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Original Research

Study to evaluate the correlation between coagulation factor, glycemic control and the severity of diabetic foot ulcers among South Indian population: A case control study

Santhini Gopalakrishnan[1], Anuradha G[1], Sumathy S[1], Sandeep Unnikrishnan[2]* Dhanvarshini S[3]

[1] **Department Biochemistry, Chettinad Academy of Research and Education, Chennai, India**
[2] **Department Plastic surgery, Chettinad Super Specialty Hospital, Chennai, India**
[3] **Ms Dhanvarshini, Chettinad Academy of Research and Education, Chennai, India**

*Correspondence to: Sandeep Unnikrishnan, Department of Plastic Surgery, Chettinad Super Specialty Hospital, Kelambakkam, Chengalpattu District, Tamil Nadu - 603103, India. E-mail: santhini.dept@gmail.com, Phone: 8754484353

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Abstract

Background: Diabetes mellitus is the most common metabolic disorder characterized by metabolic abnormalities and long term complications. Diabetic foot ulcer(DFU) is an important complication of diabetes mellitus. It contributes to major source of morbidity and mortality among chronic diabetic patients. **Aim:** To find out the utility of fibrinogen, Prothrombin time (PT) and Activated Partial Thromboplastin Time(APTT) as diagnostic markers to assess the severity of Diabetic foot ulcer. **Materials and Methods:** 60 subjects admitted with diabetic ulcer of foot 60 control subjects with diabetes mellitus but without DFU were included in the study. Fibrinogen, Prothrombin time(PT) and Activated Partial thromboplastin time(APTT) were measured in the samples collected from the subjects. **Results:** The mean level of fibrinogen among cases was 452.3±136.4 and among cases was 306.5=/-79.6 respectively and there was statistically significant difference between the two groups. (p value=0.0001). Prothrombin time (PT) showed mean value of 16.68±4.2 sec among cases and 12.5±1.6 sec among controls and the difference was statistically significant (p value=0.0001). APTT showed mean value of 36.2±7.07 sec among cases compared to 34±7.3sec among controls but the difference was not statistically significant (p value=0.24). ROC analysis shows that the area under the curve for HbA1c fibrinogen is 0.758 and 0.760 respectively. **Conclusion:** Fibrinogen and prothrombin time is found to have a significant difference between DFU patients and control subjects in the diagnosis of diabetic foot ulcer.

Keywords: Diabetic Foot Ulcer, Fibrinogen, Prothrombin time, Activated Partial Thromboplastin time.

Background and Aims

Diabetes mellitus is the most common endocrine disorder characterized by metabolic abnormalities and long-term complications. The Diabetes Federation of India has estimated that the number of diabetes patients in India will be about 550 million by 2030 [1, 2].

This disease is associated with many microvascular and macrovascular complications. Eighty percent of deaths among diabetic patients are contributed by thrombotic events, while cardiac problems contribute up to 75%, with the remaining 25% caused due to peripheral vascular events and cerebrovascular complications [9].

Patients with diabetes have a 25% chance of developing diabetic foot ulcers in the future [3]. It is a complication with a mortality rate of approximately 16%, an amputation rate of 25%, and a 3-year mortality rate after amputation is 37% [4].

Diabetic Foot Ulcers (DFU) is a major complication in chronic diabetics with severe consequences [5]. Diabetic foot ulcers development occurs due to the simultaneous effects of many risk factors among which peripheral neuropathy and peripheral arterial disease play a major role [6, 7, 8]. Peripheral arterial disease affects the tibial and peroneal arteries in the calf region. Endothelial dysfunction and smooth muscle cell abnormalities develop in the peripheral arteries due to the uncontrolled hyperglycemic status, which contributes to the development of DFU. Diabetes is a pro-coagulant state where there is an increase in the levels of pro-coagulants like fibrinogen and other clotting factors such as I, VII, IX, XII and Von will brand factor [9]. So, in recent years, the role of hemostatic factors in the development of microvascular and macrovascular complications in diabetes has achieved great interest. Plasma fibrinogen is an important component of the coagulation cascade as well as a major determinant of blood viscosity and blood flow, which in turn is a major determinant of the progress of diabetic foot [10]. Fibrinogen is an inflammatory marker linked to the development of atherosclerosis, thrombosis, and vascular complications in Diabetes mellitus. The complications caused by increased fibrinogen levels are cardiovascular disease, cerebrovascular accidents, and peripheral vascular diseases [11].

