



Duke Bleeding Time in Young Adults at Aljufra University, Libya

Ishag Ibrahim Gumma Sheriff^{1,2*}, Fadel Ali Salem³, Heja Abdulsalam Farj³, Ibrahim Abdulaati Abdullah³, Manal Omar Othman³ and Kindak Ali Mohammed³

¹Department of Laboratories, Physiology Unit, Faculty of Medical Technology, Aljufra University, Houn, Libya.

²Department of Physiology, Faculty of Medicine, Alfashir University, Alfashir, Sudan.

³Department of Laboratories, Graduate Unit, Faculty of Medical Technology, Aljufra University, Houn, Libya.

Authors' contributions

This work was carried out in collaboration among all authors. Author IIGS designed the study, performed the statistical analysis, wrote the protocol, instructed & supervised the entire research and wrote the first draft of the manuscript. Author FAS helped in wrote the abstract and an introduction.

Authors MOO and KAM assisted in literature-review and results wrote. Authors HAF and IAA synergized in wrote sections of materials & methods and discussion & references respectively. Authors FAS, HAF, IAA, MOO and KAM performed the BT for data collection. All authors read and approved the final manuscript.

Article Information

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Complete Peer review History: <http://www.sdiarticle4.com/review-history/52197>

Original Research Article

Received 18 September 2019

Accepted 20 November 2019

Published 25 November 2019

ABSTRACT

Objectives: To determine the bleeding time (BT) among Medical Technology students of Aljufra University at Houn town. This in turn aimed to be used as a pilot work for a reference standard value of the BT overall the country when a large number of individuals checked in further studies. Also designed to be as a graduation project for pursuing a bachelor degree in Medical Technology.

Study Design: A descriptive cross-sectional.

Place and Duration of Study: Department of Laboratories (Physiology and Graduate Units), Faculty of Medical Technology, Aljufra University, Houn, from February to July 2019.

Methodology: Apparently healthy 154 students (54 males, 100 females; age range of 18-25 years) were enrolled in the study. A questionnaire of diseases and conditions e.g spleen, liver, iron deficiency, bleeding disorders, infections, smoking, current medication, genetic disorders, vitamins deficiency, endocrine disorders, tuberculosis, peptic ulcer, malignancies, renal, & anticoagulants was submitted as a template for the BT. Duke's method of skin pricking needles, cotton, alcohol, gloves, filter papers, and mobile watch for time monitor was followed due to its reliability and simplicity with quick results. The statistical package for social science, SPSS-23 was used in data analysis.

Results: The BT was estimated in all research respondents. The mean BT was found to be 1.14 ± 0.70 expressed in minutes and seconds. The minimum, maximum, and median values were 0.15, 4.15, & 1.08 minutes respectively. The effect of gender on mean BT was statistically significant ($P < .05$) with higher values in females than males. In contrast, there was negative correlation between age and mean BT (-0.1) indicating old students (>20 years) recorded higher values, but statistically insignificant ($P > .05$). Smoking did not show any significant impact on BT ($P > .05$).

Conclusion: The results confirm that both males & young people record lower values of BT comparing to females and elderly ones. Therefore, the bleeding time should be viewed as an essential test that evaluates blood coagulation profile.

Keywords: Reference values; bleeding time; Duke method; Aljufra; Libya.

1. INTRODUCTION

When bleeding occurs as example of blood vessels injury, the platelets and plasma clotting proteins act in a cascade pattern to form a hemostatic plug that develops to a clot. This blood loss preventing process tends to arrest the bleeding within normal physiological duration. Therefore, determining the bleeding time is a test of evaluating the efficacy of platelets and plasma clotting factors [1]. The bleeding time also can be defined as the time duration taken to stop the bleeding by the activated hemostatic mechanisms when a small slit is made in the skin. Therefore, The BT is an excellent test for the vascular platelet phase of hemostasis, which depends on an intact vasoconstriction response to a small vessel and on an adequate number of functionally active platelet cells [2]. Platelet disorders can be in numbers as in case of thrombocytopenia (congenital, increase destruction, and consumption) or in function (inherited or acquired as aspirin ingestion [1]. On the other hand, one example of the clotting factor disorders is hemophilia of types A, B, & C which are resulted from disorders in factors VIII, IX, and XI respectively [3].

