ORIGINAL ARTICLE
ASSOCIATION OF ADIPONECTIN WITH TOTAL LEUKOCYTE COUNT IN PATIENTS OF CORONARY ARTERY DISEASE

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Background: Hypoadiponectinemia and raised total leukocyte count have been associated with coronary artery disease. The aim of this study was to investigate association of serum adiponectin levels with total leukocyte count in patients of coronary artery disease belonging to Khyber Pakhtunkhwa. **Methods:** This cross-sectional/analytical study consisted of two groups. Group A contained 100 patients of coronary artery disease while group B contained 100 healthy controls. Consent of the study subjects was obtained, their history was recorded and fasting blood samples were analyzed for serum adiponectin level, total leukocyte count (TLC), serum lipid profile which included serum total cholesterol (T-C), triglyceride level (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Adiponectin level was determined with ELIZA method, TLC was estimated on automated haematology analyzer and lipid profile was determined using enzymatic colorimetric method. SPSS version 21 was used to analyze the data. **Results:** Subjects with coronary artery disease when compared to healthy subjects showed significantly high level of total leukocyte count (9.26±1.488 vs. 6.37±4.052) and low level of serum adiponectin (4.3±0.80 vs. 9.6±3.69). Moreover, serum lipid profile showed low HDL-C (30.04±9.1 vs. 43.64±7.3) and rose triglyceride (220.1±67.7 vs. 181.86±41.4), total cholesterol (229.3±37.01 vs. 189.4±32.7), and LDL-C (153.78±38.53 vs. 109.16±33.91) levels. Significant negative association of adiponectin with TLC (r -0.826 with $p<0.01$) was observed in the study subjects. **Conclusion:** We observed elevated level of total leukocyte count and reduced level of adiponectin in subjects with coronary artery disease. Moreover, hypoadiponectinemia correlated negatively with TLC levels.

Keywords: Adiponectin, Total Leukocyte Count; Coronary Artery Disease

INTRODUCTION

Coronary artery disease (CAD) is one the most frequent causes of morbidity and/ or mortality in developed and the developing world. The global prevalence of CAD is expected to double from 1990 to 2030, 82% load being contributed by the developing countries.[1,2] Chronic inflammation plays a pivotal pathogenic role in the development of coronary artery disease. Elevated levels of circulating inflammatory markers, such as total leukocyte count, increase the risk of CAD. Raised total leukocyte count indicates inflammation and may act as a risk factor for CAD due to its role in the phagocyte dependent vascular injury, atherosclerotic vascular disease, rupture of atherosclerotic plaque and thrombus formation. Several studies have reported the association of total leukocyte count with the incidence and adverse outcome of CAD.[3–5]

Adiponectin is a protein hormone secreted by white adipose tissue and is one of the agents which may be involved in the protection of coronary vessels from atherosclerosis. Serum adiponectin concentrations range from 3-30 µg/mL, and is observed to be reduced with obesity.[6] Adiponectin tends to inhibit inflammation and atherosclerosis by decreasing monocyte adhesion with endothelial cells through many mechanisms, including: i. reduced expression of sVCAM-1 (vascular cell adhesion molecule), sICAM-1 (intracellular adhesion molecule) and E-selectin (endothelial-selectin) ii. reduced nuclear factor β iii. reduced foam cell formation and, iv. reduced endothelial migration/proliferation of vascular smooth muscle cells.[7] This vasculo-protective role of adiponectin has made the researchers to regard it as an important biomarker of CAD risk.[8–10] The aim of the present study is to find the levels of total leukocyte count and adiponectin and find possible association between the two, in cardiovascular patients of Khyber Pakhtunkhwa (KPK).

MATERIAL AND METHODS

This was a cross-sectional/analytical study. The study was carried out on two groups. Group A consisted of hundred subjects with coronary artery disease and included patients with first attack of myocardial infarction (not more than previous ten days). Group B consisted of hundred healthy subjects and included those who had no major health issues including hypertension, diabetes mellitus, coronary artery disease, liver, thyroid or kidney disease. The study subjects were randomly selected from the out-patients departments of the following three tertiary care hospitals of Peshawar; Lady Reading Hospital (LRH), Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC). Written consent was obtained from all subjects and the study was approved by the Ethical Committee of Khyber Medical College, Peshawar. A well-designed questionnaire was used to record complete history and physical examination details, i.e., blood pressure, BMI (body mass index: weight in Kg/height in m^2) etc.

We collected 5 mL fasting blood sample from the study subjects. Total leukocyte count was carried out on blood taken in EDTA tubes while serum was used to analyze lipid profile and adiponectin levels.

Triglyceride (TG) and serum total cholesterol (TC) were determined using enzymatic colorimetric method; the kits were obtained from Elitech-Sees, France. Kit obtained from Diasys Holzheim was utilized for calculating high density lipoprotein cholesterol (HDL-C) colorimetrically. Friedewald's formula[11] was used to calculate low density lipoprotein cholesterol (LDL-C). Serum adiponectin was measured with enzyme linked immunosorbent assay technique (ELIZA), using Human Adiponectin ELISA kit (Biovendor Cat. No. RD 195023100, Germany). TLC was measured with the help of automated haematology analyzer.