Prothrombin time and Activated partial thromboplastin time are two tests used to assess the extrinsic and intrinsic pathways of coagulation. They are analyzed to find out bleeding or clotting tendencies. Studies have shown that levels of these markers are raised among diabetic patients when compared to normal individuals. The diagnosis of diabetic foot ulcers is mainly based on clinical and radiological methods. Although the literature states variations in coagulation profile in Diabetic foot ulcers, there has not been much research on them in this part of the country.

This study aims to find out the levels of fibrinogen, PT, and APTT among DFU patients, and to find out whether there is any correlation between plasma fibrinogen levels with the levels of HbA1c in diabetic foot ulcers subjects among South Indian population.

Material and Method

Ethical approval of the study protocol

Prior to commencing the study, the approval of the Ethical Committee of the Institute was obtained. It was a case control study conducted from June 2019 to August 2019 for a time period of three months.

Study population: The study population was selected from among patients attending to the Surgery Department of Chettinad Academy of Research and Education.

Study design and patients: This was a case control study with 60 diabetic foot ulcers patients of both sexes, aged between 30–70 years, who attended the Surgery OPD of Chettinad Hospital and Research Institute (CHRI) and 60 age and sex matched patients with H/O Diabetes mellitus but without diabetic foot ulcer as controls.

Inclusion criteria: Subjects of both sexes admitted with C/O diabetic foot ulcers at the Department of Surgery, Chettinad Hospital and Research Institute, Kelambakkam.

Exclusion criteria

1. Those patients with H/O intake of anti platelet drugs, lipid lowering drugs.
2. History of prior treatment with corticosteroids.
3. Patients with hematological or euplastic disorders.
4. Those who had undergone recent surgeries, hyperthyroidism, cancer, pregnancy.

Laboratory, anthropometric and clinical data collection

After obtaining consent, DFU subjects were grouped based on the Wagner's classification of diabetic foot with the help of the treating plastic surgeon. Height and weight were

measured and BMI was calculated using the formula: Weight in Kg/(Height in Meters)^2. 5 ml of blood was collected from each patient in sodium citrate tube of light blue colored and violet-colored vacutainers. Fibrinogen, PT and APTT, and HbA1c were measured on the same day. Samples were stored at –20°C for the duration of the study.

Fibrinogen was measured by the turbidimetric method in a CA-50 machine in the pathology laboratory. Reference interval – 246–306 mg/dl.

Prothrombin Time (PT) and activated partial Thromboplastin Time (APTT) were measured in Pathology Laboratory using the photo optical method in a Sysmex CA-50 machine. The reference interval for prothrombin time is 11–12 seconds and for APPT it is 26–40 seconds. Hemoglobin levels were measured using cyan-met hemoglobin method in the Beckman Coulter Automated Machine in the pathology laboratory.

HbA1c was estimated in the D-10 machine based on the high performance liquid chromatographic (HPLC) principle in the biochemistry laboratory. Reference interval: 4.6–5.4%.

The DFU subjects are classified based on the severity of diabetic foot is by Wagner's classification which is as follows:

Class 1: Foot at risk;
Class 2: Superficial ulcers;
Class 3: Deep ulcers without osteitis;
Class 4: Deep ulcers with osteitis;
Class 5: Localized gangrene;
Class 6: Extensive gangrene

Statistical analysis

Sample size was calculated under the guidance of a statistician. SPSS software version 21 was used to analyze the results. SPSS version 21 was utilized for the statistical analysis. Paired t-test was used to compare the variables between the cases and controls. Pearson's correlation analysis was done to analyze the correlation between HbA1c and fibrinogen, PT and APTT. The diagnostic performance was assessed using receiver operating characteristic curve (ROC curve). P value less than 0.05 was considered to be significant.