Duke's method of bleeding time test is based on pricking the fingertip skin deeply with aseptic needle and noting the time every 15 seconds until bleeding arrests [4]. It is reliable, rapid, and accurate results method that can be performed with no risks if tested under sterile conditions.

The objective of this cross-sectional study is to determine the normal range of bleeding time at Aljufra University Medical Technology College students, Houn (centrally to south located town, Libya). A sample size of 154 respondents (54 males and 100 females) with age ranging from 18 to 25 years was enrolled in the research work randomly. Ethical approval was obtained from faculty administration and research committee; whereas the volunteers informed through an announcement as well as an oral communication. The materials used include cotton, alcohol, gloves, aseptic needles, filter papers, and mobile watch. A questionnaire also might be filled to exclude conditions and diseases affecting the normal bleeding period. Laboratory technician also seen to be necessary for helping in data collection and facilitating the access to the materials when requested. The Statistical Package Program for Social Science (SPSS) is selected for data analysis with probability ($P \leq .05$). Data should be subjected to normality test for classifying whether normally distributed (parametric sample, $P > .05$) or abnormally distributed (nonparametric sample, $P < .05$). Then parametric (e.g., T-test) and nonparametric (e.g., Mann-Whitney-U test) tests are applied.

The expected results, thought to be within the normal physiologic ranges of bleeding time and express the reference community values to not less than 95% as confidence interval. The values in males are supposed to be lower than those in

females due to bone marrow stimulating effect of the testosterone hormone (a hematopoietic hormone).

1.1 Duke Bleeding Time

If a prick is given in the skin with a needle, bleeding occurs and continues for some time and then stops. The time elapse between skin puncture and the arrest of bleeding is called bleeding time. This is achieved by many methods as Duke's of the following procedures:

- Making a deep puncture on skin of fingertip under aseptic conditions and monitor the time.
- Touching the blood drops each 15 seconds gently with a filter paper.
- Noting the time when no trace of blood on the filter paper.
- After bleeding has stopped, count the spots of blood to be referred as bleeding time per minutes and seconds.
- Normal bleeding time ranges from 1 - 4 minutes [4]. The normal bleeding times also found among females and males as 155 sec. (2.35 min.) and 96 sec. (1.36 min.) respectively [5]; as well as normal amount less than 3 minutes was studied by [6].

1.2 Platelet Formation-Thrombopoiesis

About 40% of the circulatory platelets are stored in the spleen (Fig. 1).

1.3 Platelet Characteristics

Thrombocytes are smallest blood cells of oval, spiky, and granulated in shape. The normal count of platelets ranges from 200,000 – 500,000 per microliter of blood. The count increases (ie. Thrombocytosis) in conditions as splenectomy. They are non-nucleated of about a week as half-life. The platelets aggregate at the site of vascular damage to prevent the bleeding. The megakaryocytes from the bone marrow are precursors that produce this type of blood cells [3].

1.4 Platelet Functions

Hemostasis is a prevention of blood loss. Blood coagulation takes place in three essential steps: prothrombin activator, prothrombin activator catalyzes conversion of prothrombin to thrombin,

and conversion of fibrinogen to fibrin [7]. The platelets take part in a sequence of actions in hemostasis as the following summary:

- Adhesion to subendothelial exposed collagen fibers depending on von-Willebrand factor (part of clotting factor VIII).
- Aggregation to form a hemostatic plug that converted to a clot.
- Releasing of a hemostatic reaction agents, e.g.:
 - Thromboxane A₂ (TXA₂) + serotonin → vasoconstriction.
 - TXA₂ + ADP → more aggregation depending on Ca²⁺ & fibrinogen.
 - Local thrombin (further platelet aggregation) & conversion of fibrinogen to fibrin → catalyzation of coagulation.
 - Phospholipase A₂ (PLA₂) is activated by collagen & thrombin.
 - Arachidonic acid is released from platelet plasma membranous phospholipids by the action of PLA₂.
 - Then the arachidonic acid is converted to unstable prostaglandins (PGs), PGG₂, PGH₂ by cyclooxygenase.
 - These PGs are transformed to TXA₂ (powerful vasoconstrictor) by a synthase enzyme. The TXA₂ exerts its function by inhibiting the adenylate cyclase that increases cytoplasmic free calcium of platelets.
 - Similar synthesis pathway happens in endothelial cells with conversion of PGH₂ to prostacyclin (vasodilator & potent inhibitor of platelet aggregation by an opposite mode of action to TXA₂). The prostacyclin may prevent deposition of platelets on normal endothelium and prevents formation of plug near area of vascular damage [8].
- In recent discoverers, platelets augment vascular inflammation and remodeling of the arterial wall resulting in formation of atherosclerotic plaques due to formation of inflammatory hot spot at the arterial wall. Other recent clinical studies also found that intensified and prolonged anti-platelet therapy improves clinical prognosis of patients with atherosclerotic disease [9].

