

## HAEMATOLOGICAL AND HAEMOSTATIC PARAMETERS AMONG TYPE 2 DIABETES PATIENTS IN A GOVERNMENT MEDICAL COLLEGE, SOUTH INDIA: A CROSS-SECTIONAL STUDY

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### Abstract

**Background:** Type 2 diabetes mellitus (T2DM) remains one of the non-communicable metabolic disorders associated with serious thrombotic outcomes and risk of cardiovascular disease, which can be fatal. Therefore, the goal of this study was to compare the levels of haemostatic and hematological parameters in T2DM and non-diabetic subjects. The study also determines the relationship between haemostatic parameters and hematological parameters among the T2DM subjects. **Materials and Methods:** We recruited a total of 200 participants for the study, including 100 individuals with diabetes and 100 individuals without diabetes. We collected blood samples to analyze factors such as full blood count, Factor V, VII, and tissue plasminogen activator inhibitor-1 (TPA I-1). A test of significance of means was carried out using the one-way analysis of variance test, while relationships were tested using Pearson correlation and logistic regression. **Result:** The results revealed significantly higher levels of Factor V, VII, and TPA I-1 among participants with diabetes when compared with those without diabetes. However, the participants with diabetes showed significantly lower levels of red cell parameters and red cell indices. The people with diabetes also had significantly higher levels of all white blood cells (WBC), platelets, and differential leukocytes, except for lymphocyte and eosinophil levels. Moreover, there was a significant positive correlation between Factors V and VII, TPA I-1 and Factor VII, TPA I-1 and platelets, Factor VII and Haematocrit (HCT) levels in diabetic subjects. **Conclusion:** Conclusively, the correlation between pro-coagulant and hypofibrinolytic factors may be accountable for the hypercoagulability and thrombotic events that characterize T2DM, thereby providing an insight into factor-specific management of the disease with haematological parameters routinely assisting in predicting factor levels thereafter, increasing the ease of prognosis of T2DM.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic condition associated with raised levels of blood glucose due to the body's inability to produce any or enough of the hormone insulin or effectively utilize the insulin produced by the body.<sup>[1]</sup> It is considered to be a heterogeneous group of metabolic disorders characterized by several defects in the regulation of carbohydrate, fat, or protein metabolism.<sup>[2]</sup> Diabetes mellitus is characterized by hyperglycemia, glycosuria, hyperlipidemia, and negative nitrogen

balance in the body.<sup>[3,4]</sup> If hyperglycemia is not well controlled, it can damage many organs and cause serious and life-threatening health problems like heart disease (CVD), nerve damage (neuropathy), kidney damage (nephropathy), losing a limb, and eye disease (mainly affecting the retina) that causes vision loss and blindness.<sup>[5,6]</sup>

Diabetes can be classified into four general categories that include type 1 diabetes mellitus (T1DM) which is caused by autoimmune pancreatic beta-cell destruction usually leading to absolute insulin deficiency; type 2 diabetes mellitus (T2DM) which is caused by a progressive loss of adequate

beta-cell insulin secretion frequently on the background of insulin resistance; other specific types of diabetes due to other causes (such as monogenic diabetes syndromes, diseases of the exocrine pancreas and drug or chemical-induced diabetes) and gestational diabetes mellitus is pregnancy-induced diabetes that could be diagnosed in the second or third trimester of pregnancy, not overt diabetes before pregnancy.<sup>[7,8]</sup>

It's very important to stress that type 2 diabetes mellitus (T2DM) is strongly linked to a higher risk of thrombotic events. Cardiovascular disease and thrombosis remain the major cause of death of patients with T2DM.<sup>[9,10]</sup> Blood platelets play a pivotal role in the blood clotting process by mediating the primary phase of hemostasis. Their involvement in atherogenesis and thrombotic complications has been previously well documented.<sup>[11,12]</sup> Researchers have found that platelets from diabetic patients, especially those with vascular instability and angiopathy, are more activated at rest and respond more strongly to stimuli, which is also known as platelet hyper-reactivity.<sup>[13]</sup> Altered platelet morphology and function have been reported in patients with diabetes.<sup>[14]</sup> It is reported to be connected to the pathogenesis of cardiovascular disease in T2DM patients, thereby precipitating coagulation disruption and harassment of fibrinolysis. This leads to an imbalance between haemostatic factors in plasma and endothelial cell surface, characterized by hypo fibrinolysis.<sup>[15]</sup> The intervention of coagulation factors and co-factors can be molecularly marked by the assessment of some factors of the extrinsic and common coagulation pathway, which includes Factors I, II, V, VII, VIII, and X.<sup>[15,16]</sup>