Table 1: Paired T test done to find out the difference in the variables between the DFU cases and the normal healthy controls (p value<0.05 is significant).

Variables Demographic details	Cases		Control		p value
	Mean	SD	Mean	SD	
Age(Years)	56.85±10.8	10.8	54±10	10	0.14
Sex- Female (%)	23%		30%		—
Male (%)	67%		70%		—
Height(cm)	168.67±4.64	4.64	167.2	3.90	0.059
Weight(cm)	79.43	7.12	69.30	4.00	<0.0001*
BMI	27.8	1.51	24.63	0.8	<0.0001*
Duration of diabetes(years)	7.78	5.44	6.87	4.66	0.3
Laboratory parameters					
Hemoglobin (g/dl)	12.25	2.47	11.6	2.3	0.56
HbA1c (%)	9.5	2.7	6.7	2.5	0.05*
Prothrombin time (PT) (sec)	16.68	4.4	12.5	1.6	0.0001*
Activated Partial thromboplastin time (APTT) (sec)	36.16	7.07	34.45	7.3	0.24
Fibrinogen (mg/dl)	452.3	136.4	306.65	79.6	0.0001*

HbA1c –Glycated hemoglobin.

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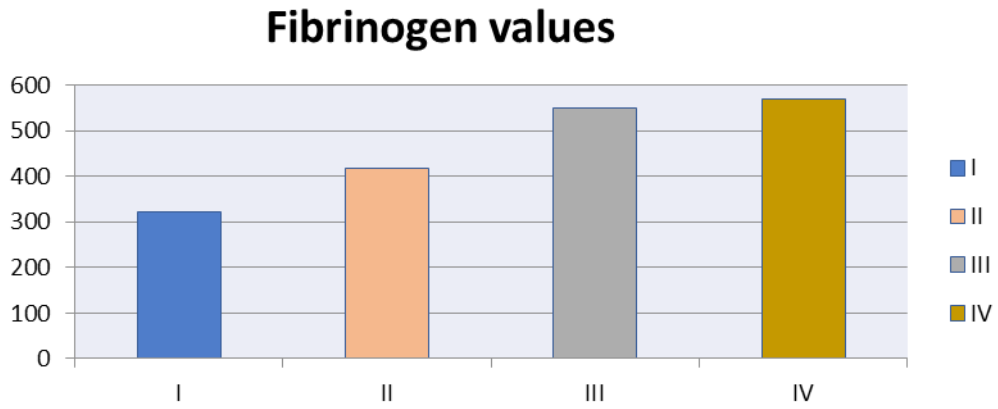

Fibrinogen levels (mg/dl) –Grade -1-320.56, Grade-II-417.25, GradeIII-548.99, Grade IV-570.5

Figure 1: Shows the mean value of fibrinogen (mg/dl) in the different grades of diabetic foot ulcers.

Results

Demographic variables and laboratory variables between the cases and controls were compared using paired test (Table 1). Among the cases, 12 belonged to Grade I group, 18 to Grade II, 25 to Grade III, and 5 to Grade IV group. The mean fibrinogen levels in Group I was 320.56±105.25, Group II was 417.25±82, Group III was 548.99±122.2, and Grade IV was 570.4±150.68 respectively (Fig. 1). The fibrinogen levels were found to be higher as the grade of DFU increases. The mean level of fibrinogen among cases was 452.3±136.4 and among controls was 306.5±79.6 respectively, and there was statistically significant difference between the two groups (p value = 0.0001). Prothrombin time (PT) showed mean value of 16.68±4.2 sec among cases and 12.5±1.6 sec among controls and was statistically significant (p value = 0.0001). APTT showed mean value of 36.2±7.07 sec among cases compared to 34±7.3 sec among controls but the difference was not statistically significant (p value = 0.24). (Fig. 2**)** depicts the levels of PT, APTT, and fibrinogen among DFU cases and controls. Pearson's correlation analysis between HbA1c and fibrinogen levels showed that there was significant positive correlation between HbA1c and fibrinogen levels among DFU patients with correlation coefficient (r value of 0.759) (Fig. 3). The correlation