An initial adhesion of platelets to subendothelial exposed collagen at the injured site is followed by platelet-platelet adhesion. The initial adhesion is mediated by binding of the exposed collagen to its platelet membrane

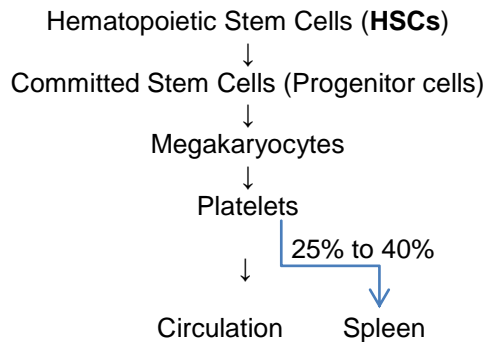


Fig. 1. Formation of platelets [3]

About 2/5 of manufactured platelets normally stored in the spleen

glycoprotein-VI (GPVI) receptor resulting in collagen-induced activation of platelets. The activation also achieved by other agonists as ADP, leading to a series of events that responsible for increasing ICF Ca^{2+} , that is derived from its stores and the ECF [10].

- Other functions of platelets:
- Appear to take part in the repair after vascular injury.
- Platelet-derived growth factor acts as mitogenic factor for smooth muscle, fibroblasts, and glial cells.
- Chemotactic for neutrophils and macrophages [8].

1.5 Clotting Factors

The plasma clotting factors are inactive proteins. They are I (Fibrinogen), II (Prothrombin), III (Thromboplastin), IV (Calcium), V (Proaccelerin, labile factor, accelerator globulin), VII (Proconvertin, SPCA, stable factor), VIII (Antihemophilic factor, AHF), antihemophilic factor A, antihemophilic globulin (AHG), IX (Plasma thromboplastic component (PTC), Christmas factor, antihemophilic factor B), X (Stuart-Prower factor), XI (Plasma thromboplastin antecedent (PTA), antihemophilic factor C), XII (Hageman factor, glass factor), XIII (Fibrin-stabilizing factor, Laki-Lorand factor), HMW-K(High-molecular-weight Kininogen, Fitzgerald factor), Pre- K_a (Prekallikrein, Fletcher factor), K_a (Kallikrein), PL (Platelet phospholipids). They are activated in a cascade way through two main paths, intrinsic and extrinsic [3].

1.6 Anticlotting Mechanism

Blood tendency to clot is balanced by anticlotting mechanism; including two reactions, i.e. platelet-

aggregating effect of thromboxane A_2 (TXA_2) and platelet anti-aggregating effect of prostacyclin. Anticoagulants can be classified into two groups:

1.6.1 Endogenous anticoagulants

- Antithrombin-III: Circulating protease inhibitor of serine proteases of clotting factors (II, IX, X, XI, and XII).
- Heparin: Naturally produced and facilitates antithrombin-III action.
- Thrombomodulin: Produced by endothelial cells and forms a complex with thrombin to activate protein C, forming activated protein C (APC) that lyses fibrin and degrades fibrinogen in various reactions.

1.6.2 Exogenous anticoagulants

- Chelating agents, i.e. remove ionized calcium from the blood by forming insoluble salts, as oxalate & EDTA.
- Coumarin derivatives (ie. Dicoumarol & warfarin), they inhibit vitamin-K action, ie. which is a coagulant).
- Aspirin, inhibits cyclooxygenase enzyme that activates production of TXA_2 [3].

1.7 Plasma Ionized Calcium

The ionized Ca^{2+} is essential for promotion or acceleration of all blood coagulation reactions. Ionized calcium binds with a platelet producing substance to form thromboplastin that in turn converts prothrombin into thrombin as in diagram of clotting mechanism [11].

