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CORRELATION OF PLASMINOGEN ACTIVATOR INHIBITOR AND ANGIOTESIN-CONVERTING ENZYME GENES WITH MISSED MISCARRIAGE IN THE FIRST HALF OF PREGNANCY

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Abstract
Background: Recurrent miscarriage stands as a significant public health issue, affecting 2%-5% of women in their childbearing years. It is characterized by the occurrence of three or more consecutive pregnancy losses before reaching viability, encompassing all miscarriages from conception to the 20th week of gestation. Thrombotic disorders are identifiable in 40–50% . Our Aim is To study the relation of the common polymorphisms in Angiotensin converting enzyme and plasminogen activator inhibitor-1with recurrent miscarriage.
Patients and Methods: This comparative observational prospective case-control study was carried out at Duhok Hospital for Obstetrics and Gynecology during the period from January 1st to September 1st, 2023, it included 100 married women in age range (18-42 years), as 50 women with a history of three or more miscarriages during the first half of pregnancy and fifty women as controls with a history of at least one successful pregnancy. Data were collected through a questionnaire form and blood tests targeting these thrombophilia genes (Angiotensin converting enzyme and plasminogen activator inhibitor-1).
Results: This study showed that women with history of Recurrent miscarriage had a significant association with plasminogen activator inhibitor l with (P= 0.009), but no association with angiotensin-converting enzyme genes, the (P= 0.275). In addition, the recurrent miscarriage has a significant association with parity ($P \leq 0.001$), while did not associated with each BMI and supplement taking.
Conclusions: The genetic marker PAI-1 is playing a discernible role in recurrent miscarriage while ACE gene does not show any significant impact.
Keywords: Angiotensin-converting enzyme, Plasminogen activator inhibitor-1, recurrent miscarriage.

INTRODUCTION

1.1 Background:

Recurrent miscarriage is one of the top public health concerns, accounting for 2- 5% of women of childbearing age [1]. Recurrent miscarriage is defined as the loss of three or more consecutive pregnancies before viability and includes all pregnancy losses from the time of conception through 20 weeks gestation [2-4]. The most commonly reported causes of miscarriage are structural chromosomal abnormalities in one of the partners, uterine abnormalities, randomly elevated homocysteine levels, and antiphospholipid syndrome [5,6].

Thrombophilia is described as a susceptibility to arterial or venous thrombotic complications due to defects in the hemostatic system, which may be acquired, such as antiphospholipid syndrome, or inherited [7,8]. Adverse pregnancy outcomes such as pregnancy failures (i.e, sporadic and recurrent miscarriage, later fetal loss), preeclampsia, and HELLP syndrome are associated with thrombotic mechanisms and thrombophilia [9]. Thrombotic disorders are detectable in 40–50% of recurrent

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miscarriage cases [10-12].
Clinically recognized miscarriage occurs in approximately 15-25% of all pregnancies. It is estimated that less than 5% of women experience two consecutive miscarriage and that only 1% of women experience three or more miscarriage [13].
Possible causes of recurrent miscarriage could be anatomical, immunological, genetics, endocrine, environmental and thrombophilic factors. It is known that the ACE gene contains a polymorphism consisting of either the insertion (I) or the deletion (D) of a 287 bp Alu repetitive sequence in intron [14]. In particular, the D allele and DD genotype are associated with elevated ACE levels [15-17]. Homozygotes for the I allele may have only half the plasma ACE level compared to homozygotes for the D allele, whereas the ID heterozygotes have an intermediate value[18] . The uteroplacental RAS assumes a crucial role in the regeneration of the endometrium post-shedding, decidualization, implantation, and placentation. Furthermore, the local RAS actively contributes to prostaglandin production, the release of estradiol, and the control of blood flow to the placenta and uterus [19].
PAI-1, a single-chain glycoprotein, member of the serine protease inhibitor superfamily, is one of the most important inhibitors of fibrinolytic activity in plasma. PAI-1 is the major inhibitor of tissue-type plasminogen activator (t-PA) and urine-type plasminogen activator (u-PA). The increased expression of PAI-1 in vivo suppresses fibrinolysis, consequently leading to pathological fibrin deposition and tissue damage. A number of previous studies have examined the association of the polymorphisms PAI-1 4G/5G and ACE I/D with infertility, Recurrent miscarriage and serious pregnancy complications [20].
In the review of literature of the surrounding area like Iran and Sudan, Considering all their results, the pooled data indicated that ACE I/D and PAI-1 4G/5G polymorphisms were associated with an increased risk of recurrent miscarriage. Moreover, these polymorphisms were associated with recurrent miscarriage risk by the number of previous miscarriages. These significant findings suggest that investigation might be adequate for ACE I/D and PAI-1 4G/5G polymorphisms in association with recurrent miscarriage and should be part of the standard examination for all women with recurrent miscarriage [21].
According to the Knowledge there is no study about the association between the ACE I/D and PAI-1 4G/5G polymorphisms and recurrent miscarriage in Duhok, so this study was done to investigate them.
Patients and Methos: A comparative prospective observational case-control study design was adopted. The research took place at Duhok Hospital for Obstetrics and Gynecology, spanning from January 1st to September 1st, 2023. The Ethical Committee of the Duhok Directorate of Health and the Scientific Committee of Duhok Hospital of Obstetrics & Gynecology formally endorsed the study proposal . Each woman invited to participate was verbally briefed about the study, with the assurance that the data would be used exclusively for the study's purposes.

