International Journal of Molecular Biology
ISSN: 0976–0482 & E-ISSN: 0976–0490, Volume 3, Issue 1, 2012, pp.-36-39
Available online at http://www.bioinfo.in/contents.php?id=34

DIAGNOSTIC UTILITY OF hs-CRP IN CORONARY HEART DISEASE

REKHA BHAGWAT, ARPITA GUPTE AND YADAV K.S.

Department of Biochemistry and Biotechnology, Pad. Dr. D.Y. Patil Medical College, Hospital & Research centre, Navi Mumbai-706
*Corresponding Author: E-mail- bhagwatrekha@gmail.com

Received: February 7, 2012; Accepted: February 21, 2012

Abstract- In Indian population highest numbers of patients are suffering from Coronary heart disease (CHD). It is projected to be 40 million Indians may suffer due to Coronary heart disease by the year 2020 [1].
Present study revealed that conventional markers for CHD are in-sufficient to predict the risk. The measurement of hs-CRP level & clinical indication may predict as a diagnostic marker for early detection of Coronary heart disease. The present study was carried at Padamashree Dr D Y Patil Hospital & Research Centre, Navi Mumbai. Fasting blood samples were collected from male and female patients attending Cardiology & Diabetic Clinics. The study designed between the different groups as diabetic, diabetic with hypertension and Myocardial Infraction & normal healthy individuals between the ages 25-60 years. It was concluded that hs-CRP level was increased significantly in diabetic, diabetic with hypertension and MI, though their lipid levels were within normal range.
Key words- hs-CRP- lipid profile- diabetic mellitus diabetic mellitus with hypertension- myocardial infraction

Citation: Rekha Bhagwat, Arpita Gupte and Yadav K.S. (2012) Diagnostic Utility of hs-Crp in Coronary Heart Disease. International Journal of Molecular Biology, ISSN: 0976–0482 & E-ISSN: 0976–0490, Volume 3, Issue 1, 2012, pp-36-39.

Introduction

Coronary Heart Disease (CHD) is postulated to be the number one killer disease by 2020 amongst Indians. Traditional risk factors like smoking, hypertension, diabetes are reported to account for fifty percent of prevalence and severity of the disease.[2] Currently established CHD risk factors are useful in identifying and monitoring the therapy of individuals at increased risk. Conventional risk factors such as Serum Glutamate Oxaloacetate Transaminase (SGOT), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglycerides (TG) etc. do not completely account for the increase in premature CHD in people from the Indian subcontinent. Lipoprotein profile has been investigated extensively in recent years, which is found to be deranged in large proportion of CHD patients. [3] However, a significant proportion of patients have a normal lipoprotein profile. Hence a need for study of novel risk factors or markers arises. The present research work attempts to assess the high sensitivity hs-CRP as a cardiac marker for early detection of CHD. Earlier the studies have been done on Indian population particularly from North and South India showing the correlation among cardiac markers as independent risk factors of CHD [1,2,3] Considering the above mentioned facts, the present study is undertaken amongst the population residing in and around Navi Mumbai.

The high sensitivity CRP (hs-CRP) test measures very small amounts of CRP in the blood and is ordered most frequently for seemingly healthy people to assess their potential risk for heart problems. It measures CRP in the range from 0.5 to 10 mg/L. The regular CRP test is ordered for patients at risk for infections or chronic inflammatory diseases. It measures CRP in the range from 10 to 1000 mg/L. Discovered by Tillett and Francis in 1930 [4] however, discovery of hepatic synthesis demonstrated that it is a native protein. [4,5,6] It was observed that hs-CRP was significantly elevated in patients dying suddenly with severe CAD, both with and without acute coronary thrombosis, and correlated with immune-histochemical staining(HIS) intensity and number of thin cap athroma [7]

Roy et al, in 2005, did a comparative study of markers of inflammation for the assessment of cardiovascular risk in patients with acute

chest pain.[8] Estimation of albumin concentration, leukocyte count and hs-CRP analyses measured. Results were obtained with lower albumin concentration, higher leukocyte count and high level of hs-CRP. This study concludes that leukocyte count is an independent predictor of ACS and high hs-CRP levels is an independent predictor of clinical outcome in ACS patients.

