Study the Association of C-Reactive Protein and Risk of Cardiovascular Disease in Heart Patients above the Age of 50 Years

Irum Naureen1, Aisha Saleem2, Muhammad Naem3, Zainab Naem4, Attique Nawaz5

1Assistant Professor, School of Zoology, Minhaj University Lahore, Pakistan
2M. Phil Researcher, School of Zoology, Minhaj University Lahore, Pakistan
3Researcher, School of Zoology, Minhaj University Lahore, Pakistan

DOI: 10.36348/sjbr.2021.v06i10.001 | Received: 06.09.2021 | Accepted: 19.10.2021 | Published: 23.10.2021

*Corresponding author: Dr. Irum Naureen

Abstract

Coronary heart disease, non-communicable disease has become epidemic and increasing in world crisis. There is escalation in developing countries and known to be the death cause in developed countries. Due to the absence of proper guidance and preventive measures its risk factors are increasing day by day. 30-40% of deaths in Pakistan are due to cardiovascular disease. Study of C-reactive protein relationship with cardiovascular disease risk factors is essential for heart disease patients. C-reactive protein is an inflammatory marker it’s helpful tool for the recognition of risks in developing heart disease. C-reactive protein assessed by Enzyme Linked- Immunosorbotant assay method is more effective in comparison with other risk factors of cardiovascular disease such as obesity, blood pressure, diabetes mellitus, cholesterol, Triglycerides, LDL cholesterol and HDL cholesterol. Written consent filled by the patients was studied to understand the relative relation of C-reactive protein with other parameters. Systolic and diastolic blood pressure mmHg was measured at 5 minutes of interval for the average analysis. By statistical analysis on Graph Pad Prism we have found the prevalence of C-reactive protein in heart patients in reference to their physical activity, smoking status and stress. Statistical means of heart patients and control were compared to each other and there see mainly increase in level of CRP, LDL cholesterol, Triglycerides, diabetes mellitus and total cholesterol. High density lipoprotein cholesterol was low in patients and higher in control because this is the good cholesterol. Total number of heart patients is 50, and 15 controls. C-reactive protein in heart patients is (4.594±0.2099**) at a 95% of confidence interval whereas in control it was 1.42±0.1776. Statistical value of total cholesterol is 246.1±7.081*** in heart patients and in control (173.7±7.909). There was significant association of all parameters with coronary heart disease.

Keywords: C-reactive protein, cardiovascular disease.

INTRODUCTION

Cardiovascular disease involves many different conditions and to understand there as on for its burden we should be familiar with the circumstances and risk factors associated with coronary heart disease, stroke, rheumatic heart disease, inflammatory heart disease, hypertensive heart disease and other configuration of heart disease. Studying the risk factors help in guidance, diagnosis, therapy, and disease control [1].

Cardiovascular disease is the supreme cause of morbidity and mortality in Pakistan. Pakistan is a developing country undergoing a series of environmental, social, and structural changes trying to enhance the developing process to fulfill all needs required for the increased level of urbanization and transition from rural to urban areas [2]. An increase in development comes associated with the disease. An insufficient amount of prevalence data is present in Pakistan related to cardiovascular disease.

By the end of 2030, the number of deaths associated with CVD will rise to twenty-three million because thirty to forty percent of all deaths in Pakistan are due to the consequences of CVD. Lack of awareness about heart disease and its risk factors cause a greater number of deaths [2]. Cardiovascular disease which is related to the abnormality within the vessels for the circulation. In reality, the combination of cancer and HIV does not cause these many deaths globally than cardiovascular disease does, as 100 times as many people a year are being killed which is also not true in war accidents.

