ORIGINAL ARTICLES

Childhood Solid Tumors and Antithrombin III

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ABSTRACT

Background: Blood coagulation influenced by solid tumors by causing hypercoagulable states. The effect of such changes in hemostatic system on tumor development and spreading is still a matter of discussion and the present interpretations remain hypothetical for the host hypercoagulability means a latent danger of acute thrombosis or disseminated intravascular coagulation. Antithrombin III (AT III) is a single chain glycoprotein consisting of 432 amino acids, synthesized in the liver, and of molecular weight 5800 Daltons. The normal plasma AT III concentration is 100-150 μg/ml and its biological half life is 68 hours in normal subjects. AT III is the major inhibitor of serine protease of coagulation cascade which in the presence heparin becomes a very potent inhibitor of coagulation. Persons predisposed to thrombosis are found to have elevated concentration of thrombin-anti thrombin III (TAT) complex and decreased plasma AT III activity.

Objective: Evaluate circulating plasma AT III activity level before and after chemotherapy in some childhood extra cranial solid tumors. Study Design: Forty consecutive children suffering from different kinds of solid tumors admitted to Oncology Unit of Zagazig University Hospitals were included in the study. They were 24 males and 16 females, of ages ranging from 1 to 15 years. In addition, thirty child with the same age and sex matched healthy children served as control group. All children were subjected to full history taking, thorough clinical examination, and routine investigations (CBC, liver function, prothrombin time (PT) and partial thromboplastin time (PTT)), as well as determination of serum AT III activity before and after chemotherapy for patients. Both verbal and written consents were taken from parent(s) of participating children.

Results: AT III plasma activity levels were significantly lower in patients than in control group and before induction of chemotherapy than after. Liver transaminases (ALT and AST), PT and PTT were significantly higher in all patients than control and before chemotherapy than after. A significant negative correlation between plasma AT III activity level and alanine transaminase (ALT), PT and PTT was observed in our study (p < 0.05).

Conclusion: Measurement of AT III in solid childhood malignancy can detect any thromboembolic risk for the patient and in addition it can reveal information about the course of the disease.

Key words: Antithrombin III activity – childhood solid tumors- hypercoagulable states.

Introduction

Cancer represents the second common cause of death in pediatric age after accidents. It constitutes 11% of causes of death in childhood worldwide (Wojtukiewicz et al., 2007). Leukemias make up most childhood cancer, approximately (25%) followed in frequency by tumors of CNS (20%), Neuroblastoma (7%), Non-Hodgkin lymphomas (6%), Wilm's tumor (6%), Hodgkin lymphomas (5%), Rhabdomyo-sarcoma (3%) and numerous rare tumors compromise the remainders (Almost and Smith, 1999).

AT III is an α-2 glycoprotein consisting of 432 amino acids, synthesized in the liver and of molecular weight 5800 Daltons (Smith et al., 1996). AT III is the most important inhibitor of the coagulation pathway. It inactivates thrombin by formation of a covalent bond resulting in an inactive 1:1 stoichiometric complex between the tow, involving interactions of the active serine of thrombin and an arginine reactive site on AT III (Jemal et al., 2010). AT III is also capable of inactivating other components of the coagulation cascade including factors IXa, Xa, Xla and XIIa as well as plasmin (Roemisch et al., 2002). In vivo, the anticoagulant effect of AT III is enhanced by its interaction with endogenous, endothelial cell- associated heparin sulfate proteoglycans (HSPGs) and by pharmacologic agents such as heparin and low molecular weight heparin (Askura et al., 2008).

The plasma AT III activity level in adults is approximately 12.5 mg/dl. The physiological range of AT III activity level in normal human plasma is quite narrow between 85% and 120% of normal for bioassay and
between 75-125% for immunoassay (Askura, 2007). It is known that plasma AT III activity level is low in newborns (30-50 % of adult levels) and becomes normal around six months of age while in adults, it decreases with age (Falanga, 2007). AT III deficiency is inherited as autosomal dominant fashion and thus affects males and females equally. The prevalence of the deficiency state in general population is approximately one in 2,000 to 5,000 persons (Spencer, 2000). Acquired AT III deficiency have been found in patients with liver diseases, renal diseases, hemolytic uremic syndrome and L-asparaginase drugs administrated in acute leukemia (Manco-Johnson, 1989 & Maclean and Tait, 2007).

Hemostatic abnormalities including thrombosis and hypercoagulability are present in a majority of patients with malignancy (as many as 60%) and are the second most common cause of death after infections (Patnaik and Moll, 2010). Thrombotic episodes may precede the diagnosis of cancer by months or years, thus representing a potential marker for occult malignancy (Nagaraja et al., 1999). Malignancy could affect coagulation by variable mechanisms as decreased hepatic synthesis of natural anticoagulant like AT III and protein C, abnormal activation of coagulation cascade through tissue factor release, decreased platelet aggregation and activity, and procoagulant release from plasma membrane vesicle of tumor cells (Donati and Falanga, 2001).

