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Original Research Paper

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STUDY OF FIBRINOLYTIC ACTIVITY AND CLOTTING TIME IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN

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ABSTRACT The incidence of cardiovascular diseases differs between men and women ,in part because of differences in risk factors and hormones . This sexual dimorphism means a lower incidence in atherosclerotic disease in premenopausal women which subsequently rise postmenopausal women to eventually equal that of men. Although extensive evidence indicates that estrogen is responsible for markedly decreased cardiovascular risk in premenopausal women, the mechanism through which it exerts its protective effect is not adequately explained. Thrombosis is recognized to play important role in the onset cardiovascular disease.

The present study was undertaken to compare fibrinolyic activity and clotting time in premenopausal and postmenopausal women.

The present study was conducted on 50 premenopausal (30+5 years) and 50 postmenopausal women (50+5years). The fibrinolytic activity was measured by euglobulin clot lysis time and clotting time was measured by capillary glass tube method. Both the parameters were compared by unpaired "t" test and the results showed significant decrease in the fibrinolytic activity in postmenopausal women ($p<0.001$) as compared to premenopausal women.

However , there is no statistically signifivant change in the clotting time ,between the subjects of tow groups($p>0.05$).

Thus , it can be suggested that decreased fibrinolytic activity in postmenopausal women could be the reason for increase in the incidence of cardiovascular disease.

KEYWORDS : PRE MENOPAUSAL,POST MENOPAUSAL,CLOTTING TIME,FIBRINOLYTIC ACTIVITY,OESTROGEN

INTRODUCTION

Chronic non- communicable diseases are assuming increasing importance among adult population in both developed and developing countries. The prevalence of chronic diseases diseases is showing an upward trend in most countries and for several reasons this trend is likely to increase. Among the chronic diseases, cardiovascular diseases are the leading cause of death in developed countries. Developing countries are also showing increased incidence and require taking appropriate steps to avoid epidemics of this disease. The disease is labeled as "Modern Epidemic" disease[1].

Ischemic heart disease is fast acquiring the globe and thus becoming the globe and thus becoming pandemic on nature. Though the incidence of ischemic heart diseases is increasing throughout the world, the incidence differs between men and women. This difference is seen partly because of differences in risk factors and partly due to hormones. This sexual dimorphism means a lower incidence in atherosclerotic diseases in premenopausal women which subsequently rise in postmenopausal women to eventually equal that of men. This is attributed mainly because of action action of oestrogen. There are extensive evidences which prove that oestrogen is responsible for markedly decreased incidence of cardiovascular disease in premenopausal women. The mechanism through which oestrogen exerts its protective role is not explained adequately[3].

Until recently the atheroprotective effects of estrogen were attributed, principally to its effect on lipid profile. However, these alterations account for one third of the observed clinical benefits of oestrogen[4] . In addition, oestrogen also improves carbohydrate metabolism and vascular reactivity. Though the benefits of oestrogen are plenty, they do not fully account for marked decrease in incidence of ischemic heart disease[3].

Recent clinical evidences suggest that thrombosis plays an important role in the onset of cardiovascular disease. Thrombosis, though is a potential risk factor for cardiovascular disease, it is also important for the daily wear and tear of the smaller blood vessels due to local injury. Though it is protective in nature and essential for life, it should be controlled and checked. This done by fibrinolytic system, which continuously removes fibrin deposits along the blood vessels. Thus, there exists a balance between two systems which helps to maintain the blood in blood in fluid state. If this balance is disturbed due to any reason, it leads to formation of atheromatous plaques,which may predispose to cardiovascular events. It is possible that oestrogen may exert its protective role by increasing fibrinolytic activity.

The present dissertation deals with the comparison of fibrinolytic activity and clotting time in premenopausal and postmenopausal women, to assess the relationship between oestrogen status and fibrinolytic potential, a determinant of thrombotic risk.

MATERIALS AND METHODS

Blood was collected from 100 students, of these 50 were premenopausal women of age group (30 + 5 years) and 50 were premenopausal women of age group (50 + 5 years). In case of premenopausal women, those with regular cycles were selected. The blood was collected between 9:00 a.m. – 10:00 a.m. to avoid diurnal variation, if any, The students were instructed to avoid physical exertion and fatty breakfast prior to blood sampling. While selecting the subjects the exclusion criteria were-

- Pregnancy
- Any menstrual disorders like dysmenorrheal, menorrhagia etc
- Any cardiac disorders like ischemic heart disease, congestive cardiac failure etc.
- Any gynecological disorders like polycystic ovarian disease, fibroids etc.
- History of drugs like steroids, hormonal preparations e.g. oral contraceptive pills, hormone replacement therapy etc.

