ORIGINAL ARTICLE

C-REACTIVE PROTEIN LEVEL IN CORONARY ARTERY DISEASE AND ITS CORRELATION WITH SERUM D-DIMER

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Background: C-reactive protein concentration has continuous associations with risk of coronary artery disease, ischemic stroke and death from several cancers. In addition, several studies have shown that CRP could be used to predict first ever myocardial infarction and stroke in healthy subjects, as well as outcome in acute setting. High levels of another biomarker, D-dimer, have been found to be independently associated with occurrence of coronary events. **Methods:** This correlational study was carried out at the Department of Cardiology, Ayub Teaching Hospital Abbottabad, in collaboration with the department of Biochemistry Postgraduate Medical Institute Lahore from 15th July 2013 to 15th May 2014. Patients aged 30 years or more of either gender having coronary artery disease was included in the study. Their serum D-dimer levels and C-reactive protein levels were measured for correlation with coronary artery disease. **Results:** A total of 50 patients of CAD were included in this study. Out of these 30 (60%) were males and 20 (40%) were females. Elevated CRP levels and D-dimer levels were noted in all of these patients. Pearson correlation coefficient test was performed on both CRP and D-dimer levels. Pearson correlation coefficient was calculated to be r= -0.1522 and when a p value was calculated, it was found to be 0.292 which implied that the results were not significant. **Conclusion:** This study showed that there is no correlation between CRP levels and D-dimer levels in patients with Coronary Artery Disease.

Keywords: CRP; Coronary Artery Disease; Hypertension; Smoking; Family history; Diabetes Mellitus; D-dimers

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INTRODUCTION

The Coronary Artery Disease (CAD), which is also known by the term Coronary heart disease or CHD, is characterized by a reduction in the lumen of the blood vessels supplying blood and oxygen to the myocardium: the coronary arteries. This reduction in blood & oxygen supply to myocardium leads to development of symptoms of angina characterized by chest pain, discomfort and tightness in chest. The pain of angina usually radiates across the chest to different structures including throat, jaw, neck, shoulders and the arms. It also radiates to back in some cases.[1]

In United Kingdom, 94,000 deaths occur each year due to coronary artery disease.[2.] Higher levels of C-reactive protein were noted in patients diagnosed with coronary artery disease when compared with healthy people.

The C-reactive protein is one of the acute phase reactants which belong to the pentraxin group. Almost all of CRP in the body is produced by the hepatocytes and this production is under the control of cytokines. Therefore it is safe to assume that the measurement of C-reactive protein levels in routine may help the physicians in an improvised stratification of the risk of developing Coronary Artery Disease (CAD).[3] The levels of C-reactive protein in blood have a persistent relationship with the risk of CAD and ischemic cerebral stroke.

D-dimer is one of the important FDPs containing two D domains and one E domain of the original fibrinogen molecule. Elevated D-dimer level has been found to play a role in predicting future cardiovascular events such as acute myocardial infarction and death due to cardiovascular causes.[4] An increase in fibrinolytic activity in patients with atheromatosis, reflected by the increase in D-dimer concentrations in the severe atheromatous group in subjects with CAD diagnosed by coronary angiography, suggests a greater fibrin deposition with consequent action of the fibrinolytic system.[5]

In sensitivity test for detecting coronary artery disease events, D-dimer and glycoprotein were much more sensitive than other parameter.[6] Elevated levels of D-dimers have been found to be present following administration of streptokinase as part of treatment protocol to patients diagnosed with myocardial infarction.[7] Age affects the level of D-dimer in plasma and the plasma levels of D-dimers have been found to increase with age, irrespective of the presence or absence of coronary artery disease (CAD).[8]

The aim of this study is to correlate the levels of C- reactive protein and D-dimers, which are markers of coronary artery disease, in Pakistani

community in order to asses and thereafter to take appropriate measures to help decrease the risk of mortality in this population.