United Nation high-level meeting on Non-Communicable Diseases has allowed discussing further the prevention of heart disease, stroke, cancer, and diabetes. Non-communicable diseases are another major factor for global deaths. Emphasized on, there diction of poverty, health equity, sustained economic value, and human security. Implementing is the most urgent reduction in tobacco use. By 2040 worldwide the tobacco use might get limited to about less than 5% [3]. CVD risk factors are very common in Bangladesh because the statistical exposure has reached to 99.6% for males and 97.9% for females. This happens only because of a lack of knowledge and awareness. CVD account for 17.3 million yearly deaths hence by the end of 2030 this will raise to 23.6 million [4].

It represents 30% of all global deaths, where 60-80% of these deaths occur in low and middle-income countries. In the Indian population, the major death cause is coronary artery disease, chronic obstructive pulmonary disease, and stroke. Economic value plays an important role in the treatment of disease. The availability of expensive treatment can not contribute to the treatment and prevention of disease because not every citizen can afford this [4].

Chronic inflammation as it’s the risk factor for cardiovascular diseases. It was assessed by measuring the hsCRP concentration among peoples [5].

---

**Figure 1:** Atherosclerosis condition: plaques deposited in arteries [15]

**Figure 2:** Primary CRP production from liver and its regulation to the context of inflammation, pCRP dissociates into monomeric CRP which in return activates leukocytes, platelets and endothelial cells for complement C1q binding [7]
Hypertension

High blood pressure (hypertension), long term force of blood to artery walls leads to the high blood pressure when plaques get accumulated and interfere with the smooth flow of blood resulting to thickening and stiffening of the heart as it has to work harder to pump blood, and if left untreated it leads to more risks of CVD [6]. CRP is correlated with systolic blood pressure, pulse pressure, and hypertension thus involving in the inflammatory pathogenesis of hypertension. Hypertension and prehypertension are correlated with inflammatory markers such as Tumor Necrosis Factor-α (TNF-α), C-Reactive Protein (CRP) and Interleukin-6 (IL6) [8].

Prehypertension involves a low level of high-density lipoprotein cholesterol, higher levels of insulin resistance, blood glucose, low-density lipoprotein total cholesterol, and higher body mass index, triglycerides, abnormalities of glucose metabolism [7].

Lipids Profile

Obesity is the condition when fat gets deposited within the walls or in body parts and this excess weight increase strain on the heart that results in raised blood pressure and blood cholesterol including triglyceride levels and lowers high-density lipoprotein cholesterol levels [10]. Cholesterol esters, cholesterol, phospholipids, and triglycerides are hydrophobic in nature, having the possibility to get transported to other tissues in the form of lipoproteins, Low-density lipoproteins (LDL), Chylomicrons (CM) and high-density lipoproteins (HDL). Abnormality in lipoproteins level is a prevalence of obesity and heart problems. Due to high cholesterol, the annual deaths recorded are 56% and 18% by ischemic heart disease and stroke which accounts for approximately 4.4 million deaths [8].

Diabetes Mellitus

Diabetes is known to be the metabolic syndrome by the absence or non-functional insulin, the hormone that regulates the sugar level in our body and this ultimately increases the risk for CVD [9]. Type 2 Diabetes so-called the complication of CVD has a great impact on our socio-economic and increases mortality and morbidity worldwide. International Diabetes Federation estimated that worldwide, 387 million people had type 2 diabetes in 2014 and this number will rise to 592 million in 2035 [12].

Smoking

Cigarette smoking is very common which harms our body health. Smoking has closely linked with heart disease and cancer. The secondary effect of cigarette smoking is an increase in oxidative stress that gives rise to vascular inflammation. CRP is released into the bloodstream when our immune system is disturbed due to inflammation. IL-6 and CRP signify the potential growth and progression of many malignancies [14].

RESEARCH DESIGN / METHODOLOGY

The study was conducted in Punjab Institute of Cardiology hospital in Lahore and a total of 65 subjects, 15 control and 50 heart patients above the age of 50 years, were studied. The patients had undergone by physical examination, mental examination, and biochemical tests.

