HIGH SENSITIVITY C-REACTIVE PROTEIN, PARAOXONASE 1 AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN MYOCARDIAL INFARCTION

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Abstract

Background: Atherosclerosis leading to myocardial infarction is an inflammatory disease. The inflammation is caused by factors like ox-LDL, Lp(a), cytokines, triglycerides; modified, synthesized or released in various disorders including diabetes and hypertension. The C-reactive protein (hs-CRP) is one of the markers of inflammation. The HDL is involved in reverse cholesterol transport hence HDL-C is said to be protective marker. Paraoxonase (PON1) is an HDL associated enzyme, which helps in prevention of LDL oxidation.

Objectives: The present study was carried out at Swami Ramanand Teerth Rural Medical College, Ambajogai, with an aim to find out the role of High sensitivity C-reactive protein (hs-CRP), Paraoxonase 1 (PON1) and High density lipoprotein cholesterol(HDL-C) in myocardial infarction (MI). The hs-CRP, arylesterase activity of PON1 and HDL-C were estimated in fifty diagnosed cases of myocardial infarction and were compared with fifty age and sex matched healthy controls.

Results: Serum levels of hs-CRP were significantly raised in myocardial infarction than controls [2.87±0.89 mg/L vs. 0.86±0.24 mg/L, P < 0.001]. Serum PON1 activity decreased significantly in myocardial infarction as compared to healthy controls [34.04±17.25 KU/L vs. 47.63±16.12 KU/L, P <0.001]. Serum HDL-C levels were significantly decreased in myocardial infarction than in controls [32.28±6.80 vs. 28.87±6.40*, P <0.05].

Conclusion: The hs-CRP showed positive correlation with HDL-C (+0.301) and showed positive but weak correlation with PON1 (+0.133). No correlation was found between PON1 and HDL cholesterol.

Keywords: C reactive protein, high density lipoprotein cholesterol, Myocardial infarction

1. Introduction

Cardiovascular disease is one of the leading causes of morbidity and mortality all over the world. In India, the prevalence of ischemic heart disease (myocardial infarction) among adults (based on clinical and ECG criteria) was estimated at 96.7 per 1000 population in the urban and 27.1 percent in rural areas. In 90% of them the main underlying cause is atherosclerosis of coronary arteries.

The atherosclerosis is an inflammatory disease and the development of atherosclerotic plaque is a complex and self reinforcing interaction between lipid accumulation and modification, and its effect on the endothelium, smooth muscle cells and macrophages. Several acute phase proteins, cytokines and intracellular adhesion molecules are said to be potential novel markers for cardiovascular risk assessment. The CRP (C reactive protein) is an acute phase inflammatory marker and is found to be the best predictor of future coronary events. C-reactive protein was originally observed by Tillet and Francies. It is synthesized in liver. Plasma half life of CRP is approximately 19 hrs. The CRP reaches peak on 2nd day and returns to baseline after 5th day of inflammation. High sensitivity assay for CRP have now been developed to detect minute quantities of CRP, hence termed hs-CRP assay. The hs-CRP also activates compliment, causes T cell mediated endothelial cell destruction, expression of adhesion molecules and stimulates macrophages to produce tissue factor.

In addition to CRP, lipids like LDL-C and HDL-C are thought to be sine qua non of cardiovascular disease. The oxidized LDL causes endothelial cell dysfunction and foam cell formation leading to plaque formation which is the hallmark of atherosclerosis. Though main emphasis in literature is given on its role in reverse cholesterol transport, recent studies reveals diverse and heterogeneous
functions of HDL like prevention of LDL oxidation, protease inhibition and complement activation. Parthasarathy et al first reported that HDL protects oxidative modification of LDL with HDL associated enzymes paraoxonase. Paraoxonase (aryldialkylphosphatase 3.1.8.1) family contains three members PON1, PON2 and PON3. PON1 and PON3 are associated with HDL and synthesized in liver, whereas PON2 is found in many tissues.

Myocardial infarction (MI) is a multifactorial disease and various biomarkers play a complex role in MI. The present study is aimed to know the role of hs-CRP, PON1 and HDL-C in MI.

2. Materials and methods
The present study was carried out at Swami Ramanand Teerth Rural Medical College, Ambajogai. Fifty diagnosed cases of myocardial infarction (WHO criteria) were compared with fifty age and sex matched healthy controls. Patients of acute and chronic inflammatory diseases were excluded by history and pathological investigations like total leucocytes count and differential leucocytes count. The fasting blood sample was collected after day five of hospital admission in MI patients, as hs-CRP returns to baseline by day five in MI. The high sensitivity C-reactive protein was measured by immunoturbidometric method using Spinreact diagnostic kit. Arylesterase activity of PON 1 was measured colorimetrically using phenyl acetate as substrate. Total cholesterol, HDL-C, and Triglycerides were estimated by routine enzymatic methods. LDL-C was calculated by Friedewald formula. The statistical analysis of data was done by unpaired Student t-test. The Pearson correlation coefficient was used for correlation between hs-CRP, PON1, and HDL-C. All results were expressed in mean±S.D.