Hypo fibrinolysis can also happen when the expression of tissue plasminogen activator inhibitor-1 (TPAI-1) goes down. TPAI-1 is an inhibitor and an important regulator of the fibrinolytic system, and it can also be used as a molecular marker to show how the system is working.<sup>[17,18]</sup> Previous investigations,<sup>[19,20]</sup> have reported numerous changes in hematological parameters among T2DM patients, and these include structural and functional alterations alongside changes in the metabolism of platelets, white blood cells (WBC), red blood cells (RBC), and the coagulation system, which manifest in various forms.

Although there have been various studies on the assessment of the effect of T2DM on the coagulation and fibrinolytic system, factor-specific studies are still few,<sup>[21,22]</sup> and inconsistent amongst the same population, coupled with the fact that there is barely any information on the relationship between hematological parameters and coagulation/fibrinolytic factors in T2DM subjects.<sup>[23,24]</sup> This study was therefore aimed at comparing the levels of haemostatic and hematological parameters of T2DM and non-diabetic subjects.

The study also determines the relationship of haemostatic parameters with hematological parameters amongst the T2DM subjects.

## MATERIALS AND METHODS

**Study Setting and Design:** We conducted the study using a cross-sectional approach, and it is approved by Ethical Committee of government medical college in Mahabubabad, Telangana. Duration of study January 2024 to December 2024. A total of 200 participants, consisting of “100” participants with T2DM and “100” randomly selected participants without T2DM, were referred to as the control group.

For participants with diabetes, the inclusion criteria for the study were diagnosed patients with T2DM attending the diabetic clinic in the health facility who were willing to participate in the study. For the control group, the inclusion criteria for the study were supposedly healthy individuals that have no diabetes in the community where the health facility is located and who were willing to participate in the study. Exclusion criteria were patients on alcohol, antiplatelet, and anticoagulant drugs for hypertension and coagulation disorders.

**Data Collection:** Participants' socio-demographic data were collected using a structured questionnaire. To carry out haematological and haemostatic analysis, a 9 ml blood sample was collected from the cubical vein of each participant using a 10 ml syringe.

Following blood collection, the blood was divided into two portions, consisting of 4.5 ml each. One 4.5 ml portion dispensed into sample bottles with 0.5 ml of trisodium citrate and another 4.5 ml portion into sample tubes with EDTA (Ethylene Diamine Tetra Acetic Acid). A blood cell count autoanalyzer (Abbott Cell-DYN Emerald 22) used the blood sample in EDTA bottles for a full blood count. The blood sample in the trisodium citrate bottle was mixed well and centrifuged at 2000 g for 15 minutes. The plasma that was produced was then spun again at 2000 g for 15 minutes to get platelet-poor plasma. This was then separated and kept at -80°C until ELISA kits were used to analyze haemostatic factors (Factors V, VII, and TPAI-1). Factors V and VII were estimated using Elabscience: E-EL-H0764 and Elabscience: E-EL-H0768, respectively; Elabscience: E-EL-H2104 was used for TPAI-1 estimation.

**Statistical Analysis:** All data were analyzed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) software. Data were presented using descriptive and inferential statistics. The generated data were presented as means ± standard deviation. A comparison of means was carried out using the one-way analysis of variance (ANOVA), while relationships were tested using logistic regression and Pearson correlation. All

analyses were carried out at a probability level of 0.05.

## RESULTS

**Socio-demographic Characteristics of the Subjects:** Socio-demographic characteristics of the study subjects revealed that a greater number of participants with diabetes and those without diabetes

span between ages 51-60 years (42%) and 41-50 years (30%), respectively. Most of the participants in both categories had tertiary education. Generally, there was a preponderance of male participants in both categories. 39% of the participants had been diagnosed with diabetes for more than eight months. The participants were treated with antihypertensive (amlodipine and nifedipine) and STATIN (rosuvastatin) drugs [Table 1].