- Any endocrinal, malignant, systemic disorders like thyroid disorders, liver disorders etc.

- The exclusion was done with the help of detailed history. Informed written consent was obtained from all subjects. The fibrinolytic activity of plasma was estimated by measuring Euglobulin clot lysis time and clotting time was measured by capillary glass tubc method (Dale's method).

- The following equipment and reagents were required

Reagents: 3.8% sodium citrate
0.01% acetic acid
Borate solution (buffer)0.025M calcium chloride

Equipments: Sterile dry syringes and needles
Clean dry 15 ml centrifuge tubes
Graduated pipettes (0.5ml ,1 ml, 10ml)
Filtcr paper
Centrifuge machine
Fine glass rod
Water bath & thermometer
Capillary glass tubes and stopwatch (for clotting time)

Euglobulin Clot Lysis Time5,6
Euglobulin fraction is believed to measure the plasminogen activator activity of blood sample, unimpeded by the presence of plasmin inhibitors. It contains nearly all plasminogen activators, all plasminogen and about 20% of fibrinogen of parent plasma, antiplasmin remaining in the supernatant.

Euglobulin fraction is prepared by acidification of plasma with acetic acid.

Method: - 4 cc of venous blood sample is mixed with 1 cc of sodium citrate solution and the tube is kept in ice bath. The sample is centrifuged in angle centrifuge at 3000 r. p. m for 10 minutes to obtain plasma.

1ml of plasma is mixed with 9ml of acetic acid and kept in ice for I minutes. Later the resulting precipitate is separated by centrifugation at 300 r. p. m for 15 minutes. Discard the supernatant and the inner surface of in tube is dried with filter paper. Add 0.5 mi of borate solution to dissolve the precipitate. The resultant solution is clotted by adding 0.5 ml of 0.025 Mc calcium chloride to 0.3 ml of the dissolved Euglobulin fraction. The time to outer formation is recorded and the tubes are placed in water bath at 37 degree calcium. The tube were inspected at intervals and the lysis time determine when the clot had completely lysed. When the completion of lysis seemed mearer, the tubes were inspected every 5 minutes.
The Euglobulin lysis time was recorded and tabulated.

Clotting Time (Capillary Glass Tube Method)6
1 cc of venous blood was collected in dry bulb and made to ascend into a capillary tube by capillary action. The stopwatch was started immediately after appearance of blood in the syinge. A small picce of capillary tupe was broken every 15 seconds carefully to see if fibrin threads were formed betwccn the two ends. The time interval between the appearance of blood in syringe and formation of fibrin threads in the capillary tube was taken as clotting time.

Observation And Results
In the present study , the fibrinolytic activity and clotting time in premenopausal and postmenopausal women are determined and the results obtained are compared with each other. The subjects are divided into tow groups, premenopausal and postmenopausal. In the premenopausal women, the age group of the subjects is 30+5 years and in postmenopausal women , it is 50+5 years. For both the parameters the mean value, standard deviation and difference in means are calculated. Students unpaired 't' lest is used to find out whether the difference in means for each parameter is significant or not. A 'p' value of less then of less than 0.05 is considered to significant.

The observations and results of the present study are as follows:
1. Among the 100 subjects selected for the study , 50 belong to the premenopausal group and 50 to the postmenopausal group.
2. The 50 subjects of the premenopausal group 30+5 years.
3. The 50 subjects of the postmenopausal group are 50+5 years.
4. There is significant decrease in the fibrinolytic activity of postmenopausal as compared to premenopausal women ($p<0.001$). In premenopausal women clot lysis time is 121.8+31.79 minutes, while in postmenopausal women it is 189.1+22.68 minutes.

There is no significant change in the clotting time between the subjects of the two groups ($p>0.05$). In premenopausal women, women it is 210.9+32.28 seconds.

