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COMPARE THE LEVELS OF THESE MARKERS IN THALASSAEMIA MAJOR, THALASSAEMIA INTERMEDIA AND NORMAL HEALTHY CONTROL

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Abstract:

Background: *Thalassaemia is an inherited haemoglobinopathy due to quantitative defect of globin chain synthesis. Clinically thalassaemia is divided into thalassaemia major (TM), thalassaemia intermedia (TI), and thalassaemia minor. Different studies provide evidence of increase incidence of thromboembolic events especially in TI. So plasma levels of thrombin-antithrombin complex (TAT) and prothrombin fragment1+2 (F1+2) are supposed to be increased in these individuals.*

Objective: *Compare the levels of these markers in thalassaemia major, thalassaemia intermedia and normal healthy control.*

Methodology: *A comparative cross sectional study was conducted in Thalassaemia Prevention Centre of Sir Ganga Ram Hospital and Lahore General Hospital. A total 24 normal healthy population, 24 thalassaemia Major(TM) and 24 thalassaemia intermedia patients participated in the study. Plasma levels of TAT complex and F1+2 were measured of all blood samples. Data was recorded and analyzed.*

Results: *The mean age of controls was 13.6 ± 8.1 years, and of cases with major and Thalassaemia intermedia was 13.94 ± 8.69 years and 13.64 ± 8.5 years respectively. The mean age was statistically same in all three groups, p-value > 0.05. Among controls and TI groups 11 (45.83%) males and 13 (54.17%) females were included, among cases with thalassaemia major, 14 (58.33%) males and 10 (41.67%) females were included. Mean thrombin-antithrombin complex among controls was 3.4±0.8, among cases with thalassaemia major was 5.0±1.9 and thalassaemia intermedia was 6.4±2.7 with statistically significant mean difference among the study groups (p-value=0.001). Mean prothrombin F1+2among controls was 0.94±.19, among cases with thalassaemia major was 1.10±1.7 and thalassaemia intermedia was 1.36±0.27 with statistically significant mean difference among the study groups (p-value=0.001), with highest values of these markers in TI.*

Conclusion: *A hypercoagulable state was established in thalassaemia major and intermedia as compared to control group revealed by higher levels of TAT and F1+2 in both thalassaemia groups. While the mean Thrombin-antithrombin complex (ng/ml) and mean Prothrombin F1+2 (nmol/L) levels were highest in TI with p-value < 0.05.*

Keywords: *Thalassaemia major, thalassaemia Intermedia, plasma levels, thrombin-antithrombin complex, Prothrombin fragment1+2 (F1+2)*

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1.1 INTRODUCTION:

Thalassaemia is a heritable haemoglobinopathy, caused by quantitative defect of Globin chain synthesis. Clinically Thalassaemia major (TM) is a severe subtype of disease and is associated with regular blood transfusions. The other clinical subtype i.e. Thalassaemia intermedia (TI) is disease of moderate severity. Patient's hemoglobin is maintained between 7-10 gm/dl and they do not need regular blood transfusions.(Hoffbrand and Moss, 2011)

More than 5000 children with thalassaemia major are born in Pakistan every year. In 1992, Khatak and Saleem found a prevalence of 5.4% thalassaemia in Pakistan.(Baig et al., 2008)Regular blood transfusions and iron chelation therapies result in increased life expectancy of these patients.(Ladis et al., 2005) This increased life expectancy leads to the appearance of new and serious complications of chronic disease like thromboembolic events.(Senanayake and Lamabadusuriya, 2001, Cappellini et al., 2000, Zalloua et al., 2003b)

Thalassaemia is associated with chronic hypercoagulable state which can result in thromboembolic phenomenon occurring more frequently in TI than TM.(Succar et al., 2011) However frequency of these events has not yet been established as only few studies are available.

In an Italian study thromboembolic events were observed in 4% TM patients and 9.6% of TI patients, these thromboembolic events were mainly affecting CNS, other areas of localization of thromboembolism are pulmonary, portal and mesenteric vessels (Pignatti et al., 1998). Cappellini conducted a cohort study on TI patients and observed that about 29% of TI patients developed different thromboembolic events such as portal vein thrombosis, pulmonary embolism or deep venous thrombosis during follow up period of 10 year (Cappellini et al., 2000). A study in Mediterranean area and Iran has shown that thromboembolic events occur 4.38 times more commonly in TI than TM, with more venous events occurring in TI and more arterial events occurring in TM.(Taher et al., 2006b)

In thalassaemia pathogenesis of chronic hypercoagulable state is complex. Increase number of platelets in activation status, with high aggregation potential and increase number of defective red blood cells (RBCs), with outer membrane's procoagulant phospholipids expression, are main factors that lead to the hypercoagulable state. Other factors include increased expression of endothelial adhesion molecules, increase in circulatory microparticles and deficiencies of anticoagulant proteins (Cappellini et al., 2010).

