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Gender - Does it affect BT (Bleeding Time) or CT (Clotting Time): A cross sectional study in medical students

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Abstract: *Background*: Bleeding time (BT) and Clotting time (CT) assess the integrity of haemostatic mechanisms. Haemostasis is the arrest of bleeding. Bleeding time depends on the effectiveness of vasoconstriction as well as platelet plug formation, while Clotting time depends upon the effectiveness of clotting mechanism. Earlier studies done on this showed the conflicting results and conclusion. *Objectives:* The objective of this study was to identify whether there is any gender difference as between Bleeding time and Clotting time. *Method:* This was a cross sectional study including 433, undergraduate medical students of age group 17 to 20 years. Bleeding time (by Duke's filter paper method) and Clotting time (by Wright's capillary tube method) was determined after obtaining an informed consent from the students. *Result and conclusion:* Our study revealed the value of CT was little higher in males but this difference was not statistically significant. Also BT was higher in females as compare to males but again not statically significant.

Keywords: Bleeding time, clotting time, medical students, Duke's filter paper method and Wright's capillary tube method.

Introduction

Platelets are 2-4μ in diameter. They are nonnucleated. The shape of platelet depends on their state of activity. In inactive state, platelet are disk shaped but when they are activated, eg, during hemostasis they are spherical. They are called 'formed elements' as they are merely cell fragments and not complete cells. Platelets live for 7-10 days [1]. Their precursor cells are megakaryocytes. The small size and shape of platelets enables them to move along the sides of vessels where they can persistently control vessel consistency [2].

Platelets have various functions in different pathophysiologic processes such as haemostasis, thrombosis, coalulation, vascular regeneration, inflammation processes like atherosclerosis, host defense, and tumor metastasis [2]. In other words, as soon as vascular damage occurs and destructs the natural barrier of endothelial cells, platelets are activated rapidly and form an obstructive plug in the damaged area. This process occurs in a set of reactions between platelets and subendothelial matrix (platelet adhesion) and among platelets themselves (platelet aggregation). In contrast to platelet aggregation, the primary adhesion process does not need the metabolic activity of platelets. However, this process results in platelet activation and the activated platelets synthesize the thromboxane A2 and release their granule contents [3]. All of these platelet responses are formed to rapidly create a haemeostatic clot to block the injured area in order to prevent hemorrhage. Platelet dysfunction or decreased platelet count will thus increase the risk of bleeding [2]. Any abnormality in platelet functions would result in clinical bleeding with different severity. In most cases, patients may develop dermal or mucosal bleeding or excessive bleeding after trauma or surgery procedures [4-5].

Bleeding time (BT) and clotting time (CT) are determined to assess the integrity of hemostatic mechanisms. Hemostasis is the stoppage of bleeding. It is a complex process that involves three major steps vasoconstriction, platelet plug formation and

coagulation or clot formation. Bleeding time depends on the effectiveness of vasoconstriction and platelet plug formation whereas clotting time mainly depends on the effectiveness of the clotting mechanism. BT is the time from the onset of bleeding till the stoppage of bleeding and CT is the time from onset of bleeding till the clot formation. Clotting time (CT) is the time interval from onset of bleeding to formation of first fibrin thread. Normal value of CT is 5 to 8 minutes [1]. CT is affected by clotting factors. Defect or absence of one or more clotting factors can cause prolonged CT [6].

Nowadays, BT is widely used not just for evaluation of platelet function but also to assess the effects of medications and medical devices (such as cardiopulmonary bypass or dialysis machines) on homeostasis status. Moreover, BT test does not require expensive equipments or an intravenous (IV) line. It is not affected by the method of sampling and anticoagulants, either. The results of this test are prepared immediately and need very few amount of blood. Despite several studies on BT /CT and its extensive utilization for physiologic assessment of platelet function in human body and hemostasis, there are many conflicts and challenges about these tests. One of them is its wide reference range (2-10 minutes) which has been caused by different races and ages and different areas throughout the world. Therefore, BT/CT changes due to various causes may not be detectable. It is hence necessary to determine normal BT/CT in each geographical zone. It is also important to note that the last guideline of platelet function test was written in the late 1980s. The present study may thus help to renew the existing guidelines.

Available literature shows conflicting findings regarding the various factors influencing BT/CT. Moreover Indian studies with adequate sample size are lacking. Accordingly, the aim of this project was to determine the normal range of BT/CT.in medical students & compare with known indices & to study association of BT /CT with gender.

Material and Methods

This was a cross sectional study carried out in the Department of Physiology in a medical college in Pune, India. Prior approval was taken from the Institutional ethical committee. The duration of the study was 4 years. The study is undertaken in 433 apparently healthy 1st year medical students, during the academic year 2010-2014. Students having hemostatic disorders are excluded from the study. By Duke's method, the bleeding time was recorded. Finger of a subject is sterilized with spirit and pricked with sterilized needle. Time of pricking was noted. Take the stain of the punctured point on a filter paper on 30 second and keep taking stain of blood in 30 second intervals until the bleeding stoped. The time of no stain has come was noted properly; it was the bleeding time of the subject.

The Capillary Tube method clotting time was recorded. It involves collecting blood in a capillary tube that does not contain anticoagulant. The timer was started when the blood first enters the tube. The outside of the tube was carefully wiped and every 30 seconds a piece of the tube was broken. The time was recorded when a strand of fibrin appears between the two pieces of capillary tube as mentioned by Ghai C L (1999) [7]. Finally BT and CT of both the genders were compared and statistical analysis was done. Prior to the study, ethical committee approval was taken from college authorities. The purpose and procedure of the study was explained to each subject. Written informed consent was taken from all the participants.