Dr. Soinio and colleagues reviewed data from 1045 patients with type-2 diabetes aged 45 to 64 years who were originally enrolled into a study of atherosclerotic disease. Over a 7-year follow up period, 157 patients died from coronary heart disease and 254 had a fatal or nonfatal CHD event. Mean hs-CRP levels were significantly higher in men who died of CHD or who had a fatal or nonfatal myocardial infarction (MI) than in men who did not.[9] According to results obtained patients with hs-CRP levels >3.0 mg /dl had a higher CHD mortality rate than patients with hs-CRP < 3.0 mg /dl. The results were similar when only the patients who have no history of MI were analysed. The independent risk posed by elevated hs -CRP levels in diabetic patients suggests that inflammation plays an important role in fatal CHD events among the high risk population.

Takahashi Kenji et al in 2006 , have shown in their study that fine inflammation is closely related not only to insulin resistance but also to macro-angiopathy in type-2 diabetic patients, and hs-CRP can be useful marker for evolution of patho-physiology in type-2 diabetes mellitus or vascular lesion.[10]

Nyandak et al in 2007, have compared the levels of hs-CRP in a patients with angiographically documented acute coronary syndrome (ACS), chronic coronary artery disease (CAD) and patients with normal coronary angiograms.[11] According to results presented by these researchers there was statistically significant hs - CRP elevation in angiographically confirmed CAD patients (p=0.00 4). It was found that hs-CRP levels were much higher in acute coronary syndrome patients compared to patients with normal coronary angiograms (p=0.003). The hs-CRP levels were found to be in direct proportion to extent source of CAD
(p=0.004) . The significant correlation was observed between the extent of CAD and hs-CRP levels. This showed that hs-CRP has positive correlation with the disease burden in CVD patients

A pilot study was carried out by Suman B. Sharma in 2008 on hs-CRP and oxidative stress in young CAD patients in India. Conclusion of above study states that elevated hs-CRP levels along with dyslipidemia and oxidative stress added to the predictive value of premature CAD in Indians [12]

Individual assessment of conventional parameter may not give enough evidence for confirmation of diagnosis at an early stage of CHD. Hence it is hypotheses that hs-CRP as inflammatory marker with lipid profile may add great value for diagnosis of disease at an early stage.

Material and Methods

Sample Selection: Forty one Type-2 diabetic patients, 40 Diabetic with Hypertension patients and twenty MI patients within an age group of 25 to 60 years were selected for the study. Pregnant females, chronic liver, kidney disease and known cases of acute and chronic infection were excluded from the study.

The subjects with newly diagnosed type I & II diabetes diagnosed as per WHO criteria, fasting plasma glucose greater than 126 mg / dl and 2 h post lunch glucose greater than 140 mg /dl and Blood pressure more than 80/120 mm Hg. The angiographically confirmed MI patients were also included in the present study. The control group included forty healthy non- diabetic and non MI subjects recruited from the OPD clinic of Dr. D. Y. Patil Hospital and Research centre Nerul, Navi Mumbai who had come for routine health check-up other than diabetes. Informed consent was obtained from all study subjects and the institutional ethical committee has approved the study.

Results and Discussion

Fasting blood samples were collected in plain bulbs without any anti-coagulant. Serum was separated and used for the study. Blood samples were analysed for triglycerides, cholesterol, HDL-cholesterol and LDL- cholesterol levels using Automated-Dimension RxL Clinical Chemistry Photometric analyser. hs-CRP was estimated using ELISA kit.

Table 1- Group-wise & Gender-wise analysis of recruited data

Sr No	Age group	DM		DM with HT		MI		Control	
		M	F	M	F	M	F	M	F
1	< 40 years	01	02	02	0	01	01	20	10
2	>40 years	24	14	16	22	12	06	06	04

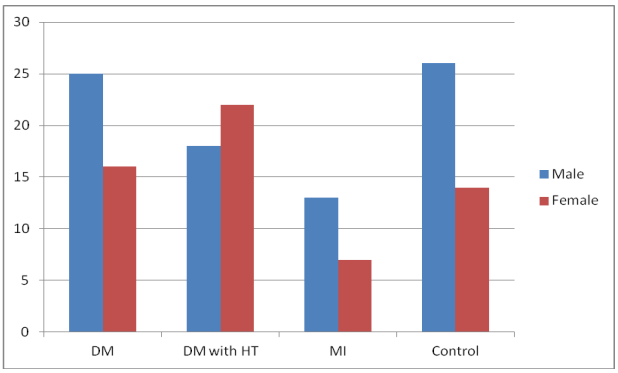

Fig. 1- Bar diagram of Group-wise & Gender-wise analysis of recruited data

It is observed from above data that men are more prone to develop CHD than female. This is also observed that as age of diseased increases the frequency of disease increased. We divided our data in two group i.e. less than 40 years and above 40 years. In this study 6.93% patients are bellow age of 40 years and 92.07% are above 40 years. Fasting TG, Cholesterol, HDL and LDL was measured as mg/dl and hs-CRP as mg /L.