**Table 1: Socio-demographic characteristics of the study subjects.**

	Socio-demographic	Participants' Categories	
		With Diabetes (n=100)	Without Diabetes (n=100)
Age (years)	<20	-	3 (3%)
	21-30	1 (1%)	25(25%)
	31-40	18 (18%)	41 (41%)
	41-50	30 (30%)	20 (20%)
	51-60	42 (42 %)	11(11%)
	>61	9 (9%)	-
Gender	Male	59 (59%)	65 (65%)
	Female	41 (41%)	35 (35%)
Marital status	Single	2 (2%)	32 (32%)
	Married	95 (95%)	68 (68%)
	Primary	3 (3%)	-
Duration (months)	<2	8 (8%)	-
	2-3	22 (22%)	-
	4-5	16 (16%)	-
	5-6	12 (12%)	-
	7-8	3 (3%)	-
	>8	39 (39%)	-
Hypertensive drugs	Patients on antihypertensive drugs	75 (75%)	3 (3%)
	Patients not on antihypertensive drugs	25 (25%)	97 (97%)
STATIN	Patients on STATIN	65 (65%)	4 (4%)
	Patients not on STATIN	35 (35%)	96 (96%)

**Coagulation and Antifibrinolytic Levels of the Participants:** In general, the average levels of all coagulation and antifibrinolytic factors that were

looked at were significantly higher ( $p \leq 0.05$ ) in people who had diabetes compared to people who did not have diabetes [Table 2].

**Table 2: Coagulation and antifibrinolytic factors in the study participants**

Concentration (ng/ml)	Participants' Categories		p-value
	With Diabetes (n=100)	Without Diabetes (n=100)	
Factor V	738.96 ± 12.33	421.38 ± 4.89	<0.001
Factor VII	774.82 ± 8.62	430.40 ± 7.56	<0.001
TPAI – 1	20.84 ± 3.71	7.26 ± 1.58	<0.001

Note: All values are mean concentrations, while values in parentheses are standard deviations of means. TPAI-1 represents tissue plasminogen activator inhibitor-1.

Among participants with diabetes, significantly variable of white blood cell count, platelet count, and some other differential leucocyte parameters

( $p < 0.05$ ) except for neutrophil count and eosinophil count were observed compared to the non-diabetic participants with diabetes.

**Table 3: Haematological characteristics of the study participants**

Concentration (ng/ml)	Participants' Categories		p-value
	With Diabetes (n=100)	Without Diabetes (n=100)	
Haematocrit (%)	36.92 ± 1.36	43.48 ± 0.39	<0.001
Haemoglobin conc (g/dl)	12.13 ± 1.22	14.68 ± 1.78	<0.001
Mean cell volume (fl)	83.36 ± 3.26	85.82 ± 3.22	<0.001
Mean cell haemoglobin (pg)	26.25 ± 1.56	28.96 ± 1.81	<0.001
Mean cell haemoglobin concentration (g/dl)	33.28 ± 5.45	32.92 ± 4.26	0.603
Red blood cell count (x10 <sup>12</sup> /L)	4.46 ± 0.05	5.01 ± 0.02	<0.001
White blood cell count (x10 <sup>9</sup> /L)	75000 ± 12.89	59000 ± 11.58	<0.001
Platelet count (x10 <sup>9</sup> /L)	320000 ± 36.78	289000 ± 31.58	<0.001
Neutrophil count (%)	55.47 ± 1.94	53.18 ± 1.58	0.043
Lymphocyte count (%)	39.51 ± 2.11	44.28 ± 3.26	<0.001
Monocyte count (%)	3.92 ± 0.15	1.89 ± 0.2	<0.001
Eosinophil count (%)	0.89 ± 0.06	0.92 ± 0.02	0.153
Basophil count (%)	1.42 ± 0.5	0.15 ± 0.01	<0.001

There was a significant positive correlation between levels of TPAI-1 and platelet count ( $p < 0.001$ ), Factor VII, and Haematocrit level ( $p < 0.001$ ) among the participants with diabetes.

