

Interaction between Serum Thrombomodulin and Serum CRP Levels in Predicting Ischemic Heart Disease: A story in reverse direction

MUHAMMAD AKBAR MUGHAL¹, MUHAMMAD ADIL RAMZAN², SYEDA ASIYA PARVEEN³

ABSTRACT

Aim: To find out the association between serum Thrombomodulin (TM) & serum C-reactive protein (CRP) in identifying individuals at risk of ischemic heart disease (IHD) in Pakistani population

Methods: This cross sectional study was conducted on population of Karachi, Pakistan. A total of 96 subjects were randomly selected and were divided into 3 groups i.e., group A= (control group), group B= (Myocardial Infarction [MI] Patients), group C (Unstable Angina [UA] patients). Each group consists of 32 subjects/patients respectively. The diagnoses were made on the basis of history, E.C.G. & serum Troponin I levels. Serum thrombomodulin (TM), Serum C reactive protein (CRP) were determined by ELISA Kit methods. Independent sample t-test was conducted for comparison of serum thrombomodulin (TM) level & serum C reactive protein (CRP) levels between these groups. Pearson Correlation Co-Efficient were used to calculate the co relation between Serum thrombomodulin (TM) and Serum C reactive protein (CRP) levels

Results: Serum Thrombomodulin levels were significantly higher in control group (p-value 0.00), whereas, Serum CRP, BMI, Blood pressure, serum L.D.L. cholesterol and serum total cholesterol levels were significantly higher in diseased group.

Conclusion: The Present study suggests that serum TM levels were high in control group in comparison with diseased group, whereas serum CRP levels were high in diseased group, suggesting inverse relationship between serum TM & CRP in predicting ischemic heart disease

Keywords: Thrombomodulin (TM), C Reactive Protein (CRP), Myocardial Infarction (MI), unstable angina (UA).

INTRODUCTION

The inner lining of all the blood vessels comprised of single layer of flattened, orthogonal cells called as endothelium. It plays a key role through the release of various mediators in the equilibrium of hemostasis, fibrinolysis, regulation of vessel tone and permeability as well as synthesis of growth factor¹. Endothelial cells protect vessel not only by providing a mechanical lining but also by controlling a vascular tone through the release of vasodilators such as nitric oxide (NO) and prostacyclin. It also plays a role in the process of clotting by releasing certain prothrombotic agents including von Willebrand factor, factor V, plasminogen activator inhibitor and tissue factor. These cells also produce a number of anti-coagulant factors. In healthy environment there is a balanced secretion of these products which maintains the integrity of the surface, ensuring protection of the vessel wall and providing a healthy flow. Vascular

damage disturbs the balance and initiates the process of atherosclerosis^{2,3,4}. Therefore a defense mechanism against thrombosis occurs via natural anticoagulant. The major natural anticoagulants involved are:

- Protein C pathway, which inhibit factor Va and VIIIa.
- Tissue factor pathway inhibitor, which blocks tissue factor-factor VII initiation of coagulation.
- Heparin: antithrombin pathway, which is most effective at inhibiting thrombin but also inactivates factor IXa, Xa, XI
- Thrombomodulin: a natural anticoagulant that inhibits thrombin formation^{5,6}.

Thrombomodulin (TM) is a transmembrane protein expressed on the surface of the endothelial cells⁷. TM binds with thrombin and forms a complex that activates Protein C. This activated protein C after its detachment from TM binds with platelet surface protein S where it degrades activated factor V and VII thus inhibiting the blood clotting mechanism. Therefore TM plays a key role in preventing thrombus formation⁸.

Chronic inflammation causes release of several inflammatory markers such as CRP, interleukin 6 and

¹Department of Physiology Karachi Medical & Dental College (K.M.D.C.) & Abbasi Shaheed Hospital Karachi

²Department of Medicine, Abbasi Shaheed Hospital & Karachi Medical & Dental College (KMDC)

³Department of Bio-chemistry, Karachi Medical & Dental College. Correspondence to Dr. Muhammad Akbar Mughal, Email: allahoghani@hotmail.com, contact no; +923332167259

18, tumor necrosis factor α , ICAM-1, E-selectin and acute phase reactants related to coagulation pathway. All are increased in blood predicting the cardiovascular events⁹⁻¹⁴.

