ORIGINAL ARTICLE

PLASMINOGEN ACTIVATOR INHIBITOR-1: IS IT A RISK FACTOR FOR RECURRENT MISCARRIAGE?

Samina Mohyuddin[1*], Syed Tousif Ahmed[2], Adnan Mustafa Zubairi[3], Shahina Ishtiaq[4], Farah Ahmed[5], Faridah Amin[6]

[1]Ziauddin University Hospital
[2]Department of Physiology, Ziauddin University Hospital
[3]Department of Chemical pathology Ziauddin University Hospital
[4]Department of Obstetrics and Gynecology, Ziauddin University Hospital
[5]Department of Community Health Sciences, Ziauddin University Hospital
[6]Department of Research, Ziauddin University Hospital

ABSTRACT

Background: About 50% cases of recurrent miscarriages are idiopathic. The association of fibrinolytic defects with recurrent pregnancy failure is a novel research avenue and was first recommended in early 1990s. High Plasminogen activator inhibitor-1 (PAI-1) levels are found to be associated with recurrent miscarriage in various studies. The association of Plasminogen activator inhibitor -1 with recurrent pregnancy loss and its various variables are detrermined.

Methods: This study was conducted at Ziauddin University Hospital Karachi, from Feb 2014 to Nov 2014. All non pregnant and non-obese women with history of consecutive two or more miscarriages, with no co-morbid diseases, visiting a gynecologist were included. Studied variables included were age, BMI, no. of miscarriages, time of miscarriage and plasma PAI-1 levels. The data was expressed in terms of median and percentages with a confidence interval of 95%. Analysis was done on SPSS version 20.

Results: The median age of females was found to be 27.34± 5.09 years. Out of seventy five females with history of recurrent miscarriages, (81.3%) (n=61) women had Plasma PAI-1 levels within normal range whereas (18.7%) (n=14) had value greater than normal. The median plasma PAI-1 was found to be 29.6±22.16 ng/ml.

Conclusion: PAI-1 was not found to be a risk factor for recurrent miscarriages in a local population of Karachi. Furthermore, studies on a large sample size need to be undertaken to assess the role of PAI-1in our population.

KEY WORDS: Recurrent pregnancy loss. Plasminogen activator inhibitor-1, Fibrinolysis

INTRODUCTION

Miscarriage is the most common dilemma of early pregnancy [1]. It is the expulsion of products of conception before 20th week of gestation or if the weight of the fetus is 500 grams or less. It can either be induced or spontaneous [2]. Induced abortion is further classified as illegally induced miscarriage and therapeutically induced miscarriage whereas spontaneous miscarriage is pregnancy loss before 20 weeks of gestation without interference [3]. The rate of early loss of pregnancies is found to be 12 to 15% [4]. In Pakistan, Miscarriage occurs in 10-15% of pregnancies. The reported prevalence of spontaneous miscarriage in Pakistan is 8% with an even higher rate of subclinical embryonic loss [5].

Recurrent pregnancy loss (RPL), defined as pregnancies (consecutive three or more) resulting in spontaneous miscarriage of the fetus before 20 weeks of gestation, effects about 1-5% of women who conceive [6] , However the American society of reproductive medicine defines RPL as, clinically proved two or more consecutive pregnancy losses [7].

The cause of spontaneous miscarriage is multifactorial, for instance, it can be caused by uterine anatomical abnormalities, chromosomal anomalies, hormonal imbalance, thrombophilia and abnormalities in fibrinolytic system [8]. The association of fibrinolytic defects with recurrent pregnancy failure was first suggested in the early 1990s, but very limited research is available to prove the association. It is known that fibrinolytic abnormalities results in decreased trophoblast invasion and increased fibrin deposition in placental circulation causing implantation failure [9]. Plasminogen activator inhibitor 1 (PAI-1) is a component of the serine protease inhibitor (SERPIN) super family secreted by hepatocytes, endothelial cells, adipocytes, and megakaryocytes into the circulation, plays a central role in regulating fibrinolytic system [10]. During early stages of implantation, it plays an important role in the regulation of human trophoblastic adhesion, migration and invasion [11]. The gene of Human plasminogen activator inhibitor-1 (PAI-1), located on the promoter region of long arm of chromosome 7, is composed of 9 exons and 8 introns (12.2 Kb) [12]. 4G allele homozygosity of the PAI-1gene is related with increased transcription of this gene, consequentially there is enhanced gene expression and high plasma PAI-1 concentrations, while in heterozygotes and 5G homozygotes, PAI-1 levels are found to be lowest [13]. Thus PAI-1 4G variant results in increased PAI-1

