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# Study on Physiological Variations in Certain Hemostatic Tests in University Students of Pakistan.

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**Abstract:** The present study was conducted to assess physiological variations in certain hemostatic tests, namely clotting time (CT) and prothrombin time (PT), among apparently healthy male and female university students in Pakistan, and to evaluate the relationship of these parameters with age, body weight, gender, and type of residence. Additionally, the study explored whether salinity% of platelet-free plasma could serve as an indirect indicator of fibrinogen concentration. A total of 184 students aged 20–30 years, enrolled in various four-year undergraduate programs, were included in the study. Body weight, temperature, pulse rate, and blood pressure were recorded prior to blood collection. Blood samples were collected aseptically and analyzed for clotting time using the Lee and White method (CT1) and the slide method (CT2), while prothrombin time (PT) was determined using Soluplastin reagent. Plasma salinity% was measured using a hand-held refractometer. Statistical analysis indicated that CT1, CT2, PT, and salinity% did not differ significantly ($P \geq 0.05$) among different age and body weight groups. Regression analysis showed positive correlations of age with CT1 ($r = 0.378$) and CT2 ($r = 0.292$), while body weight was positively correlated with CT1 ($r = 0.308$) ($P \leq 0.05$). Males and day scholars exhibited significantly higher CT1 values ($P \leq 0.05$). Salinity% showed weak positive correlations with CT1 ($r = 0.164$), CT2 ($r = 0.047$), and PT ($r = 0.147$), although these associations were not statistically significant. These findings provide baseline information regarding physiological variations in selected hemostatic parameters among young Pakistani adults.

**Keywords:** Clotting Time, Prothrombin Time, Hemostasis

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## Introduction

Hemostasis refers to the mechanism to prevent and stop bleeding, that is, to retain blood in a ruptured blood vessel. It is the initial process of wound healing. This entails the process of coagulation whereby blood is transformed into a gel. Whole blood vessels play the key role in regulating the ability of blood to form clots [1]. The original coagulation pathway is now called tissue factor (TF) and was secreted by injured vessels to transform Prothrombin into thrombin in the presence of calcium. Thrombin subsequently transformed fibrinogen into fibrin which led to the formation of a blood clot [2]. Short exposition of hemostatic tests which were conducted to investigate physiological differences in both male and female students is provided.

Clotting time testing is performed to identify and diagnose a bleeding disorder or excessive clotting disorder and to monitor the efficacy of the blood-thinning medication (anticoagulant) warfarin in preventing blood clots [3].

Among the many methods of measuring coagulation (clotting) time (CT), the method of Lee and White is the most mentioned [4]. Lee and White clotting time (LWCT), which was initially applied to observe patients with hemophilia, have been applied to observe the effectiveness of the anti-venom therapy [5]. Prothrombin time is the duration required by blood to clot. Normal Prothrombin time ranges *between* 10-15 seconds [6].

In 1935, A.J. Quick invented the PT test, which is the coagulation time (in seconds) of a platelet-poor plasma mixture with the addition of tissue factor (thromboplastin) and calcium chloride and was used to study coagulopathy related to obstructive jaundice [7].

The simplest test used for measuring the clotting time of blood is slide method. The devices are user-friendly and cheap. Many tests can be performed within a short period by putting a new slide to each case. The changes do not require any lens to be observed. The technique is good enough to be used on a regular basis [8].

Fibrinogen (Fg) conversion rate of to the insoluble product fibrin (Fn) is one of the important determinants in the hemostasis [9]. It is known that calcium ions stimulate the fibrinogen to form a fibrin clot in the company of thrombin [10]. Solution of fibrinogen precursor increases the salinity concentration, resulting in more transparent fibrin that degrades at slower rates [11].

Brix% symbol= Bx. is the amount of sugar in an aqueous solution. The strength of the solution expressed as a percentage by mass is one degree of Brix, which is 1 gram of sucrose per 100 grams of solution. When dissolved solids are present in the solution besides pure sucrose, the Bx. only estimates the dissolved content of solid [12]. Hemostasis depends on salinity and brix percentage.

