Original Article

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Pathology Section

Elevated Factor VIII Levels and Shortened APTT in Recurrent Abortions

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ABSTRACT

Introduction: Thrombotic disorders have been found to be associated with recurrent abortions. Several risk factors have been identified. APTT reflects the common pathway and intrinsic pathway of coagulation cascade and hence is a good marker for thrombotic work. Elevated factor VIII: C has also been identified as risk factor for recurrent miscarriage. This study aims at identifying association of elevated factor VIII levels, shortened APTT and recurrent abortions in Indian population as little has been studied about this and the literature available is also based on studies done in European population. This study also aims to find whether shortened APTT can be an independent risk as well.

Materials and Methods: Women referred to the obstetrics department with a history of early recurrent early pregnancy loss (at least three pregnancy losses before 13 weeks of gestation) were included in this study. Exclusion criteria were elevated CRP levels, positive antiphospholipid antibodies, endocrine, immunological or anatomical cause of embryo demise. A total of 68 cases of recurrent abortion were included in this study, 68 normal pregnant females (<15 weeks of gestation) were also included as controls with no history of abortion. The age group of the cases as well as control was 20-45 years. Activated partial thromboplastin time and factor VIII assay (one stage APTT based) were done on the blood samples.

Results: Increased factor VIII levels were seen in 25 cases (36.4%); 19 cases showed shortened APTT (27.3%); 12 cases showed both increased factor VIII levels as well as shortened APTT (18%). All risk factors were negative in 36 cases (52.9%). None of the controls showed elevated factor VIII levels or shortened APTT. The mean APTT values of the control subjects was 31.01 and cases were 27.01 (p=0.001). The mean factor VIII levels of case were 152.85% and control 144.953% (p=0.012).

Conclusion: There was significant association between recurrent abortions and elevated factor VIII :c levels and shortened APTT. Shortened APTT was also identified as an independent risk factor.

Keywords: Thrombosis, Coagulation studies, One stage factor viii assay, Pregnancy

INTRODUCTION

Recurrent abortions are traumatic to an expectant mother, both physically and psychologically. Several causes have been identified for recurrent pregnancy loss, inherited and acquired haemostatic abnormalities being important risk factors for it.

The coagulation abnormalities have been traditionally investigated by two tests for decades – the prothrombin time (PT) and activated partial thromboplastin time (APTT). Although both tests reflects the common pathway, PT reflects coagulation factors of the extrinsic pathway (i.e., tissue factor and factor VII), whereas the APTT reflects abnormalities of the intrinsic pathway (i.e., factors VIII, IX, IX, and XII) [1]. Prolongation of APTT is due to deficiency of factors of intrinsic pathway or common pathway or any inhibitors [1]. Therefore a shortened APTT may be due to excess of the coagulation factors. The finding of short APTTs in pregnant women can also in part be explained by increase in FVIII [2-4]. Several groups have also identified elevated levels of factor VIII as an independent risk [2-4].

This study is aimed at identifying association of elevated factor VIII: C and shortened APTT in recurrent miscarriages. Previous studies have been done mostly in European population. So, this study also aims in identifying the risk factors in Indian population.

MATERIALS AND METHODS

Women referred to the obstetrics department with history of early recurrent abortion, atleast three pregnancy losses before 13 weeks of gestation were eligible to be included in this study. Endocrine immunological or anatomical cause of embryo demise, elevated CRP levels or positive antiphospholipid antibodies was excluded from this study. Blood samples were collected from the patients after 6 weeks of abortion, during their review visit to obstetrics department. A 2.7 ml of blood was collected in tubes containing 0.3 ml of 3.2% trisodium citrate (ratio 9:1). The samples were centrifuged at 1500g for 15 minutes to prepare platelet poor plasma. The platelet count of platelet depleted plasma was $<5x10^9$/L. The following tests were done:

1. **Activated Partial Thromboplastin Time:** Lupus sensitive APTT reagent which is commercially available (tulip diagnostics) as a freeze dried preparation containing cephalin and a particulate activator (silica) in a buffered medium is used. One part of the plasma is mixed with one part of activator and phospholipids, incubated at 37°c and one part of calcium cholride is added and the time to clot is noted. A control sample was also run at the same time using blood from normal subject. The normal APTT by this method is 28-35 seconds. A greater than 5 second's difference from the control is considered abnormal. A shorter than normal APTT indicates an activated sample or a hyper coagulable state.

   Correction studies: When the APTT is prolonged, correction studies were done to differentiate whether the prolongation is due to an inhibitor or a factor deficiency. This is done by mixing equal volumes of the test plasma and control pool plasma and repeating the test. When inhibitors are present the prolonged timing will not correct.

2. **Factor VIII Assay (One Stage Aptt Based):** The principle of this assay is based on the ability of reference plasma and test plasma to correct the prolonged APTT of plasma deficient in the factor VIII that is being assayed. For the assay, reference

plasma with known content of all the factors and factor VIII deficient plasma with factor level less than 1% activity must be available. One part of test plasma is mixed with one part of factor VIII deficient plasma (Stago) and one part of APTT reagent (CK PREST, STAGO), incubated at 37ºc, one part of calcium chloride is added and the time taken to clot is noted. Abnormal system control plasma with a factor VIII level of 32-46% and normal system controls with factor VIII level of 87-121 % are subjected to the test simultaneously with patient's sample. Normal levels of factor VIII are 50-150%.

