Determination of normal range of bleeding time in rural and urban residents of Borujerd, Iran: A pilot study

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Abstract

BACKGROUND: Bleeding time test is used to assess the function of platelets in human body. The aim of this project was thus to estimate the sample size required to determine the normal range of bleeding time (BT) in Borujerd (a city in Iran). A pilot study was designed to determine the range of normal BT in a small group of normal people. The total sample size for the next study was then calculated according to the results.

METHODS: In order to determine the sample size, a total of 33 volunteers participated in this study. The normal range of BT was determined by Ivy method. Written informed consents were obtained from all participants and their clinical history was recorded. The sampling was performed once for each participant. However, the results were interpreted by two observers. The study protocol was approved by the Ethics Committee of the research center at Lorestan University of Medical Sciences (Iran).

RESULTS: In this study, 33 normal participants (20 women and 13 men) were divided into four age groups of 35-44, 45-54, 55-64 and over 64 years old. Maximum and minimum BTs in men were 209 (in the age group of 35-44 years) and 150 seconds (in the age group of over 64 years), respectively. On the other hand, the corresponding values in women were 194 (in 55-64 year-old subjects) and 145 seconds (in women over 64 years of age). Considering the aforementioned results, the total sample size for the next study was determined to be 580 normal subjects by two-sample t-test power analysis at a power of 0.91816.

CONCLUSION: There was a significant difference between the normal range of BT in participants of Borujerd and previously recorded range in other studies. Moreover, normal BT in men decreased by aging. This study did not show any special order in increasing or decreasing BT in women.

Introduction

Human platelets are small and discoid-shaped cells with dimensions of about 2-4 by 0.5 micrometers and mean volume of 7-11 femtoliter.[1] They are the second frequent group of cells in normal circulation (150-450 × 10⁹ per liter). Platelets are non-nuclear cells that originate from megakaryocytes and stay in blood flow for approximately 10 days. The small size and shape of platelets make 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 homeostasis, thrombosis, conglutination, 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

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(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 homeostatic 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]

Bleeding time (BT) test is used for assessment of platelet function in human body. Nowadays, this test 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. The most important advantage of BT test is its ability to evaluate normal body homeostasis and the role of vessels in this process. 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.[2-14]

Despite several studies on BT and its extensive utilization for physiologic assessment of platelet function in human body, there are many conflicts and challenges about this test. 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 changes due to various causes such as aspirin consumption may not be detectable. It is hence necessary to determine normal BT in each geographical zone. It is also important to note that the last guideline of platelet function test was written in the late 1980s.[2] The present study may thus help to renew the existing guidelines. Accordingly, the aim of this project was to determine the normal range of BT and its relationship with different factors in the population of Borujerd (a city in Iran).

Materials and Methods

This study was conducted on volunteers who provided written informed consents. The previous medical history of all participants was recorded in a questionnaire by a trained nurse in the rural health and treatment center and by a physician in the urban health and treatment center. All subjects with acquired or congenital coagulation disorders were excluded from the study. Consumption of some

medications such as aspirin, indomethacin, phenothiazine, and antihistamines may disrupt the platelet function (by affecting platelet membrane) and result in prolonged BT. Therefore, volunteers were questioned about using the aforementioned drugs in the previous week.

BT of the participants was determined according to the Ivy method. A group of sample takers had been previously trained and calibrated to use the Ivy method based on the national standard protocol in order to minimize the observer error. The collected data was then recalculated by another observer and the final values were recorded in the main research center at Lorestan University of Medical Sciences (Iran).

The Ivy Method

In this method, a blood pressure cuff is placed on upper arm and is then inflated to 40 mm Hg. An incision with a length of 8 mm and depth of 1 mm is made by a lancet in the anterior section of the underside of the forearm in an area without superficial veins. The time from the beginning of incision until the termination of bleeding is considered as the BT. A standard filter paper should be used every 30 seconds to draw off it until the blood completely stops. The normal BT values run in the range of 2-9 minutes. The risk of bleeding increases with BT values more than 10 minutes.