Recent studies suggest that refractometric measurements such as Brix or salinity percentage reflect the total dissolved solids present in plasma, including proteins and other macromolecules. Plasma proteins, particularly fibrinogen, contribute significantly to the refractive index of plasma. Previous studies have indicated that refractometric readings may correlate with plasma protein concentration, including fibrinogen precursors [9, 11]. Although refractometry does not directly quantify fibrinogen, it may provide an indirect indication of changes in plasma protein composition. Therefore, evaluation of salinity% alongside basic coagulation tests may offer exploratory insight into physiological variations in hemostatic status. The study was aimed towards assessment of physiological variations in certain hemostatic tests (clotting time and prothrombin Time) in apparently health male and female students at a Pakistani university as affected by age, body weight, gender and type of residence. Furthermore, it aimed to ascertain if salinity% of platelet-free plasma could be used as an indicator of fibrinogen concentration

## Methods

### Study setting and populations

The study was carried out at the Department of Physiology, The Islamia University of Bahawalpur, apropos to ethical approval by the Advance Study and Research Board of the university (034/Physio/2022). Students (n=184) enrolled in various 04-year Bachelor of Studies academic programs, between the age of 20-30 years were incorporated in the study. Body weight, temperature, pulse rate and blood pressure were determined for each individual prior to blood collection. Exclusion criteria included high temperature, history of any non-steroidal drug intake and hemostatic disorder.

Prior to sample collection, all participants were informed about the objectives and procedures of the study, and written informed consent was obtained from each participant. Participant confidentiality and anonymity were strictly maintained throughout the study in accordance with institutional ethical guidelines.

Participants were recruited through voluntary participation using a convenience sampling strategy from university students aged 20–30 years**.** Inclusion criteria included apparently healthy individuals with no known bleeding disorders, chronic illnesses, or current medications that could affect coagulation parameters. The study population therefore represents a healthy young adult subset, and the findings may not necessarily be generalized to other age groups or clinical populations.

**Blood collection and analyses**

Blood collection was carried out aseptically in 5mL sterilized disposable syringes and was divided into two aliquots. About 2mL of blood was taken in blue-topped sodium-citrate PT tubes (Henso, China) for plasma collection to determine PT. Remaining 3mL of blood was taken in sterilized, plain, pre-warmed (37°C) glass tubes (1mL in each tube) for determining the clotting time through Lee and White Method (CT1) as per recommended protocols [13].

For determining the clotting time through slide method (CT2), a drop of fresh blood was placed on a clean sterilized glass slide for 30 seconds. A needle was passed through it in different directions to notice the formation of fibrin fiber thread [14].

In order to determine PT, platelet-poor plasma was attained from citrated blood samples. The samples were centrifuged at 600G for 10 minutes; supernatant was separated and again centrifuged at 1300G for 20 minutes [15]. The final supernatant thus attained was used for PT determination. PT was determined using soluplastin (Weiner Lab, Rosaio, Argentina) as per manufacturer's instructions.

Salinity% was taken by placing a 50 µL drop of platelet-poor plasma on the bulb of a Digital Brix Refractometer (SR-95 Digital, Medline Scientific, UK; S.No. 0884577).

The digital refractometer was calibrated with distilled water prior to measurements to ensure accuracy. All coagulation measurements were performed by trained laboratory personnel following standardized procedures to minimize inter-observer variability. Blood samples were processed promptly after collection to reduce pre-analytical variability, which may influence coagulation parameters.

**Statistical analysis**

The data analyses were carried out using SPSS (SPSS for Windows Version12, SPSS Inc., Chicago, IL, USA). The mean (±SE) values for all the studied attributes (CT1, CT2, PT and salinity%) were calculated. For the purpose of analysis, the individuals (n=184) were divided into three age groups *viz*. up to 20 years (n=43), 21-25 years (n=58) and above 25 years (n=62), and three body weight groups *viz*. less than 58 kg (n=62), 58-80 kg (n=55) and above 80 kg (n=67) using K-clustering technique. The difference between all study groups (age-wise, body-weight wise, gender-wise and residence-wise) was determined through GLM using multivariate analysis with LSD as post-hoc test at a significance of $P \leq 0.05$. Furthermore, multiple regression analysis was carried out in order to deduce interrelationships between various studied attributes.

**Results**

Overall mean values (±SE) for various hemostatic tests as affected by age and body weight in Pakistani university students (n=184) is presented in Table 1. All the parameters were statistically non-significant ($P \geq 0.05$) between different age and body weight groups. The regression analyses showed that age was positively correlated to CT1 (r= 0.378) and CT2 (r= 0.292) whereas body weight was positively correlated to CT1 (r= 0.308) at a significance level of $P \leq 0.05$ (Table 2). Regarding gender and type of residence, males and day scholars had significantly ($P \leq 0.05$) higher values for CT1 as depicted in Figure 1 and 2, respectively.