MATERIAL AND METHODS

This correlational study was conducted in accordance with the current Good Clinical Practices (GCP). The clinical data collection and sampling was done at Cardiology Unit Department, Ayub Teaching Hospital Abbottabad, in collaboration with the Department of Biochemistry Postgraduate Medical Institute Lahore.

Fifty subjects were enrolled in the study. The study population comprised of adult patients selected through convenient sampling method, both males and females, who were at least 30 years old at the time of participation in this study. Patients who had symptoms and signs suggestive of coronary artery disease, patients who had electrocardiographic changes e.g., ST-segment elevation, ST-segment depression, unstable angina, and patients with Stable angina who had established coronary artery disease on angiography were included in the study. After obtaining the consent of patients to participate in the study, a vein was identified for drawing blood from the patients. The right brachial vein was selected in all patients and blood from left brachial vein was drawn only when the right brachial vein was not accessible. Five millilitre (ml) of blood sample was drawn within 4–6 hours after admission using 5 ml disposable syringe from the study groups by vein puncture under standard aseptic conditions. Two ml of blood was put into PT bottle containing 3.2 w/v sodium citrate as anticoagulant. The blood was then centrifuged at 2500 rpm for 10 minutes to obtain plasma and D-dimer levels were then determined using D-dimer kit. Remaining 3 ml of blood was allowed to clot in gel tube. It was then centrifuged and was used to measure C-reactive protein (High sensitivity) level with kit method. D-dimer test was performed on plasma and serum levels of C-reactive protein (High sensitivity) were measured. The D-dimer and C-reactive protein (High sensitivity) test were done as per protocol of kit manufacture and results were duly recorded.

RESULTS

This study recruited fifty patients from the Cardiology Unit Department of Ayub Teaching Hospital Abbottabad who had any of the inclusion criteria as identified in the study design. Of the 50 patients, 30 patients (60%) were males and 20 (40%) were females. Mean age of the study participants was 57.76 years and a standard deviation of 13.32. Mean arterial pulse was 79.68 per minute with a standard deviation of 12.66. Mean Systolic Blood pressure was noted to be 140.40 mmHg with a standard deviation of 31.26. Mean Diastolic Blood Pressure was 94.6 mmHg with a standard deviation of 21.20 (Table-1)

Mean CRP levels in the study population were 7.32±3.56 mg/L while mean D-dimer levels in the population were 2729.63±2445.06. Lowest CRP levels were 0.23 mg/L and maximum CRP levels were 13.00 mg/L. Likewise, lowest D-dimer levels in the study subjects were 228.0 ng/ml and highest D-dimer levels were 10000 ng/ml. (Table-2).

Mean CRP levels in males were 7.21 mg/L with a standard deviation of 3.85. In females, the mean CRP levels were higher as compared to males: 7.47 mg/L with a standard deviation of 3.19 (p=0.253). Mean D-dimer levels in males were 2594.19 with a standard deviation of 2382.82. On the other hand, mean D-dimer levels in females were 2932.81 with a standard deviation of 2584.42 (p=0.466). Among smokers, mean CRP levels were 7.19 mg/L with a standard deviation of 4.07. Nonsmokers had a mean CRP level of 7.37 mg/L with a standard deviation of 3.37 (p=0.132). Mean D-dimer levels in smokers were 2345.36 with a standard deviation of 1332.69 while mean D-dimer levels in non-smokers were 2894.33 with a standard deviation of 2791.31 (p=0.462). Mean CRP levels among hypertensives were 7.11 mg/L with a standard deviation of 3.74 while non-hypertensive patients had a mean CRP level of 8.05 with a standard deviation of 2.92 (p=0.814). Among hypertensive patients, mean D-dimer levels were 2800.20 with a standard deviation of 2735.22. The mean D-dimer levels among non-hypertensive patients were 2479.46 with a standard deviation of 880.88 (p=0.281). Those patients who had a positive family history of ischemic heart disease had mean CRP level of 8.01 mg/L with a standard deviation of 4.21. On the other hand, mean CRP levels in patients with a negative family history of Ischemic heart disease were 7.18 mg/L with a standard deviation of 3.47 (p=0.416). Similarly, mean D-dimer level in patients with a positive family history of ischemic heart disease was 4390.02 with a standard deviation of 3676.28 and mean D-dimer level in patients with any such history was 2413.37 with a standard deviation of 2048.65 (p=0.470). Patients with any type of diabetes mellitus had a mean CRP level of 8.10 mg/L with a standard deviation of 2.86 (p=0.505). These patients had a mean D-dimer level of 1835.13 with a standard deviation of 1140.84. Non diabetics had a mean CRP level of 6.88 mg/L with a standard deviation of 3.88 and a mean D-dimer level of 3232.80 with a standard deviation of 2830.18 (p=0.281). Patients who had a past history of Ischemic Heart Disease had a mean CRP level of 9.14 mg/L with a standard deviation of 2.75. The mean D-dimer level in the same group of patients was 2762.16 with a