Platelets of thalassaemic patients are in activated state and have aggregation potential as proved by different studies .There is increased urinary excretion of ThromboxinA2 (TXA2) and prostacyclin (PGI2) which confirms increased activation of platelets in these patients(Eldor et al., 1999). Platelet derived microparticles with high procoagulant potential are significantly increased in thalassaemic patients as assessed by measuring the platelet factor-3 like activity.(Pattanapanyasat et al., 2007). In 1997 Ruf and others, found a significant correlation between annexin-V bound RBCs and the number of activated platelets in TM patients. This data implies that defective RBCs in thalassaemia initiate platelet activation.(Taher et al., 2008)

In defective RBCs oxidation of globin chains result in formation of hemichromes, which can react with RBCs' membrane components, such as spectrin, band 3, ankyrin etc.(Rund and Rachmilewitz, 2005) These RBCs become rigid, deformed and express more negatively charged phospholipids like phosphatidylserine on their outer membrane. Increase percentage of annexin-v positive RBCs indicates increased expression of phosphatidylserine with procoagulant activity.(Labib et al., 2015)

Protein C and protein S, naturally occurring anticoagulants present in the blood, are decreased in thalassaemia.(Hassan et al., 2010, Bhattacharyya et al., 2007)Soluble endothelial adhesion proteins like ICAM, VCAM-1, E-selectin and P-selectin are raised in thalassaemic patients, indicating endothelial activation which is a feature of hypercoagulable state.(Kanavaki et al., 2009) Raised levels of circulating plasma tissue factor and microparticles are also thrombogenic (Habib et al., 2008).

TM is less hypercoagulable state than TI partly due to regular blood transfusions. In 1996 Chen S. and others found that in TM, cohesiveness and aggregation of defective RBCs reduces by regular blood transfusions. So transfusion therapy can reduce the proportion of defective RBCs and can protect against different thromboembolic events (Taher et al., 2015). Regular transfusions correct anemia and suppress erythropoiesis in TM.(Thein and Rees, 2011)On the other side in TI transfusion naivety and splenectomy result in increase nucleated red cells (NRBCs) and increased platelet counts. Both these factors are associated with more thromboembolic events in TI than other types of thalassaemia. NRBCs count is highest in TI with splenectomy than non

splenectomized TI patients (Taher et al., 2010a).

MATERIALS AND METHODS:

2.1 Objectives

Compare the levels of these coagulation activation markers in thalassaemia major, thalassaemia intermedia and normal healthy control.

2.2 Hypothesis

Thrombin-antithrombin complex and Prothrombin fragment F1+2 levels are higher in thalassaemia intermedia than in thalassaemia major.

2.3 Ethical consideration

Informed consent was taken from all the participants of the research data collection and blood sampling. They were assured that being a part of study would not affect the course of their treatment and it would be beneficial to such patients. All the information gathered for research purpose was kept confidential.

2.3.1 Study design

A cross sectional comparative study was conducted.

2.3.2 Study setting

Study population was selected from registered thalassaemic patients of Government Thalassaemia Prevention Centre, Sir Ganga Ram Hospital and Sundus foundation. Research tests were performed in Lahore general hospital, Lahore.

2.3.3 Sample size

24 for thalassaemia major, 24 for thalassaemia intermedia and 24 for healthy individuals were included in the study.

2.3.4 Study duration

Study was conducted from 01.12.2016 to 01.12.17 after approval of synopsis

2.3.5 Sample technique

Simple random sampling technique was used.

2.3.6 Inclusion criteria

- Diagnosed cases of Thalassaemia major and thalassaemia intermedia.
- Age 3 to 35 years
- Blood sampling for research purpose was done just before blood transfusion in thalassaemia major and thalassaemia intermedia patients.

2.3.7 Exclusion criteria

- Patients with severe liver disease.
- Those with family history of thrombosis.
- Patients taking drugs that affect haemostasis like anticoagulant drugs, thiazide diuretic and sedatives.
- Patients with co morbid conditions.
- Pregnant females and those on oral contraceptives.

2.3.8 Study groups

Total study population of 72 was divided into 3 groups

2.3.8.1 Group 1

24 diagnosed β-thalassaemia intermedia patients (non transfusion dependent anemia). These patients had increased haemoglobin F on Hb electrophoresis/HPLC but clinical growth was normal and they maintained their haemoglobin between 7-9g/dl without regular blood transfusion dependence.