Normal range of TG is 25 – 200 mg/dl, Cholesterol is 125 – 200 mg/dl, HDL- cholesterol is 30 – 65 mg/dl and LDL- cholesterol is 80 – 130 mg/dl. Normal range of fasting hs-CRP is up to 3 mg /L. The statistical analysis was done using SPSS 16 and ANOVA. Mean & standard deviation for each parameter is summarized in table2.

It is observed from Fig. 2 that Cholesterol, triglyceride LDL-cholesterol and hs-CRP is consistently increases in MI, DM with HT and DM with respect to Control group. It is also observed that approximately one-third of the patients form diseased group shows normal levels of lipids but increased levels of hs-CRP. When we

studied individual cases it is observed that these patients were suffering with Diabetic, Diabetic with hypertension and diagnosis is confirmed by physician.

Table 2- Statistical analysis of Mean and Standard deviation of all study groups

Parameter	DM (n=41)		DM with HT (n=40)		MI (n=20)		CONTROL (n=40)	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Cholesterol	194.73	43.95	172.92	39.99	171.90	42.56	141.50	16.39
Triglyceride	181.36	103.97	143.90	50.53	141.90	45.07	107.20	21.69
HDL	41.28	9.26	40.60	7.85	47.29	12.42	49.66	11.50
LDL	108.85	32.80	102.89	35.03	97.53	36.02	77.55	14.72
hs-CRP	3.59	2.81	5.44	4.18	4.11	3.68	1.22	0.63

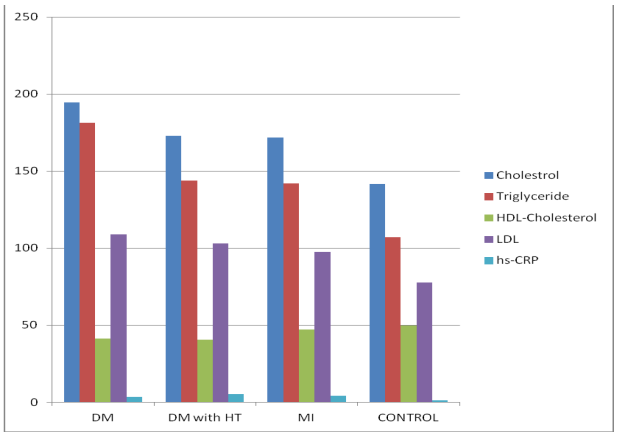

Fig. 2- Bar diagram of Mean value of Cholesterol, Triglyceride, HDL-Cholesterol, LDL- cholesterol and hs-CRP in .all study groups

It is observed from Fig2 that Cholesterol, triglyceride LDL- cholesterol and hs-CRP is consistently increases in MI, DM with HT and DM with respect to Control group. It is also observed that approximately one-third of the patients form diseased group shows normal levels of lipids but increased levels of hs-CRP. When we studied individual cases it is observed that these patients were suffering with Diabetic, Diabetic with hypertension and diagnosis is confirmed by physician.

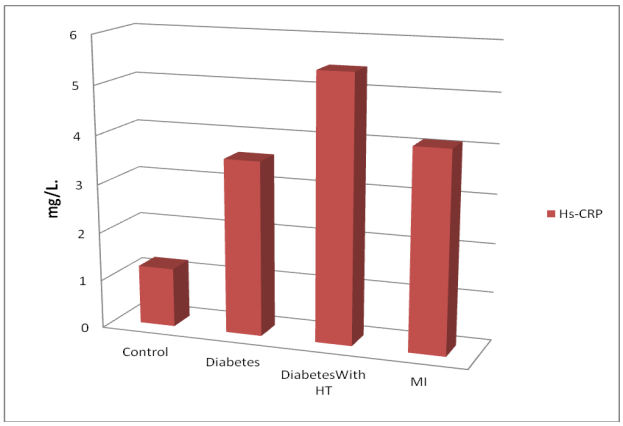

Fig. 3- Bar diagram of levels of hs-CRP in DM, DM with HT, MI & control groups

It is observed from bar diagram3 that hs-CRP concentration increases in diabetes, diabetes with hypertension and MI.

Table 3- Analysis of Variance (ANOVA) for all study groups.