standard deviation of 2791.20. In patients who did not have a past history of ischemic heart disease, mean CRP and D-Dimer levels were 6.80 mg/L and 2720.46 respectively. The standard deviations for CRP level and D-dimer level were 3.63 and 2378.72 respectively. The *p* values were (p=0.551) & (p=0.387) for CRP and D-dimers respectively. Mean CRP levels in people with obesity was 7.33 mg/L with a standard deviation of 3.72. The mean D-dimer level in obese patients was 2270.75 with a standard deviation of 1159.46. Mean CRP level in patients who did not have obesity was 7.31 mg/L and a standard deviation of 3.53 was noted. Mean D-dimer levels in these patients were 3010.88 with a standard deviation of 2956. The *p* values were (p=0.301) & (p=0.357) for CRP and D-dimers respectively (Table-3 I, II).

When the study sub-groups were further analysed to determine the correlation of CRP and D-dimers, it was found that there was significant correlation between D-dimer and CRP in the female study population (p=0.006). There was no correlation between CRP and D-dimers with respect to the smoking status, presence or absence of Diabetes, Hypertension, family history of ischemic heart disease and obesity. (Figures 1-2)

Table-1: Descriptive statistics of age, arterial pulse and blood pressure (n=50)

Descriptive Statistics	Mean	SD
Age	57.76	13.318
Arterial Pulse in beats per minute	79.68	12.658
Systolic Blood Pressure	140.40	31.26320
Diastolic Blood Pressure	94.6000	21.20935

Table-2: Descriptive statistics for CRP and D-Dimer levels

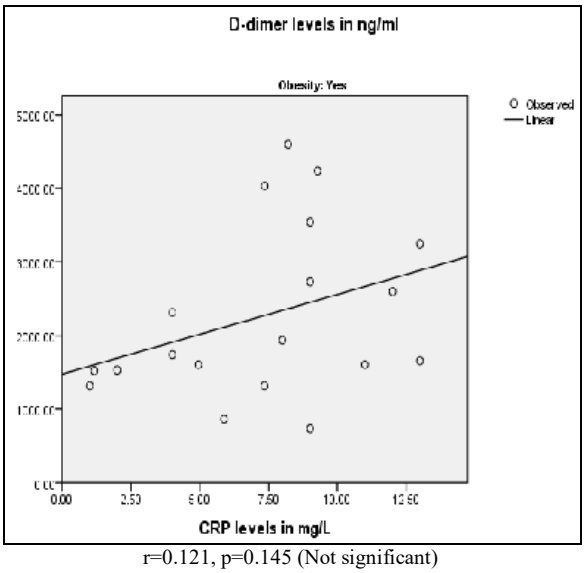
	CRP levels in mg/L	D-dimer levels in ng/ml
N	50	50
Minimum	0.23	228
Maximum	13.00	10000
Mean	7.32	2729.64
Std. Deviation	3.57	2445.06

