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RESEARCH ARTICLE

SERUM INTERLEUKIN-6 AND ALKALINE PHOSPHATASE AS PREDICTORS FOR SEVERITY AND PROGNOSIS OF CORONARY ARTERY DISEASE

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ABSTRACT

Background: Coronary Artery Disease results from accumulation of atheroma that reduces blood flow leading to ischemia and later infarction. Cardiac enzymes like cardiac troponin I and creatinine kinase MB are measured to further establish the diagnosis but not its prognosis. IL-6 increases at an early stage of atherosclerosis and peaks during myocardial infarction. Alkaline phosphatase is responsible for dephosphorylation of pyrophosphate, a calcification inhibitor which leads to calcification of blood vessels.

Aim: This study is to investigate serum levels of IL-6 and ALP and their connection to the severity of CAD and also to determine if IL-6 and ALP are correlated or independent predictors of the severity of CAD.

Methods: Fasted blood samples were collected to test for IL-6 and Alkaline Phosphatase from August 2016 to February 2017 in 80 patients with positive results of coronary angiographies at Qilu Hospital of Shandong University and divided into four groups; control (20), stable angina (20), unstable angina (20) and acute myocardial infarction (20).

Results: IL-6 and CAD showed a significant relationship with a p value of 0.018 (P value < 0.05). Additionally, ALP and CAD showed a very significant relationship (P < 0.001).

However, IL-6 and ALP yielded a negative correlation coefficient value (r= -0.032) and p value was 0.779 (P > 0.05).

Conclusion: Results from our study showed that there is a significant correlation between both IL-6 and ALP to the severity of CAD. However, there is no relationship between IL-6 and ALP as their role in CAD progression is independent of each other.

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INTRODUCTION

Coronary artery disease (CAD) is the most common reason for heart related deaths is most parts of the world with an increasing prevalence. The concept of CAD is quite complex and in reference to symptoms, it is known to manifest in three stages. These stages; Stable Angina Pectoris (SAP), Unstable Angina Pectoris (UAP) and Acute Myocardial Infarction (AMI) have different clinical manifestations of CAD, and they are related to varying prognostic outcomes (McCarthy *et al.,* 1990). The differences in clinical manifestations seem to have a connection with the presence of stable, vulnerable or ruptured coronary plaques. To make a definitive diagnosis of Acute Coronary Syndrome (ACS) in patients with myocardial infarction, cardiac enzymes such as cardiac troponin I (CTNI) and Creatinine Kinase MB (CK-MB) are measured (Slater *et*

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al., 1987). Researchers have been on the lookout for biomarkers that will predict severity and prognosis of CAD, which will later determine the managements that will reduce mortality and morbidity. Inflammatory cytokines such as Interleukin-6 play a major role in plaque formation, vulnerability and rupture (Croker, 2012). IL-6, a multifunctional cytokine not only has a vast range of effects in inflammation but also regulates the processes associated with the immune system. It is involved in the acute phase reaction by reason of its ability to increase in levels both at the site of injury such as endothelial injury and in serum. However, IL-6 has a dual effect because it acts as a defence mechanism at some levels but in chronic inflammation it acts as pro-inflammatory cytokine (Gabay, 2006). IL-6 is the principal stimulator of most acute phase proteins, whereas the other associated cytokines influence subgroups of acute phase proteins (Gauldie, 1987). It is significantly involved in physiological and pathological processes which leads to induction of C - reactive protein (CRP) (Lind, 2003). Recent studies suggest that IL-6 is a better and a more specific marker for CAD than CRP. Further studies have shown that IL-6 values were independent from CRP, but not vice versa, suggesting that IL-6 increases at an earlier stage of atherosclerosis but reaches its peak at vulnerability and rupture of plaque (Lee, 2007). Alkaline Phosphatase, a hydrolase enzyme is responsible for dephosphorylating many types of molecules. It is present in many tissues types and is also established as a biomarker in bone related diseases, hepatobiliary diseases and end stage kidney disease (Kunutsor *et al.,* 2015). Serum ALP liberates inorganic phosphate by catalysing the hydrolysis of organic pyrophosphate which is responsible for inhibiting vascular calcification (Park *et al.,* 2013) Pyrophosphate is known to protect vascular integrity, but a chronic elevation of ALP may promote vascular calcification (Sahin *et al.,* 2014). Previous studies state a synergy between IL-6 and ALP with reference to wound repair (Gallo *et al.,* 1997). IL-6 could induce the release of ALP due to its inflammatory property as both proteins may be secreted by the liver. Our objective in this study was to investigate serum levels of IL-6 and ALP and their relationship to severity of CAD and to determine if IL-6 and ALP are co-dependent predictors of the severity of CAD.

