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Original Article

Physiology Section

Fibrinolytic Activity of Blood and its Determinants in Healthy Medical Students

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ABSTRACT

Background: Decreased fibrinolytic activity results in longer fibrinolysis time of blood and an increased propensity for hypercoagulable states of blood. Subjects with less fibrinolytic activity are prone to coronary artery disease, stroke and thromboembolic phenomena.

Aim: The study aims to identify the impact of gender, dietary habits, body mass index, physical activity level and menstrual cycle on fibrinolytic activity in healthy subjects.

Settings and Design: Cross-sectional study on randomly selected 206 healthy medical students aged 17 to 23 years.

Materials and Methods: Anthropometric measurements, dietary habits, physical activity level and menstrual history were obtained. Fibrinolysis time of fasting venous blood sample was determined by Euglobulin Lysis Time (ELT) method.

Results: Highly significant gender difference was noted in mean fibrinolytic activity (p=0.002). Mean fibrinolytic activity also showed a significant relationship with BMI (p=0.001) and with different phases of menstrual cycle in females (p=0.004). However, such relationship was not observed with diet and physical activity (p>0.05) in boys and girls.

Conclusion: Gender difference, body mass index and menstrual cycle phases have influences on the fibrinolytic activity of blood. This might be due to cyclical changes in the sex hormones levels, endometrium-derived plasmin and plasminogen activators and excess production of plasminogen activator inhibitor type-1 (PAI-I) in visceral adipocytes.

Keywords: Fibrinolysis time, Coronary artery disease, Hypercoagulable states, Menstrual cycle, Stroke

INTRODUCTION

Ischemic heart disease (IHD) and stroke are emerging as major causes of mortality and morbidity worldwide [1].Its incidence is on the rise in India, particularly in younger population because of the lifestyle and dietary changes preceded by rapid economic growth and urbanization [2].

The disease is multi-factorial in origin and may be central to decreased fibrinolytic activity of blood with a long fibrinolysis time [3]. This is found to be well correlated with proneness towards stroke, IHD, thrombo-embolic diseases and is an independent predictor of both fatal and nonfatal IHD [4-6]. Thus assessment of fibrinolysis time is one of the most important markers of cardiovascular health [7].

Fibrinolysis time is the time required for lysis of clot in circulation [4]. The process is completed within 2-4 hours with a reference range of 90 minutes to 6 hours [8]. Longer fibrinolysis time indicates poor fibrinolytic activity and propensity for thrombus formation in the circulation [4]. A delicate balance is required between procoagulants and fibrinolytic system for normal haemostasis [4].

Evidences from the literature have shown that fibrinolysis time is increased by risk factors like obesity, diabetes, increased total lipids, dietary habits and lifestyle that eventually increase the proneness of an individual to thrombotic tendencies significantly [8]. However, vegetarian diet and regular physical activity have shown favorable outcome on it [9,10]. Menstrual cycle phases have also been reported to affect fibrinolytic activity in women during their reproductive years [11-13].

In the present study we tried to find out the impact of gender, dietary habits, body mass index, physical activity level and menstrual cycle on fibrinolytic activity of blood in a small segment of Indian population. This may be helpful in early detection of proneness for thromboembolic episodes in Indian population in which incidence of IHD and stroke is on the rise.

MATERIALS AND METHODS

After getting clearance from the institutional ethical committee a cross-sectional observational study was carried out on 206 randomly selected healthy medical students aged 17-23 years of Sri Aurobindo Medical College and Post Graduate Institute, Indore, India. Written informed consent was taken from the participants. Fasting venous blood sample was collected in the morning hours (7-9 A.M.) as per the standard protocol [14]. Anthropometric measurements were recorded and body mass index (BMI) was calculated by the formula Weight (kilogram)/Height (meter^2).

A pre-validated proforma was given to the participants to get information about dietary habits, physical activity level and menstrual history. The participants were divided into Lacto-vegetarians (their diet comprises of foodstuffs from plant sources and dairy products) and Semi or Partial-vegetarians (their diet comprises of foods from plant sources, fish, chicken, eggs and dairy products) on the basis of dietary history [15]. Physical activity level was also determined and subjects were classified into inactive, moderately inactive, moderately active and active categories [16].

Detailed menstrual history was obtained in female subjects and accordingly their fibrinolysis time was determined for three consecutive menstrual cycles.

Euglobulinlysis time (ELT) method [8] was used to determine fibrinolytic activity of blood. In this method plasma was separated from the venous blood, acetic acid was added and then incubated on ice for 15 minutes. A Euglonbulin precipitate is formed which contains plasminogen, plasminogen activators and fibrinogen. This precipitate was separated by centrifugation and is dissolved in borate buffer. Within few minutes after addition of thrombin reagent a clot was formed. Dissolution of this clot was observed at every 15 minutes interval to give Euglobulin Clot lysis time .The reference range of fibrinolysis time in this method was from 90 minutes to 6 hours [8].

STATISTICAL ANALYSIS

Statistical analysis was done and Mean and standard deviation were calculated. Comparison between the groups was done by student's t-test and analysis of variance test (ANOVA). P-value of 0.05 or less was regarded as statistically significant. All Statistical Analysis was performed on Graphpad-5 statistical software.