Corresponding Author: Samina Mohyuddin*

which may compromise placental formation and trophoblast invasion associated with reduced fibrinolytic activity[14]. Similarly, a meta analysis concluded analysis of eleven studies regarding RPL and PAI-1 gene polymorphism showed that women who carried the PAI-1 4G/5G polymorphism did not have a high risk of RPL [15]. High plasminogen activator inhibitor-1 (PAI-1) level was found in French women suffering from unexplained early recurrent miscarriage, which might cause impaired plasmin-dependent fibrinolysis resulting in recurrent miscarriage by promoting fibrin deposition in early placental circulation [16]. In Pakistan research is lacking in this regard and no data is available regarding association between PAI-1 and RPL, so the primary objective of this study was to find out association of Plasminogen Activator Inhibitor- 1 with recurrent miscarriage and its different variables, for instance age, BMI and time of miscarriage in our population.

METHODS

This study has been duly approved by the Ethics Review Committee of the Ziauddin University Hospital. All patients were informed in their local language about the purpose of the study and they gave their informed written consent before they were further assessed.

Selection Criteria
Seventy five (75) non pregnant healthy females of reproductive age (15-44) having history of two or more consecutive miscarriages, due to unknown cause, visiting gynecologist at Ziauddin Hospital Karachi, Pakistan were included in this study. Women with known genital tract anatomical abnormalities, endocrinological dysfunction, pregnancy, menstrual irregularities, coagulation disorders, autoimmune disease, antiphospholipid syndrome, diabetes mellitus, hypertension, obesity, liver function abnormalities, inflammatory pelvic disease, use of medications that effect liver function or the blood coagulation system, (estrogen- containing medications), history of abortion of ectopic or molar pregnancy, and polycystic ovary disease were excluded from this study. Informed consent was taken and Performa was filled containing information regarding age, BMI, blood pressure, ethnicity, no. of miscarriages, family history and consanguinity of the subjects.

Blood Sampling
For all patients complete medical history was obtained. Morning blood Samples were collected from seventy five females in 8.5 ml vacutainer tubes containing sodium Citrate as an anticoagulant (BD Diagnostics NJ 07417 USA) [17]. The plasma was aliquot and stored at -30°C till tested. To determine plasma PAI-1 levels, aliquots of plasma samples were thawed and PAI-1 levels were determined by using Human PAI-1 ELISA kit.

PAI-1 Analysis
The Human PAI-1 ELISA is an enzyme-linked immunosorbent assay for the quantitative measurement of Human PAI-1 [18].This assay utilizes a quantitative sandwich enzyme immunoassay technique to measure PAI-1. A polyclonal antibody for PAI-1 has been pre-coated against a microplate. PAI-1 in standards and samples is sandwiched by the immobilized antibody and another biotinylated polyclonal antibody against PAI-1, recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured [18].

STATISTICAL ANALYSIS

Data was entered on Microsoft excel 10 and was imported to SPSS version 20 after editing for analysis. For categorical data frequencies and percentages and for numerical data mean median and standard deviation were calculated. Chi square test was used to find an association between categorical variables. P value less than 0.05 was considered significant.

RESULTS

In the current study, the median age of females with history of recurrent miscarriages was found to be 27 years. Similarly, the median PAI-1 in plasma of females with history of recurrent miscarriages was found to be 22.9ng/ml (Table.1). Moreover, Plasma PAI-1 level of majority of women was found to be within normal range (2.4ng/ml-43.8ng/ml) (81.3%) (n=61) while fourteen subjects (n=14) (18.7%) had value greater than normal (>43.8ng/ml).In this study, 34.7% (n=26) of women had no alive child whereas 29.3% (n=22) of females had only one alive child and 36% (n=27) of females had two or more alive children. In this study we compared age, BMI, ethnicity, gestational age at the time of miscarriage and no. of miscarriages with PAI-1. No. significant difference was found in PAI-1 between different groups of recurrent pregnancy loss.Table.1.

