumax clia strip reader (model-4100) machine using the immune-enzymometric chemiluminescence assay in human serum. The sensitive methodologies for the high sensitivity CRP assays are used in routine way that may be helpful to determine the inflammation which is due to the coronary heart disease or any cardiovascular trauma. In this method, CRP caliberator and the specimen of patient or control is added and mixed to treptavidin coated well. Biotinylated monoclonal and enzyme labelled antibodies are also added and the reactants get mixed well. The reaction may takes place between various antibodies of CRP and native CRP and then sandwich complex obtained. The complex then binds with streptavidin coated to the well. When the incubation period completes, the enzyme CRP antibody linked conjugate is separated from the unbound enzyme CRP conjugate by aspiration or decantation. The activity of enzyme which is present on the surface of the well is quantitated by reaction with a suitable substrate to produce light. Student T test was statistic test is applied for comparison of two groups.

RESULTS

Out of 150 subjects 100 were established coronary heart disease patients and 50 were control of age 40–60 years. All the subjects were interviewed in detail regarding the presence of various etiological and causative factors which may be responsible for the causation of coronary heart disease. Results revealed that 74% of patients have higher serum CRP values (<3.0 mg/dl) indicating the importance and significance of inflammation in coronary heart disease. As seen in controls only 4% were having high CRP levels. So inflammation may be considered the causative factor involved in coronary heart disease (Table-1). In order to find out the validity of test it was presented by specificity and sensitivity tests. The test is 74% sensitive and 92% specific. The overall accuracy found to be the 80% which is merely acceptable so we can somehow use this test for screening purpose. The positive predictable value is 94% and negative predictable value is 63% (Table-2).

Table-1: Comparison of c reactive protein between coronary heart disease and control healthy subjects

Cut off levels of CRP (ug/ml)	Cases	Control	Frequency	Mean±SD		t-test p-value
				Cases	Control	
<1(low risk)	10	27	37			
1-3 (normal)	16	19	35	22.096±23.6604	1.288±1.2153	<0 .001
>3 (high risk)	74	4	78			
Total	100	50	150			

Table-2: Calculation for specificity and sensitivity.

	Coronary heart disease Condition +ve	Condition –ve		
CRP +ve	TP=74	FP = 4	78	PPV=74 / 78=94%
	FN=26	TN = 46	72	NPP=46 / 72=63%
CRP –ve	100	50	Overall Accuracy= 74+46/150=120/150=80%	
	Sensitivity= 74/100=74%	Specificity=46/50=92%		

DISCUSSION

CRP is the constant factor similar to blood pressure and total cholesterol level.[11,12] This is its chief quality which makes it a reliable predictor for CHD.[13] In industrialized countries the coronary heart disorders are most common and leading causes of death and morbidity. A total of three quarters of a million deaths in United States about more than 40% of all deaths are due to cardiovascular diseases.[14] As it is evident that inflammation plays an important role in causation of atheroma and coronary heart disorders. If the level of serum CRP is increased then it may show strong association with elevated risk of future coronary heart events in healthy individuals, in

patients of unstable and stable angina and in acute myocardial infarction. As atherosclerosis is the common significant factor for causing coronary artery disease in which the atheromatous fatty plaque formation occurs and its rupture will cause transient, partial, or complete narrowing and occlusion of coronary arteries. Several other risk factors including hyperlipidemia, hypertension, obesity, smoking, and positive family history, the C reactive Protein has considered to play a very important role in this account. It is a prototype acute phase protein promoted and stimulated by cytokines (immune-modulating agents).[15] In our current study the CRP was found to show significant association with coronary heart disease (<0.001) when statistic student *t*-test applied to cases and control, their mean values and standard deviation observed were 22.096±23.66 and 1.288±1.2153 respectively. By comparing to other studies of Thakur *et al*, it was again found having significant association of CRP with coronary heart disease. The sample size of that study was 200. One hundred and fifty were coronary heart disease patients and 50 were control healthy subjects. The diagnosis was also confirmed by clinical presentation as well as other investigations like positive treadmill test, ECG changes, and positive Echocardiograph changes. The detection range for high sensitivity CRP is about ≤5 mg/l. The mean value for high sensitivity CRP in coronary heart patients was 1.7±0.75 and in controls is 0.93±0.35. When student *t*-test applied, the *p*-value was found to be <0.001 which was highly significant.[15] Another study done by Irfan *et al* was observed in which their results were in favour of positive association of coronary heart disease with c reactive protein. In this study 100 subjects were assessed who had already been suffering from coronary heart syndrome observed with results of mean age 59.26±11.04, 36% were women and 64% were men. They observed that 43% of patients have non ST segment myocardial elevation and 34% had unstable angina and 23% had ST segment myocardial elevation. The mean value of hs- CRP was calculated to be about 4.26±1.42 mg/dl having highest value in subjects having eccentric or irregular lesion and in patients with macroscopic thrombus (*p*=0.01).[16] One of the studies done in northern Pakistan population by Ahmed *et al*, on high reference value for high sensitivity CRP for the first time determined the reference range and different factors associated with high sensitivity CRP could lead to different strategies to estimate the coronary heart disease risk and their long term consequences in our northern Pakistan population. This study reached the conclusion that the reference range for high sensitivity CRP is merely high in *Pathans* as compared to Punjabis and this had