The current study was therefore undertaken to measure the plasma AT III activity level in some solid tumors of children before and after chemotherapy as a marker for in vivo activation of the coagulation system.

Subjects And Methods:

This study was carried out in Pediatric Department, Hematology and Oncology Unit, of Zagazig University Hospitals.

Thirty patients participated in the study of ages ranging from 1-15 years (X±SD: 8.4±4.35) and were divided into three groups:

- **Group I:** 18 children (12 males and 6 females) of mean age 8±4.3 years newly diagnosed with neuroblastoma.
- **Group II:** 14 children (8 males and 6 females) of mean age 8.7±4.2 years newly diagnosed with Wilm's tumor.
- **Group III:** 8 children (4 males and 4 females) of mean age 8.66±5.3 years newly diagnosed with rhabdomyosarcoma.

The diagnosis of solid tumors was based on clinical examination and histopathologic findings in biopsy.

- Thirty healthy children (18 males and 12 females) of mean age 8.5±4.1 years were studies as control group.

Exclusion criteria:

Children with CNS tumors, leukemia, lymphomas and those who died during induction of chemotherapy. All children included in the study were subjected to the following:

1) Full history taking and thorough clinical examination with stress on the presence of pallor, organomegaly, abdominal masses, lymphadenopathy and other constitutional manifestations.
2) Routine laboratory investigations including complete blood count (CBC) using cell counter (cell-Dyn 1700), PT, PTT, AST and ALT.
3) Imaging studies (for patient groups) including abdominal ultrasonography for detection of organomegaly, masses and ascites, CT brain for detection of CNS infiltration and chest X-ray for detection of pulmonary infiltrate and mediastinal masses.
4) Bone marrow aspiration biopsies for detection of bone marrow infiltration (for the study patients at time of diagnosis).

Measurement of plasma AT III activity:

2ml of venous blood was taken. The plasma was separated as soon as possible to prevent hemolysis. The samples were centrifuged for 10 minutes at 3000 rpm and the supernatant plasma was stored at 2-8°C until assayed.

Plasma AT III activity was performed with commercial kits (Human Antithrombin III Bindarid Radial Immunodiffusion kit, Birmingham, UK) using the principal that antigen diffusion radially from cylindrical well through an agarose gel containing an appropriate mono-specific antibody will form antigen-antibody complexes which, under the right conditions, will form a precipitin ring as a linear relationship exist between the square of the ring diameter and the antigen concentration. So, by measuring ring diameters produced by a numbers of samples of known concentration, a calibration curve can be constructed. The square of precipitin ring diameters was plotted along the vertical (y) axis and AT III concentration should be along horizontal (x) axis (Nand and Mesmore, 2009).
Statistical Analysis:

Data were presented as Mean ± standard deviation (X±SD) or percentage (%). Mean between two groups were compared using paired student's t test. The given data were compared between groups using ANOVA. Linear correlation and regression were used to test the correlations between plasma AT III activity level and some laboratory tests in all patients. Data was carried out with the Statistical Package for Social Science, version 11.0 for windows (SPPS, Chicago, Illinois USA). P-values less than 0.05 were considered statistically significant.

Results:

Analysis of demographic clinical characteristics revealed that hepatomegaly was significantly higher in children with neuroblastoma than in other patient groups (Table1).

In comparison to normal controls, all patient groups showed highly significant decrease in plasma AT III activity level and a highly significant increase in AST, ALT, PT and PTT (p<0.001) at time of diagnosis (Table 2).

Table 3 shows that there were statistically significant differences between patient groups before treatment and those after as regard AT III and other laboratory investigations.

Plasma AT III activity levels correlated significantly with ALT, PT and PTT values but not with AST (Table 4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (n=18)</th>
<th>Group II (n=14)</th>
<th>Group III (n=8)</th>
<th>Control (n=30)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT III</td>
<td>82.4±3.5</td>
<td>81.8±2.9</td>
<td>92±2.46</td>
<td>30.01</td>
<td>&lt;0.001 *</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47.7±7.7</td>
<td>43.1±3.1</td>
<td>46.5</td>
<td>98.79</td>
<td>&lt;0.001 *</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18.7±1.7</td>
<td>13.8±2.43</td>
<td>0.057</td>
<td>118.5±6.3</td>
<td>&lt;0.001 *</td>
<td></td>
</tr>
<tr>
<td>PT (sec)</td>
<td>28.5±1.55</td>
<td>118.5±6.3</td>
<td>0.057</td>
<td>28.75±1.25</td>
<td>&lt;0.001 *</td>
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</tbody>
</table>

