Introduction: 
Coronary artery disease (CAD) is the most common cause of angina and acute coronary syndrome. It is also a common cause 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

Role of serum $\beta_2m$ 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 $\beta_2$-microglobulin ($\beta_2m$) is a newly identified biomarker that has been found to increase in patients with coronary artery disease.

Aims: To determine the role of $\beta_2m$ 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 $\beta_2$-microglobulin ($\beta_2m$) 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: $\beta_2$microglobulin level was found higher ($\geq 3/$ml) in coronary artery disease patients which was statistically significant ($p<0.001$). $\beta_2$-microglobulin was also correlated with number of diseased coronary vessels ($r=0.562$, $p<0.001$). Mean $\beta_2m$ level was found $4.48\pm0.95 \mu g/ml$ with range from $3-6.1 \mu g/ml$ and the mean SYNTAX score was found $16.27\pm8.99$ with the range from $1$ to $44$. Pearson’s correlation coefficient was done between $\beta_2m$ level and SYNTAX score. Then the result is $r=0.547$ and $p<0.001$. Therefore, there was a positive correlation between $\beta_2m$ level and SYNTAX score. The area under the receiver-operator characteristic (ROC) curves $\beta_2m$ 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 $\beta_2$-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, $\beta_2$-microglobulin.
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. It is suggested that \( \beta_2 \) may be identified as a marker of inflammation. It has been found to increase in patients with coronary artery disease.

\( \beta_2 \)-microglobulin is the light chain in the major histocompatibility complex (MHC) class I molecules. It is widely distributed in nucleated cells in the body. The large amount of \( \beta_2 \) is synthesized by lymphocytes and regulated by interferons and proinflammatory monocytes. Under normal conditions \( \beta_2 \) production is about 0.13 mg/h/kg. About 0.9 to 2.5 mg/L of free \( \beta_2 \) is found in the serum of healthy subjects after shedding from the cell membrane. Ninety percent of \( \beta_2 \)-microglobulin is eliminated via glomerular filtration and almost completely reabsorbed by the proximal tubules. \( \beta_2 \)-microglobulin may participate in the inflammatory process of atherosclerosis. It acts as a chemoattractant for mononuclear cells and potential initiator of inflammation. It may be involved in development of vascular dysfunction and aortic stiffness in atherosclerosis. It is also related to direct alteration of vascular structure, immunity and response to hypoxia.

\( \beta_2 \)-microglobulin was identified as a risk marker for coronary heart disease in a proteomic study on 50 different proteins. Risk stratification is a key issue in treatment of atherosclerosis. Risk stratification was evaluated by Integrated Discrimination Improvement (IDI) and Net reclassification improvement (NRI). It has been reported that \( \beta_2 \)-microglobulin improved risk stratification for major cardiovascular events is much better than high sensitivity C-reactive protein (hs-CRP). Several studies have shown that \( \beta_2 \) concentration were significant non-renal predictors of cardiovascular outcomes, renal outcomes and mortality. It is reported that \( \beta_2 \)-microglobulin influence tumor cells such as leukemia and myeloma. \( \beta_2 \)-microglobulin is also raised in some viral infections such as human immune-deficiency virus (HIV) and cytomegalovirus. Collagen disease may cause deposition of \( \beta_2 \)-microglobulin within joint. It is related to carotid intima thickness in haemodialysis patients. Serum \( \beta_2 \) also predicts cardiovascular events in patients with chronic kidney disease. Recent studies have shown that \( \beta_2 \) is elevated in peripheral artery disease.

\( \beta_2 \)-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. \( \beta_2 \)-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 \( \beta_2 \)-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 \( \beta_2 \)-microglobulin with coronary artery disease may indicate the severity of CAD. From previous studies, along with this study can reveal the utility of \( \beta_2 \)-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 angiogram between March 2017 to February 2018. Patients of renal dysfunction (creatinine level >1.3 mmol/l), patients with Human immunodeficiency viral (HIV) disease, Multiple Myeloma, Leukemia and Collagen diseases were excluded from the study. Total 74 patients were enrolled. According to coronary angiogram Fifty eight patient were diagnosed as significant coronary 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 and only medical university in Bangladesh. It is concerned with national policy making along with hospital services. This hospital has high quality consultations service and follow standard diagnostic protocol. Many patients from all over the country 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:
Measurement of β₂-microglobulin was 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°C until analysis and β₂-microglobulin was estimated in 4 successive occasions. Calculation of SYNTAX 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 were tested 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, Bangabandhu Sheikh mujib Medical University (BSMMU), Dhaka from March 2017 to February 2018. Total 74 patients were enrolled according to inclusion and exclusion criteria. Serum β₂-microglobulin level was measured. The SYNTAX score was calculated by online calculator to assess the severity of coronary artery disease. Finally made association between serum β₂-m level with the number of diseased coronary vessels, with the SYNTAX score and determine the value of β₂-m 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 (range 26-76 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)

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

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

Table-II
Distribution of serum β₂-m level into angiographically diagnosed significant and non-significant CAD patients (n= 74)