The preset study was conducted at 8.30 am in the morning for the convenience of students. Two faculty members of our study performed the tests for bleeding and CT and collected data from the student at the same time. The analysis of data was done by Epi info version 7.1.2.0. software. Unpaired T test & Kruskal Wallis test were used to find out association. P value <0.05 was considered statistically significant.

Results

Total 433 subjects took part in the study, out of which 250 (57.7%) were females & 183 (42.3%) were males. Both groups were comparable in terms of age, gender, weight and for association purpose BT & CT was assesed against gender. The mean CT was 3.6 and 3.9 minutes in females and males respectively. The value of CT was little higher

in males but this difference was not statistically significant. The mean BT was 2.54 minutes and 2.56 minutes in females and males respectively. BT was higher in males as compared to females but again not statically significant.

Table-1: CT was assed against gender (Clotting time)

Gender	Factors			
	Mean	SD	DF	P value
Female	3.6550	0.8633	431	0.1305*
Male	3.9710	2.2296		

*After applying unpaired T test, Barlett's test for inequality was applied. Its P value found to be < 0.0001. So Unpaired T test is not suitable here. Therefore Kruskal Wallis test was applied here with its P value = 0.1305 which is non significant.

Table-2: BT was assed against gender (Bleeding time)

Gender	Factors			
	Mean	SD	DF	P value
Female	2.5436	0.5085	431	0.7104
Male	2.5623	0.5289		

Table-3: Summary of various studies regarding the role of gender on BT/CT in medical students

Name of researcher	Sample size	Location of study	Difference in male female	
			BT	CT
Mahapatra et al [8]	740	Orissa	Not significant	Not significant
Our study	433	Maharashtra	Not significant	Not significant
Roy B et al [3]	261	Nepal	Female > male	Female > male
Kumar S.S. et al [9]	222	Kerala	Female > male *	Female >male *
Sasekala M. et al [10]	100	Chennai	Female > male *	Female >male *

*statistically significant

Discussion

The first test for evaluation of platelet function is BT test. It is still one of the most important tests to assess platelet function and primary homeostasis [8]. While normal BT usually varies between 2-10 minutes (in some studies 2-9 minutes), it may increase to more than 30 minutes in severe platelet deficiency [11-12]. Although Bowie and Owen described BT for the first time [13] but the studies of Duke on performing BT test on ear lobule and his reports about the usefulness of whole blood transfusion in controlling clinical bleeding and returning BT to the normal range had an important role in introducing the test [14-15].

After that, Ivy, a surgeon who studied patients with jaundice, performed this test in the anterior part of the arm by producing a standard pressure above the area using sphygmomanometer cuff [16]. For a long time, both of these methods were commonly used in clinical practice. The most important advantage of BT test is its ability in examining normal body homeostasis and the role of vessels in this process. In addition, expensive equipments are not needed to perform this test [11].

Gender is one of the factors that affect BT, i.e. greater values are observed in females. We could also provide some additional details. Factors such as skin temperature, exercise, anxiety, incisions longer than the standard incision, and excessive cleaning of the test area, [17] individual differences of participants, the kind of devices used, and age were also found to alter BT. Other researchers have shown that BT decreases by increasing age. Furthermore, some kinds of foods, vitamins and spices like ginger, curcuma, onion, vitamins E and C, and garlic, produce abnormal platelet aggregation and BT. Medications such as aspirin and beta-lactam antibiotics, non-steroidal anti-inflammatory drugs, cardiovascular medications, psychotropic medications, analgesic drugs and narcotics, chemotherapy medications,

antihistamines would also cause abnormal BT. Studies done to find the effect of temperature, concluded that maintaining normal body temperature in surgical procedures is essential for normal platelet function [18-19].

In the present studies all the students BT/CT was within the normal range. When compared for gender, no statistical significant difference was observed as shown in table 1 & 2. Our findings were similar with other study [8]. However conflicting findings given by other studies as shown in table 3 [10, 20-21]. They showed females had prolonged bleeding and clotting time compared to males. BT was more in females can be due to the presence of estrogens & raised CT in them may be due to decrease the level of fibrinogen in the plasma. Similar findings by Kumar et al [9] with higher BT /CT in females than males. The probable reason of it, may be due to the differences of soft tissue, and hormonal effect on blood vessels. Presence of more amounts of estrogen in females may suppress platelet function and prolongs BT. BT in males was shorter when compared with females as testosterone increases synthesis of thromboxane A2 and facilitates platelet aggregation. Again higher CT in females may be due to presence of estrogen which prolongs clotting time by decreasing plasma fibrinogen levels [22].

Further, it was reported that testosterone inhibits platelet aggregation and this effect was dependent on endothelial nitric oxide synthesis. Using endothelial cell cultures, they demonstrated that androgen directly acts at the endothelial level. It is known that endothelial NO released into the vascular lumen serves as an inhibitor of platelet activation and aggregation. They showed that testosterone inhibited platelet aggregation and this effect was dependent on endothelial NO synthesis. In summary, testosterone modulates vascular EC growth and platelet aggregation through its direct action on endothelial NO production [23]. When we focused on the recent studies we found that the less sample size could be the reason for their findings as ours and Mahapatra et al findings were consistent with larger sample size. Despite several investigations on BT & CT and extensive usage of this test for evaluation of physiology of platelets in human body by specialists, there are still many conflicts and challenges about this test.

Conclusion

The role of gender in BT (bleeding time) & CT (clotting time) is still unclear. Our study with bigger sample size has definitely helped in clearing some issues.Our study suggests that there is no statistical difference in BT and CT with respect to gender. However we recommend further detailed study in this aspect by other newer advanced techniques & larger sample size, along with the levels of estrogen and Von Wilebrand Factor.

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