Comparison Of Fibrinolytic Activity And Clotting Time In Premenopausal And Postmenopausal Women

Variables	Group	Unpaired T test					
	Premenopausal	Postmenopausal					
Fibrinolytic activity in minutes	MEAN	SD	MEAN	SD	T - valve	P-valve	Deffe rence
	121.8	31.79	189.1	22.68	-14.10	0.007 9	Signif icant
Clotting time in seconds	208.8	29.32	210.9	32.28	0.395 8	0.693 1	Not signif icant

DISCUSSION
Fibrin deposition both, intravascular and extravascular frequently occurs in health and disease and disease. The resolution of such deposits is achieved in vessels through a basic repair mechanism involving the enzymatic dissolution of insoluble fibrin polymers, a phenomenon referred to as "fibrinolysis". This phenomenon is controlled and regulated for the most part by the activity of normally circulating plasma proteolytic system termed as plasminogen-plasmin system. It is also called as fibrinolytic system, which comprises of plasminogcn, plasmin, plasminogen activators and inhibitors. Plasmin, a proteolytic enzyme is formed from inactive Plasminogen. This conversion is brought about by plasminogen activator. It is important that fibrinolysis should be closely controlled and checked as is coagulation, which is broight about by plasminogen activator inhibitors and the mechanism of activation of plasminogen.
There are main theories for fibrinolysis in vivo.

The Sherry Hypothesis[64]:
Sherry and his group proposed that as fibrin is formed, plasminogen is preferentially adsorbed preferentially to the fibrin and is available in quantity within a thrombus where, when activated, it is comparatively free from antiplasmins. Thus when plasminogen activator enters the circulation, diffuses into a clot the plasminogen in the clot is converted to plasmin, And lysis follows. This is referred to as gel Phase of plasminogen. Out side the clot, plasminogen free in the circulation (The sol phase) meetsplasminogen activator and is convertedto plasmin, but is immediately inactivated by the antiplasmins in the circulation.

The Ambrus And Markus Hypothesis[65]:
Ambrus and her associates postulated the concept of a

reversible Plasmin- antiplasmin complex which dissociated in the presence of fibrin,because fibrin competed for plas with antiplasmin. In this way plasmin became specifically localized to fibrin after the dissociation of the plasmin-antiplasmen complex and achieved substrate specificity.

The Oxford Hypothesis [66]:

Allington , Chesterman and Sharp have proposed that plasminogen activator in the circulation binds or adsorbs to the fibrin of a thrombus, and that circulating plasminogen is converted to plasmin within the thrombus, and which brings about fibrinolysis. In contrast to the Sherry hypothesis they proposed that activator is fixed in thrombus and plasminogen circulates through the thrombus. The requirements for successful thrombolysis according to this hypothesis have recently been rested by Sharp (1975) and depend on.

1. The presence of activator which binds to from fibrin;
2. The persistence of plasminogen in the blood perfusing the thrombus;
3. The unrestricted action of plasmin generated on the fibrin.

Fibrinogen activity depends upon plasmingen activator levels. The plasminogen activator is mainly present in the walls of blood vessels, where it is synthesized by the endothelial cells. Thus, increased fibrinolytic activity Is seen in highly vascular, such as meanings, myometrium of uterus, choroid of eye , arterial and vinous adventitia [67], prostate ,thyroid , heart, ovaries adrenals, lymph nodes[68]. Activators are also present in blood cells, i. e, erythrocytes, leucocytes and platelets [68].

The release of plasminogen activators is brought about , not only by fibrin deposition, but also by other factors. These include emotion or stress of any kind ,exercise, electric shock, epinephrine and vasoreactive stimuli such as vasopressin and histamine [69]. However, dietary ingredients especially fat inhibit fibrinolysis.

Fibrinolytic activity shows diurnal variation[68]. Thus time of blood collection is important . The present study involved collection of blood between9.00am to 10.00am in all subjects. Exercise and fatty diet also affect fibrinolytic activity. This fact should be taken into consideration . Thus, the subjects were instructed to avoid physical and fatty breakfast prior to blood collection to avid variations.

Various studies show difference in fibrinolytic activity between males and females and the difference could be because of the influence of hormones [68]. Different studies carried out by Cash[30], McNicol and Doglas32, showed that fibrinolytic activity was greater in females as compared to males, thus suggesting that estrogen possibly increases fibrinolyitc activity .