In this study all incisions were made by a lancet and standard filter papers were selected to draw off the blood. All sample takers were provided with similar digital chronometers to measure time. Blood was drawn off the forearm every 30 seconds. The collected blotting papers were encoded. They were then sent to the research center along with the filled time tables to be reevaluated by the second observer. Due to painfulness of this technique, the process was performed just once for each participant. However, all results were recorded by two observers, i.e. one person recorded the results during the test and the other one interpreted the results and recorded them in the related forms. When there were differences in sample reading, we considered the mean of the two results. The standard forms were filled, and the results were then entered into statistical software for analysis. All sample takers were trained for performing the test.

The ethics committee of the university research center confirmed this project.

Sample Size Determination and Data Analysis

Considering similar data in published studies, a pilot study was performed and according to the results, a sample size of 580 (290 male and 290 female) subjects was calculated for the next study by variance determination at a power of 0.91816. In the final study, 580 subjects were selected by cluster sampling

method. Data was entered into SPSS$_{11.5}$ (SPSS Inc., Chicago, IL, USA). Frequency distribution of data was assessed with a confidence interval of 95% to determine the mean measured time in the participants.

The results of this study showed that the mean of BT in men and women were 182.69 and 168.7 seconds, respectively. The results of BT in gender groups were analyzed using two independent sample t-test in PASS software. A sample volume of 580 persons, including 290 women and 290 men, was estimated by a power of 0.91816 (Table 1).

Results

According to this pilot study, society variance was determined by performing the test on 33 subjects who were selected by the clustering method. This was an epidemiologic analysis by Lorestan University of Medical Sc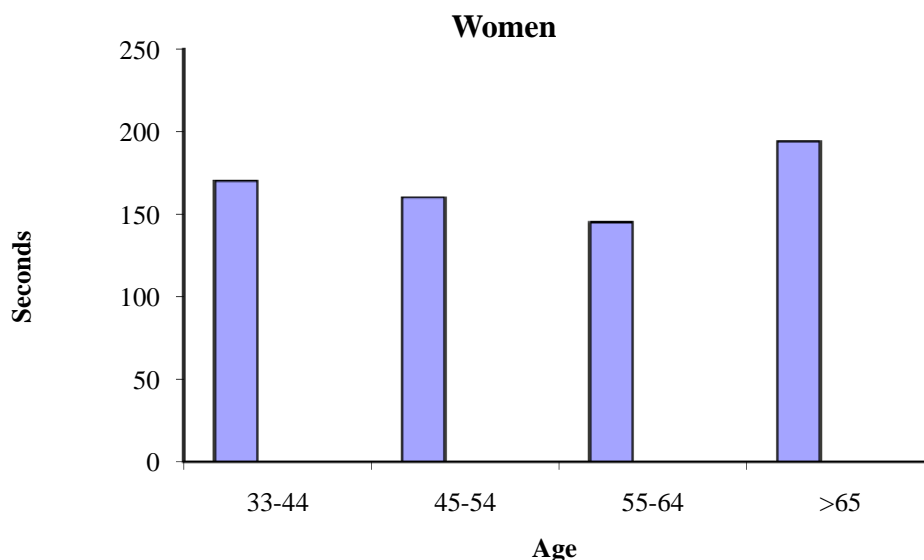iences in Borujerd. The participants in the pilot study (20 women and 13 men) were divided into four age groups of 35-44, 45-54, 55-64, and over 64 years old.