Table1 PAI-1 in plasma of females with history of recurrent miscarriages

	PAI-1		
Variable	**%**	**n=75**	**P-value**
No. of miscarriages 2 miscarriages	46.7	35	0.09
3 or more miscarriages	53.3	40	

BMI underweight (<18.5 kg/m2)	13.3	10	0.752
normal weight (18.5 - 24.99kg/m2)	52	39	
over weight (24.99 - 29.99kg/m2)	34.66	26	
obese (>30 kg/m2)	0	0	
Ethnic Group			0.302
Pathan	69.3	32	
Punjabi	9.3	7	
urdu speaking	9.3	7	
Sindhi	12	9	
Time of miscarriages			0.556
1st trimester miscarriage	66.7	54	
2nd trimester miscarriage	12.3	10	
1st and 2nd trimester miscarriage	13.6	11	

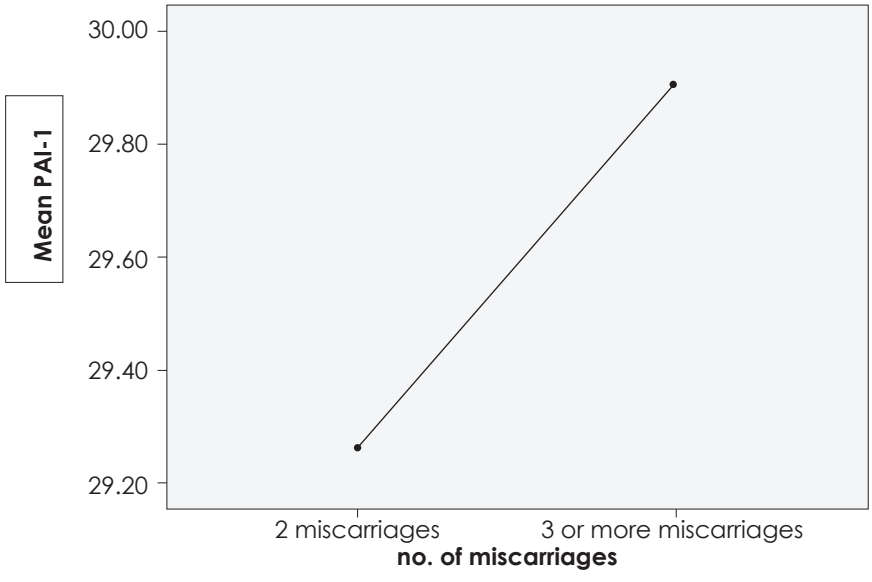

Figure1: Represent relation of mean PA1-1(ng/ml) with no.of micarriages.

DISCUSSION

The objective of the current study was to find out association between PAI- 1 and recurrent pregnancy loss. Most of the study subjects belonged to Province KPK (69.3%). We compared different characteristics of women (age, BMI, ethnicity, no. of miscarriages and gestational age of the fetus at the time of miscarriage) with their plasma PAI-1 levels . No significant difference was found in PAI-1 level among all of the above variables. Findings could be due to small sample size or similar ethnicity.

In the current study mean age of females with history of recurrent miscarriage is found to be 27 years. Similarly, a study conducted in India also reported same mean age. [19]. In addition, high PAI-1 concentration in females suffering from first unexplained early recurrent miscarriage is reported [16] One more study stated that PAI-1 levels were associated with placenta-associated obstetric complications, including recurrent miscarriages [20]. In contrary to that, in this study, majority of females (n=61) (81.3%)had Plasma PAI-1 levels within normal range, the variation in outcome could be due to diversity in inclusion criteria, i.e. several researchers chose to include females with two consecutive abortions and some included females with history of three or more pregnancy losses. [15]

A positive correlation was found between PAI-1 activity and BMI (p=0.25) among females with history of RPL [21], Whereas in the current study, we could not find a significant difference in PAI-1 level among different groups of BMI (underweight, normal weight, and overweight) (p=0.471), the difference in results might be due to exclusion of obesity from the current study. Furthermore we could not establish significant difference in PAI-1 level among females with history of two miscarriages and three or more miscarriages (p=0.09). Further studies are required with inclusion of large sample size and different ethnicities to find an association between PAI-1 and recurrent miscarriage in our population. Furthermore, integrating different proposals i.e. variation in gene and DNA variants, protein expression and epigenetis to carry out research on molecular factors for RPL will be beneficial [21].

CONCLUSION

PAI-1 is not found to be a risk factor for recurrent miscarriage in a local Pakistani population. Furthermore PAI-1 should be studied on a large sample size and various other ethnicities as there are controversies regarding increased PA1-1 as a cause of recurrent miscarriage in some population.

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