Original Article

Consequences of Different Solid Malignancies in Stage I and II on The Level of Antithrombin III A Natural Anticoagulant

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Objective: To elaborate the association between the levels of natural anticoagulant antithrombin III (AT) in already diagnosed cases of different solid malignancies at stage I or II.

Methods: A cross sectional study was carried out in the Department of Pathology, Lahore General Hospital and Sheikh Zaid Hospital Lahore. Diagnosed cases of solid malignancies were selected from INMOL Hospital Lahore. 52 subjects of already diagnosed cases of solid malignancies were selected according to the selection criteria. They were divided in 2 groups, group 1 encompassed 26 subjects of stage I solid malignancy, while group 2 comprised of subjects who were diagnosed as stage II solid malignancy. Automated Coagulometer Sysmex CA 600 was used to measure AT levels and the subsequent data was recorded. Data was statistically analyzed using SPSS 20.

Results: The mean AT level in group I was 128.1±16.4 and in group II was 111.6±12 respectively. Results reveal that there was significant difference in mean AT level between the two groups (p value < 0.001).

Conclusions: It was concluded that the AT level is decreased in solid malignancies even in initial stages. Mean AT in subjects of group II was significantly lower (p value <0.001) as compared to subjects of group I. So, as the stage of malignancy advances AT level decreases and number of AT deficient subjects increases.

Keywords: antithrombin III (AT), solid malignancies, stage I and II.

Introduction

Cancer is a major public health problem worldwide. Among the cancer prevalence, maximum is due to solid tumors.[1] A solid tumor is an abnormal mass of tissue that usually does not contain cystic or liquid areas. Solid tumors can be benign in nature or they can be malignant.[2] Malignancy can be classified according to their primary site of origin or by histologic or tissue types. Grading of tumor is determined by the abnormality of the cells with respect to the surrounding normal tissues. Similarly malignancies can be staged by different staging methods. The most frequently used method is called TNM staging method which classifies according to tumor size (T), degree of nodal involvement or regional spread (N) and distant metastasis (M). In our study we used malignancies in stage I and in stage II, as stage I signify tumor that is limited to the tissue of origin and stage II shows limited local spread only.[3]

Malignancy is a well-known hypercoagulable state. All the patients with malignancy display a hypercoagulable state, which includes platelet activation, blood coagulation, complement activation, vasodilation and inflammation. Malignant cells can activate the clotting system directly thereby generating thrombin or indirectly by stimulating mononuclear cells to synthesize and express a variety of procoagulants. This often results in thrombosis, the second leading cause of death in patients with malignancies.[4] A thrombus that is formed is a result of alterations in the blood products especially platelets, clotting factors, the endothelium and turbulence and stasis of blood flow.[5]

A vital naturally produced thrombin inhibitor is Antithrombin III (AT). It forms thrombin-antithrombin TAT complexes through irreversible reaction with resultant inactivation of thrombin.[6,7] In malignancy the hemostatic balance of clotting and fibrinolysis is shifted to thrombosis due to the deficiency in the inhibitor molecules AT, PC and PS.[8]

Coagulation of blood is extremely coordinated so that it is constantly altered and impacted by procoagulants and anticoagulants. It is really essential to maintain a balance between the different components of coagulation so they work in harmony. So this study was conducted in order to evaluate the plasma levels of AT at stage I and II in patients of solid malignancies. It may lead to early detection or primary prevention of the complications such as thrombosis, which might be helpful in decreasing the morbidity as well as mortality of these patients by prompt assessment of fall in the level of AT. However, the literature is still insufficient to indicate

if AT level can be used in screening programs for early detection or primary prevention of the complications.

Method

A cross sectional study has been performed. The study was performed on 52 subjects. There were 2 groups each comprising of 26 subjects. Group 1included already diagnosed cases of solid malignancies in Stage I and group 2 encompassed diagnosed cases of solid carcinomas in stage II. Cases were classified on the basis of imaging and histopathology according to the tissue type. This study incorporated Breast CA (42.3%), Lymphoma (17.3%), Female Genital Tract CA (9.6%), Gastrointestinal CA (7.7%), Bone Tumor (6.7%) and Male genital tract CA (6.7%) as presented in figure 1. Group 1: 26 diagnosed cases of stage I solid malignancy that has neither entered deeply into adjacent tissues nor has it spread into other parts or lymph nodes, also known as early stage cancer. Group 2: 26 diagnosed cases of stage II malignancy indicating limited local spread. This stage shows malignancies which are localized and not spread to the other parts of the body.

Subjects were selected with the age range of 19-85 years. Patients already having deep venous thrombosis and pulmonary embolism, hemostatic disorders, inflammatory bowel disease, severe acute infectious disease, connective tissue disorders and diabetes mellitus were excluded. Patients with known inherited thrombophilia on the basis of history and any past evidence of thrombosis were also excluded as well as patients on oral contraceptive pills (OCPs) or anticoagulants, pregnant females and those with history of stroke and /or neurosurgery within past 6 months were not included in the study.