Physical and Mental Examination

The physical and mental examination was conducted by filling up the detailed self-reported questionnaire containing all information about their age, sex, body mass index, physical activity, stress, and smoking status [12]. The body mass index of each patient was calculated from weight in kg divided by their height in meter squares and weight [10,11]. Systolic blood pressure and diastolic blood pressure were obtained by the sphygmomanometer device. Three readings of systolic and diastolic blood pressure were recorded at 5 minutes of interval for the average analysis. For the mental examination of the patient, their stress levels were analyzed to recognize if there is any threat or worries that are affecting the mental health of a patient.

Biochemical test

Venous blood was collected to measure the levels of C-reactive protein, Total cholesterol TC, Low-density lipoprotein cholesterol LDLc, High-density lipoprotein cholesterol HDLC, Triglycerides TG, and diabetes mellitus DM.

Total Cholesterol

Total cholesterol levels were spectrophotometrically analyzed by using a commercially available kit (Analytic on Biotechnologies AG, 4046, Germany) in mg/dl unit.

Low-density lipoprotein cholesterol

Patients were told to fast 8 to 10 hours before the accuracy test. Friedwald calculation was used for estimating the low-density lip protein cholesterol.

Diabetes mellitus:

Sugar levels of each patient were measured using a glycated hemoglobin test. The test was done after the fasting of 8 hours to get a precise amount of sugar levels and measured in mg/dl unit. Its range was compared with the normal control number.

HDL cholesterol

HDL-C precipitation reagent (Analyticon Biotechnologies AG, 410, Germany) was used and HDL-C was estimated with the help of the aforementioned Analyticon kit.

Triglycerides

Triglycerides are called fats that are deposited their amount in our body is also very important as their increased level in our blood helps to indicate the risks
for CVD and peripheral atherosclerosis. Triglyceride tests do not require fasting blood for testing. Triglycerides are measured in mg/dl unit same way on a Hitachi 704 Analyzer.

**C-reactive protein analysis by ELISA Kit**

The hsCRP ELISA is intended for the quantitative determination of C-reactive protein (CRP) in human serum. The quantitative determination of C-reactive protein from human serum is brought up by hsCRP ELISA.

Patient blood samples were collected by venipuncture technique and serum is separated from packed cells 60 minutes after collection. The kit carries its components and has 96 wells to which we dispense 10 µl of CRP standards, diluted controls, and serum for their analysis. 100 µl of prepared CRP enzyme conjugate reagent is poured into each well. Contents are mixed well for 30 seconds as it is very important to enhance their reaction. Incubated for 45 minutes at the room temperature of (18-25°C) and the mixture is then removed by flicking plate into a waste container. 5 times distilled water was used to wash and flick the micro titer wells and then the wells were sharply struck to absorbent paper to remove there residual water droplets and unbound labeled antител bodies. 100 µl of tetra methyl Benzene TMB solution was dispensed into each well followed by 5 second mixing and incubating for 20 minutes, developed blue color in wells. Reaction into each well was stopped using the 100 µl HCL Stop solution which changes color to yellow. Absorbance was measured by spectrophotometrically at 450nm as CRP concentration is directly proportional to the color intensity of the testing sample.

**Statistical Analysis**

Applying unpaired t-test statistics and finding out the mean, standard deviation, and p-value for each parameter. An associated graph was made on an Excel program using the frequency table for categorical data. P-value gave the possible significance effect of CRP to each parameter.