Parameter Vs hs-CRP	DM (n=41)		DM with HT (n=40)		MI (n=20)		CONTROL (n=40)	
	t-Value	P-Value	t-Value	P-Value	t-Value	P-Value	t-Value	P-Value
Triglyceride	14.223	0	17.243	0	14.243	0.001	15.643	0.002
Cholesterol	21.402	0.001	25.702	0.001	15.452	0.001	17.452	0.001
HDL-Cholesterol	26.106	0.002	25.106	0.003	25.666	0.004	27.766	0.001
LDL-cholesterol	15.255	0	17.255	0.001	18.551	0.001	16.441	0.002

Table 4- Statistical analysis of Karl Pearson Correlation Coefficient (KPCC) and Spearman's Correlation Coefficient (SCC) of all study groups.

Parameter	DM (n=41)		DM with HT (n=40)		MI (n=20)		CONTROL (n=40)	
	KPCC	SCC	KPCC	SCC	KPCC	SCC	KPCC	SCC
Cholesterol	0.668	0.695	0.544	0.694	0.943	0.916	0.656	0.604
Triglyceride	1	0.889	1	0.988	1	0.789	1	0.788
HDL	0.774	0.695	0.797	0.552	0.600	0.693	0.663	0.656
LDL	0.746	0.743	0.873	0.655	0.843	0.916	0.773	0.651
hs-CRP	0.826	0.634	0.754	0.721	0.563	0.651	0.774	0.721

In control group all 40 subjects including both male and female showed normal level of TG. 3out of 26 males and 1 out of 14 female showed lower levels of Cholesterol (< 200 mg/dl) respectively, 2 males showed higher HDL- cholesterol (> 65 mg/dl) whereas HDL-cholesterol in all females reported within a normal range. The LDL- cholesterol levels found lower in 5 males out of 26 whereas it was same trend observed in females (< 80 mg/dl)., hs-CRP values were found within the normal range.

Out of 25 male diabetics, 9 had high TG (>200 mg/dl), 12 had Cholesterol >200 mg/dl whereas 2 had Cholesterol <125 mg/dl, 2 had HDL- cholesterol <30 mg/dl, 3 with high and 5 with low LDL-cholesterol levels. In females 3 out of 16 showed TG >200 mg/dl. Cholesterol was found within normal range in all 16 females. Only 1out of 16 had HDL- cholesterol >65 mg/dl whereas LDL -cholesterol level was shown >130 mg/dl in 5 females out of 16 diabetic cases. In all dabetic subjects ,Considering other parameters it was observed that 31.7% subjects were having higher levels of hs-CRP.

Out of 20 MI sujects 13 are males & 7 are females. 3 out of 13 male subjects showed TG >200 mg/dl whereas all 7 female subjects had normal levels of TG whereas 3 males and 1 female had Cholesterol >200 mg/dl. HDL-cholesterol level in 2 males was found <30 mg/dl, only 1 male showed HDL- cholesterol >65 mg/dl whereas all the females had a HDL- cholesterol in a normal range. Out of 20 subjects 2 male and 1 female showed higher LDL-cholesterol levels whereas 5 males and 1 female showed lower LDL- cholesterol levels. Statistical data in present study showed that 45% patients were having high level of hs-CRP than normal range.

Out of 40 diabetic with hypertension 18 are males & 22 are females. 2 out of 18 males and 2 out of 22 females showed high TG levels, 4 males and 2 females showed high cholesterol levels,

whereas 3 males and 2 females had low cholesterol. The HDL-cholesterol level in 3 males and 1 female was found to be low. The LDL-cholesterol level was found to be low in 4 male and 6 females whereas 6 males and 5 females showed higher levels than the normal range. In findings, 62.5% subjects were having higher hs-CRP. Observations further revealed that female subjects were having higher level of hs-CRP than male subjects

Students 't' test was used to compare the mean of the variables. Pearson's correlation coefficient was used to look for association among the risk factors. All groups were combined for Pearson's correlation analysis. Analysis was performed with the SPSS 16 statistical software package and p values < 0.05 were considered as the level of significance.

Hs-CRP level in all the study groups were found elevated as compared to control subjects (fig. 2). hs-CRP showed a significant and positive correlation with the lipid profile amongst the study groups ($p< 0.05$) as seen from the Table 3 and 4.

Pearson's coefficient of relation showed high degree positive correlation for the variables ($p< 0.05$). Spearman's correlation coefficient was also applied to test the correlation between marker and conventional risk factors which showed high degree positive correlation for variables ($p<0.05$) as shown in Table 4.

