ORIGINAL ARTICLE

Frequency of Acquired Antithrombin III Deficiency in patients with Disseminated Intravascular Coagulation

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ABSTRACT

Background: Disseminated intravascular coagulation (DIC) is an acquired syndrome that is characterized by activation of coagulation resulting in the formation of intravascular fibrin deposit and finally thrombotic occlusion of smaller vessels.[1] The blood supply to the vital organs is compromised and thus in association with metabolic and hemodynamic derangements, it eventually contributes to the multiple organ failure[2]. DIC shows a continuum in clinical pathological severity which is characterized by the increasing loss of compensated control of intravascular activation of coagulation[3].
Aim: To find out the frequency of acquired Antithrombin deficiency and correlation of AT deficiency with coagulation profile.
Study design: Cross sectional study was carried out at Sheikh Zayed Medical Complex Lahore from December 2011 to June 2012.
Methods: Total of 90 patients of DIC who met the selection criteria were included. The age of the patients included in the present study ranged from 10-65years with a mean standard deviation 44.0±10.8 years and median of 46 years. Male to Female distribution was 60%and 40% with male to female ratio of 1.5:1.Blood specimens were taken and used for the estimation of Antithrombin level by chromogenic auto analyzer method and for the measurement of coagulation profile. Hb, TLC, PLT count, PT, ApTT& D-dimers tests were also performed on all the patients.
Results: Twenty one (23.3%) patients were deficient in AT and 30(33.3%) had abnormal coagulation profile. D-dimers were raised in all the patients with DIC as it was the inclusion criteria for selection of patients. The Hb levels were measured and ranged from 6.5g/dl -12.9g/dl with a mean haemoglobin of 8.8g/dl. Total leucocyte count was performed on all the selected patients and their values ranged from 17 - 41x10^6/Lwith a mean TLC of 26.5 x $10^{6.}$±10.1x10^6. All patients had a platelet count of <100x10^9/L. It was observed that PT was prolonged in 33.3% whereas ApTT levels were deranged in 5.5% of pts.
Conclusion: 1) A negative correlation was found between acquired AT deficiency and the patients of DIC who also had deranged coagulation profile which included PT APTT. 2) Thrombocytopenia with a platelet count of less than 100x10^9/L was observed in all the 90 patients with DIC and appeared as a consistent feature of DIC regardless of the underlying condition.
Key words: Disseminated Intravascular Coagulation, Antithrombin III, and Coagulation profile.

INTRODUCTION

Disseminated intravascular coagulation (DIC) is characterized by systemic activation of blood coagulation system, which results in generation and deposition of fibrin, leading to microvascular thrombi in various organs[1]. The blood supply to the vital organs is compromised and thus in association with metabolic and hemodynamic derangements, it eventually contributes to the multiple organ failure[2]. DIC shows a continuum in clinical pathological severity which is characterized by the increasing loss of compensated control of intravascular activation of coagulation[3].

There is no single laboratory test or a combination of tests available,sensitive and specific enough, to allow a definitive diagnosis of DIC. In most cases the diagnosis can reliably be made by taking into consideration the underlying disease and a combination of laboratory findings[4]. The laboratory diagnosis of DIC thus uses a combination of these tests because no single test result alone can firmly establish or rule out the diagnosis. The tests commonly used for assessment of DIC are platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen level, fibrin degradation products (FDP) assay and D-dimer assay, As DIC is a dynamic process, repeat measurements are necessary. Baseline tests of hemostasis may initially provide evidence of coagulation activation and later provides evidence of

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consumption of coagulation factors, but their individual diagnostic efficiency is limited[5].

Antithrombin III(ATIII) is an important physiological regulator of blood coagulation that affects extrinsic, intrinsic and common pathways of coagulation.[6] It inactivates several enzymes of coagulation system[7]. Antithrombin (AT I) absorbs thrombin on to fibrin after thrombin activates fibrinogen, Antithrombin II(ATII) is a cofactor in plasma which together with heparin interferes with the interaction of thrombin and fibrinogen and AT III inactivates thrombin.[8]The normal concentration of AT III in plasma is approximately 0.12mg/ml [9]Its primary action is to inhibit the activated coagulation factor IIa(thrombin) and Xa[10]. It also plays a role to inhibit of inflammation within the area of damaged vascular endothelium. All major physiologic anticoagulants appear to be affected in patients with DIC. Low circulating levels of AT is associated with poor outcome[11].Acquired AT III deficiency is due to consumption, which is observed in situations where activation of coagulation system is inappropriate, i.e. are DIC ,microangiopathic haemolytic anemias due to endothelial damage(haemolytic uremic syndrome) and veno-occlusive disease(VOD) in patients undergoing bone marrow transplantation[12].

