The Factor VIII:C plasma activity level in females with primary unexplained infertility and recurrent miscarriage

Hind S. Al-Mammury[1] , Haithem A. Al-Rubaie[2], Bassam M. Hameed[1]

1- Department of Pathology- College of Medicine- Al-Nahrain University- Baghdad,IRAQ

2-Department of Pathology- College of Medicine- University of Baghdad- Baghdad,IRAQ.

Abstract

Background:

Recurrent miscarriage and infertility are intriguing problems with growing clinical concern.Among the causes of thrombophilia; elevated plasma VIII:C activity level emerges with a rising concern as a possible risk factor of repeated miscarriage and subsequent infertility.Factor VIII is activated in the intrinsic pathway of coagulation cascade of secondary hemostasis.

Objective:

To evaluate plasma VIII: C activity level in females with repeated miscarriage and primary unexplained infertility in order to determine if Factor VIII:C over-activity is a probable cause of such conditions.

Patients, Materials and Methods:

This study was performed on strictly selected90 females, aged 20-45 year. The females wereassignedinto three groups (34 females with unexplained primary infertility, 41 females with history of repeated miscarriage(after exclusion of other gynecological and obstetrical causes) and 15 healthy age-matched females were selected as a control group. Plasma VIII:C functional activity was assayed.Blood samples for general hematological studies (Packed cell volume PCV, Prothrombin time PT, and activated partial thromboplastin time APTT) were obtained manually for all included females. C-reactive protein assayed in all samples to exclude the inflammatory causes of elevated factor VIII, which is regarded as an acute phase protein.

Results:

Factor VIII data were expressed as a mean of percentage of activity (±SD). A significant (*p=0.009) higher plasma activity level of factor VIII:C was demonstrated in infertile females(111.29%±31.66) and females with recurrent abortions (118.63%±39.54); Compared with the healthy control females (80.23%± 22.23), PCV, PT and PTT measurements demonstrated no statistical significance in infertile and recurrent abortion groups when compared to the control group (p>0.05). There was no statistically significant difference between the three groups regarding the PCV, PT and PTT measurement.

Conclusions:

Plasma Factor VIII:C activity level was higher in females with unexplained primary infertility and those with history of recurrent abortion. This elevation suggested of being independent risk factor of such conditions; after exclusion of other multiple causes leading to thrombophilia, abortion and infertility.

Key words: factor VIII:C, miscarriage, infertility, thrombophilia.

Introduction:

Recurrent miscarriage (three or more spontaneous consecutive fetal losses before the 20th week of gestation)[1] is a serious reproductive problem impacting 1–2% of couples [2, 3]. Furthermore;female infertility defined as the inability to produce offspring that impacting 10-15% of couples. This could be primary (with no history of previous conception) or secondary (e.g. due to recurrent fetal loss)[4, 5].

Impairment of the placental perfusion may result in various gestational pathologies, including first- and second-trimester abortions; intrauterine growth retardation and fetal death[6,7].During pregnancy, critical changes in blood coagulation may predispose to abortion due to thromboembolism, since hemostatic disorders including thrombophilia may lead to obstruction of placental vessels, thus impeding the placental perfusion.

The etiology of recurrent miscarriages could be endocrine, genetic, immunological and anatomical abnormalities, however nearly half of the cases are idiopathic[4, 8].

Lately, attention has been diverted toward the possible association of both early and late pregnancy loss with either acquired or inherited defects of coagulation pathway, specifically thrombophilic defects predisposing to the development of deep venous thrombosis, namely Factor V Leiden, prothrombin gene mutations, hyperhomocysteinemia and antiphospholipid antibodies[9].

Sporadic studies suggested that elevated levels of plasma FVIII:C may represent an independent risk factor for thromboembolism[10, 11].Factor VIII: is a coagulation factor in the intrinsic pathway, synthesized within the liver, but requires Von Willebrand factor (vWF)(that synthesized in megakaryocytes and endothelial cells) for normal stability in the plasma. Factor VIII facilitates the activation of factor X in coagulation cascade[12].

A gradual dose–response relationship between factor VIII: C levels and risk of venous thrombosis was demonstrated, as well as an increased risk of recurrent venous thromboembolismin patients with elevated FVIII: C plasma levels[13]. This suspected to be one of the risk factor for thromboembolism[14], thus act as an etiological factors of recurrent abortions and infertility in females due to placental perfusion impedance caused by thromboembolism[15].