CRP is a major inflammatory cytokine that functions as a nonspecific defence mechanism in response to tissue injury or infection. Present study showed higher level of hs-CRP in subjects with Diabetes, MI and Diabetes with hypertension than in control group. Increasing hs-CRP level suggests the chronic inflammation that leads the progression of atherosclerosis and atherothrombosis, a causative factor for CHD. It is also etiologically involved in the pathogenesis of diabetes and is considered as an important cardiovascular risk marker in patients with diabetes mellitus. [14] Many evidences indicate that inflammation of hs-CRP may lead to the development of type II diabetes and the progression of atherosclerosis. [15] Gender wise analysis from this study shows that in diabetic group, female subjects showed higher level of cholesterol and hs-CRP than male subjects. The same observations were found in diabetes with hypertension group. In MI group, however cholesterol level was reported to be higher in male subjects than in females; hs-CRP levels reported almost similar in both male and female subjects. According to study carried out by Soinio Laakso et al in 2006, [9] the independent risk posed by elevated hs-CRP levels in diabetic patients suggest that inflammation plays an important role in CHD. Research by Takahashi et al and Pfutzner Forst in 2006 [10,11], noted that elevation of hs-CRP was associated with increased risk of type-2-diabetes development in patients with all levels of metabolic syndrome. The comparative study between type-2-diabetic patients and non diabetic patients revealed that type-2-diabetic patients were at higher risk for CHD when classified into low, intermediate, and high risk group based on hs-CRP levels [15].

The statistical analysis of all the parameters showed that hs-CRP level in diabetic patients was 3 times higher than control group. Whereas it was 5 times and 4 times higher in the subjects with diabetes with hypertension and MI patients respectively. Further studies on hs-CRP as cardiac marker for early detection of CHD is required in different risk groups of Indian population.

Conclusion

It is concluded that there is positive correlation between conventional lipid parameters and hs-crp in CHD. The level of hs-CRP measured in control, diabetic, diabetic and hypertensive and MI and it was found that degree of significance is observed. It is summarised that the results obtained in this study states that hs-CRP and conventional lipid parameters can be used to predict the risk of CHD.

Aknowledgement

We would like to express our gratitude to Dr. Priya Patil- Cholera, Dr. Rashmin Cholera, Dr. Shirish Patil, Mr Prabhakar Bhagwat for encouragement and inspiration. I am obliged to patients, technical and paramedical staff of this institution who helped us to peruse this work. I thankful to Dr Milind Hanchate, Madhavbaug Cardiac Centre who helped us lot.

Reference

[1] Gupta O.P., Phatak S. (2003) *Rev articl*e. 23(2), 37-50.
[2] Rajasekhar D., Saibaba K.S.S., Srinivas R., Latheef S.A.A., and Subramanyan G. (2004) *In J. clini Bio.*, 19(2), 53-59.
[3] Burman A., Jain K., Gulati R., Chopra V., Agrwal D.P., Vasisht. (2004) *JAPI,* 52, 99-102.
[4] Tillett W.S., Francis T. (1930) *J. Exp. Med.* 52(4), 561-71.
[5] Thompson D., Pepys M.B., Wood S.P. (1999) *Structure* 7(2), 169-77.
[6] Peter J. Kennelly, Murray, Robert F., Victor W. Rodwell, Kathleen M. Botham (2009) *Harper's illustrated biochemistry*.
[7] Burke Allen P., Russell P. Tracy, Frank kologies, et al. (2002) *Circulation*, 105, 2019-2023.
[8] Roy D., Quiles J., etal. (2006) *The N. England Journal of Medicine*, 109, 317-321.
[9] Soinio M., Mamiemi J., Laakso M., et al. (2006) *Care*, 29,329-333.
[10] Kenji T., Takahero S., (2006) *Japan- Science link Japan*\, 68, 119-122.
[11] Pfutzner A., Forst T. (2006) *Diabetes Technol Ther*, 8(1), 28-36.
[12] Nyandak T., Gogna Arun, Bansal S., et al. (2007) *Indian Journal of Clinical Biochemistry*., 8(3), 217-221.
[13] Sharma S.B., Garg S., Garg V.A. and Dwivedi S. (2008) *Indian Journal of Clinical Biochemistry*, 23(4), 334-336.
[14] Khan D.A., Qayyum S. (2009) *J. Med Sci.*, 25, 776-781.
[15] David M. Capuzzi and Jefferey S. Freeman (2007) *Clin Diabetes*, 25, 16-22.