Table-3(i): *p* values for CRP and D-dimers in the study population

Categories	CRP levels (Mean±SD)	*p* value
Male	7.21±3.85	
Female	7.47±3.19	0.253
Smokers	7.19±4.07	
Non-Smokers	7.37±3.37	0.132
Hypertensives	7.11±3.74	
Non-Hypertensives	8.05±2.92	0.814
Family Hx. Of IHD	8.01±4.21	
No Family Hx of IHD	7.18±3.47	0.416
Diabetes Mellitus	8.10±2.86	
No Diabetes Mellitus	6.88±3.88	0.505
Past Hx of IHD in patients	9.14±2.75	
No Past Hx of IHD in patients	6.80±3.63	0.551
Obesity	7.33±3.72	
No Obesity	7.31±3.53	0.301

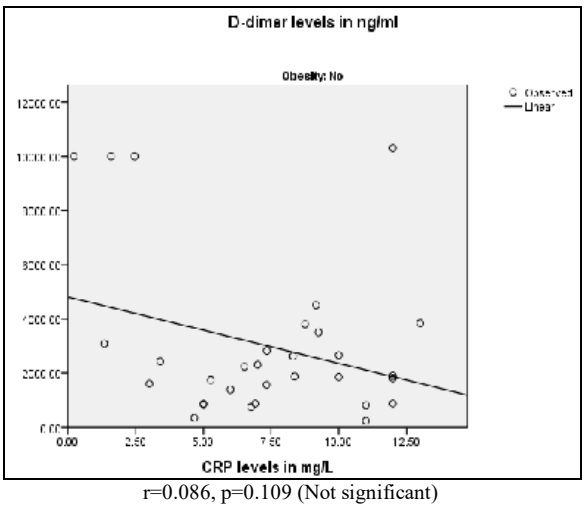
Table-3(ii): *p* values for CRP and D-dimers in the study population

Categories	D-dimer levels (Mean±SD)	*p* value
Male	2594.19±2382.82	
Female	2932.41±2584.42	0.466
Smokers	2345.35±1332.69	
Non-Smokers	2894.33±2791.31	0.462
Hypertensives	2800.20±2735.22	
Non-Hypertensives	2479.45±880.87	0.281
Family Hx. Of IHD	4390.02±3676.28	
No Family Hx of IHD	2413.37±2048.65	0.470
Diabetes Mellitus	1835.13±1140.84	
No Diabetes Mellitus	3232.80±2830.18	0.281
Past Hx of IHD in patients	2762.16±2791.19	
No Past Hx of IHD in patients	2720.46±2378.71	0.387
Obesity	2270.75±1159.46	
No Obesity	3010.89±2956.83	0.357



r=0.121, p=0.145 (Not significant)

Figure-1: Correlation of CRP with D-dimers in obese patients



r=0.086, p=0.109 (Not significant)

Figure-2: Correlation of CRP and D-dimers in non-obese patients

DISCUSSION

C-reactive protein (CRP), a biomarker and an acute phase reaction protein is one of the plasma components of the response to inflammation in body. A number of studies have connected this molecule with the risk of future cardiovascular disease events in a person (Danesh *et al.*).[9] High serum levels of CRP were recorded in patients with a history of cigarette smoking and obesity (Danesh *et al.)*[10]

The mean CRP and D-dimer levels were higher in the patients who reported that they were non-smokers and people who were not obese. However, since they also had co-morbidities and were admitted in hospital, the higher levels of CRP in these patients could be explained on the basis of presence of other conditions that might have affected serum levels of these biomarkers.