Results regarding the association of salinity% with various hemostatic attributes of the study (CT1, CT2 and PT) revealed that though salinity% was positively correlated to CT1 (r= 0.164), CT2 (r= 0.047) and PT (r= 0.147), However, these association are statistically non-significant (Figure 3).

**Table 1. Overall mean values (±SE) for various hemostatic tests as affected by age and body weight in university students (n=184)**

| Factors | | CT 1 (Minutes) | CT 2 (Minutes) | PT (Seconds) | Salinity (%) |
|---|---|---|---|---|---|
| **Age** | Up to 20 Years (n=43) | 7.9±0.2 | 2.98±0.2 | 12.7±0.4 | 8.0±0.2 |
| | 21-25 Years (n=58) | 8.3±0.2 | 3.4±0.1 | 13.2±0.3 | 7.4±0.3 |
| | Above 25 Years (n=83) | 9.0±0.4 | 3.5±0.5 | 13.0±0.7 | 8.6±0.4 |
| **Body Weight** | Less than 58 kg (n=62) | 7.9±0.2 | 3.2±0.1 | 12.7±0.3 | 8.0±0.2 |
| | 58-80 kg (n=55) | 8.7±0.2 | 3.4±0.2 | 13.4±0.2 | 7.3±0.4 |
| | Above 80 kg (n=67) | 8.0±0.5 | 2.9±0.4 | 13.5±0.7 | 7.7±0.5 |
| **Overall** | | **8.2±0.1** | **3.3±0.1** | **13.0±0.2** | **7.7±0.2** |

CT1= Clotting Time through Lee & White Method, CT2= Clotting Time through Slide Test, PT= Prothrombin Time

**Table 2. Regression analysis between age, body weight and various hemostatic tests in university students (n=184)**

| Factors | Tests | r Value | Adjusted R square | P value |
|---|---|---|---|---|
| **Age** | CT 1 | 0.378 | 0.126 | 0.05* |
| | CT 2 | 0.292 | 0.067 | 0.03* |
| | PT | 0.163 | 0.008 | 0.23 |
| | Salinity % | 0.156 | 0.006 | 0.25 |
| **Body Weight** | CT 1 | 0.308 | 0.127 | 0.03* |
| | CT 2 | 0.076 | 0.03 | 0.58 |
| | PT | 0.196 | 0.02 | 0.15 |
| | Salinity% | 0.112 | 0.006 | 0.41 |

*Significant at P≤0.05; CT1= Clotting Time through Lee & White Method, CT2= Clotting Time through Slide Test, PT= Prothrombin Time

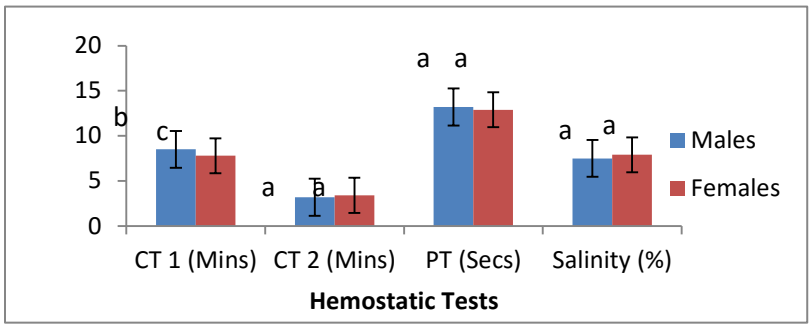


**Figure 1. Effect of gender on various hemostatic tests in university students (n=184).**
Values are mean (±SE). Different letters are different at P≤0.05 within males (n=70) and females (n=114) CT1= Clotting Time through Lee & White Method, CT2= Clotting Time through Slide Test, PT= Prothrombin Time

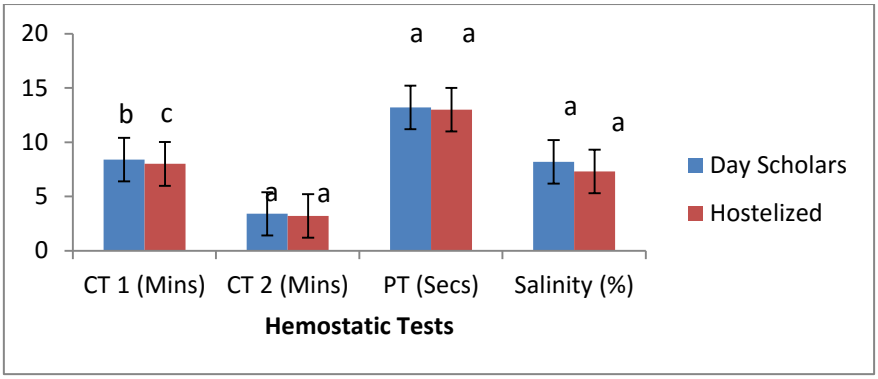


**Figure 2. Effect of residence on various hemostatic tests in university students (n=184)**. Values are mean (±SE). Different letters are different at P≤0.05 within day scholars (n=99) and hostelite students (n=85) CT1= Clotting Time through Lee & White Method, CT2= Clotting Time through Slide Test, PT= Prothrombin Time