2.3.8.2 Group 2

24 diagnosed β-thalassaemia major patients (transfusion dependent anemia). These patients had Hb F up to 95-100% on Hb electrophoresis/HPLC and require regular blood transfusion for maintenance of Hb up to 10g/dl

2.3.8.2 Group 3

24 normal healthy age and sex matched control group, these individuals had no history of thromboembolic phenomenon in family

2.4 Data collection

2.4.1 Clinical history:

A detailed clinical history of all individuals taking part in study was taken.

Previous laboratory investigation were noted

2.4.2 Sample collection:

Blood samples of all individuals were collected in blue topped vacutainer containing 3.2% disodium citrate as an anticoagulant. 1.8ml blood in 0.2ml sodium citrate solution i.e. 9:1 ratio was taken and within half hour, centrifuged at 3000rpm for 10 minutes to separate plasma. Plasma samples were separated in aliquot and these aliquots were stored at -20º. Tests were performed within one month of sample collection.

RESULTS:

3.2: AGE DISTRIBUTION AMONG STUDY GROUPS:

The mean age of controls was 13.6 ± 8.1 years, and of cases with thalassaemia major and Intermedia was 13.94±8.69 years and 13.64 ± 8.52 years respectively as shown in TABLE.3. The mean age was statistically same in all three groups with p-value 0.97. There is no significant difference in the age of the three groups.

TABLE 3.1: Age Distribution among Study Groups:

Study groups	Total number (n)	Mean Age (years)	S.D	p-value
Control (group1)	24	13.6	8.1	0.97
Thalassaemia Major (group2)	**24**	13.94	8.69	
Thalassaemia Intermedia (group3)	**24**	13.64	8.52	
Total	**72**	13.76	8.32	

Key: group1= Control, group 2= thalassaemia major, group 3= thalassaemia intermedia,
SD= standard deviation

3.3: AGE GROUPS DISTRIBUTION IN ALL GROUPS

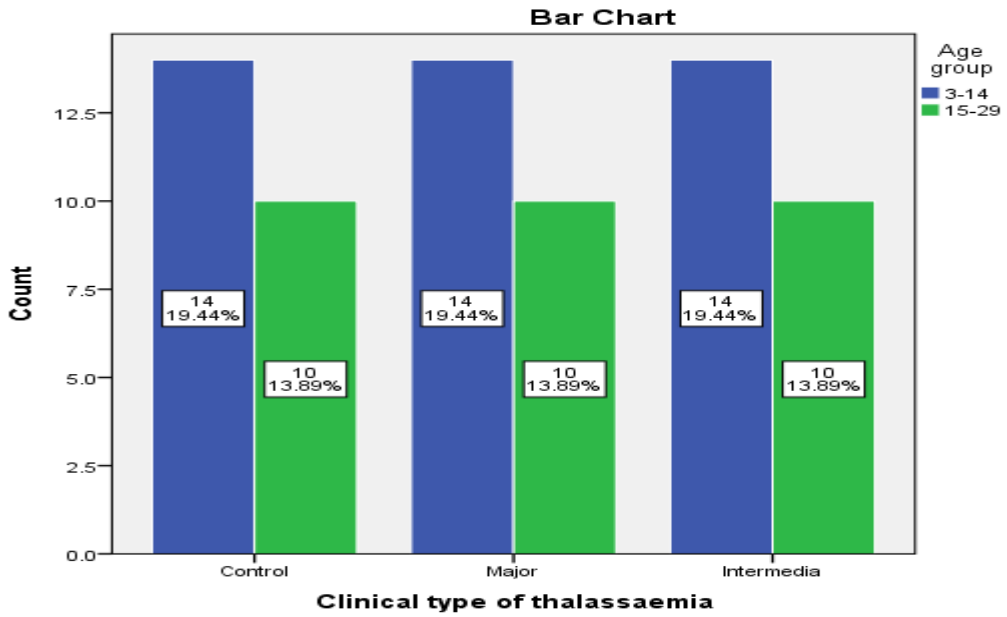

FIGURE 11: Age Distribution in All Groups

As shown in bar chart above, 14(19.44%) adults and 10(13.84%) children of total population are present there in each study group. In each study group population14 (58.33%) subjects were less than 15years old children and 10 (41.67%) were between15-29 years old adults i.e. statistically insignificant.