Data analyses showed that the maximum BT was 209 seconds in men who aged 35-44 years old. The minimum BT was 150 seconds in men older than 64 years. In women however, the maximum (194 seconds) and minimum (145 seconds) BTs were observed in the age range of more than 64 and 55-64 years old, respectively. The results demonstrated that there was not a significant relation between BT and different age groups among women. However, BT was prolonged in subjects older than 64 years (Figure 1). The normal range of BT in this study (2.5-3.5 minutes) was significantly different from the international normal range. Moreover, according to this study, BT decreased by increasing age in men (Figure 2). In order to provide more details, the ranges of BT are presented in figures 3 and 4.
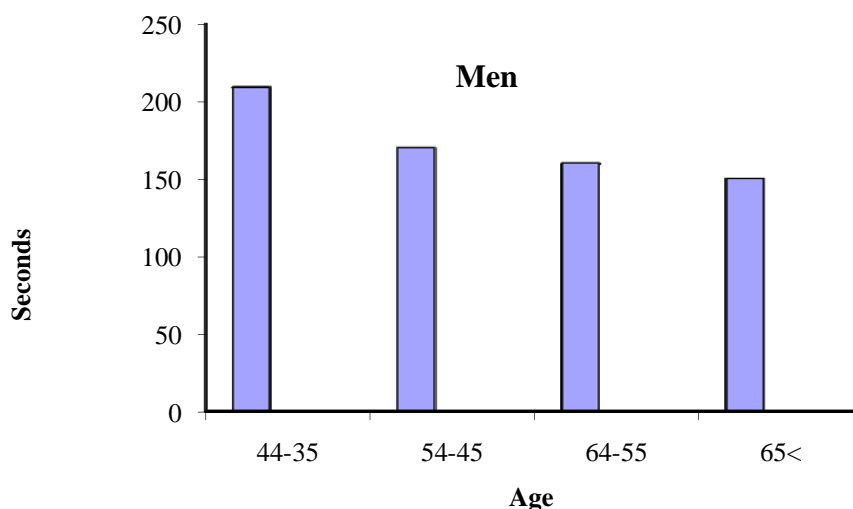
Table 1. Estimated sample size according to different powers (the used numbers in the estimated sample size was 290 males and 290 females with 0.91816 powers.

Two-Sample T-Test Power Analysis									
Allocation									
S2	S1	Mean2	Mean1	Beta	Alpha	Ratio	N2	N1	Power
55.7	43.9	182.7	168.7	0.08184	0.05000	1.000	290	290	0.91816

The estimated numbers are 290 males and 290 females with 0.91816 powers.

Figure 1. Bleeding time in different age groups of women

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Figure 2. Bleeding time in different age groups of men

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] BT is the period of time required to stop hemorrhage after an incision. 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.[2,3]

Although Bowie and Owen described BT for the first time,[9] 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.[10,11] 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.[12] For a long time, both of these methods were commonly used in clinical practice.

As mentioned previously, BT test is used to evaluate platelet function in human body. Despite the shortcomings of this test such as weak reproducibility, invasiveness, insensitivity, time consumption, and inability to predict bleeding risk in patients under surgery, it is currently used extensively for evaluation of platelet function.[13] Moreover, BT test is used to assess the effects of medications and medical devices on homeostasis status. 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.[2,14]

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,[15] 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. While the results of this study were in concordance with this theory, the results were not similar in women. Other factors which may prolong BT include white blood cell (WBC) and red blood cell (RBC) count, chronic kidney disease, anemia, connective tissue disorders (such as Ehlers-Danlos syndrome). 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 (penicillin and cephalosporin), non-steroidal anti-inflammatory drugs, cardiovascular medications, psychotropic medications (amitriptyline and haloperidol), analgesic drugs and narcotics, chemotherapy medications, antihistamines would also cause abnormal BT. Finally, diseases such as Bartter syndrome, atopic asthma, hay fever, and Wilms tumor may alter BT, as well.[3,4]