Venous blood was drawn aseptically in a light blue top vacutainer that contains 3.2% sodium citrate anticoagulant. 2.7 ml blood in 0.3ml sodium citrate solution was taken in 9:1 ratio and centrifuged immediately to separate plasma. Samples were centrifuged for 15 minutes at 2000. Sera were stored at -80oC in freezer. Frozen plasma was thawed within 10 minutes at 37oC and homogenized by carefully mixing without foam formation. Test was carried out for AT within hours after thawing.

AT was measured applying Innovance AT kit on Siemen's fully automated Blood Coagulation Analyzer Sysmex CA 600 as per the standard procedure written in the literature of the kit by optical detection and percentage detection method.

Results

Shapiro Wilk test was applied to confirm the normality of the data. The data was not of normal distribution so Kruskal Wallis test was applied to compare the mean AT among groups. Results revealed that difference appeared significant in mean levels of AT among study groups. Mean AT in group 2 was significantly lower as compared to group 1. As

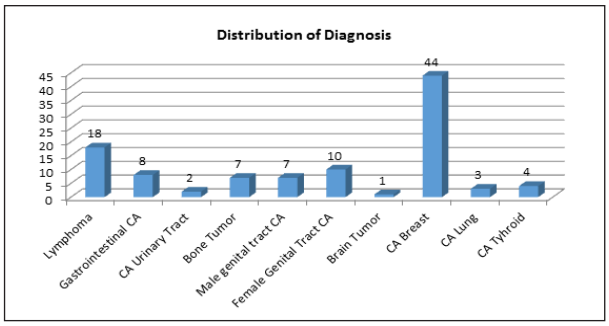

Fig-1: Malignant neoplasm distribution in study groups.

Table-1: Percentage of at deficient subjects in the study groups.

Deficiency AT	Group-I	Group-II
Yes	13 (50.0%)	23 (88.5%)
No	13 (50.0%)	3 (11.5%)
Total	26 (100%)	26 (1000%)

Key: using Chi-square test, p value <0.001 (significant)

Table-2: Showing results of study groups.

	ANTITHROMBIN III				
Group	Mean±SD	Median (Inter-quartile rage)	Minimum	Maximum	p-value
Goup-1	128.1±16.4	126.1 (122.1 - 131.2)	107.0	195.3	<0.001
Goup-2	111.6±12.8	112.3 (101.1 - 121.7)	88.9	134.6	<0.001

**. The mean difference is taken as significant at 0.05 level.*

Table-3: Showing comparison of at level between two groups.

		COMPARISION			
Sr. No	Groups		Mean difference	Std. Error	p-value
1	1	2	16.47308*	5.91234	0.003*

**. The mean difference is taken as significant at 0.05 level.*

the stage of malignancy increases Antithrombin III level decreases. **(Table-1)** The study participants were divided into two groups according to tumor stage. Out of 26 patients in stage II, 23 (88.5%) were found AT deficient. Out of 26 patients 13 (50.0%) were found AT deficient in stage I. Chi-square test showed a distribution difference in AT deficiency among study groups. As the stage of tumor advances number of AT deficient subjects increases. **(Table-2)** The mean AT level in group1 and 2 was 128.1 ± 16.4 and 111.6 ± 12.8 respectively. p value was calculated < 0.001 which was significant.**(Table-3)**Detailed comparison between the two groups is given in the table mentioned below. The mean difference in the level of AT between two groups was found to be significant (p value 0.003).

Discussion

Purpose of the current study was finding out deficiency in AT level, a natural anticoagulant and a core inhibitor of coagulation cascade in initial stages of solid malignancies as it is substantially increasing the risk of thrombosis that can affect the morbidity and mortality. In the current study 23 (88.5%) patients were found AT deficient in stage II and 13(50.0%) patients were found AT deficient in stage I with p value <0.001 (significant). The balance between the fibrinolytic system and coagulation cascade can shift to a prothrombotic state in malignancy due to deficiency in the inhibitory molecules such as AT.[8-12] The patients of cancer experience an elevated risk of developing thrombosis mostly in the initial three months after diagnosis.[13] AT is one of the major factors that inhibit the tumor progression and it is down regulated in tumors, so promoting their progression.[14] The plasma levels of AT correlate with tumor prognosis, aggressiveness and staging of various neoplasias.[15] AT deficient individuals have the highest risk of developing first venous thrombosis.[16] The mean level in plasma of various natural anticoagulants including AT are significantly lower in carcinoma.[11,16-18] T he current study strongly indicates a direct association between falling levels of AT with the increasing stage of solid malignancies. Further research is required in the line that antithrombin III level may be assessed for screening and prognostic purposes as this cannot only prevent disease progression but will be helpful to reduce economical burden of the diagnosis and treatment.

Conclusion

50.0% cases of group 1 i.e., 13 out of 26 cases were AT deficient and 88.5% patients of group 2 i.e., 23 out of 26 were found AT deficient. Among 52 patients of solid malignancies in stage I and II, 36 were AT deficient. This study proves that level of AT is decreased even in initial stages of solid malignancies. As the stage of solid malignancy advances, AT level decreases and number of AT deficient subjects increases.

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