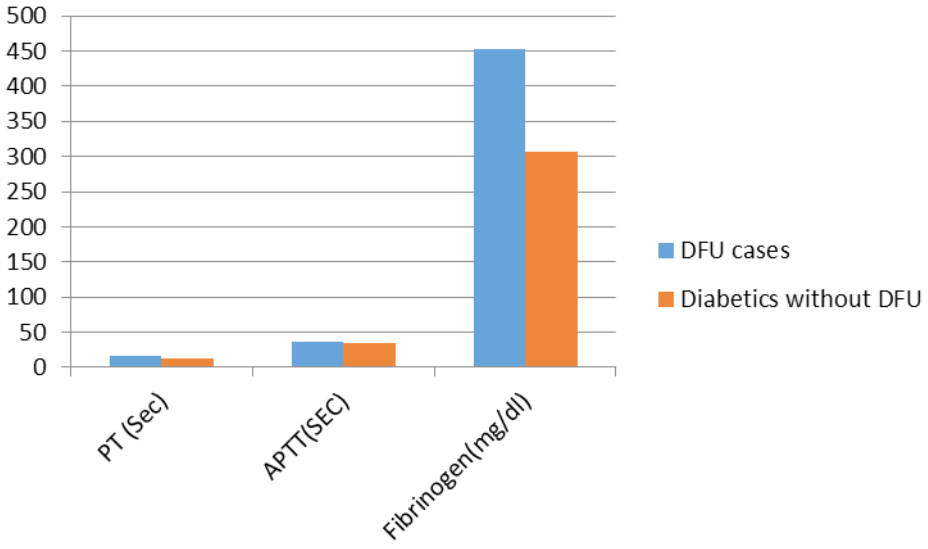

Figure 2: Shows the mean value of PT, APTT and fibrinogen among the DFU patients and diabetes patients without DFU.

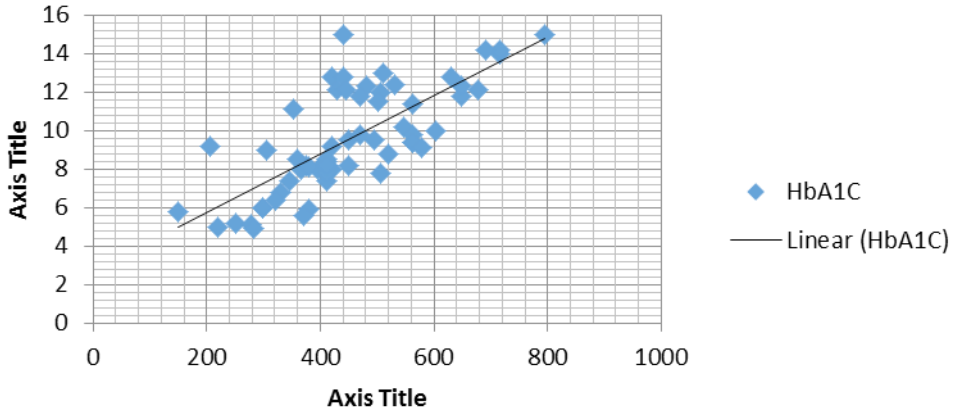

Figure 3: Scatter plot between HbA1c and fibrinogen levels among DFU patients.

Table 2: Shows the correlation analysis of variables (HbA1c and PT, HbA1c and APTT, HbA1c and Fibrinogen) (p value <0.05 is significant).

Correlation analysis	PT (r value)	p value	APTT (r value)	P value	Fibrinogen (r value)	p value
HbA1c	0.184	0.162	0.017*	0.89	0.75	0.00*

PT-Prothrombin Time, APTT-Activated Partial Thromboplastin time, HbA1c-Glycated Hemoglobin.