A previous study evaluated the effectiveness of different doses of aspirin on BT among 70 healthy subjects with different ages and genders. The participants had not consumed any drugs during the 3 weeks prior to the study. BT was measured and recorded twice, once after fasting and once 1.5-2 hours after taking aspirin, by Ivy method in the anterior section of the forearm. The results demonstrated an inverse relation between BT and age. In all age groups (except lower age groups), prolonged BT occurred at lower doses of aspirin. The mean BT in different age groups was 13.4 seconds before aspirin consumption.[16]
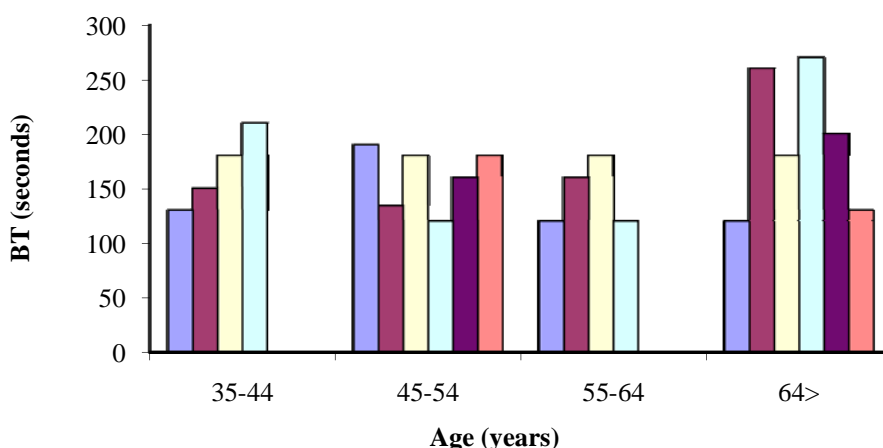
Figure 3. Bleeding time (BT) in each female subject (n = 20) stratified based on age
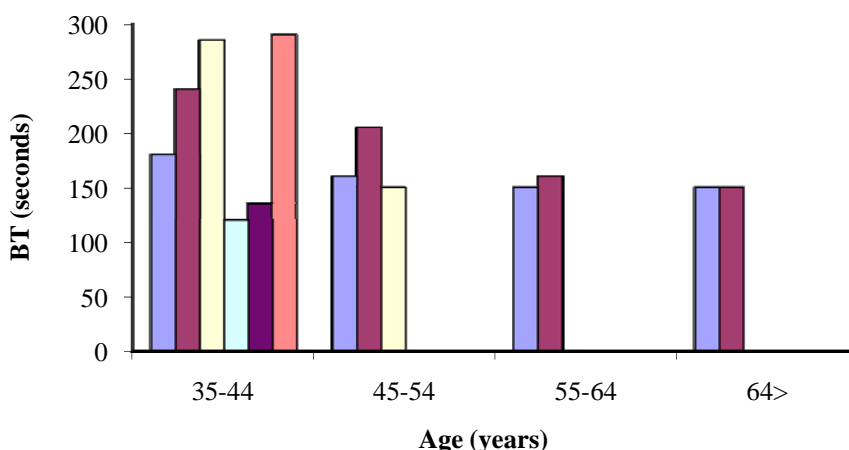

Figure 4. Bleeding time (BT) in each male subject (n = 20) stratified based on age

In another study, BT was measured in 100 normal participants and 136 patients with different diseases. BT was approximately 4.5 ± 1.5 minutes in subjects with normal platelet count. However, prolonged BT was associated with platelet dysfunction due to using aspirin, uremia, or von Willebrand's disease. On the other hand, BT values lower than normal resulted from enhanced homeostatic ability of young platelets, i.e. in idiopathic thrombocytopenic purpura or bone marrow recovery after chemotherapy.[17]

In a retrospective study on 569 persons with a history of clinical bleeding or a similar experience among their close relatives, there were significant inverse relationships between BT and platelet count parameters, hematocrit, logarithm of von Willebrand's antigen, and the extent of its activity. A reverse, independence, and significant relationship was also reported between BT and age of patients.[18]