METHODS AND MATERIALS

Blood samples were collected continuously from August 2016 to February 2017 in 80 patients with positive results of coronary angiographies at Qilu Hospital of Shandong University. Subjects included 20 cases of acute ST elevation myocardial infarction, 20 cases of unstable angina, 20 cases of stable angina and 20 cases of control group with normal results of coronary angiographies. Among them were 45 males and 35 females, with an average age of 61.2 ± 9.6 years. All subjects performed diagnostic criteria developed by the American College of Cardiology (ACC) and the American Heart Association (AHA), excluding all infectious diseases, tumours and connective tissue disease based on history, signs and laboratory tests. Upon admission, blood samples were collected from selected subjects after at least 6 hours of fasting to test for IL-6, ALP, CK-MB and CTNI. The next day, more peripheral blood samples were taken to determine blood lipids, blood glucose and other serological indicators. At the same time, 5 ml of left peripheral venous blood was collected, centrifuged at 3000r / min for 10 minutes and serum separation was done at -80 ℃ cryopreservation. Interleukin 6 and alkaline phosphatase levels were determined by ELISA method using interleukin 6 and ALP kits purchased from Shanghai Xitang Biotechnology Co., Ltd. strictly following instructions of the kit, the microplate reader was detected in accordance with Therom SCIENTIFIC 3001 type from United States. Using SPSS 23.0 statistical software package for statistical analysis. The data of the normal distribution were expressed as mean ± standard deviation. The count data were analysed by $\chi 2$ test and ANOVA variance analysis was used to compare the data. Correlation test using Pearson correlation analysis, $P < 0.05$ was statistically significant.

RESULTS

Patient Demographics

The patient demographic results showed that a total number of 80 subjects were included in the study and divided into four groups. The Control group (n=20), Stable angina (n=20), Unstable angina (n=20) and Acute myocardial infarction (n=20), having a mean age of (61.2 ± 9.6). The subjects were composed of 45 males (56.25%) and 35 females (43.75%), this showed a unique pattern as the number of males per group took a crescendo form and females, vice versa. Also, the percentage of patients with corresponding chronic diseases like hypertension continued to increase as the group went from normal subjects (30%) to AMI (70%). Smoking habit also showed an increasing pattern from normal to most severe. The peripheral white blood cells and neutrophil count of AMI group were significantly higher than those of UAP group, SAP group and control group (Table 1).

Table 1. Patient Demographics

Group	AMI(n=20)	UAP(n=20)	SAP(n=20)	Control (n=20)
Age	63.5 ± 10.2	62.6 ± 8.0	58.5 ± 8.2	60.1 ± 11.2
Gender/Male	15 (75.0%)	13 (65.0%)	10 (50.0%)	8（40.0%）
Smoking History	11 (55.0%)*	11 (55.0%)*	8(40.0%)*	5(25.0%)*
Hypertension	14（70.0%）*	12(60.0%)*	9(45.0%)*	6（30.0%）
Diabetes	7 (35.0%)*	16 (80.0%)*	3 (15.0%)*	4（20%）
Family History	9 (45.0%)*	6 (30.0%)*	2(10.0%)*	5 (25.0%)
TG(mmol/l)	2.03±0.97 *	1.90±0.95	1.53±0.71	1.27±0.50
CHO(mmol/l)	5.20±1.50*	4.86±1.05	4.552±0.91	4.36±1.16
LDL-C(mmol/l)	3.01±1.21*	2.82±0.86	2.59±0.81	1.62±0.75
HDL(mmol/l)	1.13±0.31	1.13±0.25	1.18±0.38	1.18±0.36
GLU(mmol/l)	8.47±2.80*#	5.79±1.32	5.44±1.14	5.50±0.66
WBC ($\times 10^9$/L)	7.90±3.36*#$	7.15±2.39#	7.01±2.164	6.01 ±1.68
NEU%	71.65±12.8*#$	62.64±7.40#	62.00±11.21	62.18±9.67

Compared with the control group * P <0.05; Compared with SAP group # P <0.05; Compared with UAP group $ P <0.05.

Association between Serum IL-6, ALP and CAD Severity

Correlation between IL-6, ALP and CAD severity

Obtained results showed significant correlation between IL-6 and CAD (P value < 0.05) indicating that change in severity of CAD is associated with serum IL-6 increase. ALP showed more significant correlation with severity of CAD (P < 0.01). This also indicates that changes in ALP levels can lead us to predict the extent of CAD. Table 2

Table 2. Association between Serum IL-6, ALP and CAD Severity

Group	IL-6	ALP
AMI(n=20)	0.257±0.528*	0.160±0.078**
UAP(n=20)	0.171±0.348*	0.088±0.159**
SAP(n=20）	0.076±0.049**	0.07±0.026**
Control(n=20)	0.042±0.027**	0.061±0.026**

