

## ANTITHROMBIN LEVELS IN HEALTHY PAKISTANI MALES

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### ABSTRACT

**Objective:** To determine the antigenic concentration and percent functional activity of antithrombin in healthy Pakistani males.

**Methodology:** Over a period of one year, 50 healthy male volunteers divided into two age groups were tested for antithrombin levels by radial immunodiffusion (RID), at the Departments of Pathology King Edward Medical College and Postgraduate Medical Institute, Lahore. None of them were suffering from any acute or chronic disease, nor were taking any medications. Plasma samples were used to determine the antigenic and derived percent activity from NOR-Partigen\* plates supplied by Dade Behring.

**Results:** The younger group of healthy male volunteers (n=25), mean age 23.5 years showed a higher antithrombin concentration 46.7mg/dl or 155% functional activity; while the older volunteers (n=25), with mean age 44.0 years, showed 42.4 mg/dl concentration or 142% functional activity. The p value was insignificant between the groups (p> 0.05).

**Conclusions:** The younger healthy individuals in our population show higher antithrombin concentration and functional activity, which tends to decrease insignificantly with age.

**KEYWORDS:** Antithrombin, Males, Age factors.

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### INTRODUCTION

The definition of haemostasis has been broadened to encompass both the imbalances between the anticoagulant and procoagulant factors which result in either a bleeding tendency or that lead to a hypercoagulable state. Throm-

bophilia is a term used to describe an increased tendency to develop a thrombotic episode. This is usually a laboratory based finding and may not be manifested clinically. It often becomes problematic to document such procoagulant states and is therefore necessary to identify all such variables that may be indicative of a thrombotic tendency.<sup>1</sup>

Thrombophilia may be acquired wherein there is a deficiency or a defect in the natural anticoagulants or it may be a temporary defect that occurs during surgery, trauma, pregnancy or drug therapy. Those abnormalities that have been associated with inheritance and manifesting a more than normal risk of venous thrombosis include reduced anticoagulant activity as a result of deficiency of the natural anticoagulants such as antithrombin, protein C or protein S.<sup>2,3</sup> Others include heritable

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abnormalities leading to the development of a procoagulant state as a result of Factor V leiden or G20210A prothrombin.

Thrombin is the main enzymic agent of the coagulation system. All naturally occurring anticoagulants, principally 'antithrombin', regulate thrombin activity and function by direct inhibition of the formed thrombin and by down regulating its production by inhibition of FVa and FVIIIa by the protein C and protein S systems.<sup>4,5</sup>

Antithrombin (previously also called antithrombin III) is believed to be synthesized mainly in the liver.<sup>6</sup> Antithrombin is a serine protease inhibitor,<sup>7</sup> which plays its inhibitory role by not only complexing with thrombin, but also by inhibiting the clotting factors (factors IX, X, XI, XII & tissue factor VII).<sup>8</sup> Heparin and vascular proteoglycans act as a catalyst in the antithrombin-serine proteinase complex formation greatly enhancing the reaction rate.<sup>9</sup>

Antithrombin concentration in normal plasma ranges between 12-15mg/dl or 2.5-4.0 $\mu$ mol/l,<sup>10</sup> however it varies with race, age, sex as well as methodology used.<sup>11,12</sup> Amongst females between 19-60 years a slightly lower value has been found as compared to women older than 60. In males, it is said to decrease with age especially after 60 years of age.<sup>13</sup> As regards the clinically deficient value, at least 50-70% of the normal antigen and percent functional activity is believed to be essential to ensure effective inhibition of blood coagulation.<sup>14,15</sup>

Two types of inherited antithrombin deficiencies have been recognized. Type I comprises of a quantitative reduction of qualitatively normal antithrombin.<sup>16</sup> Type II deficiency involves qualitatively abnormal antithrombin protein. In both types however, antithrombotic activity is reduced to or below 50%.<sup>15</sup> Acquired deficiency may occur due to pregnancy, drugs especially oral contraceptives, severe liver disease, disseminated intravascular coagulation (DIC), nephrotic syndrome, inflammatory bowel disease and in patients on heparin therapy.<sup>17</sup>

We carried out this study to see the mean concentration in our healthy male population. We

were also interested to know if age had any effect on antithrombin activity. The cut off point of 40 years for segregation between the two groups was chosen, as generally the incidence of thrombotic episodes increases sharply beyond 40.

## METHODOLOGY

*Study Population:* Over a period of one year, 50 healthy male volunteers divided into two age groups were tested for antithrombin concentration and derived percent activity by RID. Fifty percent of the volunteers were aged below 40 years and fifty percent were older than 40. Subjects were not suffering from any acute or chronic disease nor were they on any sort of medication.

*Sample Collection:* Samples were collected by clean venepuncture from the antecubital vein or a clear and prominent vein on the dorsal aspect of the hand. Blood was withdrawn by means of a 5.0 ml disposable syringe fitted with a silicone coated needle. After detaching the needle, 4.5 ml blood was transferred to a 100mm x 10mm polystyrene round bottom tube containing 0.5 ml of 3.8% sodium citrate as anticoagulant. The tube was instantly capped, and gently rotated both horizontally and vertically. Plasma was obtained by centrifugation and used for antithrombin estimation in batches.