3.4 GENDER DISTRIBUTION IN ALL GROUPS

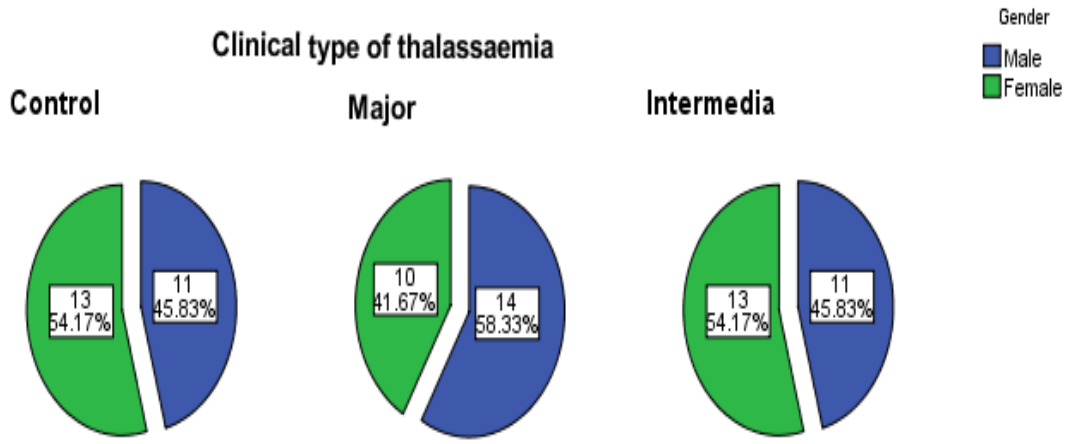

FIGURE10: Gender Distribution in All Groups

In the pie chart above it is shown that among controls group 11 (45.83%) males and 13 (54.17%) females were included, among cases with thalassaemia major 14 (58.33%) males and 10 (41.67%) females and among cases with thalassaemia intermedia 11 (45.83) males and 13 (54.17%) females were included. Gender distribution was equal in all groups.

3.5: COMPARISON OF TAT COMPLEX IN STUDY GROUPS

Mean and SD thrombin-antithrombin complex among controls was 3.4±0.8ng/ml (2-5ng/ml), among cases with thalassaemia major mean and SD value was 5.0 ±1.9ng/ml (1-9ng/ml) and thalassaemia intermedia was 6.5±2.7ng/ml (2-11.3ng/ml). There was statistically significant difference among mean TAT between all three study groups (p-value=0.001). TI patients have highest mean TAT complex levels. More than 50% of patients of TI and TM had TAT levels more than 5ng/ml i.e. above the upper limit of normal healthy control value.

TABLE 3.2: Comparison of TAT Complex in Study Groups

	Study groups	Total number (n)	Mean (ng/ml)	±SD	p-value
TAT complex	**Group1**	24	3.4	±0.78	0.001
	Group 2	24	5.0	±1.93	
	Group 3	24	6.5	±2.76	
	Total	72	5.0	±2.35	

Key: group1= Control, group 2= thalassaemia major, group 3= thalassaemia intermedia, TAT= thrombin antithrombin complex, p value between all 3 groups= 0.001 (significant)