Table 3: Comparative study among patients group before and after chemotherapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (n=18)</th>
<th>Group II (n=14)</th>
<th>Group III (n=8)</th>
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</thead>
<tbody>
<tr>
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<td>92±2.46</td>
</tr>
<tr>
<td>Paired- t</td>
<td>&lt;0.001 *</td>
<td>&lt;0.001 *</td>
<td>12.52</td>
</tr>
<tr>
<td>Paired- t</td>
<td>&lt;0.001 *</td>
<td>&lt;0.001 *</td>
<td>&lt;0.001 *</td>
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<td>AST (U/L)</td>
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<td>13.8±2.43</td>
<td>0.057</td>
</tr>
<tr>
<td>44.7±7.8</td>
<td>28.75±1.25</td>
<td>&lt;0.05 *</td>
<td></td>
</tr>
</tbody>
</table>
Discusison:

Various coagulation and fibrinolysis disorders due to the disease itself or to the therapy are known to occur in children with solid tumors. Therefore, the major inhibitors of coagulation, AT III, protein-C and protein-S, had been investigated in pediatric patients with solid tumors (Askura et al., 2008 and Bick, 2003). AT III is the most important physiologic inhibitor of the coagulation pathway. It not only inhibits thrombin, as its name implies, by preventing fibrin formation but also has a broad spectrum of inhibitory actions against several enzymes in the hemostatic pathway (Olds et al., 1994).

Activation of coagulation results in formation of thrombin which in turn inactivated by its major inhibitor AT III leading to formation of stable complex. The generation of thrombin also acts as a tissue hormone stimulating the proliferation of malignant cells (Nand, 1987).

In this study, neuroblastoma accounts for about 46% of solid tumors in the study patients and this is in consistent with Van De Water et al., (1985) who reported that neuroblastoma is the most common extra-carnial childhood solid tumor. Metastatic disease is common in neuroblastoma which is explained by presence of organomegaly, lymphadenopathy and chest findings. Similar results were reported by De Ross et al., (2001).

Swelling in the head and neck is common in rhabdomyosarcoma and this explains the presence of proptosis in 16% of our cases. This result was in agreement with Maris and Matthay (1999). In patients with Wilm's tumor, the commonest presentation is abdominal mass (80%) which is consistent with Dagher and Helman (1999).

In our study, all patient groups had significantly lower ATT III activity levels before treatment compared to controls (p < 0.001). This result was in keeping with the study by Ali (2008) who reported AT III deficiency in malignancies at time of diagnosis possibly as a result of alteration in liver synthetic function. Also, Donati and Falanga (2001) reported that the mean AT III levels in patients with malignancy before therapy are low which is due to the presence of elastase-like-protein that is capable of degeneration of AT III. These results were in agreement with our results.

In this study, there were highly significant difference between patients with solid tumors before therapy and control group as regard PT and PTT (p < 0.001). These results were in agreement with Sabah Sallah et al., (2004) who explained the prolonged PT and PTT on the basis of hepatic dysfunction caused by infiltration of the liver by malignant cells with decreased factors II, V, VII and IX and increased circulating fibrin monomers. Also in our series, all patient groups had highly significant rise of AST and ALT levels before treatment compared to controls.

In the present study, there were highly significant rise of AT III activity levels in all patients after treatment compared with levels at time of diagnosis (p < 0.001). These results were in agreement with Edwards et al.,(1993) who explained the increase in plasma AT III activity level due to increase of albumin level, at remission. After induction of chemotherapy, there was a significant decrease in AST and ALT levels compared to levels before therapy (p < 0.05). These findings can be useful as a prognostic criteria for follow up, and also can be useful in the treatment of coagulation disorders associated with malignancies in children (Sabah Sallah et al., 2004).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma AT III activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>0.273</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>0.421</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>0.512</td>
</tr>
<tr>
<td>PTT (sec)</td>
<td>0.359</td>
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</tbody>
</table>

AST: Aspartate transaminase, ALT: Alanine transaminase, PT: Prothrombin time, PTT: Partial thromboplastin time, *: Highly significant, **: Significant
Upon correlations of AT III activity and some laboratory data, we found a significant negative correlation of AT III with ALT, PT and PTT. This may be attributed to the liver synthetic dysfunction resulting from the cancer and liver damage resulting from anatomical or metabolic complications of malignancies that affect hemostasis (Sabah Sallah et al., 2004).

In conclusion, there are varying changes in parameters of coagulation-fibrinolysis system in the form of changes in AT III activity levels in plasma of pediatric patients with solid tumors specially those with liver dysfunction. Decreased levels of AT III activity in untreated cases of solid tumors can be explained by liver infiltration resulting in liver dysfunction. So, we recommend further large scale studies to prone these points with evidence of thrombosis and/or bleeding as well as monitoring of cancer patients using routine coagulation test and carefully followed by measurement of AT III level activity especially in patients with liver dysfunction.

References