<table>
<thead>
<tr>
<th>β₂-m level (according to Beta-2-Microglobulin ELISA Kits reference)</th>
<th>Significant CAD (≥50% vessel stenosis)</th>
<th>Non-significant CAD (&lt;50% vessel stenosis)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=58)</td>
<td>(n=16) Subjects</td>
<td>Subjects</td>
<td></td>
</tr>
<tr>
<td>High (≥3.0 µg/ml)</td>
<td>58</td>
<td>3</td>
<td>0.001s</td>
</tr>
<tr>
<td>Normal (&lt;3.0µg/ml)</td>
<td>0</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

s = significant, p-value reached from Chi square test
Here β₂-m 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 β₂-m level according to diseased coronary vessels among the respondents (n=74)

<table>
<thead>
<tr>
<th>Severity of CAD</th>
<th>Percentage</th>
<th>β₂-m(µg/ml) Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-significant CAD (n=16)</td>
<td>21.61</td>
<td>2.92±0.41</td>
<td>0.001s</td>
</tr>
<tr>
<td>Significant CAD (n=58)</td>
<td>78.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single vessel disease(SVD) (n=12)</td>
<td>16.21</td>
<td>4.19±0.67</td>
<td></td>
</tr>
<tr>
<td>Double vessel disease(DVD) (n=26)</td>
<td>35.13</td>
<td>4.63±0.62</td>
<td></td>
</tr>
<tr>
<td>Triple vessel disease(TVD) (n=20)</td>
<td>27.02</td>
<td>5.11±0.66</td>
<td></td>
</tr>
</tbody>
</table>

Result: shows the distribution of β₂-m level according to diseased coronary vessels among the respondents. It was observed that β₂-m level was gradually increase with the number of diseased coronary vessels.
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. Several biomarkers like C-reactive protein, 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. Atherosclerosis leading to coronary artery disease is complex in origin. Morbidity of coronary artery disease is generally related to the extent of vascular lesions. In this regard, the clinical risk factors are considered to be useful in predicts the severity of atherosclerosis. 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 $\beta_2$-microglobulin ($\beta_2$m) and cardiovascular disease remain under research. This is the first study that revealed the role of $\beta_2$-microglobulin ($\beta_2$m) for predicting the coronary artery disease in Bangladesh. $\beta_2$-microglobulin (11.8kD, protein) also known as $\beta_2$m is component of major histocompatibility complex (MHC) class I molecules. It lies below $\alpha$ chain and beside the $\beta_3$ chain on the cell surface. It has no transmembrane region. It lies on the all nucleated cell. Under normal conditions $\beta_2$m production is about 0.13 mg/h/kg. About 0.9 to 2.5mg/L of free $\beta_2$m is found in the serum of healthy subjects after shedding from the cell membrane. Ninety percent of $\beta_2$-microglobulin ($\beta_2$m) is eliminated via glomerular filtration and almost completely reabsorbed by the proximal tubules. A large amount of $\beta_2$m 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 $\beta_2$m level. Then it can be modified by advance glycation end products. This glycosylated $\beta_2$m 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. $\beta_2$m also induce apoptosis and necrocytosis in fibroblast and vascular endothelial cell. Apoptotic or necrotic cells can release

Table-IV

Distribution of the respondents by $\beta_2$m level and SYNTAX score ($n=74$)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-significant CAD Mean±SD ($n=16$)</th>
<th>Significant CAD Mean±SD ($n=58$)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_2$m (µg/ml)</td>
<td>2.91±0.65</td>
<td>4.48±0.95</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SYNTAX score</td>
<td>0.13±0.34</td>
<td>16.27±0.99</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Unpaired student t-test

Table-IV shows the distribution of the respondents by $\beta_2$m level and SYNTAX score. Here $\beta_2$m 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 $\beta_2$m the best area under curve, which are significantly associated to identification of Coronary artery disease.

Fig-1: Receiver-operator characteristic (ROC) curve of $\beta_2$m for prediction of Coronary 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.
intracellular enzyme and cytokine to recruit inflammatory cells that cause inflammatory reaction. In this pathway β₂m can initiate inflammation.30 β₂m is also associated with carotid intima thickness22 and influence arterial stiffness.31 Both indices are related to subclinical target organ damage of cardiovascular system. β₂m was found to be associated with cardiac valvular calcification32 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 β₂m level gives a reflection of severity of coronary artery disease. β₂-microglobulin level was found higher (≥3/ ml) in coronary artery disease patients which was statistically significant. The normal level of β₂-microglobulin (<3μg/ml) was found in 81.25% non-significant CAD patients. There was significant finding between β₂-microglobulin and CAD. It was also statistically significant (p<0.001). β₂m 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 β₂m level and SYNTAX score between significant CAD and non-significant CAD subjects. In case of non-significant CAD the mean ±SD of β₂m 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 β₂m level was 4.48±0.95μg/ml and mean ±SD of SYNTAX score was 16.27±0.99. Here significant difference was found between β₂m level and SYNTAX score among respondents (p<0.001).

Receiver-operator characteristic (ROC) were constructed using β₂m value of the patient’s and a best combination of sensitivity and specificity for coronary artery disease. It gave β₂m cut off value of 3.6 with 81.4% sensitivity and 86.7% specificity as the value for identifying the coronary artery disease.

β₂-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 β₂-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 β₂-microglobulin can be used as a noninvasive diagnostic 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 β₂-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.

References:
Role of serum b2m in predicting severity of Coronary artery disease


23. Kluft, C. Identifying patients at risk of coronary vascular disease: the potential role of inflammatory markers.*European Heart Journal Supplements*, 6 (suppl C), 2004; C21-C27.