2. MATERIALS AND METHODS

Ethical clearance was obtained from faculty administration as well as faculty research committee. The informed consent was obtained

directly from volunteers. The study was conducted at Houn town, Aljufra Municipality, Libya with a descriptive cross-sectional design. Samples were collected randomly in a population of 154 volunteers including both sexes (54 males, and 100 females). The respondents were classified into two age groups, ≤20 & >20 years.

2.1 Methodology

Needles, cotton wool, alcohol, filter papers, gloves, and a mobile watch for time monitoring were used. A questionnaire containing personal data, diseases, and conditions related to bleeding time as spleen, liver, iron deficiency, smoking, genetic disorders, vitamins deficiency, bleeding disorders, current medication, peptic ulcer, malignancies, tuberculosis, endocrine disorders, infections, anticoagulants was designed and filled as a template for the BT.

Duke’s method of bleeding time was followed due to its reliability and simplicity with accurate results. Bleeding time test was performed for each volunteer in Faculty Research Laboratory as the following procedures:

- Disinfecting tip of a finger after holding it,
- Waiting a minute for alcohol to dry,
- Gently prick the skin with aseptic needle,
- Start counting 15 seconds after blood comes out to apply a filter paper,
- Repeat the process every similar time till no spot on a paper,
- Count numbers of spots to multiply in 15, and
- Express the bleeding time in minutes and seconds e.g., 1.14 (one minute & 14 seconds).

2.2 Statistic Method

Data were subjected to normality test; they were abnormally distributed (nonparametric sample, $P<.05$). Statistically, the obtained data were analyzed by using the statistical package for social science, SPSS-23, of non-parametric software tests (correlation- Spearman & Z, Mann-Whitney-U, & Explore). The results were considered significant only when $P\le.05$.

3. RESULTS

3.1 The Study Population

Total numbers of 154 individuals of age ranging from 18 - 25 years were studied, among them 100 females and 54 males as below (Fig. 2). Subjects also divided into two age groups (Fig. 3). The research respondents again categorized based on smoking, gender, and age (Table 1).

3.2 The Bleeding Time

In this study the mean bleeding time among the whole study population was 1.14 min. (minutes, second) with standard deviation of 0.70 was estimated (Table 2). Therefore, 95% as confidence interval; the respondents recorded bleeding times of 1.03 – 1.25 minutes as lower and upper bounds.

3.3 The Bleeding Time & Gender

The probability of correlation between mean bleeding time and gender was 0.00, revealing statistical significant ($P<.05$). Males have less bleeding time comparing to the females (Table 3) & (Fig. 2).

Table 1. Distribution of subjects according to sex, age & smoking

Variable/ total	Variable-1 (No)	Variable-2 (No)
Gender (154)	Males (54)	Females (100)
Age (years) (154)	≤20 (32)	>20 (122)
Smoking (154)	Smokers (29)	Nonsmokers (125)

**Data were analyzed by Explore & Mann-Whitney softwares of SPSS-23*

Table 2. Mean bleeding time & standard deviation in study population

Lower limit	Upper limit	Mean (x)	Standard deviation (SD)
1.03	1.25	1.14	0.70
Minimum	Maximum	Medium	Skewness
0.15	4.15	1.08	1.5

**Values expressed in minutes and seconds in two decimals as below. Data were analyzed by Explore software of SPSS-23*

Table 3. Relationship between bleeding time, gender, age and smoking

Test	Correlation coefficient	Probability	Relationship
Gender (Spearman)	.2	.01	positively below middle & not significant ($P < .05$)
Age (Z)	-.1	.15	Negatively below middle & not significant ($P > .05$)
Smoking (Z)	-.48	.63	Negatively below middle & not significant ($P > .05$)