All the data was analyzed with SPSS version 21 and results were expressed as means±SD (standard deviation). Variables between the two groups were compared by independent student's t test. P value less than 0.05 was considered as significant. Any possible association between adiponectin and other variables including TLC and lipid profile was established using Pearson's correlation coefficient r.

RESULTS

Table 1 shows the comparison of variables between the two groups. It can be seen that TLC levels are significantly higher (9.26±1.488 vs. 6.37±4.052, $p<0.05$) in patients with CAD than normal control.

Serum adiponectin levels are significantly lower (4.3±0.80 vs. 9.6±3.69, $p<0.05$) in patients with CAD than control group. Lipid profile shows significant abnormalities in patients with CAD. Total cholesterol (229.3±37.01 vs. 189.4±32.7), serum triglyceride levels (220.1±67.7 vs. 181.86±41.4) and LDL-C levels (153.78±38.53 vs. 109.16±33.91) are significantly higher in patients with CAD ($p<0.05$). Serum HDL-C was significantly lower in patients with CAD (30.04±9.1 vs. 43.64±7.3) with p-value <0.05.

Table-2 shows association of adiponectin level with TLC and lipid profile. The association was established using Pearson's correlation coefficient r. p-value of less than 0.05 was considered significant and that of less than 0.01 was considered highly significant. It can be seen that adiponectin has a significant negative association with TLC (r -0.826, $p<0.01$), TC (r -0.401, p 0.037) and TG (r -0.568, $p<0.01$). Serum adiponectin level also showed a significant positive association with HDL-C (r 0.874, $p<0.01$)

Table-1: Comparison of variables between the study groups

Variables	Group A (cases) n=60	Group B (control) n=60	p-value
Age (years)	62±8.8	55±3.8	<0.05
TC (mg/dL)	229.3±37.01	189.4±32.7	<0.05
TG (mg/dL)	220.1±67.7	181.86±41.4	<0.05
HDL-C(mg/dL)	30.04±9.1	43.64±7.3	<0.05
LDL-C (mg/dL)	153.78±38.53	109.16±33.91	<0.05
TLC (mm₃)	9.26±1.488	6.37±4.052	<0.05
Adiponectin(µg/mL)	4.3±0.80	9.6±3.69	<0.05

Note: *p-value <0.05 is considered as significant

Table-2: Correlation of adiponectin with TLC and other parameters in the study subjects

Variables	r	p
TLC	-0.826	< 0.01**
TC	-0.401	0.037*
TG	-0.568	< 0.01**
HDL-C	0.874	< 0.01**
LDL-C	-0.378	0.077

*Significance at 0.05 level. **Significance at 0.01 level

DISCUSSION

Research has extensively established the role of inflammation and atherosclerosis in the development and progression of coronary artery disease. Inflammation has been observed to play role in the formation, progression and instability of atherosclerotic plaque leading to adverse coronary outcomes including myocardial infarction.[12, 13]

In our study we observed markedly elevated levels of TLC in patients with MI as compared to the control group. These results are similar to the observations reported by other studies.[14-17] Raised

total leukocyte count is an indicator of inflammation because it acts as a host defence to injury. In CAD, leukocytes not only cause oxidative and proteolytic injury to the coronary arteries but also aggregate and impact the microvasculature thus leading to hypercoagulable state and electrical instability in the heart.[18,19] There are other studies which have linked elevated leukocyte count in patients of myocardial infarction with increased mortality rate as well as post myocardial infarction complications.[20–24] An increase in the recurrence of vascular disease was reported by Grau and colleagues in patients who had MI, peripheral artery disease or ischemic stroke.[25] A large meta-analysis of 7 studies comprising of 5,337 CAD patients performed by Danesh *et al* reported an association of high leukocyte count with a higher risk of coronary artery disease.[26] The above discussion throws light on the strong and consistent relationship between total leukocyte count and coronary artery disease in different populations.

In the present study, we observed significantly lower levels of serum adiponectin in patients with MI than the control subjects. This hypoadiponectinemia was also associated with dyslipidaemia and raised total leukocyte count in the study subjects. Similar results have been observed in other studies.[27–29] Liang *et al* reported an association of higher adiponectin levels in patients of MI at admission with lesser myocardial damage and better future outcomes in the study population .[30] The beneficial effects of higher adiponectin in patients with CAD are attributed to its anti-inflammatory and anti-atherogenic effects.[31,32] A meta-analysis performed by Zhengie *et al* reported no association between circulatory adiponectin levels and CAD in diabetic patients.[33] They have attributed this contradiction to a small sample size and a small follow-up duration time and recommend a larger well-structured prospective trial in future.

In this study we observed a significant negative association between serum adiponectin and TLC levels as well as with dyslipidemia.[28–30]

Limitations: This study was conducted on a relatively smaller sample size which may account for its limitations.

CONCLUSION

This study confirms the relationship of higher total leukocyte count and lower adiponectin levels in patients with coronary artery disease of Khyber Pakhtunkhwa. Moreover; this data may encourage a potential diagnostic and prognostic role of both total leukocyte count and serum adiponectin in coronary artery disease.

AUTHORS' CONTRIBUTION

SD, SA, UR: Conceptualization of the study design, Data analysis and data interpretation. Mk, MAK, RI: Data collection, interpretation. All authors approved the Final version for publication and agreed to be accountable for all aspects of the work

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