*P value < 0.01 **P value < 0.05

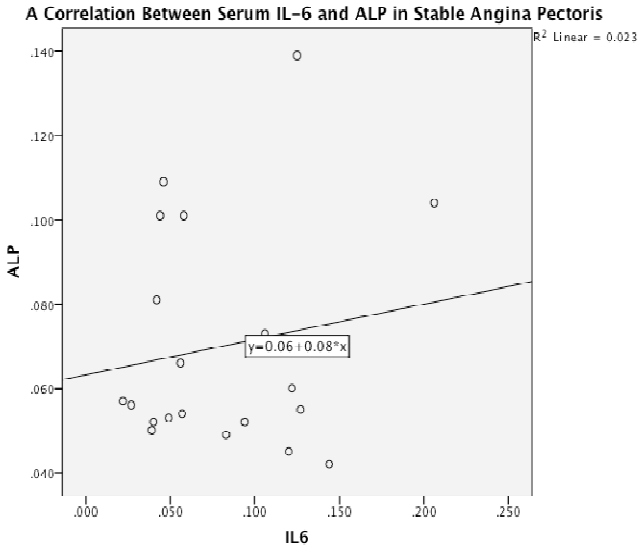

Figure 1. A Correlation between serum IL-6 and ALP in Stable Angina Pectoris

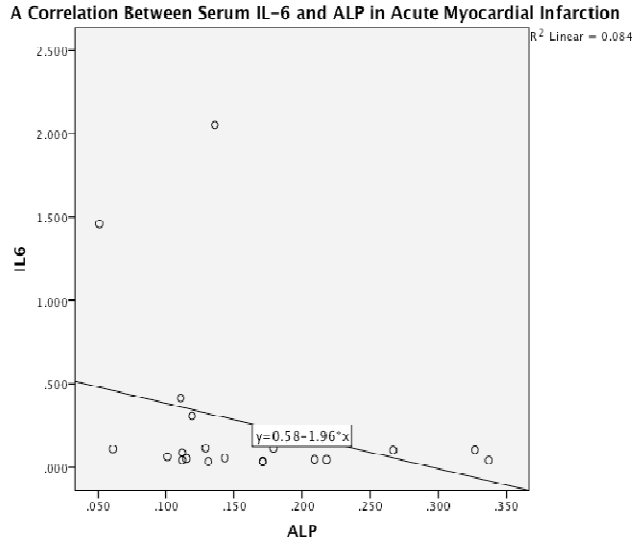

Figure 2. A Correlation between Serum IL-6 and ALP in Unstable Angina

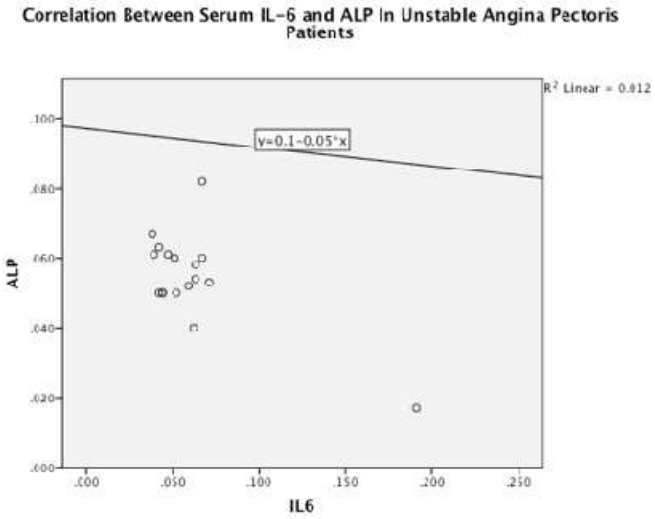

Figure 3. A Correlation between Serum IL-6 and ALP in Acute Myocardial Infarction

Association between SerumIL-6 and ALP

IL-6 and ALP correlation was calculated in SAP group (Figure 1), UAP group (Figure 2) and AMI group (Figure 3) independently. There was a positive correlation in the SAP group and negative correlation in both UAP and AMI groups. However, all three sets of correlations were defined as no linear correlation between IL-6 and ALP due to their r value (coefficient of correlation) being close to zero (SAP r=0.1513, p>0.05; UAP r=0.1113, p >0.05; AMI r=0.2898, p>0.05). Therefore, IL-6 does not affect the release of ALP and vice versa.