The aim of this study to evaluate plasma VIII:C activity level in females with repeated miscarriage and primary unexplained infertility in order to determine if Factor VIII:C over-activity is a probable cause of such conditions.

Patients, Materials and Methods:

Seventy-five females aged 20–45 years old were selected for this study, with strict criteria:History of recurrent abortion (at least three-months post abortion) and infertility, healthy, not diabetic, not hypertensive, not febrile, with no history of hormonal therapy and with no hormonal abnormalities.

The patients were divided into first group of patients selected were those with recurrent abortion this included 34 patients and a second group of patients were those with primary un explained infertility and never attains full gestation (para=0) this include 41 patients, all were counseling the gynecological Department in Al-Yarmouk Teaching Hospital in Baghdad. A third group of fifteen healthy age-matched female who are in child-bearing age, and not pregnant with no history of or current diseases, were included in this study as a control group. In both groups male partners were confirmed by the clinic as not being the cause of infertility.

Blood assays:

Packed cell volume (PCV): performed manually by the use of the micro-method, using non heparinized capillary tubes (75mm length and internal diameter of 1 mm).The samples were centrifuged at 12,000 g for 5 minutes.

Prothrombin time (PT): to assess extrinsic coagulation pathway. This assay utilized the following reagents: freeze-dried thromboplastin(STA®-Neoplastin® Cl plus 5) containing specific heparin inhibitor, low ISI. Cat. Nr. 00606.And aqueous solvent, containing calcium.PT of 10-14 seconds was set as a normal range.

Activated Partial Thromboplastin Time (APTT): to assess the intrinsic coagulation pathway. The reagents utilized were: STA® - PTT Automate and STA®-CaCl2 0.025M. APTT of 30-41 seconds set as a normal range.

Factor VIII:C Assay: Factor VIII:C activity level was measured through calculating the clotting time of the examined sample in relation to control plasma, in the presence of cephalin and activator of a system in which all the factors are present, constant and in excess except factor VIII which was deprived from the sample being tested. Reagents used were:STA® - Deficient factor VIII and STA® - PTT,Owren –Koller Buffer, pH 7.35, Frozen citrated PPP stored at -40C. The activity of Factor VIII:C was expressed as a percentage of normal control sample; 50-100% set as a normal functional activity of plasma Factor VIII:C

Statistical Analysis:

Within each group of studied subjects data are expressed as the mean± SD. Control group was tested for intra group-variability using one-way ANOVA. Computerized statistical analysis was performed using SPSS (statistical package of social sciences). ANOVA test, with the significance value set to 0.05, was used to determine if there was a statistical significance among the repeated abortion group, infertile group and age-matched control group.

Results:

1. Packed cell volume (PCV) L/L:

There was no statistically significant difference ($p>0.05$) in PCV measurements between both females with recurrent abortion (38.71l/l ±2.86) and infertility (38.71l/l ±2.14),and with control age-matched group (38.6l/l ±2.23), ($p=0.998$, 0.897 respectively) as demonstrated in (Figure 1).

2. Prothrombin time (PT):

PT measurements of infertile and recurrent abortion group were(11.71 seconds±1.03) and (11.63seconds±1.11) respectively, and control group (11.87seconds±1.13). There was no statistical significant difference ($p=0.777$) between both infertile group and control group(figure 2)

3. Activated partial thromboplastin time (APTT):

APTT values for infertile and recurrent abortion groups were(35.35 seconds±2.57), (35.80seconds±2.72) respectively and control group (35.60 seconds±3.07).There was no statistical significant difference for APTT of infertile and repeated abortion groups compared to the control group (p=0.775) as demonstrated in figure 3

4. Factor VIII: C plasma Activity level:

Factor VIII plasma percentage of activity level elevated (activity > 150%) in 29.3% of females with recurrent abortion and in 26.5% of females with infertility.The mean activity levels of factor VIII were (111.29%±31.66), (118.63%±39.54)in infertile and recurrent abortion groups respectively. A statistical significant difference (*p=0.009) between infertile, abortion groups and control group (80.23%±22.23)(figures 4, 5).