RESULTS

Basic characteristics of the study group are presented in [Table/Fig-1]. The mean fibrinolysis time is significantly (p=0.002) higher in girls as compared to boys [Table/Fig-2]. Whereas, no significant difference was observed when mean fibrinolysis time was compared between lacto-vegetarians and semi-vegetarians.

The prevalence of obese, normal weight and underweight subjects in the study group was 2%, 53% and 44% respectively [Table/Fig-3]. The mean fibrinolysis time was found to be significantly (p=0.001) higher in high BMI group and significantly (p=0.001) lower in low BMI group as compared to normal [Table/Fig-3].

Although statistically not significant, physically inactive participants had the highest mean fibrinolysis time whereas it was lowest in physically active participants [Table/Fig-3].

The present study found a statistically significant (p=0.004) increase in mean fibrinolysis time during luteal phase and significant (p=0.004) decrease in mean fibrinolysis time during menstrual phase as compared to follicular phase [Table/Fig-3].

Parameters	Boys (n= 135) Mean ± SD	Girls (n= 71) Mean ± SD
Age (year)	19.5 ± 1.88	18.7 ± 1.21
Weight (Kilogram)	54.09 ± 6.99	45.57 ± 6.27
Height (Inches)	65.72 ± 5.73	61.16 ± 3.10
BMI (Kilogram/meter^2)	19.12 ± 2.08	18.90 ± 2.41

[Table/Fig-1]: Basic characteristics of study group

Variables		Number of Cases (n)	Fibrinolysis Time in Minutes (Mean ± SD)	t-Value	p-Value
Gender	Boys	135	291.06 ± 99.62	3.051	0.002
	Girls	71	345.66 ± 156.23		
Dietary Habit	Lacto-Vegetarian	104	314.07 ± 126.20.	0.487	0.626
	Semi -Vegetarian	102	305.60 ± 123.20		

[Table/Fig-2]: Fibrinolysis time with respect to Gender and Dietary habits

Variables		Number of Cases (n)	Fibrinolysis time in minutes (Mean ± SD)	f-Value	p-Value
Body Mass Index (BMI)	Normal BMI	110	328.95 ± 55.34	6.859 (Df=2)	0.001
	Low BMI	91	288.03 ± 99.22		
	High BMI	05	340.20 ± 139.68		
Physical Activity Level	Inactive	03	340.53 ± 69.44	0.535 (Df=3)	0.658
	Moderately Inactive	90	332. 72 ± 82.31		
	Moderately Active	94	321.52 ± 132.76		
	Active	19	298.20 ± 112.20		
Menstrual Cycle Phase	Menstrual	13	259 ± 93.49	3.267 (Df=2)	0.004
	Follicular	26	348.48 ± 167.06		
	Luteal	32	384 ± 150.33		

[Table/Fig-3]: Fibrinolysis time with respect to BMI, Physical Activity level and Menstrual cycle phases

DISCUSSION

In the present study fibrinolytic activity was determined and influence of risk factors was studied. It was observed that the girls had long mean fibrinolysis time than boys and this difference was statistically significant [Table/Fig-2]. This difference between two sexes might be due to the differences in their physical activity level, BMI and hormonal changes occurring in girls during their menstrual cycle phases [17].

Obesity, sedentary life style and a preference for non-vegetarian diets results in longer fibrinolysis time and increased proneness to thrombotic tendencies. Conversely normal weight, vegetarian diets and regular physical exercise have favorable outcome on fibrinolytic activity [10,11]. In the present study an inverse and statistically significant (p=0.001) relationship was found between BMI and fibrinolytic activity [Table/Fig-3]. This may be attributed to the excessive synthesis of plasminogen activator inhibitor type-1 (PAI-1) from visceral adipocytes, which is a strong inhibitor of tissue plasminogen activator. Similar results were reported in the studies conducted in the past [18-21].

Various observational studies in the past have found that vegetarian diets increases the fibrinolytic activity and decreases the thrombogenic potential of blood by decreasing PAI-1, factor VII, and blood viscosity [4,10].On data analysis of present study we did not find any significant difference in fibrinolytic activity between lacto-vegetarian and semi-vegetarian groups [Table/Fig-2]. This might be due to lack of representative population of different dietary classes in our study sample.

A positive correlation between physical activity level of an individual and fibrinolysis time was reported in the literature [22,23]. The beneficial effect of regular physical activity is due to improvements in endothelial functions, endothelial progenitor cell activation and mobilization along with activity dependent release of muscle derived cytokines or leptins [24]. In the present study, we observed that fibrinolytic activity was higher in physically active subjects as compared to physically inactive subjects, but the difference was not statistically significant [Table/Fig-3]. It might be due to less precise reporting of physical activity by the participants since it was based on self-reported questionnaire based on recall of information [24].

