[TAME]-esterase—A new cardiovascular risk factor in smokers

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A mathematical model has been proposed to study the effect of [TAME] esterase on blood clotting time. Using this model, clotting time was found to decrease by 30% with increasing plasma [TAME] esterase activity in a group of smokers. It is hypothesized that [TAME] esterase through its effect on Hageman factor could affect clotting time. However mechanism of clotting by [TAME] esterase remains to be elucidated. It is concluded that [TAME] esterase is involved in the cascade of reactions leading to blood coagulation and increased [TAME] esterase activity could be an additional risk factor for possible cerebro-vascular accidents in smokers.

Process of blood coagulation is a complex and incompletely understood. Traditional division of the coagulation system into intrinsic and extrinsic pathways has been supplanted because tissue factor—factor VIIa complex has been shown to be a potent activator of factor IX as well as factor X (ref 2). It is now widely agreed that the principal initiating pathway of coagulation is the extrinsic pathway due to action of tissue factor and Factor VII. The critical component, which is a tissue factor, is an intrinsic membrane protein composed of a single polypeptide chain that functions as a cofactor. It is analogous to the contact system cofactor, kininogen (HK), a high molecular weight protein.

Both prekallikrein and factor XI exist in a non-covalent bimolecular complex with high molecular weight kininogen, which by attaching to surfaces facilitates the reactions of factor XIa on its substrates. Kallikrein cleaves factor XII to convert it to factor XIa thereby accelerating contact activation and also cleaves high molecular weight kininogen liberating the nonapeptide bradykinin. Factor Xa slowly converts prothrombin to thrombin.

Smoking has been shown to increase the ability of blood to coagulate. However the mechanism by which cigarette smoking leads to activation of coagulation cascades remain poorly understood. Cigarette smoking has been shown to affect the extrinsic system of coagulation. Fibrinogen levels are also related almost linearly to the average cigarette consumption per day and positively related to the length of smoking history. It has also been suggested that endothelial cell injury or activation of white blood cell induced by smoking may initiate the activation of the extrinsic coagulation pathway. The presence in cigarette smoke of material that is both allergic and capable of activating factor XII of the intrinsic system of coagulation may be important to the pathogenesis of cardiovascular and pulmonary disease associated with cigarette smoking.

N-α-tosyl L-arginine methyl ester [TAME]-esterase is a recently described component of the kinin-kallikrein system. No data are currently available regarding the role of [TAME]-esterase in smokers. Activation of [TAME]-esterase involves activation of kinin-kallikrein pathway and [TAME]-esterase activity involves a cyclic GMP, nitric oxide dependant pathway. Further, it has also been reported that fibrinogen is an important marker of possible thromboembolic events in habitual smokers.

The present study has been undertaken to determine the potential role of [TAME]-esterase in the sequence of reactions leading to blood coagulation during extrinsic and intrinsic pathway.

One hundred and eleven (111) male volunteers between the age of 20 and 87 and not suffering from asthma were recruited into the study. The sample population was divided into two groups. Smoking group which consisted of 82 subject, (X age 43.6±14.9 years). Participants smoking at least 3 non-filtered tobacco cigarettes per day for a minimum of one year were recruited. The second group consisted of 29 non-smokers, (X age 46.5±16.7 years). Upon inclusion into the study, each subject was interviewed...
using a self-designed questionnaire before blood collection. Questions related smoking habits, age and exercise were set in the questionnaire.

Venous blood (10 ml) was collected from each subject from the antecubital fossa of the forearm using disposable plastic syringe. All participants were seated in a standardised position. Blood was dispensed in plain, trisodium citrate (3.2%) and potassium-EDTA coated tubes.

For measurement of activated partial thromboplastin time (APTT), blood was collected in trisodium citrate (3.2%) tubes and immediately centrifuged at 3000 g for 5 min in a Hermle Z320 centrifuge. Resulting supernatant plasma was used within 3 hr for determination of clotting time. Activated partial thromboplastin time was determined using a commercially available kit (Biomerieux). Blood collected in EDTA coated tubes were used for determination of haematological parameters using an automated Coulter Counter (Model T-890).

Serum obtained from blood collected in plain tube after centrifugation at 3000 g for 5 min was used for measuring [TAME]-esterase activity using a micro-method1.

Statistical analysis—Data were statistically analysed using Microsoft Excel 7.0. Z-test was used to compare mean [TAME]-esterase activity, clotting time and haematological values in smokers and non-smokers. Strength of relations was assessed by linear correlation and was tested using correlation t test. P <0.05 was considered as significant. Linear regression analysis was also used for designing the mathematical model relating [TAME]-esterase to clotting time.

Arithmetic means and standard deviations of [TAME]-esterase activity, clotting time and haematological parameters are given in Table 1. Data analysis showed that mean [TAME]-esterase activity of smokers was significantly higher compared to non-smokers (P<0.05). As opposed to [TAME]-esterase activity, clotting time (APTT) was significantly lower in smokers compared to non-smokers (P<0.05). Mean [TAME]-esterase activity in smokers was 19.45 μmol/ml/hr, while in non-smokers an enzymatic level of 3.40 μmol/ml/hr was obtained. The mean clotting time (APTT) in smoker was 35 sec compared to 40 sec in the non-smoking group.