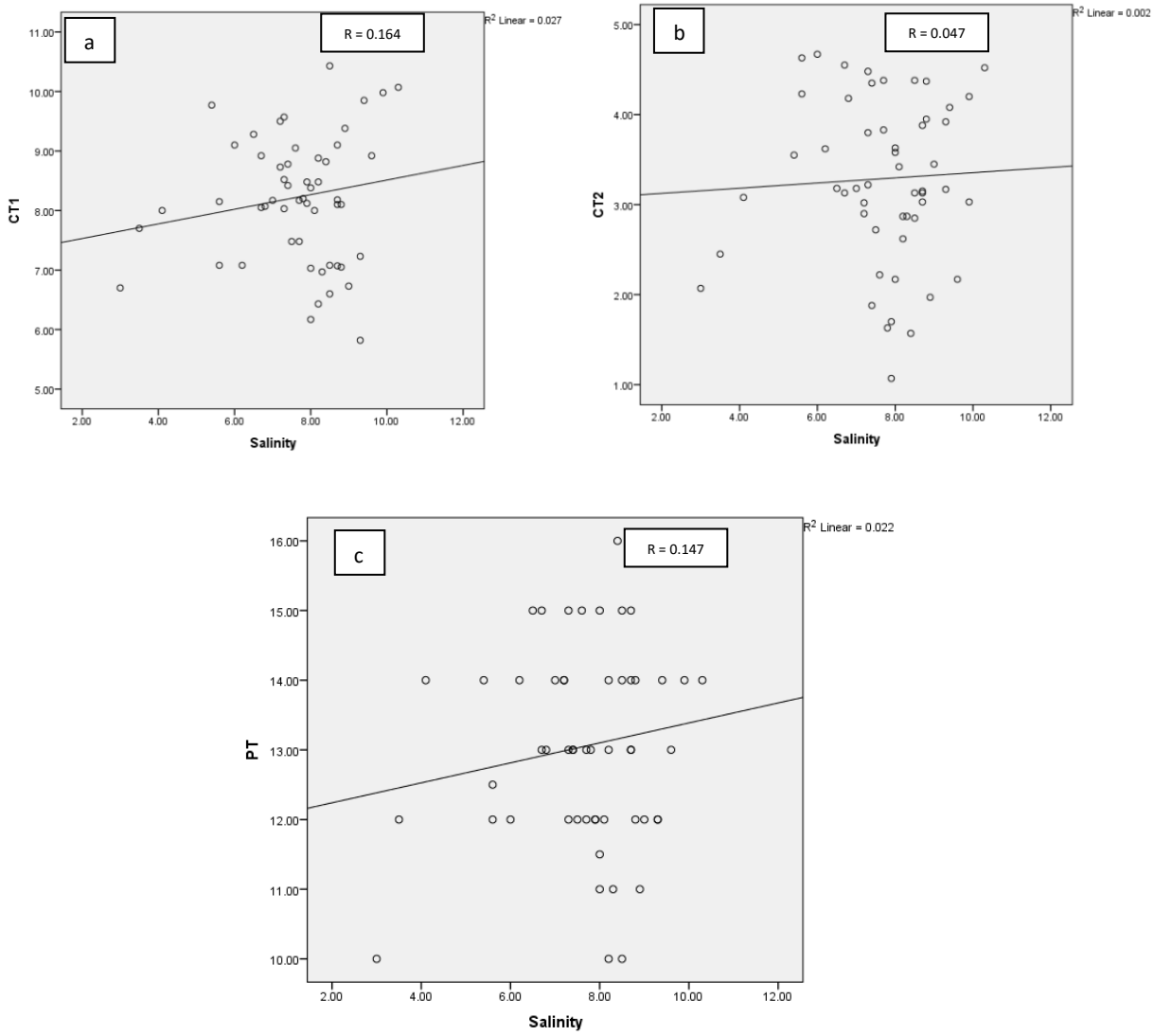


**Figure 3. Regression analysis between salinity % and CT1 (a), CT2 (b) and PT (c).** CT1= Clotting Time through Lee & White Method, CT2= Clotting Time through Slide Test, PT= Prothrombin Time