*Antithrombin Estimation:* Antigen concentration of antithrombin in the sample was determined by RID on NOR-Partigen\* plates supplied by Dade Behring. In this method, 5 $\mu$ l of the antigen solution was applied to the centre of a cylindrical well, in a thin layer of 2% agarose gel containing monospecific antihuman antithrombin produced in rabbits and layered on to plastic plates. The antigen in the sample diffused radially into the thin gel matrix. The plate was allowed to stand horizontally at room temperature for 48 hours. Each plate contained 12 wells, eleven were used for test purposes while the 12<sup>th</sup> well was used for standard of known concentration. The diameters of the precipitin rings were measured by the provided scale. Concentrations in mg/dl and percent activity were deduced from the tables provided with

the kit. The percent activity represents the functional activity of antithrombin. Student's t-test was employed to find p value between the study groups.

## RESULTS

Healthy male volunteers were divided into two groups on the basis of age, 40 years being the cut-off point. Fifty percent of the volunteers (group I) were aged below 40 years, (age range 20-39 years and mean age 23.5 years). Fifty percent (group II) were older than 40 years, (age range 40-72 years and mean age 44 years).

Table-I shows the pattern of results that emerged in the healthy males. The mean concentration observed in group-I was 46.7mg/dl which was equivalent to 155% functional activity. In group-II a lower mean concentration than group-I was observed. In this group, the mean concentration was 42.4mg/dl equivalent to 142% functional activity. The p value was greater than 0.05 and insignificant.

## DISCUSSION

*Antithrombin levels in healthy males:* The foremost and pertinent finding in our study is the higher mean concentration of antithrombin amongst younger healthy males. Amongst group I (mean age 23.5 years) it was found to be 46.7mg/dl or 155% functional activity. In group II (mean age 44.0 years) it was 42.4mg/dl or 142% functional activity.

Different studies have quoted varying normal values for antithrombin; some consider 12-15mg/dl or 2.57 $\mu$ mol/l concentration and 75-125% functional activity as normal.<sup>10</sup> Others have considered antithrombin levels of 30.0 mg/dl, as equivalent to or 100% functional activity, in the same age groups as ours, as normal.<sup>18</sup> Comparing these figures, we note that our healthy males had almost 30 to 55%

more antithrombin as compared to other races. Larger epidemiological studies are called for in our relevant environment to establish concrete baseline reference values.

*Effect of age on antithrombin levels:* The results show a tendency of decreasing antithrombin levels with advancing age. This finding is also in agreement with those of Fagerhol and Abildgaard who have reported a 17% decrease in mean antithrombin concentration between 18 - 66 years in males.<sup>19</sup> They also suggest that, although variation in concentration in serum with regard to age and sex has not been studied for many proteins, it is quite possible that a similar effect exists for other proteins as well, such as albumin, immunoglobulins, beta-lipoproteins, haemopexin and alpha-2 macroglobulin. In serum, the antithrombin concentration is about 35% less as compared to plasma.<sup>20</sup> Others have also reported a decrease in antithrombin concentration with increasing age.<sup>21</sup>

*The significant deficient value:* The deficient value of antithrombin causing symptoms is generally held to vary between 29-80% of normal concentration. In this study, as the normal mean concentration was 155%, this deficient value would mean approximately 77% activity or less, that is a 50% reduction from normal concentration at least. Initially, it was Egeberg who reported that a 50% reduction in the plasma levels of antithrombin in inherited cases was associated with a severe tendency to venous thrombosis.<sup>22</sup> He suggested that a similar degree of transient decrease in previously normal individuals might involve a correspondingly enhanced risk to develop thromboembolism.<sup>22</sup> There are studies reporting even lower levels of antithrombin concentrations associated with thromboembolic clinical episodes. Mackie et al report concentrations 25% to 66% of normal in 12 people including seven with thrombotic disease, one of whom at necropsy

Table-I: Mean antithrombin concentration range and percent functional activity in the study population.

Study Groups	Mean antithrombin concentration in mg/dl	Range in mg/dl	Functional percent activity
Group I	46.7	40.7-50.8	155%
Group II	42.4	36.0-49.0	142%

showed severe degree of arterial atheroma.<sup>23</sup> Marciniak and co-workers report 9 out of 24 subjects studied, (of a single family) having antithrombin activity 26 – 49% of normal and presenting with thromboembolic episode.<sup>24</sup> Gruenberg et al, hold 50% reduction in activity as more definitive of clinical significance.<sup>25</sup> A similar value has also been reported by Barrowcliffe and co-workers.<sup>26</sup> Antithrombin levels between 50 – 75% indicate a moderate risk, while levels less than 50% represent a significant risk.<sup>14</sup>

### CONCLUSIONS

We conclude from our findings that healthy Pakistani males have a higher concentration and functional percent activity of antithrombin as compared to other races, and that the antithrombin values decrease with advancing age.

**Limitations of the study:** As already pointed out above, the authors are conscious of the fact that the sample in our study is small to extrapolate a generalized finding of reference ranges in the Pakistani males. Our study groups of healthy males were only a part of other groups which we had studied. We feel that this finding should stimulate other researchers to conduct larger studies to establish normal values in our population.

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