3.6 ERROR BAR CHART SHOWING MEAN TAT OF DIFFERENT STUDY GROUPS

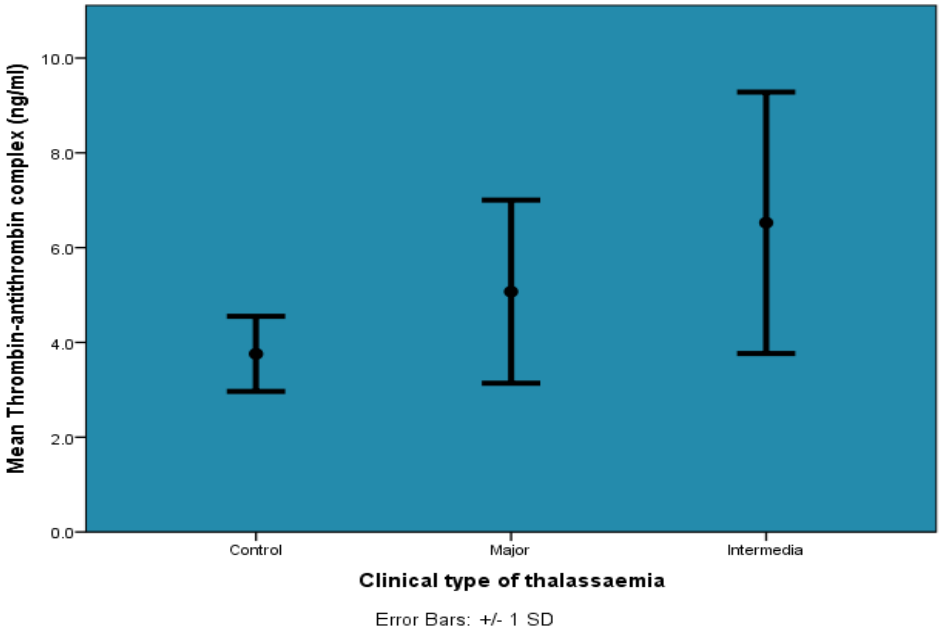

FIGURE 13: Error Bar Chart Showing TAT of Different Study Groups

Error bar chart representation of mean ±1SD of all three groups is given above. The mean of all the groups were represented by the black marker i.e. 3.5, 5.0 and 6.5ng/ml in control, TM and TI respectively. The length of the bar above and below the mean was the spread of data around the mean i.e. ±1SD. There was some overlapping among the SD of three groups but statistical test one way ANOVA showed that data is statistically significant among all the three groups

3.7: PAIR-WISE COMPARISON OF TAT COMPLEX BETWEEN GROUPS

Comparison between mean of TAT of different study groups showed that mean Thrombin-antithrombin complex (ng/ml) was significantly higher in TM and TI as compared to controls. p value between means of TM and Control was equal to 0.015 and between TI and control was 0.001. While within thalassaemic patients mean Thrombin-antithrombin complex (ng/ml) was statistically higher in thalassaemia Intermedia than in major with p-value 0.037. This means a significantly high TAT in the patients of TI as compared to TM. About 54.2% patients of TI had high TAT than normal control group.

TABLE3.3: Pair-wise Comparison of TAT Complex in Different Groups

Variables	Study groups	p-value
TAT Complex	**Group 2 vs group 3**	0.037
	Group 1 vs group 2	0.015
	Group 1 vs group 3	0.001

Key: group1= Control, group 2= thalassaemia major, group 3= thalassaemia intermedia, TAT= thrombin antithrombin complex

3.8. COMPARISON OF PROTHROMBIN F1+2 IN ALL STUDY GROUPS

Mean prothrombin F1+2 among controls was 0.94±0.19 nmol/L (0.60-1.3 nmol/L), among cases with Thalassaemia major was 1.11±1.7 nmol/L (0.80-1.4 nmol/L) and thalassaemia intermedia was 1.26±0.17 nmol/L (0.90-1.63 nmol/L). There was statistically significant difference among mean F1+2 of all three study groups (p-value=0.001). TI patients have highest mean F1+2 levels. About 16.7% patients of TI and 4.2% of TM had F1+2 value above 1.3 nmol/L.

TABLE 3.4: Comparison of Prothrombin F1+2 in All Groups

	Study groups	Total number (n)	Mean (nmol/L)	±SD	p-value
F1+2	**Group1**	24	0.94	0.19	0.001
	Group 2	24	1.11	0.17	
	Group 3	24	1.26	0.17	
	Total	72	1.10	0.21	

Key: group1= Control, group 2= thalassaemia major, group 3= thalassaemia intermedia,
F1+2= prothrombin fragment 1+2.

