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Role of serum β_2m in predicting severity of Coronary artery disease

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Abstract

Background: Coronary artery disease is the principal cause of disability and mortality worldwide. Its prevalence is increasing around world. It is about 75% of deaths occurring in developing countries like Bangladesh. It is very important to know about the inflammatory risk factors of coronary artery disease for early assessment of coronary artery disease. Serum β_2-microglobulin (^{2}2m) is a newly identified biomarker that has been found to increase in patients with coronary artery disease.

Aims: To determine the role of β_2m in predicting the severity of coronary artery disease.

Methods: This cross-sectional study was carried out in Department of Cardiology and Laboratory Medicine, BSMMU, Shahbag, Dhaka during March 2017 to February 2018. Total seventy four patients who underwent coronary angiography as per criteria where included in this study. Serum β_2-microglobulin (^{2}2m)was done before angiography procedure by indirect ELISA method and severity of coronary artery disease was assessed by extent of diseased coronary vessels and SYNTAX score.

Results: β_2-microglobulin level was found higher (≥3/ml) in coronary artery disease patients which was statistically significant ($p<0.001$). β_2-microglobulin was also correlated with number of diseased coronary vessels ($r=0.562$, $p<0.001$). Mean β_2m level was found 4.48±0.95 µg/ml with range from 3-6.1 µg/ml and the mean SYNTAX score was found 16.27±08.99 with the range from 1 to 44. Pearson's correlation coefficient was done between β_2m level and SYNTAX score. Then the result is $r=0.547$ and $p<0.001$. Therefore, there was a positive correlation between β_2m level and SYNTAX score. The area under the receiver-operator characteristic (ROC) curves ^{2}2m cut off value of 3.6 with 81.4% sensitivity and 86.7% specificity as the value for identifying the coronary artery disease.

Conclusion: Our study revealed that β_2-microglobulin effectively correlates with the severity of coronary artery disease. So it may be used as a reliable marker for assessment of coronary artery disease severity.

Keyword: Coronary artery disease, Severity of coronary artery disease, β_2-microglobulin.

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Introduction:

Coronary artery disease (CAD) is the most common cause ofangina and acute coronary syndrome. It is also a commoncause of death worldwide. The World Health Organization (WHO) has estimated that 3.8 million men and 3.4 million women die from cardiovascular disease (CVD) each year.[1]WHO also estimated that there will be nearly 20 million cardiovascular death worldwide by the year 2020 and it may increase 24 million by the year 2030.[2]Bangladeshis are unduly prone to develop CAD. It is often premature in onset, follows a rapidly progressive course and angiographically more severe.[3] The prevalence of coronary artery disease in Bangladesh is gradually increasing due to rapid urbanization, migration of people from village to the cities, change in life style and food habits. In Bangladesh, coronary disease death reach 50,708 or 6.96% of total death.[4]Some established risk factors for coronary artery disease are hypertension, diabetes

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mellitus, positive family history,dyslipidaemia, smoking, sedentary life style, obesity and psychological stress.Coronary artery disease is one of the most common manifestation of atherosclerosis. It has been suggested that inflammation is fundamentally involved in the pathogenesis of atherosclerosis.[5] It is suggested that ²2m may be identified as a marker of inflammation.[6] It has been foound to increase in patients with coronary artery disease.[7]

β_2-microglobulin is the light chain in the major histocompatibility complex (MHC) class I molecules.[8] It is widely distributed in nucleated cells in the body.[9] The large amount of ²2m is synthesized by lymphocytes and regulated by interferons and proinflammatory monocytokines.Under normal conditions ²2m production is about 0.13 mg/h/kg.[10]About 0.9 to 2.5mg/L of free ²2m is found in the serum of healthy subjects after shedding from the cell membrane.[11]Ninety percent of β_2-microglobulin is eliminated via glomerular filtration and almost completely reabsorbed by the proximal tubules.[12]

²$_2$-microglobulin may participate in the inflammatory process of atherosclerosis. It acts as a chemoattractant for mononuclear cells and potential initiator of inflammation.[13] It may be involved indevelopment of vascular dysfunction and aortic stiffness in atherosclerosis.[14] It is also related to direct alteration of vascular structure, immunity and response to hypoxia.[15]