## Discussion

The response of the animal to the environment is closely associated with the hematological parameters, which is an indicator that the environment in which organisms live, might have some influence on hematological characteristics [16]. The present study is apparently first of its kind, being reported with an aim of assessment of physiological variations in certain hemostatic tests (clotting time and prothrombin time) in apparently healthy male and female students at a Pakistani university as affected by age, body weight, gender and type of residence. Furthermore, it aimed to ascertain if salinity% of platelet-free plasma could be used as an indicator of fibrinogen concentration.

Overall mean values (±SE) for various hemostatic tests such as CT1=8.2±0.1, CT2=3.3±0.1, PT= 13.0±0.2 and salinity %=7.7±0.2) affected by age and body weight in Pakistani university students (n=184). All the parameters were statistically non-significant ($P \geq 0.05$) between different age and body weight groups.

The regression analyses showed that age was positively correlated to CT1 (r= 0.378) and CT2 (r= 0.292). Present study also showed that age was positively correlated to CT1 (Lee and White clotting time) at a significance level of $p \leq 0.05$. Previous studies also showed similar findings [17] which demonstrated that the Prothrombin time for males and females aged between 0-14 years was 13.3 sec and found to be significantly higher compared to males and females aged between 15-50 years and their prothrombin time was 12.90 sec.

Present study also showed the effect of gender (males, n=70 and females, n=114) on clotting time and it was found that Lee and White clotting time as well as prothrombin time was higher in males as compared to females.

Lee and White clotting time in males were 8.9 minutes and in females was 8.2 minutes reported in previous study [18]. Lee and White clotting time in patients after recovery was 9 minutes at Toto kabila hospital [19]. Results of this study and previous findings are same.

The present study also demonstrated the effect of residence (Day scholars, n=99 and hostelite, n=85) on clotting time and it was observed that day scholars had significantly ($p \leq 0.05$) higher values for CT1 (Lee and White clotting time). Apparently, it was first study of its kind describing the effect of residence on clotting time; to best of our knowledge no report on this parameter is available in literature.

The association of salinity% with various hemostatic attributes was studied. It was observed that though salinity% was positively correlated to CT1 (r= 0.164), CT2 (r= 0.047) and PT (r= 0.147), however, statistically non-significant.

A study showed that body weight has a non-significant positive correlation with BRIX% and salinity [20]. Moreover, in hostelites, body weight and salinity have significant positive correlation but BRIX % and clotting time has non-significant positive correlation. Further results showed that in day scholars, there is a non-significant negative correlation between body weight with clotting time, BRIX % and salinity. Results of our study and previous findings are almost same.

The present findings should be interpreted cautiously because most observed associations between salinity% and coagulation parameters were weak and statistically non-significant. Therefore, salinity% cannot be considered a validated diagnostic marker for fibrinogen or coagulation status. Instead, this parameter should be regarded as an exploratory indicator that may indirectly reflect plasma protein composition. Future studies involving direct fibrinogen assays, larger sample sizes, and more diverse populations are required to validate the potential relationship between refractometric parameters and hemostatic biomarkers.

## Conclusion

This study establishes important baseline values for key hemostatic parameters in healthy young Pakistani adults, demonstrating that clotting time and prothrombin time remain largely stable

across variations in age and body weight, with only minor influences observed. While gender and residence showed limited effects, the overall consistency of these parameters highlights their physiological stability in this population. Although salinity% exhibited weak positive associations with coagulation measures, it cannot be considered a reliable surrogate marker for fibrinogen. These findings provide a useful reference for future research and emphasize the need for further studies using direct and more sensitive biomarkers to better explore the relationship between plasma composition and hemostatic function.

**Ethics Approval and Consent to Participate**
Current study was conducted under the prior ethical approval from the departmental ethical review committee (034/Physio/2022) of the Department of Physiology, IUB. All the procedures were carried out in accordance with institutional ethical guidelines. Whole procedure was explained and written consent was obtained from the student volunteers.

**Human and Animal Rights**
All the procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee. No animals were involved in this study.

**Consent for Publication**
Written consent for publication of data *(in anonymity)* was obtained from all participants/volunteers.

**Availability of Data and Materials**
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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**Conflict of Interest**
The authors declare no conflict of interest regarding the publication of this article.

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