For the haematological indices, it was noted that platelet count, white blood cell count, % lymphocyte and lymphocyte count were significantly higher in smokers compared to non-smokers (P<0.05). However there was no such significant differences in

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smokers (n=89)</th>
<th>Non-smokers (n=29)</th>
<th>Reference Value</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>[TAME]-esterase activity(μmol/ml/hr)</td>
<td>19.5±12.7</td>
<td>3.4±1.65</td>
<td>3-10 (μmol/ml/hr)*</td>
<td>S</td>
</tr>
<tr>
<td>Clotting time (APTT) (sec)</td>
<td>35.0±3.9</td>
<td>40.0±1.5</td>
<td>39-42 sec*</td>
<td>S</td>
</tr>
<tr>
<td>WBC (×10³/l)</td>
<td>8.3±2.5</td>
<td>7.6±2.0</td>
<td>4.0-11.0×10³/L*</td>
<td>NS</td>
</tr>
<tr>
<td>RBC (×10³/l)</td>
<td>5.3±0.52</td>
<td>5.2±0.6</td>
<td>5.4-6.2×10³/L*</td>
<td>NS</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>15.4±1.3</td>
<td>15.0±1.4</td>
<td>13.5-18.5 g %*</td>
<td>NS</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>45.9±3.6</td>
<td>44.7±4.1</td>
<td>47-51 %*</td>
<td>NS</td>
</tr>
<tr>
<td>MCV (Femtoliters)</td>
<td>86.7±6.1</td>
<td>86.6±7.3</td>
<td>87-92*</td>
<td>NS</td>
</tr>
<tr>
<td>MCH (Pico gram)</td>
<td>29.2±2.8</td>
<td>29.1±2.7</td>
<td>29-32*</td>
<td>NS</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.6±1.5</td>
<td>33.6±1.6</td>
<td>34-36*</td>
<td>NS</td>
</tr>
<tr>
<td>PLT count (×10³/l)</td>
<td>214.5±37.7</td>
<td>194.6±45.8</td>
<td>140-400*</td>
<td>S</td>
</tr>
<tr>
<td>LY%</td>
<td>45.9±12.7</td>
<td>38.9±10.3</td>
<td>34-44 %*</td>
<td>S</td>
</tr>
<tr>
<td>LY count (×10³/l)</td>
<td>3.5±1.2</td>
<td>2.90±0.9</td>
<td>2.9-3.2×10³/L*</td>
<td>S</td>
</tr>
</tbody>
</table>
haemoglobin level, red blood cell count, haematocrit, mean cell volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration ($P>0.05$) in smokers compared to non-smokers.

Clotting time (APPT) showed a negative correlation it decreased with increase in [TAME]-esterase activity ($r = -0.47$). None of the haematological parameters such as white blood cell count, haemoglobin, platelet count showed any significant change when correlated with [TAME]-esterase activity.

**Prediction of clotting time with respect to [TAME]-esterase activity: A proposed mathematical model**—A model is proposed to relate clotting time (sec) to [TAME]-esterase activity ($\mu$m/ml/hr). Microsoft Excel 7.0 software was used to estimate unknown parameters. A straight-line probabilistic model was hypothesized as follows:

$$Y = \beta_0 + \beta_1 x + E$$

where $\beta_0 =$ y-intercept, and $\beta_1 =$ Estimate of slope from the graph.

Simple linear regression analysis was used which gave $\beta_0 = 38.68$ and $\beta_1 = -0.147$. Least square equation obtained was $y = 38.68 - 0.147x$.

The utility and adequacy of the hypothesized model were tested. $R^2$ value obtained graphically was close to 0.30. Thus about 30% of the variability in clotting time is explained by the [TAME]-esterase activity. This model can be used to make predictions for [TAME]-esterase activity between 1.61 and 61.0 $\mu$m/ml/hr in all subjects irrespective of smoking or non-smoking. A straight line may not provide a good model for the relationship between the mean value of clotting time and the value of [TAME]-esterase when stretched over a wider range of values.

The main findings in this study showed that [TAME]-esterase activity in smokers was higher in smokers compared to non-smokers. However the mechanism by which [TAME]-esterase activity increases in smokers is not known since no data are available regarding [TAME]-esterase activity and smoking. During the events of blood coagulation, kallikrein cleaves factor XII to convert it to factor XIIa, thereby accelerating contact activation. [TAME]-esterase is a component of the kinin-kallikrein system and moreover it is associated with the conversion of prekallikrein into kallikrein. Based on present findings a mechanism is proposed by which [TAME]-esterase brings about a decrease in clotting time. During cigarette smoking, there is an increase in [TAME]-esterase activity, which increases conversion of prekallikrein into kallikrein. This leads to an increase in cleavage of factor XII into its activated form XIIa thereby accelerating the contact activation. As all the components of the blood coagulation are linked, the end result would be a decrease in clotting time. It is concluded that increase [TAME]-esterase activity in smokers could be an additional risk factor that could lead to artherosclerosis and cardiovascular diseases.

**References**