C-reactive protein is a 115 Kda acute phase protein of the pentraxin family of Ca-dependent ligand binding protein in serum¹⁵. It is a marker of systemic inflammation and has been identified as a risk factor for coronary artery disease¹⁶⁻¹⁸. Studies on diabetic and coronary artery disease patient shows that high sensitivity C-reactive protein (CRP) was a significant predictor for cardiac events and mortality¹⁹⁻²³.

As the thromboembolic events are the major cause of death around the world, different studies conducted in different regions of the world have shown the role of thrombomodulin & CRP in the risk assessment of coronary artery disease. Therefore the aim of present study is to find out the association between serum Thrombomodulin (TM) & serum C-reactive protein (CRP) in identifying individuals at risk of ischemic heart disease (IHD) in Pakistani population

MATERIAL & METHODS

This cross sectional study was conducted at Tabba Heart Institute (THI) Karachi, Pakistan. 32 healthy subjects were compared with 32 patients with history of myocardial infarction (M.I.) & 32 patients with history of unstable angina (U.A.). All of these were recruited from April to December 2010 by random sampling. They were divided into 3 groups.

Group A (n=32): Control Group i.e., apparently healthy subjects.

Group B (n=32): Patients with history of Acute Myocardial Infarction. (Diagnosed on the basis of history, E.C.G. & Troponin I levels).

Group C (n=32): Patients with history of Unstable Angina. (Diagnosed on the basis of history, E.C.G. & Troponin I levels).

Patients with the history of unstable angina (UA), Myocardial Infarction (MI), hypertension are included in the study. Patients with the history of diabetes, smoker, chronic liver or renal disease and cancers are excluded from the study.

Blood samples of all participants were obtained in 4-5ml serum separator tube by a well-trained phlebotomist. The tubes were then centrifuged at 1500 g for 10 minutes. The obtained serum was separated and immediately frozen and stored in aliquots at -70°C pending analysis. Serum Thrombomodulin levels were later determined by an enzyme-linked immunosorbent assay. (Cusa Biotech Co. Ltd China), while the serum CRP & lipid profile were measured by ELISA (Roche Hitachi 912).

Obtained data were recorded and statistically analyzed through statistical package for social sciences (SPSS version 16). Mean and standard deviation of continuous variables were computed while frequencies for categorical variables were also determined. Independent sample t-test was used to compare the mean among 3 groups. P-value of ≤ 0.05 was considered as statistically significant.

RESULTS

Table 1 shows that BMI, systolic blood pressure and values of serum LDL, serum total Cholesterol & serum CRP were higher in diseased (MI) group as compared to normal, in contrast to serum TM levels, which were high in control group when compared with diseased group

Table 2 almost shows the similar characteristics that systolic & Diastolic blood pressure, serum LDL, serum total Cholesterol & serum CRP were fairly high in diseased (UA) group when compares with normal, in contrast to serum TM levels, which were higher in control when compared to diseased group

Table 1: Base- Line Characteristics of the patients with Myocardial Infarction and Control Group

Characteristics	Control (n=32)	MI (n=32)	p- Value
Age (Yrs.)	49.95	57.69	---
Body mass index (Kg/m ²)	24.26	26.88	0.0
Systolic blood pressure (mmHg)	124.75	137.06	0.0
Diastolic blood pressure (mmHg)	79.12	84.34	0.012
HDL-Chol (mg/dl)	40.43	37.4	0.083
LDL-Chol (mg/dl)	121.63	163.27	0.003
Cholesterol(mg/dl)	200.54	239.40	0.00
TM (ng/ml)	8.25	4.18	0.00
CRP(mg/L)	2.81	21.19	0.00

Table 2: Base- Line Characteristics of the patients with Un-Stable Angina and Control Group

Characteristics	Control (n=32)	UA (n=32)	p-Value
Age (Yrs.)	49.95	53.78	--
Body mass index (Kg/m ²)	24.26	25.49	0.082
Systolic blood pressure (mmHg)	124.75	140.53	0.0
Diastolic blood pressure (mmHg)	79.12	86.96	0.0
HDL-Chol (mg/dl)	40.43	38.09	0.206
LDL-Chol (mg/dl)	121.63	143.56	0.002
Cholesterol(mg/dl)	200.54	245.97	0.00
TM (ng/ml)	8.25	4.99	0.003
CRP(mg/L)	2.81	20.60	0.00

Pearson correlation co-efficient: Statistically significant correlation was found between thrombomodulin and CRP levels ($r = .339, p = .001$).

