

## LEVELS OF NATURAL ANTICOAGULANTS, ANTITHROMBIN III AND PROTEIN C IN DIABETES VASCULAR DISEASE

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Accelerated micro and macro vascular thrombotic disease is characteristic of diabetes mellitus. A hypercoagulable state is appreciated in this disease. We studied levels of natural anticoagulants such as Antithrombin III (AT III) and protein C which are factors to counteract the effects of hypercoagulable state. Both AT III and protein C levels were found decreased in diabetics with macrovascular disease suggesting that natural mechanism of anticlot formation are also affected in these patients, thus may be contributing to the thrombotic complications. Bahrain Med Bull 1995;17(4):

Inherited deficiencies of Antithrombin III (AT III) and Protein C are known to be associated with thrombotic diathesis<sup>1,2</sup>. In addition to congenital deficiencies acquired defects of AT III and Protein C has also been reported in clinical conditions associated with increased tendency to thrombosis<sup>3,4</sup>. AT III is a serine protease inhibitor that plays an important role in regulating blood coagulation. It inactivates thrombin and factors Xa, IXa, XIa and XIIa, thereby limiting clot formation<sup>4</sup>. Protein C is a vitamin K dependent protein which contains several gamma carboxyglutamate residues, binds Ca<sup>++</sup> and has an anticoagulant activity<sup>5,6</sup>.

We initiated this study to investigate whether patients with diabetes mellitus, who have a high incidence of thrombotic complications, have any abnormality of AT III and/or Protein C<sup>7</sup>.

### METHODS

The study was conducted on 82 (24 males and 58 females) patients with non insulin dependent diabetes mellitus (NIDDM) attending the diabetic out patient clinic at the Nehru Hospital of the Postgraduate Institute of Medical

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Education and Research, Chandigarh, India. The patients were grouped according to the presence or absence of vascular disease. Twenty five patients had microangiopathic disease (group I), 10 patients had macroangiopathic disease (group II) and 37 patients were without any vascular complications (group III). Micro vascular diseases were retinopathy, either background or proliferative (confirmed by an ophthalmologist) and nephropathy (albugin protein-urea). Other causes of proteinuria were excluded by simple tests (midstream urine, blood count, ESR, antinuclear factor and serum complement). Macrovascular disease included history of ischemic heart disease, intermittent claudication and the presence of normal peripheral pulses and ECG findings.

Blood samples were collected in ethylene diamine tetra acetate (EDTA) tubes by vein puncture from patients and normal controls. Plasma was used for the estimation of AT III and protein C. AT III activity was measured by the method

of progressive inhibition of thrombin by a clotting assay<sup>8</sup>. AT III values (%) were obtained by comparing to the clotting times of dilutions of pooled normal plasma when tested with the same concentration of thrombin. Protein C activity was measured by the method of Griffin et al by using commercial kits from Diagnostica Stago (Asrieres-Sor-Seine, France)<sup>9</sup>.

## RESULTS

The levels of AT III and protein C in different groups of NIDDM cases and normal controls are presented in Table 1.

Table 1  
Levels of AT III and protein C in patients of diabetes mellitus with vascular complications

	Group I	Group II	Group III	Normal Controls
Number of patients	35	10	37	25
AT III % (Range)	94.2±10.5 72.3-130.8	69.0±9.9 59.2-79.5	79.4±22.6 48.1±113.5	100.5±47.7 75.7±145.2
Protein C Mg/L(Range)	74.9±31.5 38.2±137.1	51.5±20.2 38.9±95.8	97.5±37.8 49.5±143.2	83.1±28.5 70.2±140.7

P value - AT III, Normal vs Group II >0.01,  
Group I vs group II >0.01  
Protein C, Normal vs Group II >0.01,  
Group III vs group II >0.01

The values of AT III in all groups of diabetes was lower than the controls. Most significant decrease was observed in diabetics with macrovascular disease. The same trend was also seen in protein C levels. Marked decrease in protein C levels was observed in the group with macrovascular disease. A significant decrease was also seen in patients with microvascular disease. In the group of diabetics without any vascular complications, there was a wide variation in the distribution of protein C levels. Several patients in this group had protein C values significantly lower than values obtained in normal controls.

## DISCUSSION

In patients with diabetes mellitus morbidity and mortality rates are increased as a result of vascular complications<sup>7</sup>. Recent evidence suggests that increased thrombotic tendency manifested by such risk factors as enhanced platelet activity and coagulation potentials as well as hypofibrinolysis may be involved in the pathogenesis of these vascular lesions<sup>10-13</sup>.

Natural anticoagulants such as AT III and protein C play important roles in regulating blood coagulation. AT III is a major inhibitor of blood coagulation and hereditary deficiency is associated with venous thrombotic disease<sup>1</sup>. Deficiency of AT III has also been observed in clinical conditions associated with increased risk of thrombosis, for example patients with nephrotic syndrome<sup>3</sup>. Measurements of AT III levels in diabetes have yielded results which varied widely from depressed, elevated to unchanged, when compared to non-diabetic controls<sup>10,14-15</sup>. Our results have shown a significant decrease in AT III levels in NIDDM cases with macrovascular complications. In the groups with microvascular disease and those without any vascular complications, the mean

levels of AT III although were found to be low, a statistical significance was not obtained in them. Most previous studies have tried to correlate AT III levels with microvascular disease such as retinopathy and no consistence tendency in the levels have been observed<sup>16,17</sup>. Our results are also similar in this group. However our finding of a significant decrease in the group with macrovascular complications calls for attention. In the presence of several other factors contributing to a thrombophilic state in diabetes mellitus whether the increased risk of thrombosis is related to the development of AT III deficiency remains speculative.

Activated protein C, a serine protease, plays an important regulatory role in blood coagulation through its ability to degrade coagulation factors Va, VIIIa and is also believed to activate plasminogen activation, thus stimulating fibrinolytic activity<sup>18-19</sup>. Reduced levels of protein C result in thromboembolic disorders. Congenital protein C deficiency occurs, but at a low frequency and results in fatal neonatal thrombosis characterised by purpura fulminans<sup>20</sup>. In heterozygotes with this condition, mild to severe thrombophilia has been reported<sup>21</sup>. Frequently the heterozygotes who have moderate protein C deficiency are asymptomatic and no thrombotic episodes are observed<sup>22</sup>. Acquired deficiency of protein C occurs in states such as anticoagulation therapy, liver disease, disseminated intravascular coagulation, neonatal period and in patients with sickle cell disease<sup>4,23-26</sup>. It appears from the results of the present study that acquired partial deficiency of protein C also occurs in patients of diabetes with vascular complications.

Plasma levels of protein C observed in our patients are higher than those reported in inherited deficiency of this factor. This is possible explanation for the absence of recurring venous thrombotic episodes and thrombophlebitis in these patients. It should be remembered that in diabetes, microvascular obstruction seems to be more important from the pathophysiological point of view than thrombosis of great vessels such as veins of the leg. Extensive endothelial damage resulting in chronic endogenous activation and consumption of protein C with accelerated clearance from plasma, are possible explanations for decreased concentrations<sup>4</sup>. By impairing fibrinolysis decreased protein C levels may further contribute to microvascular accumulation of fibrin<sup>19</sup>.

#### CONCLUSION

Blood levels of natural anticoagulants, such as Antithrombin III and Protein C, were decreased in patients with diabetes mellitus and macrovascular disease. These might be important contributing factors for thrombotic complications in this disease along with other risk factors, such as enhanced platelet activity, coagulation potentials and hypofibrinolysis.

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