β_2-microglobulin was identified as a risk marker for coronary heart disease in a proteomic study on 50 different proteins.[16] Risk stratification is a key issue in treatment of atherosclerosis. Risk stratification was evaluated byIntegrated Discrimination Improvement (IDI) and Net reclassification improvement (NRI). It has been reported that β_2-microglobulin improved risk stratificationfor major cardiovascular events is much better than high sensitivity C-reactive protein (hs-CRP).[17] Several studies have shown that β2m concentration were significant non-renal predictors of cardiovascular outcomes, renal outcomes and mortality.[18]It is reportedthat ²$_2$-microglobulin influence tumor cells such as leukemia and myeloma.[19] β_2-microglobulin is also raised in some viral infections such ashuman immune-deficiency virus (HIV) and cytomegalovirus.[20]Collagen disease may cause deposition of β_2-microglobulin within joint.[21]It is related to carotid intima thickness in haemodialysis patients.[22]Serum β_2m also predicts cardiovascular events in patients with chronic kidney disease.[23] Recent studies have shown that β_2m is elevated in peripheral artery disease.[24]

β_2-microglobulin can be measured by indirect ELISA method. The test is rapid simple and reliable. It may be available in any hospital in our country. β_2-microglobulin may act as a noninvasive tool for the assessment of severity of the atherosclerosis process in coronary artery disease.It may also play an important role in follow-up and treatment of selected cases.This study helps to assess the role of β_2-microglobulin as a non-invasive tool to improve risk stratification of patients for coronary artery disease and to guide subsequent testing and interventions. The correlation between β_2-mcroglobulin with coronary artery disease may indicate the severity of CAD.From previous studies, along with this study can reveal the utility of ²$_2$-microglobulin to assess the severity of coronary artery disease.

Method and Material:

The study population who were attending in the Cardiology Department of BSMMU for the evaluation of coronary artery disease by coronary angiogrambetween March 2017 to February 2018.Patients of renal dysfunction (creatinine level >1.3 mmol/l),patients withHuman immunodeficiency viral (HIV) disease, Multiple Myeloma, LeukemiaandCollagen diseases were excluded from the study. Total 74 patients were enrolled. According to coronary angiogram Fifty eight patient were diagnosed as significantcoronary artery disease (≥50% vessel stenosis) and remaining thirteen were diagnosed as nonsignificant coronary artery disease (normal angiography or <50% vessel stenosis) coronary artery disease. BSMMU is a tertiary hospital andonly medical university in Bangladesh. It is concerned with national policy making alongwith hospital services. This hospital has high quality consultations service and follow standarddiagnostic protocol.Many patients from all over the country are come to this hospital. That's why, this is one of the appropriate places for data collection and research work.

Ethical consideration:

Prior to the commencement of this study, the research protocol was approved by the Ethical Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University, Dhaka (Appendix-I). The aims and objectives of the study along with its procedure, risks and benefits of this study were explained to the patient in easily understandable local language and then informed consent was taken from each patient both orally and in written forms.

Laboratory analysis:

Measurment of β_2-microglobulinwas done by Indirect ELISA method.

Samples were collected before coronary angiography procedure of a patient. A total 4.0 ml venous blood was taken by venipuncture in a red capped tube and centrifuged to separate serum from cells after clot formation. Samples were stored at -20^{0}C until analysis and β_2-microglobulin was estimated in 4 successive occasions. Calculation ofSYNTAX score was done by online calculator.

Statistical analysis:

Statistical Package for Social Sciences version 24 (SPSS Inc. Chicago, II, USA) was used for all statistical analysis. Data was presented as mean ± SD. Relationships between variables wastested by Pearson correlation Coefficient analysis.

Result:

This cross-sectional study was carried out at the Department of Laboratory Medicine in collaboration with the Department of Cardiology, Bangabandu Sheikh mujib Medical University (BSMMU), Dhaka from March 2017 to February 2018. Total 74 patients were enrolled according to inclusion and exclusion criteria. Serum

β_2-microglobulin level was measured. The SYNTAX score was calculated by online calculator to assess the severity of coronary artery disease. Finally made association betweenserum β_2m level with the number of diseased coronary vessels, with the SYNTAX score and determine the value of β_2m for prediction of Coronary artery disease Receiver-operator characteristic (ROC) curve.

Table 1:The mean age of the respondents was 52.5±10.7 years (range26-76 years).Majority patients are male 61(82.4%) and 13(17.6%) patients were female. The male and female ratio being roughly 4:1.

Table-I

Age and sex distribution of the respondents (n=74)

Age (in years)	Number of patients	Percentage
20-30	1	1.4
31-40	12	16.2
41-50	18	24.3
51-60	25	33.8
61-70	14	18.9
71-80	4	5.4
Mean±SD	52.5±10.7	
Range(min-max)	26-76	
Sex		
Male	61	82.4
Female	13	17.6

Most of the patients were in 51-60 years age group and most of them were male.