**RESULTS**

Baseline clinical characteristics of 50 diseased heart patients (cases) and 15 normal (control) participants are given in Table 1. The study population included 30 males and 20 females about the mean age of 63.5 years old that are compared with the controls of 7 males and 8 females about the mean age of 62.7 years old. The mean systolic blood pressure of heart patients is found to be 147 mmHg and by applying unpaired t-test we have found the relative significance difference between various variables of the study population for example including systolic and diastolic blood pressure mm Hg, Body mass index kg/m2, serum CRP mg/l, total cholesterol mg/dl, LDL cholesterol mg/dl, HDL cholesterol mg/dl, triglycerides and diabetes mellitus/dl.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls n=15</th>
<th>Diseased n=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Age</td>
<td>62.73±2.67</td>
<td>63.52±1.275</td>
</tr>
<tr>
<td>Systolic blood pressure mmHg</td>
<td>120.1±2.25</td>
<td>147.2±4.876**</td>
</tr>
<tr>
<td>Diastolic blood pressure mmHg</td>
<td>72±1.331</td>
<td>81.72±1.248***</td>
</tr>
<tr>
<td>BMI Kg/m2</td>
<td>23.81±0.678</td>
<td>28.57±0.5093***</td>
</tr>
<tr>
<td>CRP mg/l</td>
<td>1.42±0.1776</td>
<td>4.59±0.2099***</td>
</tr>
<tr>
<td>Total cholesterol mg/dl</td>
<td>173.7±7.909</td>
<td>246.1±7.081***</td>
</tr>
<tr>
<td>LDL cholesterol mg/dl</td>
<td>116.7±3.096</td>
<td>155.9±6.409**</td>
</tr>
<tr>
<td>HDL cholesterol mg/dl</td>
<td>54.2±2.085</td>
<td>47.6±1.158**</td>
</tr>
<tr>
<td>Triglycerides mg/dl</td>
<td>114.4±5.161</td>
<td>164.9±7.26***</td>
</tr>
<tr>
<td>Diabetes mellitus mg/dl</td>
<td>122.5±3.23</td>
<td>182±8.874***</td>
</tr>
</tbody>
</table>

Studying various parameters by which the risk factors of cardiovascular disease are affected. Systolic blood pressure 147.2±4.876 mmHg is higher in heart patients as significantly different from control by statistical analyses from unpaired t-test at a 95% confidence interval with a p-value of 0.0038. Diastolic pressure 81.72±1.248mmHg is calculated significantly different with a p-value of 0.0001 where as BMI and CRP are also represented significantly different in Figures 1, 2 and 3.
Figure 3: Unpaired t-test graphical representation of systolic and diastolic pressure in controls and cases

Figure 4: Unpaired t-test graphical representation of body mass index and CRP in controls and cases

Figure 5: Unpaired t-test graphical representation of total cholesterol and LDL Cholesterol mg/dl in control and cases

Table 2: Relative frequency of physical activity in heart patients

<table>
<thead>
<tr>
<th>Physical activity</th>
<th>Frequency</th>
<th>Relative Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>34</td>
<td>0.68</td>
</tr>
<tr>
<td>Moderate</td>
<td>14</td>
<td>0.28</td>
</tr>
<tr>
<td>Vigorous</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

This study was based on 50 heart patients whose different parameters were compared with the controls and associated cardiovascular disease risk factors were determined. Cardiovascular disease risks increase when its factors are more prominent and dominate the other. The study included physical activity, smoking status, body mass index, systolic and diastolic blood pressure, CRP, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, diabetes mellitus parameters that are compared by t-test statistics. The statistical analysis demonstrated the effective significant difference between these parameters at a 95% confidence interval.

The study comprises of 60% male and 40% female whose age was in the range from 50-85 years old. The effective impact of these factors was observed in adults from 15 controls who do not exhibit any disease. The mean value of systolic and diastolic pressure in control was 120.1±2.25mmHg and 72±1.331mmHg whereas the systolic and diastolic blood pressure means in cases was 147.2±4.876**mmHg and 81.72±1.248***mmHg. The body mass index was 9% higher in heart patients from control this shows that body mass index fitness is associated with heart diseases. C-reactive protein knows to be the acute phase inflammatory marker rise in plasma within 24 to 48 hours due to acute tissue damage, trauma, and inflammation. C-reactive protein was discovered highest in heart patients 4.594±0.2099***mg/l and lower in control (1.42±0.1776) as they don’t possess any inflammation in their bodies. Heart disease is linked with inflammation in any vessels which interrupts the proper functioning of the heart results in an increased level of protein.