OBJECTIVES

To find out the

1. Frequency of acquired AT III deficiency in DIC patients
2. Correlation of AT III deficiency with coagulation profile of the patients.

MATERIALS & METHODS

This crossectional study was carried out at the Shaikh Zayed Hospital & Services Hospital Lahore during June 2012- December 2012.A self administeredquestionare was prepared in English language & was also translated to Urdu. Informed consent was obtained from the patients before taking a detail history & physical examination A total of 100 consecutive patients were interviewed 90 patients were selected for this study. The age of the patients ranged 10-65years with a mean & standard deviation 44.0±10.8years and median of 46 years with a male to female ratio of 1.5:1. Only diagnosed patients of DIC were included. Patients on anticoagulant drugs, known cases of coagulation disorders, with pulmonary embolism, with H/o of contraception, renal diseases & hepatoma were exclude.

Blood was drawn for Hb, TLC, DLC, Plt count, PT/ApTT, D-dimers & AT III levels.Only those patients who had a D-dimer level of more than1000ng/ml were selected for the analysis of AT III. Haemoglobin was estimated by SLS haemoglobin method on automated haematology analyzer (sysmex XT 2000i). Total leucocyte count was performed by flow cytometry method on automated hematologyanalyzer. Platelet count was also estimated by flow cytometry on auto analyzer. Already separated plasma was used for the estimation of PT by adding extrinsic source of thromboplastin and calcium in the plasma. The time required for clotting of plasma was recorded. The test measured the activity of those coagulation factors which were involved in extrinsic and common pathway of clotting system especially factor II, V, X. After taking 100microliter of plasma 200microliter of thromboplastin and calcium chloride was added in equal quantity at 37C.The endpoint was noted when the clot appeared.Stago diagnostic France kit was used. APTT was estimated by recalcification of plasma after the addition of standardized amount of cephalin (platelet substitute) and factor XII_activator (Kaolin). After taking 100microliter plasma, 100 microliter APTT reagent was added then incubated for 3minutes then 100microliter of calcium chloride was added. Endpoint was noted when clot appeared. Stago Diagnostic France kit was used.the graphic representation. D-dimer was performed by using latex agglutination test for the qualitative and semi quantitative determination of fibrin D-dimers in plasma by Stago diagnostic France.

A suspension of latex particles coated with monoclonal antibodies directed specifically against D-dimer was provided in the kit. One drop of latex suspension and one drop of plasma diluted 1:2, 1:4, 1:8 with the buffer as provided by the manufacturer for semi quantitative estimation were mixed and rocked gently for the length of time directed by the manufacturer, usually 2-3 minutes. If macroscopic agglutination was observed the test is designated as positive. The cut off value was taken >1000ng/ml [108.] AT III was performed by using chromogenic substrate assay, I took 2ml of plasma which was obtained by adding patient's venous blood into a glass tube containing sodium citrate and centrifuged it at 4000RMP for 10min. This obtained plasma was then run on to an auto analyzer (sysmexCA 500 series) which was calibrated by using standard human plasma for analyzing AT activity in the given sample. Statistical analysis were determined by using Microsoft Excel & SPSS version 15.

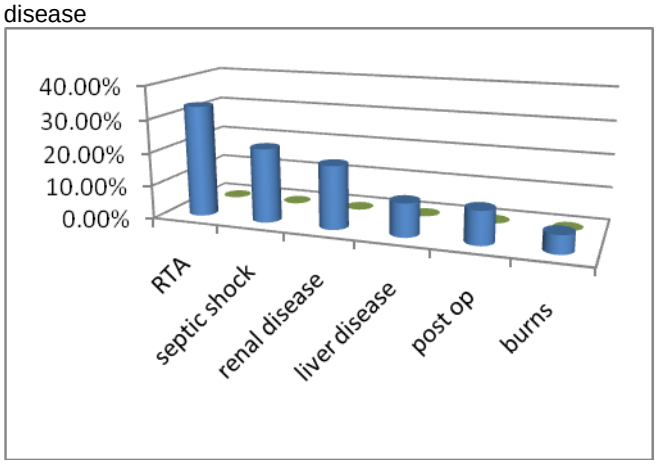
RESULTS

On the blood specimen of 90 selected patients laboratory tests revealed the results as follow:

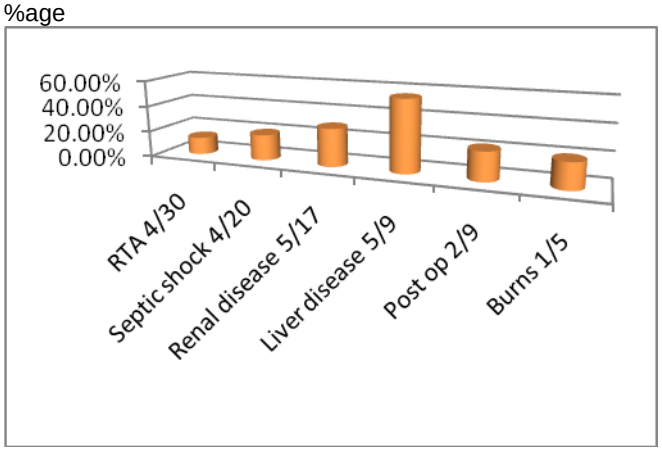
Distribution of cases by sex and its %age

Gender	No.	%age
Male	54	60
Female	36	40
Total	90	100

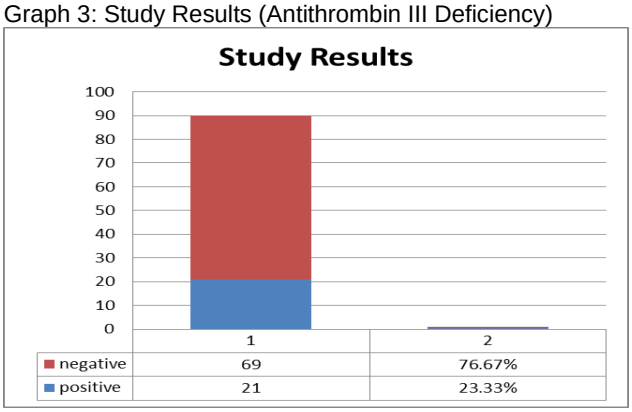
Graph 1: Distribution of cases with relation to underlying disease



Graph 2: Affected cases with underlying diseases and %age



Graph 3: Study Results (Antithrombin III Deficiency)



Qualitative measurement (present or absent) were derived on AT III analysis. AT III levels (AT deficiency) were noted low in 21(23.3%) of cases out of which 13 patients were male & 8 were females. It was observed that all clear cut cases of AT deficiency had prolonged PT/APTT. The Prothrombin time was prolonged in 33..3% of the cases. Same was the finding with APTT. The D-dimers levels were raised in all of the selected cases. The values of D-dimer 1000-2000ng/dl were found in 53(58.9%) of cases, &>2000ng/dl in 37(41.1%) of cases.

DISCUSSION

The study was conducted to determine the frequency of acquired Antithrombin(AT) deficiency in patients with DIC. Frequency of AT deficiency varies all over the world according to their demographic distribution,socioeconomic status, race, genetic makeup and diet.

In a total of 90 cases studied low levels of AT were detected in 21 cases (23.3%). The variables included in my study were age, gender, type of disease and coagulation profile.

Various studies in Pakistan and all over the world exhibit different frequency of acquired AT deficiency in association with various diseases. Some of those showed higher frequency while it was low in others.

Hb concentration was estimated in all the patients. Most of our patients(48.8%) had moderate degree of anemia(8-10g/dl).Levels of Hb reflect nutritional status, socio economic status, access to the medical facility, literacy and mode of disease. In our study population most of the patients had Road traffic accident and lost a variable amount of blood followed by the patients who presented with septic shock, renal failure liver disease, burns or postoperative cases. Hb levels however did not show any direct correlation with DIC & no other study showed this correlation.

TLC was performed on all the patients. It was found that all of our patients had variable degree of leucocytosis. None of our patient therefore presented with leucocytopenia. The direct association of leucocytosis with DIC was not found.

Platelet count was also done on all the patients. Thrombocytopenia has been reported to be associated with DIC in the literature.Thrombocytopenia has been one of the important marker of DIC, especially a platelet count lower than 100 x 10^9/L. Similar finding has been observed in our study where 100% of the patients of DIC had a platelet count of <100x 10^9/l.

In our study prolonged PT &/or APTT was observed in 33.3% of cases as compared with 19.4%

in the reference study. A higher percentage of our patients showed coagulation (PT/APTT) abnormality as compared with the reference study. A variable degree of derangement of coagulation profile (PT/APTT) has been reported in DIC patients in international studies

In a recent study carried out in India,50 patients of DIC with various underlying diseases were analyzed. The PT was found to be prolonged in 92%, APTT in 82% and PT + APTT in 82% of cases.It is much higher frequency as compared with our study[13].