DISCUSSION

Coronary artery disease is the leading cause of death in the globe, recording about 17.3 million deaths in a year, this number is expected to rise to more than 23.6million by the year 2030. Between the years from 2001 to 2011, the mortality rate as a result of heart disease has fallen to about 39 percent, but the burden and risk factors remain critically high (Sanchis-Gomar *et al.,* 2016). Atherosclerosis is the chief cause of CAD, in which atherosclerotic changes expresses itself within the walls (mainly the intima) of the coronary arteries. CAD is a progressive disease process that escalates with age and certain lifestyle choices, beginning in childhood and reveals itself clinically in the middle age to late adulthood stage of life. CAD is a complex inflammatory process characterized by atherosclerosis in the medium-sized and large epicardial coronary arteries. Atherosclerosis is the hoarding of biomaterials like macrophages (i.e. foam cells), smooth muscle cells and lipids in the coronary artery lumen. This continuous accumulation leads to a progressive narrowing and blockage of myocardial blood flow resulting in myocardial ischemia (Benjamin *et al.,* 2017). CAD is primarily the intrinsic cause of myocardial ischemia and infarction. Stable CAD is concomitant with chronic stenosis in the coronary arteries which as a result, leads to symptoms, most commonly chest pain (angina) due to ischemia at a certain workload. Stable CAD most often referred to as stable angina pectoris (SAP) is featured by unchanged or slowly progressing symptoms over time. A sudden change of symptoms is associated with unstable CAD, as a consequence of plaque rupture which leads to thrombus formation in the artery. When a plaque ruptures and sub endothelial vessel structures come into contact with blood cells, a thrombus formation is triggered, creating a dynamic occlusion in the coronary artery. Unstable CAD also commonly referred to as unstable angina pectoris (UAP) carries a high risk of myocardial infarction, thus cardiac death and this situation needs urgent medical intervention. Cardiac enzymes such as CK-MB and CTNI along sides the use of EK Gare functional in the diagnosis and risk stratification of patients with chest pain and suspected acute ACS. The cardiac troponins, in particular, has been the chief marker followed by CK-MB for patients with ACS. Indeed, cardiac troponin is central definition of AMI but CK-MB is still used to rule it out as it presence in the serum connotes active or recent AMI.

However, the above cardiac markers have been used to confirm the diagnosis of CAD but there is yet to be a biomarker to predict the stage of severity and prognosis (O'Gara *et al.,* 2013). Recent studies have shown IL-6 and ALP to be promising markers for predicting the severity of CAD due to their inflammatory properties. IL-6 release leads to intracellular signalling via two trans membrane proteins; a)

80 kDa glycoprotein ligand-binding sub- unit (IL-6R) (b) a membrane bound 130 kDa glycoprotein (Gp130) (Ueda *et al.,* 1999). IL-6 also binds to sIL-6R and as a complex, binds Gp130, this is termed ‘‘Trans IL-6 receptor binding’’. As sIL-6R increases, IL-6 receptor binding shifts towards ‘‘Trans IL-6 receptor binding,’’ and this shift in binding is implicated as a mechanism of inducing inflammation in a number of chronic inflammatory diseases such as CAD (Bhakdi *et al.,* 2004). Unlike other biomarkers that rise and fall within a period of time, IL-6 is shown to rise exponentially as the disease continues to progress, this makes it a suitable predictor for severity and prognosis. Additionally, ALP in recent studies has been stated as a contributor to CAD by vascular calcification. Atherosclerotic calcification occurs in the intimal layer and this leads to further build-up of atherosclerotic plaque. Atherosclerotic calcification involves cellular necrosis, inflammation, and lipid deposition. ALP is responsible for dephosphorylating pyrophosphate, a calcification inhibitor which later leads to calcification of blood vessels.

ALP is now gaining ground as a suitable risk marker that can predict adverse outcomes among patients that have survived AMI (Tonelli *et al.,* 2009). It may also be an independent predictor of mortality, infarction, and stent thrombosis in CAD patients post- PCI with drug eluting stents (DES) (Park *et al.,* 2012). Our study aimed to investigate serum levels of IL-6 and ALP and their relationship to the severity of CAD and also to determine if both IL-6 and ALP are correlated or independent predictors of the severity of CAD. Our main finding showed a steady increase in both IL-6 and ALP, this is significant for predicting prognosis as both proteins increase in serum as the disease progresses. This study showed a significant association between IL-6 and CAD between the 4 groups, showing a steady rise in serum levels as the mean of values in each group increases. A significant increase was noted in the AMI group in comparison to the other groups suggesting the response to plaque vulnerability and possibly, rupture. ALP also showed a slight crescendo behaviour as the group of subjects changed from normal to severe. However, the difference in levels between the control, UAP and SAP where all within normal levels with a significant increase in the AMI group. IL-6 and ALP where not significantly correlated, suggesting that they are independent markers for the severity and prognosis of CAD.

Conclusion

This study demonstrated significant correlation between IL-6 and severity of CAD and ALP with CAD. However, there is no relationship between IL-6 and ALP as their function in CAD is independent of each other. This observation suggest that even though they acts synergistically, both IL-6 and ALP does not affect each other’s function and investigating both in serum may provide a more accurate prediction and prognosis. However, more research is to be done to determine their mechanism and how to improve the quality of life in patients with poor prognosis.

Conflict of interest: The authors declare that there is no conflict of interest.

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