The lipid profile test screened the lipid concentration and levels to which it is affecting the metabolism of the body. The lipid profile involves total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. LDL-C said to be bad cholesterol and it seemed to be high in heart patients about the range of 197.2-416.4 mg/dl. By statistical analysis, there was a significant difference in control and cases with a p-value of < 0.0001. Low-density lipoprotein cholesterol was only 116.7±3.096 mg/dl indicating the absence of a massive amount of bad cholesterol in their body. High-density lipoprotein cholesterol called bad cholesterol was significantly low in heart patients as they had a massive amount of bad cholesterol in their body whose deposition resulted in the formation of plaque and leading to atherosclerosis condition. The statistical value of HDL-C in heart and controls were 47.6±1.158** and 54.26±2.085 mg/dl. Triglycerides are another form of lipids present in our blood and they are responsible for providing energy to our metabolism in between our meals when the body requires energy to fulfill its task. Triglyceride's normal range is less than 150 mg/dl and its borderline high-value range is 150-199 mg/dl above which is considered to be high in risks for cardiac diseases and other related diseases as shown in Figure 1, 2, 3 Table 1.

In our study, the mean value of triglycerides in heart patients was found to be 164.9±7.26***, designated to be higher. Diabetes mellitus was also

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Frequency</th>
<th>Relative –frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>smoker</td>
<td>1</td>
<td>0.066667</td>
</tr>
<tr>
<td>Non- smoker</td>
<td>14</td>
<td>0.933333</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>
considered to be the variable of patient characteristics because high blood vessels functions.

Diabetic patients even have chances to develop heart disease because there is a great link between these two conditions. Therefore patients’ blood sugar levels were compared with the control of blood sugar levels and there seems to be a very high significance difference at a 95% confidence blood glucose can damage blood vessels and interval and p-value of 0.0006. The calculated difference between their means was 59.56 and heart disease patients (182±8.874**), and control (122.5±3.23).

Patient physical examination was very important as their life style has a greater impact on their present condition. Vigorous physical activity includes regular exercise, running, and gaming as we know exercise keeps our body active, and heart patients are recommended to keep themselves more active to keep their heart muscle strong. Exercise prevents chest pain and other symptoms associated with heart disease and instantaneously lowering blood pressure and cholesterol. Only 4% of heart patients follow vigorous physical activity whereas 34% of heart patients do low physical activity as shown in Figure 4, 5 and Table 2.

Smoking is very injurious to health as it causes various diseases including cancer, heart disease, cancer, stroke, diabetes lung diseases, and chronic obstructive pulmonary disease (COPD), which includes chronic bronchitis and emphysema. Smoking has its adverse effects on certain eye diseases, and rheumatoid arthritis. Cigarette contains solid-phase contents like nicotine, phenols, and naphthalene affecting our lung and heart. In our study 18% of subjects were smokers and others were non-smokers. Due to the smokers, the non-smokers are also affected due to the inhalation of harmful discharged gases from a smoker as shown in Table 3.

Stress destructs our health because long-term stress eventually leads to increase levels of cortisol and this in return elevates the levels of triglycerides blood cholesterol, blood pressure, and blood sugar. Discussed earlier they all are the known risk factors of cardiovascular disease and stress involved in promoting the plaque deposition is also at a high peak. Stress could be due to any health problem, job, household work, or life calamity. Heart patients mostly take stress about their health.

**CONCLUSION**

CRP is strongly associated with the high risk for the development of the cardiovascular disease. There was significant association of all parameters with coronary heart disease. The association of C-reactive protein levels with risk of myocardial infarction is largely explained by other risk factors, and the remaining unexplained association, if it exists, is small.

Other cardiovascular disease risk factors like blood pressure, total cholesterol, low-density lipoprotein cholesterol, blood sugar, triglycerides, in heart patient’s issues and having, nerves which has a role in regulating the heart.

**REFERENCES**


15. https://www.hopkinsmedicine.org/health/conditions-and-diseases/atherosclerosis