CRP levels have been known to be increased in smokers (Ohsawa *et al).*[11] In contrast, the association between number of cigarettes smoked and elevated CRP levels was found. They noted that the odds ratio increased with the number of cigarettes smoked and the CRP levels were higher in heavy cigarette smokers in past month (O'Loughlin *et al*).[12] The CRP levels in this study were higher in non-smokers (7.37 mg/L) as compared to smokers (7.19 mg/L) but this can be explained by the fact that no effort was made to determine whether those who reported to be no smokers were actually life-long non-smokers or had quit smoking recently. In this study no significant correlation was found between CRP and D-Dimers when compared among smokers and non-smokers (p 0.12 and 0.074 respectively).

An association between elevated blood pressure and increased CRP has also been reported. (Shafi Dar *et al.* [13] but it was noted that elevated CRP levels didn't lead to the development of hypertension (Davey Smith *et al).*[14] In this study the CRP levels in non-hypertensive patients (8.05 mg/L) were elevated as compared to hypertensive study subjects (7.11 mg/L). This could be explained on the basis of the fact that the study sample was small and only 50 patients were included in the study. Plus the non - hypertensive patients were not healthy patients and they probably had other co-morbidities which affected the CRP levels in these patients. There was no significant correlation between CRP and D-Dimers in the hypertensive and the non-hypertensive study participants (p 0.28 and 0.454 respectively).

The association of diabetes mellitus either alone or in association with other diseases with increased levels of CRP has been identified in different studies Mugabo *et al.*[15] Elevated CRP levels in diabetes mellitus probably reflect the levels of low-grade inflammation (Mugabo *et al*).[15] However, CRP elevation was noted in association with other factors in diabetic patients, for example microalbuminuria and hypertension (Lima *et al*. 2007).[16] This study had a small sample size i.e., 50 patients and because patients had co-morbidities, it was difficult to ascertain if the elevated CRP levels were due solely to diabetes mellitus or presence of co-morbidities but the CRP levels in this study were found to be elevated in patients with diabetes mellitus compared to non-diabetic patients and these results were in line with earlier published studies but there was no linear correlation between CRP and D-dimer level in diabetics as well as non-diabetic patients (p 0.92 and 0.481 respectively). In this study the CRP levels were high in patients with a prior history of ischemic heart disease. It has been a well-known fact that high CRP levels are associated with cardiovascular disease but no significant relationship between CRP and D-Dimer level was noted in patients with or without past history of ischemic heart disease (p 0.38 and 0.10 respectively).

Mean CRP levels in the study population were 7.32 mg/L with a standard deviation of 3.56 while mean D-dimer levels in the population were 2729.63 ng/ml with a standard deviation of 2445.06. Pearson Correlation Coefficient test was applied on CRP and D-dimer levels to find if they were correlated and the results showed that these two biomarkers were not significantly correlated. (p-value =0.291).

A prospective evaluation of relationship between CRP, D-dimers and progression of peripheral arterial disease.[17] found that in symptomatic patients elevated baseline DD, a marker of thrombotic activity, was significantly associated with the occurrence of myocardial infarction. The study did not find a relationship between progression of PAD and baseline DD or CRP during the first 3 years.[17] On the other hand, a recent study by Wen *et al* reported that in addition to other factors, D-dimer levels $\geq$5.67 μg/mL and CRP levels $\geq$11.21 mg/L were important risk factors and independently associated with acute Aortic Dissection related in-hospital death.[18] Although my study does not confirm to the already reported association of CRP and/or D-dimers with the development of coronary heart disease, the results were promising and indicated toward a possible role for the CRP and D-dimers. These biomarkers alone have been proved to be associated with the coronary artery disease.

CONCLUSION

This study shows that there is no correlation of CRP with the D-dimer levels in patients with the coronary artery disease. The association between these two biomarkers is insignificant according to this study

except in females where a significant association was found (p 0.006). One of the main limitation of this study was that it was a small scale correlation study. Further large scale research is required to approve or disprove these findings in the region and also to describe the effect of presence of risk factors on the relationship between CRP and D-dimers.

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