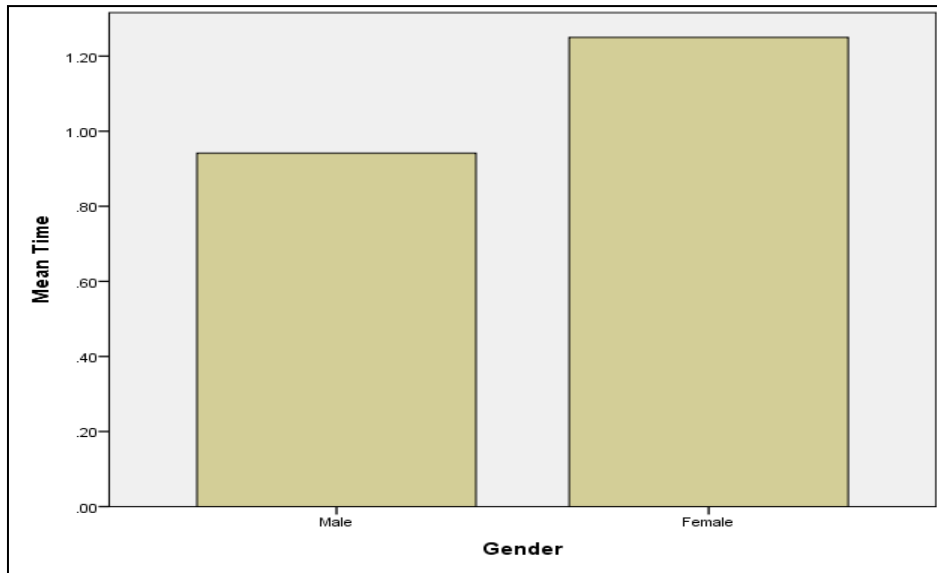


Fig. 2. Distribution of study population (154) based on gender

Males below a minute, whereas females above 1.20 min. with clear sig. effect of testosterone hormone ($P < .05$)

3.4 The Bleeding Time & Age

There was a relationship between mean bleeding time and age ($r = 0.146$) revealing that old

people have higher bleeding time (reversely associated, i.e. negative sign, but statistically insignificant ($P > .05$)) (Table 3) & (Fig. 3).

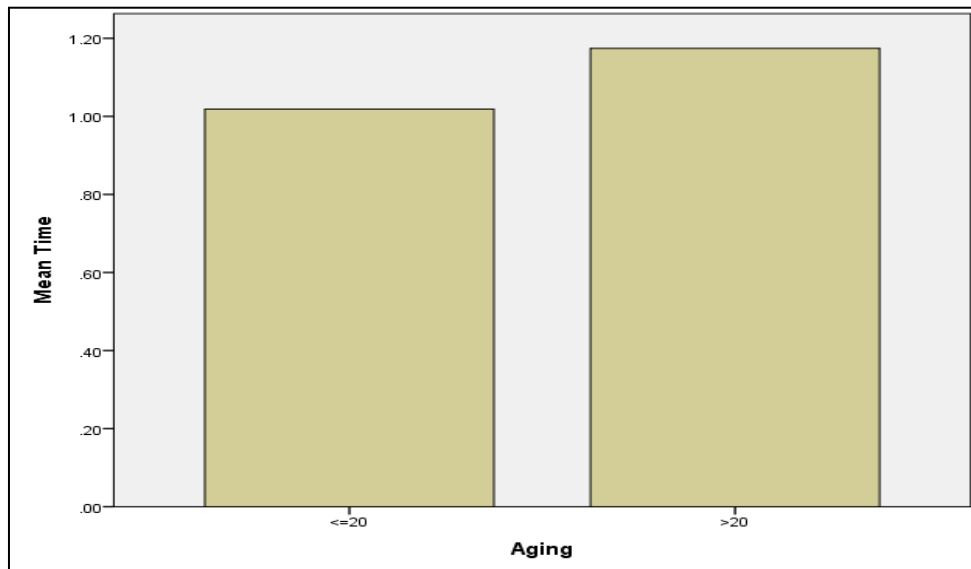


Fig. 3. Distribution of study population (154) based on age

Young students around a minute, whereas above 20 years around 1.20 min; with apparent age effect, but not sig; ($P > .05$)

3.5 The Bleeding Time & Smoking

Statistically no relationship between smoking and bleeding time ($P>.05$) (Table 3).

4. DISCUSSION

4.1 Bleeding Time

The mean bleeding time was found to be 1.14 ± 0.70 . It was lower than what reported by [6], less than 3 minutes, because of young age. On the other hand, it is similar/close to the value reported by [4], from 1 - 4 minutes.

4.2 Effect of Sex on Bleeding Time

Normal males and females of this work showed statistically significant gender related differences on the mean bleeding time. The results showed that normal males of the study recorded lower bleeding time than females (Fig. 2), due to the effect of the testosterone hormone on thrombopoiesis; and the thrombocytes play a key role in forming a clot that terminates the bleeding.

4.3 Effect of Age on Bleeding Time

The research work showed below middle relationship between age and bleeding time as revealed by Spearman test (Table 3) with a negative sign indicating that, the elderly people have prolonged bleeding time than the youngest ones, but statistically not significant ($P>.05$). This goes with what studied by [12], who confirmed that the bleeding time is less in newborns; with reference range of 1.5 – 9.5 minutes.