## DISCUSSION

In the present study, a greater proportion of the participants with T2DM were in the age range of 51–60 years. This was marginally higher than the observation of earlier researchers with similar studies.<sup>[25]</sup> A recent study reported that individuals with T2DM predominantly fall into the slightly higher age range of 60–69 years.<sup>[26]</sup> Experts believe that most non-communicable diseases, including diabetes, exhibit a change in metabolism with age. Given that aging reportedly initiates a reduction in insulin sensitivity, the tilt of the age trend toward adulthood is not implausible. Hence, glucose tolerance gradually declines with age.<sup>[27]</sup> The majority of the study participants with diabetes were observed to be overweight as against the high record of normal weight observed among participants without diabetes. Previous researchers have also observed a similar trend.<sup>[28,29]</sup>

A lot of extra weight has been linked to a higher risk of T2DM. This is because extra weight makes the body make more cytokines and adipokines, which make insulin resistance worse and lower adiponectin levels. It also leads to the deposition of ectopic fat in some body parts, most especially the liver.<sup>[30]</sup> In addition, the study observed a higher number of male participants among those with diabetes. A similar observation has been reported by earlier investigators.<sup>[31]</sup> Increased prevalence of T2DM in middle-aged/elderly men in which smoking has reportedly played a significant role, as has been reported earlier.<sup>[32]</sup> However, some studies have reported a high prevalence of diabetes among females, which is incongruous with the present study.<sup>[33]</sup>

Participants with diabetes showed significantly higher levels of Factors V and VII in the current study. A similar observation has been reported by earlier investigators.<sup>[34]</sup> The study findings, however, negated the observation, and the similar findings observed by of Erem et al,<sup>[23]</sup> who revealed no significant change in the levels of Factors V and VII in participants with diabetes when compared with those without diabetes. Generally, modification of coagulation factors has been reportedly associated with metabolic disorders, including diabetes mellitus. This alteration of the physiological mechanisms leads to a prothrombotic state. A significantly higher level of TPAI-1 was observed among the participants with diabetes in this study. A similar observation has also been reported elsewhere [34]. A threefold increase in the level of TPA I-1 has been linked to oxidative stress, which is a key factor in T2DM. This happens because of the AP-1 binding site at s-60/52 of the promoter, which is a normal finding in mutational analysis.<sup>[35]</sup>

Results of the haematological parameters revealed significantly lower levels of red cell parameters were observed among the participants with diabetes. This observation corroborates the trend reported by Arkew et al.<sup>[36]</sup> Generally, oxidative stress, which is connected with diabetes mellitus, does affect the antioxidant enzymes of the red blood cells. This condition affects the glutathione reductase level, leading to a decrease in red cell parameters, primarily hemoglobin.<sup>[37]</sup> However, significantly variable of white blood cell count, platelet count, and some other differential leucocyte parameters ( $p < 0.05$ ) except for neutrophil count and eosinophil count were observed compared to the non-diabetic participants with diabetes in this study. A similar observation has been reported by other workers.<sup>[38]</sup>

In addition, the present study revealed a significantly positive correlation between factors V and VII in both categories of participants. A similar trend has been reported by other researchers.<sup>[39]</sup> The blood contains a high proportion of TPAI-1, which platelets can accommodate. Diabetic platelets have reportedly been big and over-reactive. This might have resulted in the correlation increment observed between TPAI-1 and platelets in T2DM subjects.<sup>[40]</sup> Similarly, there was a positive correlation between the hematocrit levels and factor VII levels in diabetic subjects, which was not recorded in the non-diabetic counterpart. Research indicates that RBCs play a significant role in hemoglobins, both physiologically and pathologically. The coagulation cascade associates with the RBC and RBC-precipitated microvesicle surface phosphatidylserine, revealing its contribution to thrombotic events.<sup>[40]</sup>

## CONCLUSION

This study revealed a significant correlative increment between Factors V and VII, TPAI-1 and Factor VII in diabetic subjects. This has further established the claim that hypercoagulability is associated with T2DM. Moreover, the positive correlation observed between TPAI-1 and platelets, factor VII, and HCT can perhaps make platelets and HCT serve as routine predictors of TPAI-1 and factor VII levels, respectively. So, it becomes more likely that the goal of factor-specific therapy in the management of T2DM can be reached in a clear and straightforward way. The limitation of this research is the difficulty in getting information from some illiterate subjects.

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