3.9 ERROR BAR CHART SHOWING MEAN F1+2 OF DIFFERENT STUDY GROUPS

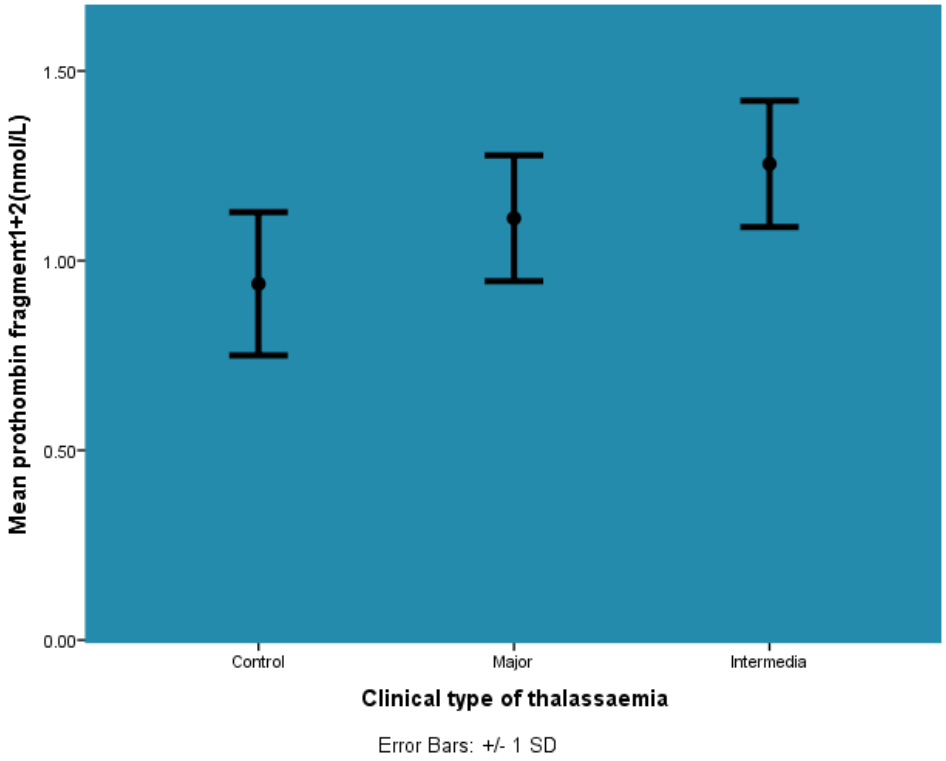

FIGURE 14: Error Bar Chart Showing F1+2 of Different Study Groups

Error bar chart representation of mean ±1SD of all three groups is given above. The mean of all the groups was represented by the black marker i.e. 0.95, 1.11 and 1.26nmol/L in control, TM and TI. The length of the bar above and below the mean was the spread of data around the mean i.e. ±1SD. There was some overlapping among the SD of three groups but statistical test one way ANOVA showed that data is statistically significant among all the three groups

3.10: PAIR-WISE COMPARISON OF F1+2 BETWEEN GROUPS

Comparison between mean of F1+2 of different study groups showed that mean F1+2 (nmol/L) was significantly higher in thalassaemic patients as compared to controls. p value between means of TM and Control was equal to 0.003 and between TI and control was 0.001. While within thalassaemic patients mean F1+2 (nmol/L) was statistically higher in thalassaemia Intermedia than in major with p-value 0.016. This shows a significantly high F1+2 (nmol/L) in the patients of TI as compared to TM. About 16% patients of TI had high F1+2 (nmol/L) than normal control group.

TABLE 3.5: Pair-wise Comparison of Prothrombin F1+2 in-between Study Groups

Variables	**comparison between Study groups**	p-value
F1+2	Group 2 vs group 3	0.016
	Group 1 vs group 2	0.003
	Group 1 vs group 3	0.001

Key: group1= Control, group 2= thalassaemia major, group 3= thalassaemia intermedia,
F1+2= prothrombin fragment 1+2.

3.11. CORRELATION BETWEEN TAT AND F1+2 IN ALL GROUPS

TAT and F1+2 both were observed to be higher in TI than TM as compared to control. A correlation was present between these two markers of thrombin generation in the study groups.

TABLE 3.6: Pearson Correlation between TAT Complex and F1+2

	TAT and F1+2 (ng/ml) (nmol/L)
Total cases	72
r	0.721
p value	0.01

Key: r= Pearson correlation, TAT= thrombin antithrombin complex
F1+2= Prothrombin Fragment 1+2, p= 0.01(Significant)

Pearson coefficient of correlation between TAT and F1+2 is equal to 0.721 with p value < 0.01 that means a strong positive correlation between both these markers of in vivo coagulation activation, is present. Both of these markers had linear relationship and both were increased in patients with hypercoagulable state.

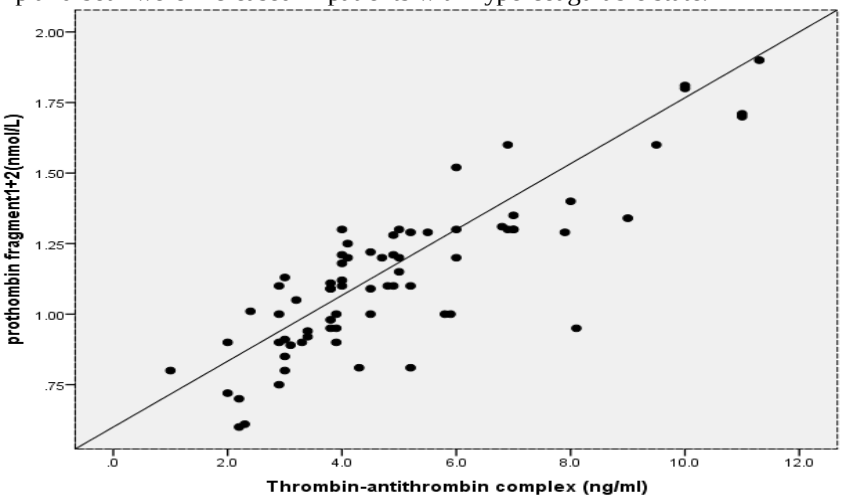

FIGURE 15: Pearson Correlation between F1+2 and TAT

Figure above shows a positive correlation between F1+2 and TAT. Value of r= 0.72 (p <0.01). A positive correlation indicates that increase in on variable is associated with increase in the level of other variable.