5. CONCLUSION AND RECOMMENDATIONS

- The mean bleeding time among research respondents was estimated to be 1.14 ± 0.70 min/sec.
- Statistically, gender differences reported, i.e. males showed lower bleeding time values than females of similar age.
- Age also showed relation to bleeding time, i.e. old students recorded higher values than younger ones, but not significant ($P>.05$) (-ve by Z) (Table 3).
- Bleeding time test is useful in giving quick screening to blood coagulation profile.
- More work is needed to establish typically representative reference bleeding time among Libyans.

- Public awareness should be raised on the necessity of the BT.
- We recommend our research BT values to be used instead of overseas standards.
- Similar study can be done in other faculties and universities.
- Ministry of health should take an initiative of distributing these results.
- Smoking showed no effect on the bleeding time.

CONSENT

All authors declare that informed consent was obtained from the volunteer students orally after an announcement approved by faculty administration & research committee in the college.

ETHICAL APPROVAL

All procedures performed in the study were in accordance with the ethical standards.

ACKNOWLEDGEMENTS

Our acknowledgements to Medical Technology College administration & research committee for giving a permission to conduct this work. Acknowledgements also extended to the faculty laboratory technicians for facilitating in access to research materials. Volunteer students who participated in the study; their nice cooperation is highly appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bashawri LAM, Ahmad MA. The approach to a patient with a bleeding disorder: For the primary care physician. *Journal of Family & Community Medicine*. 2007; 14(2):53–58. [Accessed March 2019]. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/pmc3410146/#_ffr-sectitle
2. Raber MN. Coagulation test. In: Walker HK, Hal WD, Hurst JW, editors. *Clinical methods: The history, physical and laboratory examinations*. 3rd ed. Boston: Butterworths; 1990. [Accessed 16 October 2019]. Available: <http://www.ncbi.nlm.nih.gov/books>

3. Ganong WF. Review of medical physiology. 25th ed. China: McGraw-Hall; 2016;31:554–567.
4. Ghai CL. A textbook of practical physiology. 3rd ed. Delhi: JP medical publishers.1987;175.
5. Kumar SS, VK JM, George J, Mukkadan JK. Health prospect. Journal of Public Health. 2013;12:8.
[Accessed May 2019]
Available:http://www.academia.edu/22782865/Bleeding_time_and_Clotting_Time_in_Healthy_Male_and_Female_College_Students_of_Karukutty_Village_Kerala
6. Russeau AP, Manna B. Bleeding time. Stat Pearls Journal of Clinical Sciences; 2019.
[Accessed July 2019]
Available:<http://www.ncbi.nlm.nih.gov/books/NBK537233>
7. Adair TH, Dzielak DJ, Hall JE, Lohmeier TE, Manning RD, Mihailoff G, Young DB. Pocket companion to Guyton & Hall textbook of medical physiology. 11th ed. Philadelphia: Elsevier Saunders INC; 2006;36:284–285.
8. Bray JJ, Gragg PA, Macknight ADC, Mills RG. Lecture notes on human physiology. 4th ed. Victoria, Australia: Blackwell Science Pty Ltd.1999;12:310 –312.
9. Chatterjee M, Gawaz M. Platelets in atherosclerosis. In: Gresele P, Kleiman N, Lapez J, Page C, editors. Platelets in thrombotic and non-thrombotic disorders. Springer, Cham; 2017.
[Accessed 31 October 2019].
Available:http://doi.org/10.1007/978-3-319-47462-5_66
10. Koltai K, Kesmarky G, Feher G, Tibold A, Toth K. Platelet aggregometry testing: molecular mechanisms, techniques and clinical implications. Int. J. Mol. Sci. 2017; 18:8.
[Accessed 31 October 2019]
DOI: 10.3390/ijms18081803
11. Guyton AC, Hall JE. Textbook of Medical Physiology. 11th ed. Philadelphia: Elsevier Saunders. 2006;451.
12. Parsons JC. Bleeding time. Pathology Outlines. com; 2016.
[Accessed 28 October 2019]
Available:<http://www.pathologyoutlines.com/topic/coagulationbleedingtime.html>

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Peer-review history:

The peer review history for this paper can be accessed here:
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