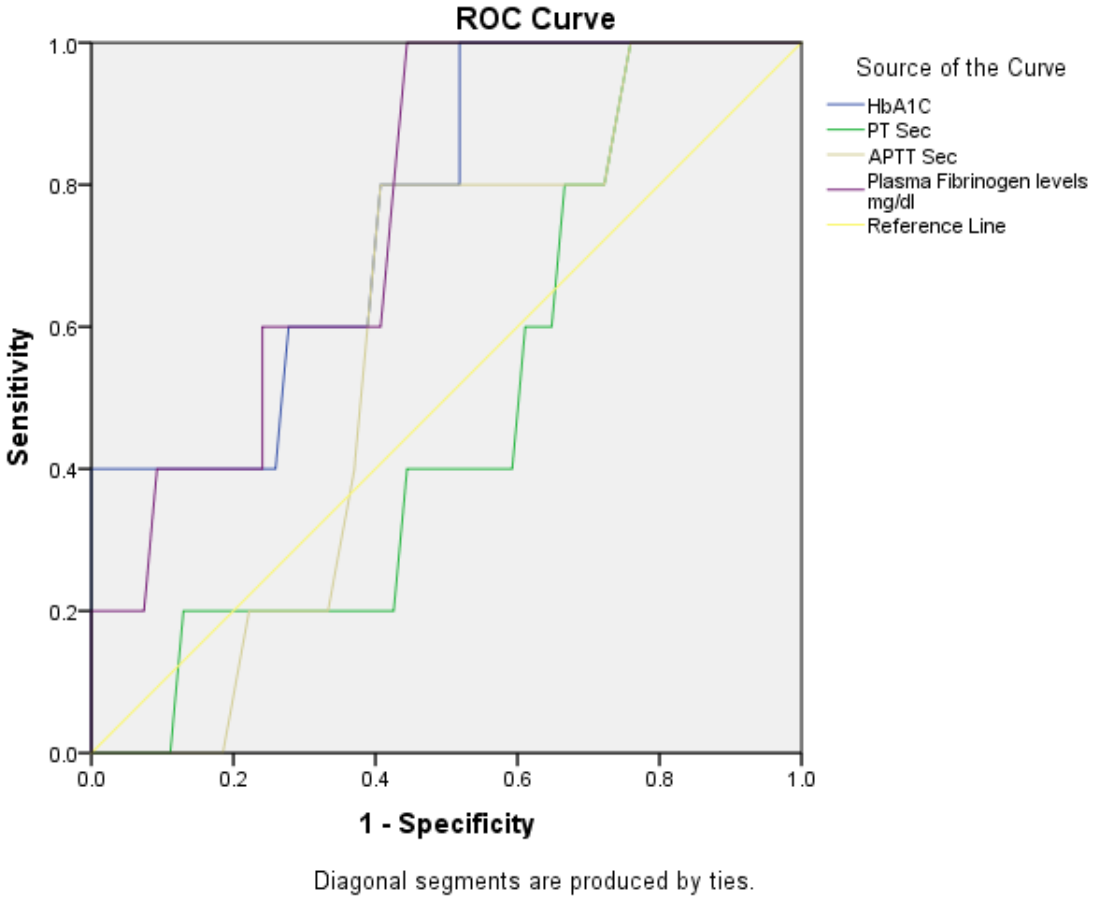

Figure 4: ROC curve of fibrinogen showing its ability to diagnose diabetic foot ulcers (DFU).