DISCUSSION

The present study suggest that raised serum TM levels in control group confers protection against ischemic heart disease, whereas raised serum CRP levels in diseased group predicts the incidence of ischemic heart disease. Different studies conducted in different regions showed conflicting results. Karakas M et al²⁴ in their study mentioned that serum CRP & serum TM levels were raised in cases as compared to controls. Thorand et al (2006)²⁵ also showed that level of TM were lowered in patients with history of myocardial infarction, whereas the level of CRP were elevated in subjects with increased TC/HDL ratio. The raised CRP in diseased group is obvious as inflammation causes formation, progression and rupture of atherosclerotic plaque^{26,27}. This feature were also mentioned by Konstantolas et al 2004²⁸ as well as Mezaki et al²⁹ & Danesh et al³⁰. The level of serum TM in ischemic heart disease & in control subject is controversial. Sir Salomma et al 1999³¹, WU 2003³², mentioned the raised TM in control as compared to diseased group, whereas Mezaki et al. (2003)²⁹, Chan et al. (2006)³³ and Huang et al. (2008)³⁴ showed the contrasting results.

Therefore in the light of these results the study should be considered on a large scale including the different ethnic groups of Pakistan in order to correlate the importance of these markers in the incidence of Ischemic heart disease (IHD).

CONCLUSION

A high serum TM level predicts as a defensive marker and high CRP in disease group predicts onset of ischemic heart disease.

REFERENCES

- Lip GYH, Blann A. Von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovasc Res* 1997; 34:255-265.
- Anderson T.J. Nitric oxide, atherosclerosis and the clinical relevance of endothelial dysfunction. *Heart Fail Rev* 2003; 8:71-86.
- Blann AD. Assessment of endothelial dysfunction: focus on atherothrombotic disease. *Pathophysiol Hemost Throm* 2003;33:256-61
- Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation* 2004; 109:11127-11132.
- Bouma BN, Mosnier LO. Thrombin activatable fibrinolysis inhibitor (TAFI) at the interface between coagulation and fibrinolysis. *Pathophysiol Haemost Thromb* 2003; 33:375-381.
- Norris LA. Blood coagulation. *Best Pract Res Clin Obstet Gynecol* 2003; 17:369-383.
- Lougheed JC, Bowman CL, Meininger DP, Komives EA. Thrombin inhibition by cyclic peptides from thrombomodulin. *Protein Sci* 1995; 4:773-780.
- Esmon CT. Thrombomodulin as a model of molecular mechanisms that modulate protease specificity and function at the vessel surface. *FASEB J.* 1995; 9:946-55
- Altman R, Rouvier J, Scazziotto A and Gonzalez C: No causal association between inflammation and Chlamydia Pneumoniae in patients with chronic ischemic arterial disease *Inflammation* 2002; 26:25-30.
- Liuzzo G, Biasucci LM and Gallimore JL et al.: The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina *N Engl J Med* 1994; 331:417-424.
- Biasucci LM, Vitelli A and Liuzzo G et al.: Elevated level of interleukin-6 in unstable angina *Circulation* 1996; 94:874-877.
- Blake GJ and Ridker PM: Novel clinical markers of vascular wall inflammation *Circulation Res* 2001; 89:763-771.
- Libby P, Ridker PM and Maseri A: Inflammation and Atherosclerosis *Circulation* 2002; 105:1135-1143.
- Wu KK, Aleksic N, Ballantyne Ch M, Ahn Ch, Juneja H and Boerwinkle E: Interaction between soluble thrombomodulin and Intercellular Adhesion Molecule-1 in predicting risk of coronary heart disease *Circulation* 2003; 107:1729-1732.
- Pepys, M.B. and Hirschfield, G.M. C-reactive protein: a critical update. *J. Clin. Invest.* 2003; 111:1805-1812.
- Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta analysis of prospective studies. *JAMA* 1998; 279:1477-1482.
- Kuo HK, Yen CJ, Chang CH, Kuo CK, Chen JH, Sorond F. Relation of C-reactive