RESULTS

A total of 68 cases of recurrent abortion were included in this study; 68 normal pregnant females (<15 weeks of gestation) were also included as controls with no history of abortion. Increased factor VIII levels were seen in 25 cases (36.4%). A 19 cases showed shortened APTT(27.3%); 12 cases showed both increased factor VIII levels as well as shortened APTT (18%). All risk factors were negative in 36 cases (52.9%). The mean APTT value of the control subjects was 31.01 s and cases was 27.01s (p=0.001) [Table/Fig-1]. The mean factor VIII levels of cases were 152.85% and control 144.953% (p=0.012) [Table/Fig-2]. None of the controls showed elevated factor VIII levels or shortened APTT. Two controls showed prolonged APTT. There was no significant relation in factor VIII levels or APTT with the chronology of parity. The age group of the cases as well as control was 20-45 years [Table/Fig-3].

APTT	N	Mean	S.D	p-value
Case	68	27.01	4.92	<0.001
Control	68	31.16	2.12	

[Table/Fig-1]: Mean APTT values of control and cases.

Factor VIII	N	Mean	S.D	p-value
CASE	68	152.85	6.48	0.012
CONTROL	68	144.93	3.29	

[Table/Fig-2]: Mean factor VIII levels of control and cases.

Factor VIII	Cases (n=68)	Controls (n=68)
Age	32.8 (20-45)	31.9 (20-45)
Gestational age	12.3 (at abortion)	11.2

[Table/Fig-3]: Mean age and Mean gestational age of cases and controls.

DISCUSSION

The causes of recurrent abortion can be classified into genetic causes, anatomic causes, immunologic causes, infectious causes, endocrine causes etc. In most of the cases causes are idiopathic [5]. In the past few years attention has been drawn to various causes like antiphospholipid antibodies, hyperhomocystenaemia, factor V Leiden mutation etc, [4]. They are postulated to cause recurrent abortions by causing vascular thrombosis [4,6]. Elevated factor VIII levels has been identified as an important associated factor in venous thromboembolism [7,8].

The present study reveled a high association between elevated factor VIII: C levels and patients with recurrent abortions (36.4%). This finding suggests a possible association between the thrombophilic condition and early reproductive failure. This study is in concurrence with available literature [9,10].

Various hypercoagulable states can lead to vascular thrombosis during early phase of fetal implantation. It has been observed that factor VIII: C rise can be seen in normal pregnancy and rises significantly in advanced pregnancy [2,3]. In this study 68 normal pregnant women (<15 weeks of gestation) with no history of abortion were taken as control and none of them showed elevated factor VIII levels. The steep increase in factor VIII levels are usually seen after 16-18 weeks [4,11] and all the controls in our study were <15 weeks pregnant.

In this study, 19 cases showed shortened APTT (27.3%); 12 cases showed both increased factor VIII levels as well as shortened APTT (18 %). Activated partial thromboplastin time reflects the activity of intrinsic and common pathway. Out of the 25 cases that showed elevated factor VIII levels, 12 cases showed shortened APTT (48%) which can be explained by the elevated factor VIII levels. In rest of the cases with shortened APTT, elevation of other factors like factor V,IX,XI, XII could be the reason [12-14]. Other reasons for shortened APTT found in literature were improper collection of blood sample, myocardial infarction, thrombo embolic event, diabetes etc [9,13]. This study emphasizes the fact that cause of shortened APTT is more complex than increased factor VIII levels. There was a statistically significant difference between mean APTT values of patients with recurrent abortions as well as pregnant controls (p-value<0.001). This indicates that there is significant association between shortened APTT and recurrent abortions

A theory has been suggested recently that "pregnancy is a Th2-dominant state. Significant levels of the Th1 cytokines, interferon-g and interleukin-2, are found in the maternal-fetal junction of a spontaneously aborting conceptus. This means that a Th1-dominant state is present in pregnancies that end in miscarriage. Subclinical infections are speculated to induce a Th1-dominant state, and the Th1 cytokines might induce hypercoagulability [5]. This supports that APTT should be used a screening test in case of a recurrent pregnancy loss.

LIMITATIONS

Factor VIII levels can be increased in acute phase reactions. Therefore patients with elevated CRP levels were not included in the study. Many acquired variables may increase the factor VIII levels, so a follow up of the elevated factor VIII levels should have been ideally included in this study.

Similarly various clinical conditions like hyperthyroidism, diabetes, cancer etc can cause shortened APTT [12]. Even inappropriate collection of specimen could lead to shortened APTT [12]. So, it should have been systematically confirmed by subsequent sampling and follow up.

CONCLUSION

Association between recurrent miscarriages and thrombophilia still remains an open issue. In this study we came to the conclusion that elevated factor VIII:C levels is an important risk factor for recurrent miscarriages and shortened APTT can be an independent risk factor. Hence APTT should be advised to all patients with recurrent miscarriages, so that prophylaxis can be done in future pregnancies

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