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between HbA1c and PT and APTT was not found to be significant (r = 0.184, p value = 0.162), (r = 0.017, p value = 0.9). The correlation coefficients between HbA1c and fibrinogen values in Grade I was (r value = 0.561) (p value = 0.072), Grade II (r value = 0.704) (p value = 0.00), Grade III (r value = 0.860) (p value = 0.00), Grade IV (r value = 0.413) (p value = 0.49). Correlation analysis between HbA1c and PT showed a negative correlation coefficient of r = 0.18, HbA1c and APTT showed a correlation coefficient of r = 0.017 but was not statistically significant (Table 2). ROC analysis shows that the area under the curve for HbA1c fibrinogen is 0.758 and 0.760 respectively, but PT and APTT doesn't show much significance (Fig. 4).

Discussion

Diabetes is a disease characterized by enhanced activation of the coagulation profile and disturbances in the integrity of the vascular epithelium. Glycated hemoglobin (HbA1c) gives us an idea of the diabetic control of patients for the last three months. The mean value of HbA1c was found to be higher among diabetic foot ulcers patients (9.5) when compared to the diabetic controls with. Glycated hemoglobin was found to have a positive correlation with plasma fibrinogen levels. This finding is in accordance to the study done by Madan *et al.* among Japanese school children, who found out a significant association between fibrinogen and HbA1c [12].

The correlation between glycemic control and fibrinogen levels in this study could be because glycated fibrinogen is less susceptible to degradation by plasmin and the relative insulin deficiency in Type-II Diabetes mellitus causes an imbalance in the protein synthesis, leading to a 29% decrease in albumin synthesis and 50% increase in synthesis of fibrinogen synthesis [13].

Our results have shown that the fibrinogen levels increased with the increase in the grade of DFU, which was on par with the studies done by Rattan et al., and Li et al., who also found out that fibrinogen levels increased with increase in severity of DFU [14, 15]. Weigelt et al., showed that there was no significant difference in the levels of fibrinogen between the different grades of DFU [16].

Fibrinogen plays an important role in thrombosis, development of subclinical atherosclerosis, formation of fibrin clot. The coagulation cascade is activated by hyperglycemia, leading to hyperfibrinogenemia. This leads to an increase in fibrinogen degradation products, which will inturn stimulate the synthesis of fibrinogen from the liver. The various mechanism by which fibrinogen has been found to promote thrombosis are that hyperfibrinogenemia increases plasma viscosity, induces aggregation of RBC, causes platelet aggregation, forms fibrin and fibrinogen degradation products (FDPs) which in turn bind LDL and sequester more fibrinogen which in turn stimulate smooth cell proliferation and migration. [17, 18]. ROC (Receiver operating curve) curve shows that fibrinogen also shows higher specificity and sensitivity to diagnose diabetic foot ulcers when compared with PT and APTT.

Prothrombin time was found to be higher than the reference interval among diabetic foot ulcers patients. APTT levels were found to be within normal limits. But when compared with the control group, the levels were lower than the cases and were found to be statistically significant. Studies done by Alao O et al are on par with this study showing prolonged PT and APTT levels among diabetic foot ulcers patients. Our study did not show much correlation between HbA1c and PT, APTT [19]. Studies by Collier et al. have shown that the levels of PT and APTT among diabetic foot ulcers patients were within the normal reference range but were lower when compared with the control group [20].

Diabetes is a pro-coagulant state. The variations among the coagulation profile have only been partially understood. Plasma fibrinogen levels were significantly elevated among diabetic foot ulcers patients, and gradually increased as the ulcer grade increased, but prothrombin time, activated partial thromboplastin time showed no particular significance. More extensive studies are required to use it as a marker for assessing the severity of DFU.

Conclusions

This study was conducted among patients with diabetic foot ulcers to assess the correlation between HbA1c and plasma fibrinogen level, PT and APTT. We found that the fibrinogen levels increased with an increase in the grade of DFU. There was a significant positive correlation between HbA1c levels and fibrinogen among Grade 2 and Grade 3 classes of DFU. Prothrombin time also showed significant difference between the two groups. Fibrinogen and Prothrombin time were found to have a promising role in the diagnosis of diabetic foot ulcers. With further studies, we can utilize these markers as screening tools to assess hyper coagulable states of diabetes mellitus.

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Conflict of interest

The authors declare no conflict of interest.

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