It is therefore analysed that PT/APTT abnormalities have a variable degree of association with DIC. D –dimer level was also performed on all the patients . In the reference study the D-dimer level was not used as an inclusion or exclusion parameter.This parameter can therefore not be compared with the reference study. D –dimer levels were reported to be abnormal in almost all the patients presented with DIC. It is however reported that D-dimer is a more reliable marker for DIC when compared with routine coagulation tests like PT and APTT[14].

In the present study 21 (23.3%) of the total patients exhibited a lower than normal levels of AT. The decreased levels of AT were rightly attributed to acquired AT deficiency associated with different underlying and ongoing conditions. The frequency of AT deficient patients in the present study was 23.3% as compared with 22.2% in the reference study[13].The difference was statistically non significant with a p value 0.89.Our findings were therefore in conformity with the reference study.

In the present study the mean age of the patients was 44.0± 10.8 years It was observed that AT deficiency was present in all the patients above 40 years of age as compared to a reference study in which AT deficiency was seen in >50 years of age[13].The age difference of the affected patients was however found to be statistically non significant (p value 0.10). On this account the results were comparable with the reference study as far as the age distribution of the patients with AT deficiency was concerned.

A similar observation has been made in other local and international studies which also report that AT levels were found to be lower in older patients as compared with younger patients.The difference was well elucidated in the Pakistani study where significantly lower levels of AT were found in patients above 60 years when compared with the patients who were less than 40 years of age

While analysing acquired AT deficiency in both genders a slightly different observation was made. Considering male patients 24.1% in the present study as compared with 18.2% in the reference study demonstrated acquired AT deficiency [13].The difference was statistically significant with a p value 0.003. This however shall be considered that the male population in the reference study was much smaller as compared with the present study.

Considering the female (22.2%) patients in the present study exhibited AT deficiency as compared with 24% reported in the reference study[13]the difference was found to be non significant with a p value of 0.76.

On correlating acquired AT deficiency with deranged coagulation, 21 (70%) patients out of 30 patients who had deranged coagulation profile (PT,APTT) also had AT deficiency.Whereas in the reference study 07 patients who had deranged coagulation profile (PT/APTT) 06 (85.7%) demonstrated acquired AT deficiency.The difference between the ratio of patients having AT deficiency among those who had deranged coagulation profile (PT,APTT) was found to be statistically significant with a p value of 0.002.

All of our patients (100%) who had AT deficiency also had prolonged PT &/or APTT as compared with 85.7% patients in the reference study[13].The level of significance was therefore non-significant with a p value 0.92. It has been reported in various studies that AT deficiency was frequently associated with a deranged coagulation profile including PT and APTT

In the present study among the 9 patients who presented with DIC associated with liver disease 55.5% patients demonstrated acquired AT deficiency. Whereas a much higher frequency (84-100%) of AT deficiency is reported among the patients with chronic liver disease[1516].

In the present study the 29.4% patients with renal disease exhibited AT deficiency whereas higher but variable percentage has been reported in the literature[1718].

Among the post operative patients included in the present study 22.2% exhibited acquired AT deficiency. In a similar study conducted at Michigan University 38% postoperative DIC patients showed AT deficiency[19].

Out of 05 patients with burns 01(20%) had acquired AT deficiency in the present study, Whereas in international studies burns have been reported to be associated with AT deficiency in 54% -71% [20] [21].The difference may be attributed to a fewer number of burn patients with DIC. In our study as compared with a much larger population of burn patients with varying degree of burns being treated in burn units were included.

Among the 20 patients with septic shock, 4(20%) showed acquired AT deficiency, whereas a significantly higher frequency of AT deficiency was

reported in DIC associated with septic shock in international studies[22].

In the present study 13.3% patients with trauma / RTA exhibited acquired AT deficiency, however in an international study AT levels were found to be reduced in patients who presented of DIC associated with trauma/RTA. Although the number of the patients suffering from DIC associated with trauma / RTA was highest among our study population yet the percentage of patients who exhibited AT deficiency was lowest in comparison with DIC associated with other disorders[23]. This may due to degree of trauma and release of procoagulants[23].

CONCLUSION

1. In this study the frequency of Acquired AntithrombinDeficiency was found to be 23.3% of the cases suffering from DIC due to different underlying conditions.
2. A negative correlation was found between acquired AT deficiency and the patients of DIC who also had deranged coagulation profile (PT, APTT). Acquired AT deficiency shall be suspected in all the DIC patients who show associated PT / APTT prolongation.
3. Thrombocytopenia appeared as a consistent feature of DIC regardless of the underlying condition.
4. The present cross sectional study of patients suffering from DIC represents a particular setting, and therefore may not reflect the exact frequency of DIC associated AT deficiency in a general population with reference to a city or region. Therefore, multicentre and coordinated studies on larger patient population are recommended.

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