Sutor believed that BT can be applied as a screening test for patients with bleeding symptoms. Besides, it can be used as a single test in children to provide the best information since in this age group, primary dysfunctions of homeostasis are more common than coagulopathy. He emphasized that the standard technique should be used and limitations of this test should also be considered.[14]

In order to evaluate the effects of temperature on closure time (CT) and BT tests, Valeri et al. studied 54 healthy female and male volunteers in the age range of 19-35 years old. Their participants did not have the history of taking any medications. The skin temperature of people was changed between 20-38°C. Blood samples were drawn from the anterior part of the arm. At each temperature, complete blood cell (CBC) count, BT, and thromboxane A2 level were assessed in serum and plasma. BT was tested in

38 volunteers and CT was performed in 16 volunteers. The results showed that at skin temperatures above 32°C, BT was more prolonged and hematocrit was lower in women compared to men. However, at the mentioned skin temperatures, BT increased significantly in both genders. This increment was consistent with decrement of thromboxane A2 concentration. On the other hand, decreasing temperature from 35°C to 22°C resulted in 3-4 folds increment of BT. There was not a significant change in BT at skin temperatures higher than 35°C. Eventually, the investigators concluded that maintaining normal body temperature in surgical procedures is essential for normal platelet function.[19] In another study, Valeri et al. made general and local hypothermia in healthy baboons and noticed a reversible platelet dysfunction due to hypothermia. However, returning the skin temperature to normal resulted in higher BT and reversed it to normal range.[20]

Khuri et al. demonstrated that BT is prolonged when peripheral skin temperature decreases during the bypass surgery.[21] In another study, it was shown that every 6-degree reduction in the temperature of anterior skin of the arm increased BT by 3 minutes.[22]

A research on the effects of different blood groups on BT in 116 participants showed that the von Willebrand's factor in the plasma of subjects with blood group O was lower than other blood groups. However, BT was similar in all groups.[23]

The results of a previous study demonstrated that during adhesion to extracellular matrix, platelets tend to be more active in patients with type 2 diabetes compared to non-diabetics.[24] Similar results were observed in patients with hypercholesterolemia and hypertension.[25-28]

Despite several investigations on BT 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. One of them is the wide normal range for this test (2-10 minutes). The significant difference of the range observed in this study confirms to the necessity of defining the normal range of BT in each geographic area. It is also important to note that the last guidelines for platelet function test were written in the late 1980s. Some measures are currently being taken to rewrite the guidelines.[2] The results of this study might also be a step toward providing the appropriate data in this field.

Acknowledgments

Conflict of Interests

Authors have no conflict of interests.