3. Results
Serum levels of hs-CRP were significantly raised in myocardial infarction than controls [2.87±0.89 mg/L vs. 0.86±0.24 mg/L, P < 0.001]. Serum PON1 activity was decreased significantly in myocardial infarction as compared to healthy controls [34.04±17.25 KU/L vs. 47.63±16.72 KU/L, P < 0.001]. Serum HDL-C levels were decreased significantly in myocardial infarction as compared to healthy controls [32.28±6.80 vs. 28.87±6.40, P < 0.05]. (Table 1.1)

The other lipid parameters like Total Cholesterol, VLDL-C and Triglyceride showed significant increase in myocardial infarction than in controls while LDL-C showed no difference (Table 1.2).

The hs-CRP showed positive but weak correlation with PON1 (+0.133) and with HDL-C (+0.301). The PON1 was weakly correlated with hs-CRP, but no correlation was found between PON1 and HDL cholesterol. (Table 1.3)

4. Discussion
The levels of hs-CRP was found to be significantly raised in MI (2.89±0.90 mg/L), than in controls (0.85±0.24 mg/L). The result is consistent with finding in previous studies by Ridkar PM, Berk et al, Yip et al. The principle cause of elevated levels of hs-CRP seems to be the persistent and subclinical chronic inflammation leading to atherosclerosis. Contrary to our expection the hs-CRP was positively correlated with HDL-C, this may be due to the modification of HDL in inflammatory states. Fogelman and colleagues have shown that HDL particles isolated from patients of coronary artery disease, failed to retard, and in fact enhanced LDL mediated inflammation. The another reason why we observed positive correlation between hs-CRP and HDL-C is that absolute HDL-C levels were not indicative of HDL functioning. As hs-CRP rises, as a response to inflammation it might be natural mechanism to raise anti-inflammatory and antioxidative components like PON1, giving positive correlation between hs-CRP and PON1. Secondly there might be a molecular factor which is involved in activation/induction of both hs-CRP and paraoxonase simultaneously. The serum paraoxonase activity was significantly lowered in patients with myocardial infarction as compared to healthy controls (34.04±17.25 KU/L vs 47.63±16.72 KU/L, p < 0.001). The dietary habits such as eating cholesterol rich diet and degraded cooking oil have been reported to decrease serum PON1 activity. Durrington P. et al showed that long term exposure to pesticides (particularly organophosphorous) may cause long term decrease in serum PON1 activity as most subjects in study are farmers by occupation, the decreased PON1 activity may be due to their chronic exposure to pesticides in farms and fields. The PON1 is located on HDL and correlation between PON1 and HDL-C was expected. But
surprisingly we found no correlation between PON1 and HDL-C. One of the reasons for this may be PON1 was also shown to be associated with non-HDL particles. More recently ability of the cells to secrete PON1 on VLDL as well as on HDL has shown in vitro Laura Rozek. et al. 18 PON1 polymorphism,20 which is reported as an independent risk factor for cardiovascular disease may have impact on PON1 activity. The PON1 is located on HDL-3 sub fraction.18 Individual in this study might have decreased HDL-3 sub fraction. This may be an indication that protection by HDL is not dependent upon absolute levels of HDL-C but rather depends on dynamic functional HDL.

There are some limitations of present study as we have estimated arylesterase activity and not paraoxonase activity of PON 1, no phenotyping and genotyping of PON1 was done and lastly sub fractionation of HDL was not done. Further studies are required in this direction.

References
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Table 1.1 Serum hs-CRP, paraoxonase (PON1) activity and HDL-C in healthy controls and Myocardial infarction (MI).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr. hs-CRP mg/L</td>
<td>0.86±0.24</td>
<td>2.87±0.89***</td>
</tr>
<tr>
<td>PON1 (KU/L)</td>
<td>47.63±16.72</td>
<td>34.04±17.25***</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>32.28±6.80</td>
<td>28.87±6.40***</td>
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P < 0.05, *** P < 0.001

Table 1.2 Lipid profile in healthy controls and Myocardial infarction (MI).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol mg/dl</td>
<td>168.74±21.63</td>
<td>183.71±40.11***</td>
</tr>
<tr>
<td>Triglycerol mg/dl</td>
<td>112.53±23.71</td>
<td>181.89±95.19***</td>
</tr>
<tr>
<td>LDL-C mg/dl</td>
<td>113.95±20.39</td>
<td>113.21±45.59</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>22.50±4.74</td>
<td>36.37±19.03***</td>
</tr>
</tbody>
</table>

P < 0.05, *** P < 0.001

Table 1.3 Correlation coefficient (r) of serum hs-CRP, PON1 and HDL-C in Myocardial infarction.

<table>
<thead>
<tr>
<th></th>
<th>hs-CRP</th>
<th>PON 1</th>
<th>HDL-C</th>
</tr>
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<tbody>
<tr>
<td>PON1</td>
<td>+0.133***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>+0.301***</td>
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*** P < 0.001