In the present study, fibrinolyic activity and clotting time of premenopausal and postmenopausal women are compared. There is significant decrease in fibrinolytic activity of postmenopausal women as compared to premenopausal women ($p<0.001$). In premenopausal women mean clot lysis time is 121.8+31.79 minutes, while in postmenopausal women it is 189.1+22.68 minutes.

The finding of present study is consistent with that of the previous workers like Gebara O. C.E.et al[3], Shahar E. et al[55], Kwang K.K.[56],whoalso found decrease in fibrinolytic activity in postmenopausal women. Thisdecrease in fibrinolytic activity was because of increased levels of plasminogen activator inhibitor-1 and decreased tissue tissue plasminogen activator activity.

Though the study carried out by K.K. Bengsten[33] showed no difference in fibrinolytic activity between premenopausal and postmenopausal women , the present assessment clearly shows decreased fibrinolytic activity in postmenopausal women.

Morriz Notelovitz[36], Mier Stampfer[39] and many others [49,50,51,72,73] in there studies showed that estrogen replacement therapy in postmenopausal women caused decrease in levels of plasminogen activator-1 and increase in the activity of tissue plasminogen activator , thus resulting in increased fibrinolytic activity .

The study carred out by Robinsen R. et al[26,27] on women with bilateral oopherectomy before natural menopause , showed decreased fibrinolytic Activity as compared to women with natural menopause , which was reversed after estrogen replacement therapy .

Above studies suggest that estrogen increases fibrinolytic activity .This is confirmed by an experimental study carried study carried out by Sobel et al[57] onaortic endothelial cell culture , in which the tissue plasminogen activator level increased and plasminogen activator inhibitor level decreased by estrogen.

According to theory proposed by Rokitansky[8], athcrosclerosis is caused by deposition of fibrin on vascular endothelium due to decreased fibrinolytic activity. Several workers like, Duguid[10],Morgen[11] and french[12] supported his theory.

Hamsten A [41], Chakrabarti [29], prins[70], Folsom[62], and Meade [71],in their studies found that impaired fibrinolysis is associated with cardiovascular disease. They found increased levels of plasminogen activator inhibitor-1.

The study carried out by Cushman M.[74],Suggested that increased levels of plasminogen activator inhibitor–1, plasmin – antiplasmin complex, fibrin D- dimer are independent risk factors for cardiovascular disease .Morris Noteloviz[37] Mendelsohn[4] and Rosenberg[35] suggested that there is increased incident of cardiovascular disease in postmenopausal women.

So it suggcsts that decreased fibrinolytic activity after menopause could be responsible for an increase in the incidence of cardiovascular diseases.

Madina et al[2] in their study found that endogenous estrogen caused decrease in the activity of plasminogen activator – 1 and fivrinogen levels , with increase in anticoagulant activity . Estrogen has a protective role against cardiovascular disease, but the exact mechanism is not known. It was proposed that estrogen might exert its beneficial effects on cardiovascular risk factors by modifying lipoproteins levels, carbohydrate metabolism, and vascular reactivity .

In present study , clotting time is also compared between premenopausal and postmenopausal women. Findings show no significant difference in clotting time between the two groups ($p>0.05$). The mean clotting time in premenopausal women in 208.+29.32 seconds, while in postmenopausal women it is 210.9+32.28 seconds.

Above findings are consistent with the studies carried out by , Petersen K.R. et al[52], Morris Notelovitz[36],who found no significant change in coagulation profile in premenopausal between postmenopausal women.

The study carrid out by ,Giardina et al[75],on young women showed no change in coagulation activity through out the menstrual cycle, thus, suggesting that endogenous estrogen and progesterone have no effect on coagulation system.
MaryFran et al[63]concluded that endogenous estrogen may

reduce cardiovascular risk via modulation of fibrinolytic factors, but not coagulation factors.

Whereas, study carried by pierre-Yves Scarabin et al[58] ,Found that after oral estrogen and progesterone therapy , there was increase in coagulation factors. But in present study there is no significant change seen in clotting time.

From about studies it can be concluded that estrogen probably caused increase in fibrinolytic activity by decreasing levels of plasminogen activator activity. The exact mechanism for this effect of estrogen is not known.

In present study there is decrease in the fibrinoltic activity in postmenopausal women. Suggesting that decreased levels of estrogen in postmenopausal women., may lead to increase in levels of plasminogen activator inhibitor-1 and decrease in tissue plasminogen activator activity.

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