DISCUSSION:

Thalassaemia is an inherited hemolytic disorder caused by a partial or complete deficiency of α- or β-globin chain synthesis. Thalassaemia is genetic disorder and Pakistan is an endemic area of different genetic mutations causing thalassaemia. With the improved management, many complications of this persistent disease are becoming apparent. Thrombosis in thalassaemic patients is one of the important complications that is becoming evident and causing increase rate of mortality and morbidity in these patients. The goal of current study was to identify the hypercoagulable state in thalassaemia patients by measuring the markers of coagulation activation and also comparing the levels of these markers in two subtypes of thalassaemia i.e. TI and TM.

As already explained in literature review thalassaemic red cells, platelets and several other factors may be involved in the in vivo coagulation activation and cause a procoagulant state in thalassaemia. This hypercoagulable state in thalassaemia in the presence of several others factors can lead to development of clinical thrombosis. Incidence of these thromboembolic events is even higher in patients of TI than TM. Thalassaemia intermedia subtype, transfusion naivety and splenectomy have been observed to be associated with more pronounced hypercoagulable state in thalassaemia.

Although various researches were performed globally, but not much focus in this regard has yet been given in Pakistan. We, therefore, aimed to compare hypercoagulable state by measuring the levels of the important plasma coagulation activation markers in thalassaemia major, thalassaemia intermedia and normal healthy controls in order to establish a hypercoagulable state in thalassaemia.

In the present study 24 patients of TI, 24 patients of TM and 24 normal healthy controls were included. All three groups were age and sex matched, no significant difference in the age and sex were observed between the groups. The Mean ±SD age of controls was 13.6 ± 8.1 years, and Mean ±SD of cases with Thalassaemia major and intermedia were 13.94 ± 8.69 years and 13.64 ± 8.5 years respectively. The mean age was statistically same in all three groups, p-value > 0.05. Among controls, 11 (45.83%) males and 13 (54.17%) females were included, among cases with thalassaemia major 14 (58.33%) males and 10 (41.67%) females and among cases with thalassaemia intermedia 11 (45.83) males and 13 (54.17%) females were included. There was equal gender distribution among the three study group, p value=0.95 (not significant).

In current study Plasma levels of coagulation activation marker TAT was observed to be significantly increased in thalassaemic population as compared to normal healthy control with p value <0.001. Among TM cases, 12(50 %) of patients were having high TAT levels than the upper limit of control population, with p value 0.01. Same observations were made by Eldor et al., 1999, he found increased levels of TAT in TM in about 50% of both children and adults study population with p value less than 0.01. Similarly Sirachainan et al., 2012 found that TAT complex levels are significantly higher in transfusion dependent TM patients than normal healthy control population. In another study Sirachainan et al., 2016, comparison was done between the TAT levels in α thalassaemia and β TM and results showed significantly increased TAT in TM patients with p value less than 0.05. In this study mean TAT in healthy was 2.1µg/L as compared to 4.7µg/L in transfusion dependent TM patients.

In present study, 13(54.2 %) TI patients have high TAT than upper limit of control population, with p value 0.001 as shown in Table 3.3. These results were almost similar as Teli et al, 2012 found increased levels of TAT in 62.5% patients of thalassaemia intermedia. Angchaisuksiri et al., 2007 measured the levels of plasma thrombin-antithrombin in thalassaemia intermedia (β/E thalassaemia) with a p-value for significantly increased TAT was <0.001.

In our study comparison of TAT levels between TI and TM patients showed that TI had higher TAT than TM patients with p value < 0.037. These results indicated that in TI the hypercoagulable state is more severe.

In present study, F1+2 the other marker of coagulation activation was also significantly increased in thalassaemic population than control population, with p value of significance < 0.001. About 1(4.2%) patient of TM had high F1+2 than upper limit of control population p value 0.003. This observation is in contrast to the study conducted by Eldor et al., 1999. In this study plasma F1+2 levels were found to be insignificant as compared to normal healthy control. In contrast to present study, a study conducted by Sirachainan et al., 2016 also found normal levels of F1+2 in transfusion dependent TM patients. But our study results were inconsistent with results of the study by Sirachainan et al., 2012. In this study significantly increased F1+2 levels were observed in TM patients as compared to normal healthy control.

In current study 4(16.7%) patients of TI had high F1+2 with significantly increased mean F1+2 levels as compared to normal healthy population p value 0.001. Similar to our study cappellini et al., 2000 also observed higher F1+2 in TI then normal healthy population with p value less than 0.05. In another study Teli et al., 2012 found significantly increased F1+2 in 8.33% cases of TI patients. Angchaisuksiri et al., 2007 concluded that thalassaemia intermedia patients had high F1+2 with p value <0.04.