19. protein to stroke, cognitive disorders, and depression in the general population: systematic review and meta-analysis. *Lancet Neurol* 2005;4:371–80
20. Nakou ES, Liberopoulos EN, Milionis HJ, Elisaf MS. The role of C-reactive protein in Atherosclerotic cardiovascular disease: an overview. *Curr Vasc Pharmacol* 2008; 6:258-270.
21. Matsumoto K, Fujita N, Ozaki M, Tominaga T, Ueki Y, Miyake S. coexistence of insulin resistance and inflammation effectively predicts cardiac disease but not stroke in Japanese patients with type 2 diabetes mellitus. *Diabetes Res Clin Prac* 2006; 74:316-321.
22. Soeki T, Tamura Y, Shinohara H, et al. Fibrinolytic factors, serum lipid and Creative protein predicting cardiac events in Japanese patients with coronary atherosclerotic lesions. *JPN Circ J* 1999;63:976–80.
23. Kinjo K, Sato H, Ohnishi Y, et al. Impact of high-sensitivity C-reactive protein on predicting long-term mortality of acute myocardial infarction. *Am J Cardiol* 2003;91:931–5.
24. Sato S, Kobayashi T, Awata N, Nakagawa T, Takeda Y. High sensitive C-reactive protein, fibrinogen, white blood cell count and the recurrences of cardiovascular diseases on five-year follow-up of PCS study. In: The 67th Annual Scientific Meeting of the Japanese Circulation Society. 2003 [abstract].
25. Shimada K, Fujita M, Tanaka A, et al. Elevated serum C-reactive protein levels predict cardiovascular events in the Japanese Coronary Artery Disease (JCAD). *Circ J* 2009; 73:78-85.
26. Karakas M, Baumert J, Herder C, Rottbauer W, Meisinger C, Koenig W, Thorand B. Soluble thrombomodulin in coronary heart disease: lack of an association in the MONICA/KORA case-cohort study. *Journal of Thrombosis and Haemostasis*. 2011; 1;9(5):1078-80.
27. Thorand B, Baumert J, Döring A, Schneider A, Chambless L, Löwel H, Kolb H, Koenig W. Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. *Thrombosis and haemostasis*. 2006;95(1):134-41.
28. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004;109:112–10.
29. Koenig W. Predicting risk and treatment benefit in atherosclerosis: the role of C-reactive protein. *Int J Cardiol* 2005;98:199–206.
30. Konstantoulas CJ, Cooper J, Warnack G, Miller GJ, Humpius SE and Ireland H. A combination of two common thrombomodulin gene (1208-1209TT del TT and A455V) influence risk of coronary heart disease: A prospective study in men. *Atherosclerosis* 2004; 177(1):97-104.
31. Mezaki T, Matsubara T, Hori T, Higuchi K, Nakamura A, Nakagawa I, Imai S, Ozaki K, Tsuchida K, Nasuno A, Tanaka T, Kubota K, Nakano M, Miida T, Aizawa Y. Plasma levels of soluble thrombomodulin, C-reactive protein, and serum amyloid a protein in the atherosclerotic coronary circulation. *Jpn Heart J* 2003; 44(5):601-612.
32. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *New England Journal of Medicine*. 2004 ;350(14):1387-97.
33. Salomaa V, Matei C, Aleksic N, Garcia LS, Folsom AR, Juneja H, Chambles LE and Wu KK. Soluble thrombomodulin as a predictor of incident coronary heart disease and
34. symptomless carotid artery atherosclerosis in the atherosclerosis risk in communities (ARIC) study: a case cohort study. *Lancet* 1999; 353.
35. Wu KK. Soluble thrombomodulin and coronary heart disease. *Curr Opin Lipidol* 2003; 14:373-375.
36. Chan SH, Chen JH, Li YH, Lin LJ, Tsai LM. Increasing post event plasma thrombomodulin level associates with worse outcome in survival of acute coronary syndrome. *Int J Cardiol* 2006; 111(2):280-285.
37. Huang J, Fu G, Yao Q, Cheng G. Relation of thrombomodulin, TFPI and plasma antioxidant in healthy individuals and patient with coronary heart disease. *Acta Cardiol* 2008; 63(2):341-346.