Studies have shown that women during their reproductive years have better fibrinolytic activity particularly during menstrual phase of menstrual cycle. Such females were reported to be relatively free from risk of developing thrombo-embolic episodes and IHD [11-13].In the present study fibrinolysis time was determined in 71 healthy females for three consecutive menstrual cycles. A significant difference was noted in fibrinolytic activity during different phases of menstrual cycle [Table/Fig-3]. Mean fibrinolysis time was lowest in menstrual phase and highest in luteal phase, and the difference was highly significant [Table/Fig-3]. Decrease in the fibrinolytic activity as evident from high value of fibrinolysis time was noted in luteal phase. This observation reflects that menstrual cycle specific hormonal changes along with changes in the concentration of endothelium derived plasmin and plasminogen activator may be responsible for this [25,26].These findings also suggest that females are more prone to develop thrombo-embolic episodes during their luteal phase because of lowest fibrinolytic activity observed during this phase of menstrual cycle.

LIMITATIONS OF THE STUDY

Small sample size devoid of representative population from all the dietary classes does not allow us to draw inferences with respect to their diet. Due to economic constraints we were unable to do hormonal estimation of female subjects for delineating different menstrual cycle phases instead of menstrual history. Similarly physical activity level was also decided on the self-reported questionnaire instead of direct measurement of physical activity. Further research involving large population may provide a greater insight into the cause effect relationship involved in hypercoagulable states of blood.

CONCLUSION

We conclude that fibrinolytic activity gets affected with many known risk factors implicated in hypercoagulable states of blood. Gender difference, BMI and menstrual cycle phases influences it significantly.

REFRENCES

[1] American Heart Association. Heart and stroke statistical update. Dallas: *American Heart Association*, 2000:1–10.
[2] Reddy KS. Cardiovascular disease in non-western countries. *N Engl J Med.* 2004;350:2438–40.
[3] Collen D, Lijnen HR. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood.*1991;78:3114–24.
[4] Gorog DA. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. *J Am Coll Cardiol.* 2010;55:2701–09.
[5] Korninger C, Lechner K, Niessner H, Gossinger KI, Kundi M. Impaired fibrinolytic capacity predisposes for recurrence of venous thrombosis, *Thromb Haemost.* 1984;52:127-30.
[6] Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet.* 1993;342:1076–79.
[7] Lisman T, Leebeek FW, Mosnier LO, Bouma BN, Meijers JC, Janssen HL, et al.Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. *Gastroenterology.* 2001;121:131–39.
[8] de Lange Z, Pieters M, Jerling JC, Kruger A, Rijken DC. Plasma clot lysis time and its association with cardiovascular risk factors in black africans. *PLoS ONE.* 2012;7(11): e48881.
[9] Meltzer ME, Doggen CJM, De Groot PG, Rosendaal FR, Lisman T. Reduced plasma fibrinolytic capacity as a potential risk factor for a first myocardial infarction in young men. *Br J Haematol.* 2009;145:121–27.
[10] Famodu AA, Osilesi O, Makinde YO. The influence of a vegetarian diet on haemostatic risk factors for cardiovascular disease in Africans. *Thromb Res.*1999;95:31-36.
[11] Feuring M, Christ M, Roell A. Alterations in platelet function during the ovarian cycle. *Blood Coagul Fibrinolysis.* 2002;13:443–47.
[12] Siegbahn A, Odlind V, Hedner U, Venge P. Coagulation and fibrinolysis during the normal menstrual cycle. *Upsala J Med Sci*. 1989;94(2):137-52.
[13] Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, et al. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab.* 2001;86(11):5389-95.
[14] Ho CH, Chwang LC. Influence of vegetarianism on fibrinolytic activity. *Proc Natl Sci Counc Repub China B.* 1993;17(1):35-39.
[15] Tonstad S, Butler T, Yan R, Fraser GE .Type of vegetarian diet , body weight, and prevalence of type 2 diabetes. *Diabetes care.* 2009;32(5):791–96.
[16] Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the european prospective investigation into cancer and nutrition (EPIC) study. *Public Health Nutr.* 2003;6:407–13.
[17] Meltzer I, Lisman T, Doggen CJ, De Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. *PLoS Med.* 2008;5:751–59.
[18] Anfossi G, Russo I, Trovati M. Platelet dysfunction in central obesity. *Nutr Metab Cardiovasc Dis.* 2009;19:440–49.
[19] Mertensl. Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. *Obesity Rev.* 2002;3(2):85–101.
[20] Lijnen HR. Role of fibrinolysis in obesity and thrombosis. *Thromb Res.* 2009;123(Suppl 4):S46–S49.
[21] Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA.*1999;282:2131-35.
[22] Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J AmColl Cardiol.* 2005;45:1563-69.
[23] Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation.* 2004;109:2181-85.
[24] Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol.* 1994;23:991-99.
[25] Gleeson NC. Cyclic changes in endometrial tissue plasminogen activator and plasminogen activator inhibitor type 1 in women with normal menstruation and essential menorrhagia. *Am J Obstet Gynecol.* 1994;171:178–83.
[26] Toth B, Nikolajek K, Rank A, Nieuwland R, Lohse P, Pihusch V, et al. Gender-specific and menstrual cycle dependent differences in circulating microparticles. *Platelets.* 2007;18(7):515-21.

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