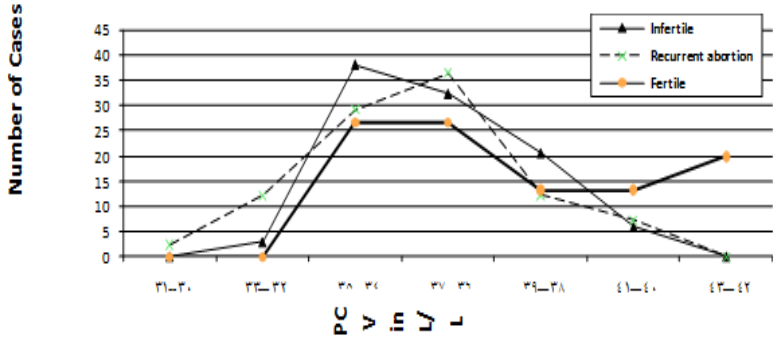

Figure 1: Packed cell volume (PCV) measurments in infertile, repeated abortion and cor group(p>0.05).

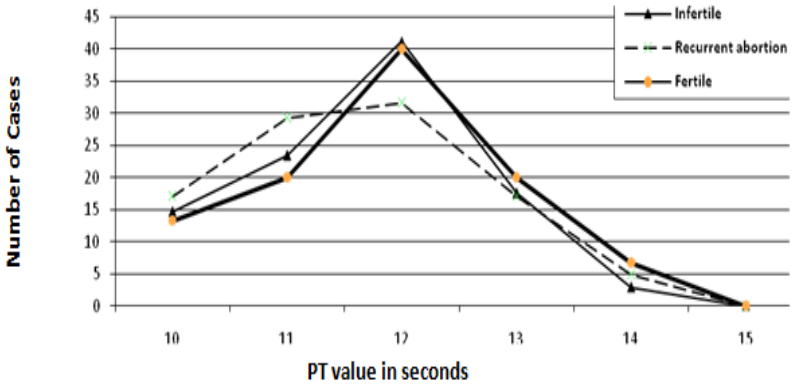

Figure 2: Prothrombin time (PT) measurments in infertile, repeated abortion and cor group. (p>0.05).

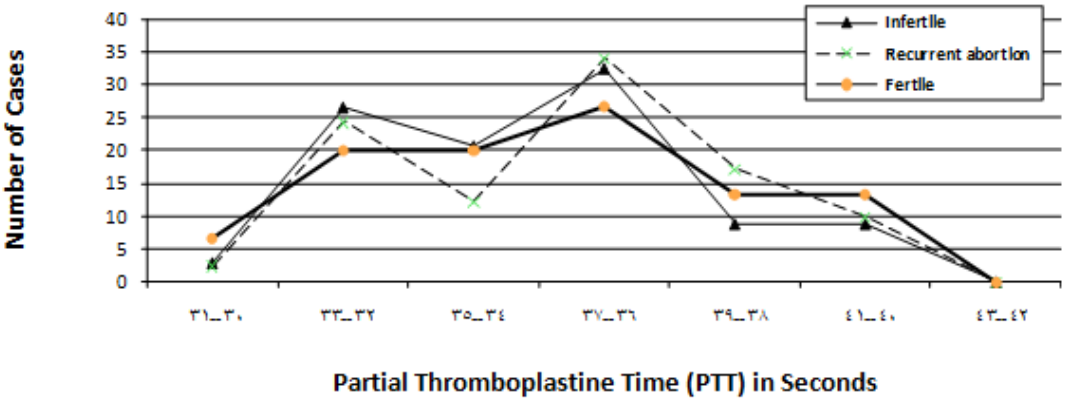

Figure 3: Partial thromboplastine time (PTT) measurments in infertile, repeated abortion and control group. (p>0.05).

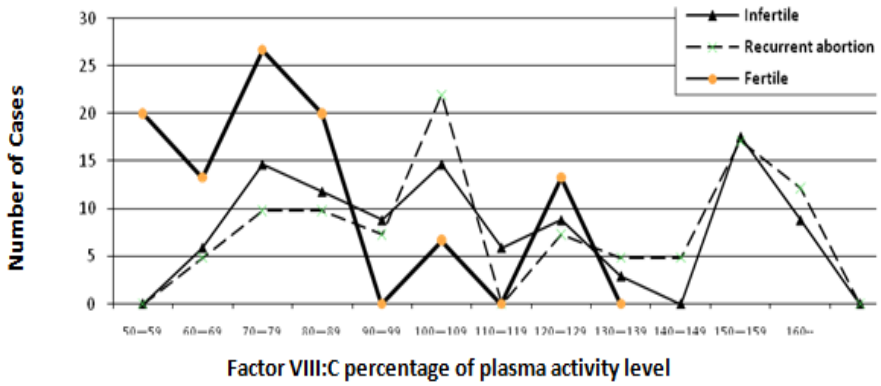

Figure 4: Factor VIII:C plasma activity level Assay in infertile, repeated abortion and control group. (p=0.009) significantly different between both infertile groups and control group..