Table-II

Distribution of serum β_2m level into angiographically diagnosed significant and non-significant CAD patients (n= 74)

β_2m level (according to Beta-2-Microglobulin ELISA Kits reference)	Significant CAD (≥50% vessel stenosis) (n=58) Subjects		Non- significant (<50% vessel stenosis) CAD (n=16) Subjects		P value
	n	%	n	%	
High (≥3.0 µg/ml)	58	100	3	18.75	0.001^s
Normal (<3.0µg/ml)	0	00	13	81.25	

s = significant, p-value reached from Chi square test

Here β_2m level was high significantly high in CAD patients than in non-significant CAD patients. The difference was statistically significant (p<0.001).

Table-III

Distribution of β_2m level according to diseased coronary vessels among the respondents (n=74)

Severity of CAD	Percentage	β_2m(µg/ml)Mean±SD	p-value
Non-significant CAD (n=16)	21.61	2.92±0.41	0.001^s
Significant CAD (n=58)	78.37		
Single vessel disease(SVD) (n=12)	16.21	4.19±0.67	
Double vesseldisease(DVD) (n=26)	35.13	4.63±0.62	
Triple vessel disease(TVD) (n=20)	27.02	5.11±0.66	

Result was expresed as mean±SD, ANOVA test

Table 3: Shows the distribution of ²2m level according to diseased coronary vessels among the respondents. It was observed that β_2m level was gradually increase with the number of diseased coronary vessels.

Table-IV

Distribution of the respondents by β_2m level andSYNTAX score (n=74)

Variables	Non- significant CAD (n=16) Mean±SD	Significant CAD (n=58) Mean±SD	P value
β_2m (µg/ml)	2.91±0.65	4.48±0.95	<0.001*
SYNTAX score	0.13±0.34	16.27±08.99	<0.001*

Unpaired student t-test

Table-I|V shows the distribution of the respondents by ²2m level and SYNTAX score. Here ²2m level was proportionately increase with the SYNTAX score.

The area under the receiver-operator characteristic (ROC) curves for the Coronary artery disease is depicted in the following Figure-1. Based on the receiver-operator characteristic (ROC) curves had ²2mthe best area under curve, which are significantly associated to identification of Coronary artery disease.

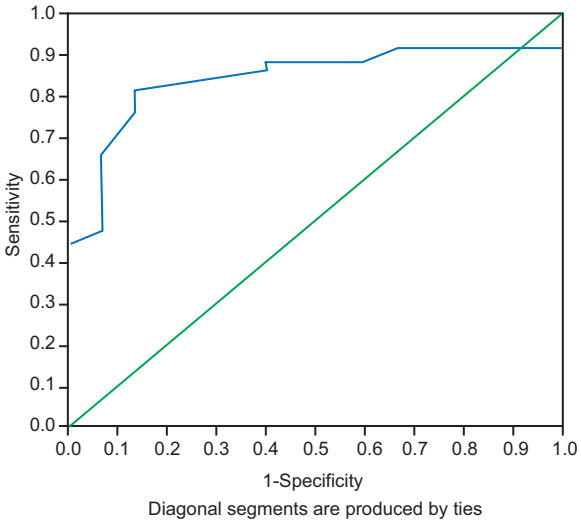

Fig.-1: *Receiver-operator characteristic (ROC) curve of $\beta_2$mfor predictionofCoronary artery disease.*

Cut off value	=	3.6
Sensitivity	=	81.4
Specificity	=	86.7
Area under the	=	0.844
P value	=	0.001
95% CI	=	0.749 – 0.938

Discussion:

Inflammation assess a new risk factor for coronary artery disease.Inflammatory markers may be expressed in the different parts of the atherogenic process. The measurement of inflammatory markers may be a potent method for identifying individuals with increased inflammation at risk of future cardiovascular events.[25] Several biomarkers like C-reactive protein[26], high sensitivity C-reactive protein (hs-CRP), intrleukin-6 (IL-6) and tumor necrosis factor (TNF) have been shown to be predictors of coronary artery disease.These markers were not associated with severity of coronary artery disease.[27]Atherosclerosis leading to coronary artery disease is complex in origin. Morbidity of coronary artery disease is generally related to the extent of vascular lesions.[28] In this regard, the clinical risk factors are considered to be useful in predicts the severity of atherosclerosis.[29] Early diagnostic facilities are more important than treatment. Protein markers have potential to enhance the understanding of disease pathogenesis and elucidate biological process that affects the disease risk. The association between ²$_2$-microglobulin (²2m) and cardiovascular disease remain under research.[7]This is the first study that revealed the role of ²$_2$-microglobulin (²2m) for predicting the coronary artery disease in Bangladesh. ²$_2$-microglobulin (11.8kD, protein) also known as ²2m is component of major histocompatibility complex (MHC) class1 molecules. It lies below ±$_1$ chain and beside the ±$_3$ chain on the cell surface. It has no transmembrane region. It lies on the all nucleated cell. Under normal conditions ²2m production is about 0.13 mg/h/kg.[10] About 0.9 to 2.5mg/L of free ²2m is found in the serum of healthy subjects after shedding from the cell membrane.[11] Ninety percent of ²$_2$-microglobulin is eliminated via glomerular filtration and almost completely reabsorbed by the proximal tubules.[12] A large amount of ²2m is shedding from the surface of lymphocyte due to generation and migration of lymphocyte in both acute chronic inflammatory response. This is leading to increase ²2m level. Then it can be modified by advance glycation end products. This glycosylated ²2m can release inflammatory agent such as IL-1, IL-6, IL-8, tumor necrosis factor-± (TNF- ±). It acts as chemoattractant for mononuclear cell and potentially initiate the inflammatory response.[13]²2m also induce apoptosis and necrocytosis in fibroblast and vascular endothelial cell. Apoptotic or necrotic cells can release

intracellular enzyme and cytokine to recruit inflammatory cells that cause inflammatory reaction. In this pathway β_2m can initiate inflammation.[30] β_2m is also associated with carotid intima thickness[22] and influence arterial stiffness.[31] Both indices are related to subclinical target organ damage of cardiovascular system. β_2m was found to be associated with cardiac valvular calcification[32] which may be one of the cardiac presentation of systemic atherosclerosis.It was observed that one- quarter 18(24.3%) of patients was in their 4th decade of life,one third 25(33.8%) in their 5th decade and 14(18.9%) in 6th decade of life. In our study, Patients were selected who underwent coronary angiography to evaluate coronary artery disease by coronary angiogram. Serum β2m of all patients were measured. Then correlated with angiographic findings, disease vessel and also SYNTAX score. High β_2m level gives a reflection of severity of coronary artery disease. β_2-microglobulin level was found higher (≥3/ml) in coronary artery disease patients which was statistically significant.The normal level of β_2--microglobulin (<3µg/ml) was found in 81.25% non-significant CAD patients. There was significant finding between β_2-microglobulin and CAD. It was also statistically significant ($p<0.001$). β_2m level was found normal (2.92±0.41µg/ml) in case of 16(21.61%) no vessel disease and gradually increase according to number of diseased coronary vessels such as 4.19±0.67µg/ml in SVD 12(16.21%), 4.63±0.62µg/ml for DVD 26(35.13%), 5.11±0.66µg/ml for TVD 20(27.02%). The difference was statistically significant ($P<0.05$).

This study also found that there was significant difference of β_2m level and SYNTAX score between significant CAD and non-significant CAD subjects.In case of non-significant CADthe mean ±SD of β_2m level was 2.91±0.65µg/ml and mean ±SD of SYNTAX score was 0.13±0.34. On the other hand, in case of significant CAD the mean±SD of β_2m level was 4.48±0.95µg/ml and mean ±SD of SYNTAX score was 16.27±08.99. Here significant difference was found between β_2m level and SYNTAX score among respondents ($p<0.001$).

Receiver-operator characteristic (ROC) were constructed using β_2m value of the patient's and a best combination of sensitivity and specificity for coronary artery disease. It gave β_2m cut off value of 3.6 with 81.4% sensitivity and 86.7% specificity as the valuefor identifying the coronary artery disease.

β_2-microglobulin test is safe, rapid, reliable, less expensive and can be measured easily by indirect ELISA method. It may be a noninvasive severity tool for coronary artery disease which may beneficial for patients. Our study revealed that β_2-microglobulin was significantly associated with coronary artery disease. So it may be used as a reliable marker for assessment of coronary artery disease. The study was done in limited time of span.Cases were collected from only one center, hence may not represent the whole population of the country. The sample size was small. Follow up assessment of the same patient could not be done. Serum β_2-microglobulin can be used as a noninvasivediagnostic screening tool for predicting the severity of coronary artery disease. Study period should be extended. Further studies are need to evaluate the potential benefits of serum β_2-microglobulin level for coronary artery disease in clinical practice.It can also help discover new cases of coronary lesions, follow-up and control of the selected cases.

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