While comparing mean F1+2 between TI and TM, a significantly high mean F1+2 with p value 0.016. The result indicates a more hypercoagulable state among the patients of TI than TM. This result was in concordance to the study conducted by Cappellini et al., 2000, in which plasma F1+2 levels were found to be significantly increased in TI than TM and in control population a p value of less than 0.05. Cappellini et al., 2000 compared the markers of coagulation activation like plasma antithrombin, F1+2 and D-dimer levels in TM and TI. A significantly increased plasma coagulation activation markers like F1+2 ($p<0.05$) and d-dimers ($p<0.001$) in splenectomized TI than TM and control groups were found. Antithrombin levels were also decreased in both TI and TM. AT levels were supposed to be reduced as it has to make 1:1 molecular complex with increased thrombin leading to the formation of increased TAT in thalassaemia.

In 2012 Sirachainan conducted a study on thalassaemia patients. Hypercoagulable state was confirmed in patients with severe β-thalassaemia major disease with high levels of plasma coagulation activation markers i.e. TAT and F1+2 than control healthy group. Then after bone marrow transplantation these levels again measured at the median follow-up time of 70.3 months. Decrease in levels of TAT, F1+2 and D-dimer confirmed that hypercoagulable state in thalassaemia was due to inherited defect of red cells.

In present study two coagulation activation markers i.e. TAT and F1+2 were in linear positive correlation with each other, r=0.72 and p value 0.01 indicated a strong positive correlation. This result is inconsistent with the study on TI by Atichartakan et al., 2002, in which both F1+2 and TAT were correlated r=76.9 and p value 0.001

The results of our study have important clinical implications. In the light of results by international studies and in our study it is evident that patients with thalassaemia intermedia are at higher risk of thrombosis. Therefore careful and consistent observation of these patients should be considered. More researches are also suggested in this area to unearth further related aspects. Each patient should be assessed individually and high risk patients should be identified early. All the intrinsic risk factors like type of thalassaemia, circulating RBCs count etc as well as extrinsic factors like splenectomy, transfusion naivety, etc should be considered in each patient individually. Regular monitoring and proper management of these high risk patients can reduce the incidence of thrombotic complications and can improve the quality of life of these patients.

Short-term antiplatelet or antithrombotic prophylaxis with heparin can be given during and after any surgical procedure, even in young thalassaemia patients. Splenectomized thalassaemia intermedia patients should be assessed and prophylaxis may be required during prolonged immobilization, surgery and pregnancy etc, when they are at increased risk of developing thrombotic manifestations. Oral contraceptive drugs should not be given to these patients. Long term use of anticoagulant drugs if required should be closely monitored as risk of bleeding are there. All these considerations can reduce the burden of this complication. Preventive and prophylactic measures if required will be very cost effective and will improve the quality of life of these patients.

CONCLUSION:

In vivo coagulation markers were increased in thalassaemic patients than normal healthy population. Comparison of F1+2 and TAT in TI and TM showed increased levels in TI. So it can be concluded that more marked Hypercoagulable state is present in Thalassaemia intermedia than thalassaemia major, as TAT and F1+2 coagulation activation markers were increased more markedly in TI than TM.

We can say that although TI is clinically milder clinical type of disease but severe complications can develop in TI. Hypercoagulable state present in thalassaemia intermedia can lead to arterial and venous thromboembolic events and pulmonary hypertension. So regular monitoring, risk factor identification and prophylactic treatment may be necessary in these patients. Splenectomy, surgery, pregnancy and other conditions that can lead to thrombosis should be closely monitored and these patients might require prophylactic medication.

5.2 LIMITATIONS OF THE PRESENT STUDY:

- Our study was conducted on a very limited number of populations.

- In Pakistan much attention is not given to TI patients, especially those TI patients who rarely need transfusion. These Patients came into attention only after they had developed complication. No electronic record and plan of management of such patients was available in hospitals
- Although Punjab Government had started Thalassaemia Prevention Centre in Lahore, which provide the facility of early prenatal diagnosis, premarital screening of carriers and family screening. But public awareness is limited about this facility.
- Many autonomous thalassaemia centres in Pakistan have their own limited numbers of patients. There is no record keeping and maintenance of these patients at one place.

5.3 WAYS TO OVERCOME THESE LIMITATIONS:

- Available data on thalassaemia should be organized at one place.
- Researches on large scale should be conducted.
- Public awareness should be increased and large scale screening should be done in population for early diagnosis especially TI and carriers.

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