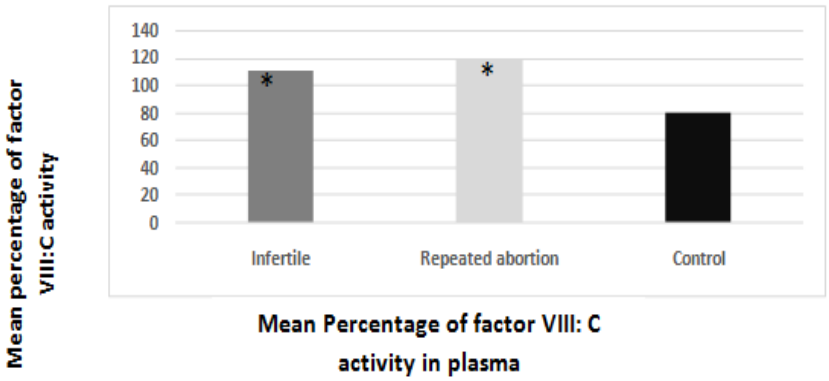

Figure 5: Mean of percentage of factor VIII:C plasma activity in infertile, repeated abortion and control group (p=0.009).

Discussion:

In this study, the PCV measurements demonstrated no statistical significant difference between the three examined groups. This might be explained by the strict selection criteria of the examined females in this study of being at least three-month post abortion; this post-abortion period suggested of being sample for the hematological alteration of gestation to return to normal levels[16]. Additionally, excluding abnormal PCV in the studied groups was important to delineate the impact of polycythemia and anemia on the coagulation pathway[17], which might interfere with this study as a confounding factor.

This study noticed a significant elevation of Factor VIII:C plasma activity (activity >150%) in 29.3% and 26.5% of females with recurrent abortion and those presented with infertility,respectively. This elevated activity (more than 150% of control plasma) was statistically significant when compared to control group.This suggests that thrombophilia due to factor VIII:C elevated activity might be a probable independent cause of such abortion or infertility, after excluding such elevation of factor VIII plasma activity of being due to inflammation or infection; which was excluded through negative C-Reactive protein. This hypothesis is supported by other published studies[18, 19].

Factor VIII is considered as one of the risk factors of thrombophilia [18]suggesting that a functional over-regulation of hemostatic balance could play a key role in determining placental vascular thrombosis diminishing its perfusion. Although the finding of placental vascular thrombosis is very rare in the products of early abortions, it could be hypothesized that hypercoagulable states due to factor VIII over activity can compromise placental perfusion during early embryo implantation, with absence of overt placental thrombosis. Physiologically, FVIII:C concentration rises as pregnancy advances, with a first increase between 11 and 16 weeks and a second, steeper one, between 16 and 18 weeks[19-21]. A gradual dose–response relationship between FVIII levels and the risk of thrombosis has been observed[14, 22, 23]. Therefore it can be argued that in predisposed women even a modest rise in the already hypercoagulable state of pregnancy can attain a hypothetical threshold sufficient for triggering the thrombotic event.

In this study elevated Factor VIII:C function might suggest that Factor VIII:C over-activity is sufficient to induce thrombophilia with subsequent placental insufficiency leading to fetal loss in women, who are already suffering from hematological disturbances accompanying the pregnancy. This solidify the need for inclusion of factor VIII:C specific assay in the workup and screening of thrombophilia in patients with recurrent abortions and infertility of unknown cause, even if the first line hematological indicators are within normal range.

C-Reactive Protein was measured as an acute-phase marker[24]and demonstrated to be negative in all patients; thus ruling out the argument that FVIII:C over-activity might be the result of an inflammatory status, as the acute-phase reaction. Therefore; an inflammatory response as a confounding factor was avoided in this study.

Conclusions:

Plasma Factor VIII:C activity level was higher in females with unexplained primary infertility and those with history of recurrent abortion. This elevation suggested of being independent risk factor of such conditions; after exclusion of other multiple causes leading to thrombophilia, abortion and infertility.

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