positive correlation with age, BMI, smoking, and gender.[17] The sensitivity and specificity of CRP in our study was observed to be 74% and 92% respectively. These tests were done to find out the accuracy and validity of test. The overall accuracy was found to be about 80% which was good enough and showed it clearly that we can use this test for screening purpose of all middle age individuals of age group of about 40–60 years. As high sensitivity CRP test are available in all standardized clinical laboratories so we can use and apply it easily for screening as well as for diagnostic purpose. Another important study done by Kulsoom and Hasnain on association of statins with atherosclerosis showed that atherosclerosis is considered to be a form of inflammatory disorder, not merely a cause of dyslipidemia. As in this study both CRP and cholesterol are measured and this provides an accurate indication of risk instead measuring cholesterol alone. So the lipid lowering drugs can be helpful in reducing the CHD risk and stroke.[18]

CONCLUSION

This study therefore concludes that C reactive protein act as a significant marker in causation of coronary artery disease and presents strong association with coronary heart disease. We can use this marker as a diagnostic tool and for screening purpose in individuals of middle age that is 40–60 years. The facilities for performing C reactive protein test are available in almost all standardized laboratories. So it is our responsibility to make awareness in common people through different awareness programmes via government means as well as by means of NGOs and through societies in general public. But there is question in doubt that either by decreasing the levels of C reactive protein which is an inflammatory marker; it may affect or prevent the coronary artery events so more research work is required in this context.

AUTHOR'S CONTRIBUTION

All the authors contributed equally.

REFERENCES

1. Cardiovascular disease. in: Kumar PJ, Clark ML. Kumar & Clark's clinical medicine. Edinburgh; New York: Saunders Elsevier; 2009.p.743–7.
2. Diseases of the coronary arteries. in: Julian DG, Cowan JC, McLenachan JM, editors. Cardiology. 8th ed. Edinburgh; New York: Elsevier Saunders; 2005.p.78–5.
3. Cardiovascular diseases. in: Ballinger A, Patchett S. Pocket essentials of clinical medicine. 4th ed. Saunder Elsevier, New York 2007.p.43–7.
4. Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S, Murphy A. Growing Epidemic of Coronary Heart Disease in Low- and Middle Income Countries. Curr Probl Cardiol 2010;35(2):72–15.

5. The cardiovascular system. in: Chandrasoma P, Taylor CR, editors. Concise pathology. 3rd ed. Mc Graw Hill, New York 2001.p.315–22.
6. Kruse AL, Luebbers HT, Grätz KW. C-reactive Protein levels: A prognostic marker for patients with head and neck cancer. J Head Neck Oncol 2010;2:21.
7. Burtis CA, Ashwood ER, Bruns DE, Tietz NW, editors. Apolipoproteins and other cardiovascular risk factors: in: Tietz textbook of clinical chemistry and molecular diagnostics. 4ed. St. Louis, Mo: Elsevier, Saunders; 2006.962–7.
8. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. J Intern Med 2008;264(4):295–314.
9. Hingorani AD, Shah T, Casas JP, Humphries SE, Talmud PJ. C-reactive protein and coronary heart disease: predictive test or therapeutic target?. Clin chem 2009;55(2):239–55.
10. Patel VB, Robbins MA, Topol EJ. C-reactive protein: A Golden marker for inflammation and coronary heart disease. Cleve Clin J Med 2001;68(6):521–24, 527–34.
11. Danish J, Wheeler JG, Hirschfield GM, Eda S, Erricksdotter G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350(14):1387–97.
12. Ridker PM. Clinical application of CRP for cardiovascular disease detection and prevention. Circulation 2003;107(3):363–9.
13. Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, et al. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women The Cardiovascular Health Study. Circulation 2005;112(1):25–31.
14. Braunwald E. Atlas of internal medicine. Philadelphia: Current Medicine LLC; 2007.
15. Thakur S, Gupta S, Parchwani H, Shah V, Yadav V. Hs-CRP - A Potential Marker for Coronary Heart Disease. Indian J Fundam Appl Life Sci 2011;1(1):1–4.
16. Irfan G, Ahmed M. High sensitivity C-reactive Protein concentration and angiographic character of coronary lesion. J Ayub Med coll Abbottabad 2008;20(3):100–3.
17. Khan DA, Ansari WM. Saleem S, Khna FA. Reference value for high sensitivity CRP in northern Pakistan population. Pak Armed Forces Med J 2009;59(4):257–8.
18. Kulsoom B. Hasnain SH. Association of Serum C-Reactive Protein and LDL:HDL with Myocardial Infarction. J Pak Med Assoc 2006;56(6):318–22.

Address for Correspondence:
Dr. Nadia Haleem, Department of Biochemistry, Ayub Medical College, Abbottabad-Pakistan
Cell: +92 322 910 0036
Email: nadiahaleem@myself.com