References

1. George JN. Platelets. Lancet 2000; 355(9214): 1531-9.
2. Harrison P. Platelet function analysis. Blood Rev 2005; 19(2): 111-23.
3. George JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. N Engl J Med 1991; 324(1): 27-39.
4. Triplett DA. Coagulation and bleeding disorders: review and update. Clin Chem 2000; 46(8 Pt 2): 1260-9.
5. Peerschke EI. The laboratory evaluation of platelet dysfunction. Clin Lab Med 2002; 22(2): 405-20.
6. Thiagarajan P, Wu KK. In vitro assays for evaluating platelet function. In: Gresele P, Page CP, Fuster V, Vermylen J, Editors. Platelets in Thrombotic and Non-Thrombotic Disorders: Pathophysiology, Pharmacology and Therapeutics. Cambridge: Cambridge University Press; 2002. p. 459-70.
7. Rand ML, Leung R, Packham MA. Platelet function assays. Transfus Apher Sci 2003; 28(3): 307-17.
8. Guidelines on platelet function testing. The British Society for Haematology BCSH Haemostasis and Thrombosis Task Force. J Clin Pathol 1988; 41(12): 1322-30.
9. Bowie EJ, Owen CA. The bleeding time. Prog Hemost Thromb 1974; 2(0): 249-71.
10. Duke WW. The relation of blood platelets to hemorrhagic disease. By W.W. Duke. JAMA 1983; 250(9): 1201-9.
11. Brinkhous KM. W. W. Duke and his bleeding time test. A commentary on platelet function. JAMA 1983; 250(9): 1210-4.
12. Ivy AC, Shapiro PF, Melnick P. The bleeding tendency in jaundice. Surg Gynecol Obstet 1953; 60: 781-4.
13. Rodgers RP, Levin J. A critical reappraisal of the bleeding time. Semin Thromb Hemost 1990; 16(1): 1-20.
14. Sutor AH. The bleeding time in pediatrics. Semin Thromb Hemost 1998; 24(6): 531-43.
15. Peterson P, Hayes TE, Arkin CF, Bovill EG, Fairweather RB, Rock WA, et al. The preoperative bleeding time test lacks clinical benefit: College of American Pathologists' and American Society of Clinical Pathologists' position article. Arch Surg 1998; 133(2): 134-9.
16. Jorgensen KA, Dyerberg J, Olesen AS, Stoffersen E. Acetylsalicylic acid, bleeding time and age. Thromb Res 1980; 19(6): 799-805.
17. Harker LA, Slichter SJ. The bleeding time as a screening test for evaluation of platelet function. N Engl J Med 1972; 287(4): 155-9.
18. Gerrard JM, Docherty JC, Israels SJ, Cheang MS,

Bishop AJ, Kobrinsky NL, et al. A reassessment of the bleeding time: association of age, hematocrit, platelet function, von Willebrand factor, and bleeding time thromboxane B2 with the length of the bleeding time. Clin Invest Med 1989; 12(3): 165-71.

19. Valeri CR, MacGregor H, Cassidy G, Tinney R, Pompei F. Effects of temperature on bleeding time and clotting time in normal male and female volunteers. Crit Care Med 1995; 23(4): 698-704.

20. Valeri CR, Feingold H, Cassidy G, Ragno G, Khuri S, Altschule MD. Hypothermia-induced reversible platelet dysfunction. Ann Surg 1987; 205(2): 175-81.

21. Khuri SF, Wolfe JA, Josa M, Axford TC, Szymanski I, Assousa S, et al. Hematologic changes during and after cardiopulmonary bypass and their relationship to the bleeding time and nonsurgical blood loss. J Thorac Cardiovasc Surg 1992; 104(1): 94-107.

22. Valeri CR, Khabbaz K, Khuri SF, Marquardt C, Ragno G, Feingold H, et al. Effect of skin temperature on platelet function in patients undergoing extracorporeal bypass. J Thorac Cardiovasc Surg 1992; 104(1): 108-16.

23. Rodeghiero F, Castaman G, Ruggeri M, Tosetto A. The bleeding time in normal subjects is mainly determined by platelet von Willebrand factor and is independent from blood group. Thromb Res 1992; 65(4-5): 605-15.

24. Knobler H, Savion N, Shenkman B, Kotev-Emeth S, Varon D. Shear-induced platelet adhesion and aggregation on subendothelium are increased in diabetic patients. Thromb Res 1998; 90(4): 181-90.

25. Carvalho AC, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. N Engl J Med 1974; 290(8): 434-8.

26. Aoki I, Aoki N, Kawano K, Shimoyama K, Maki A, Homori M, et al. Platelet-dependent thrombin generation in patients with hyperlipidemia. J Am Coll Cardiol 1997; 30(1): 91-6.

27. Lande K, Os I, Kjeldsen SE, Westheim A, Hjermann I, Eide I, et al. Increased platelet size and release reaction in essential hypertension. J Hypertens 1987; 5(4): 401-6.

28. Thomas JS, McConnell MF, Bell TG, Padgett GA. Platelet aggregation and dense granule secretion in a colony of dogs with spontaneous hypertension. J Hypertens 1992; 10(12): 1493-8.