Data Collection Tool and analysis :

Patient responses to the checklist questionnaire during interviews and file reviews by the researcher constituted the data collection. The questionnaire form (see Appendix B) included information about age, residence, educational levels, occupations, and economic status. Gravidity, parity, and the number of recurrent miscarriage were assessed. Past medical history (PMH), past surgical history (PSH), family, social, and psychological history were considered. The weight, height, and body mass index (BMI) were measured during the examination. The types of supplements were also evaluated.

Blood samples were collected and sent to the laboratory for DNA extraction and genotype analysis by Roter gene (CVD), to investigate the association between ACE insertion and deletion I/D (homozygous or heterozygous) and plasminogen activator inhibitor insertion and deletion4G/5G (homozygous or heterozygous) in women experiencing missed miscarriage. And The statistical analysis that used in present study were frequency, percentage, mean, and standard deviation as descriptive

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analysis and Chi square and Fisher exact test as inferential analysis with P value of <0.05 considered as statistically significant.

RESULTS

3.1. The Socio- demographic Characteristics of the Study Sample.

Table 3.1shows that most of the pregnant women aged between 25-30 have no recurrent miscarriage, while 37-42 stand for Recurrent miscarriage 20(40%), 15(30%) respectively, in both the large proportion, are residents of urban areas 50(100%), 36(72%) and secondary school graduate 29(58%), 23(46%), and have enough monthly household income 46(92%), 32(64%) as presented respectively, however (48%) of control are governmental employee and (52%) of those with recurrent miscarriage are housewife worked. Besides, recurrent miscarriage is associated with each pregnant woman's age, residence, occupation, and economic status with *P*-values 0.036, ≤ 0.001, 0.002, and 0.003 respectively.

Table 3.1: Pregnant Women Demographic Characteristics and their Association with Recurrent Pregnancy Loss.

		Miscarriage		
		Control F (%)	Recurrent miscarriage 3 and more F (%)	*P*- Value
Age	19-24	10 (20)	9 (18)	0.036*
	25-30	20 (40)	12 (24)	
	31-36	16 (32)	14 (28)	
	37-42	4 (8)	15 (30)	
	Age Mean (SD)	30.74 (6.159)		
Residence	Urban	50 (100)	36 (72)	≤ 0.001*
	Rural	0 (0)	14 (28)	
Educational level	Primary school	8 (16)	12(24)	0.342**
	Secondary school	29 (58)	23 (46)	
	Tertiary & above	13 (26)	15 (30)	
Occupation: Employment status	Housewife worked	10 (20)	26 (52)	0.002*
	Government employee	24 (48)	18 (36)	
	Non-governmental employee	16 (32)	6 (12)	
Economic status (Monthly household income)	Not enough	1 (2)	5 (10)	0.003**
	Enough	46 (92)	32 (64)	
	Enough and more	3 (6)	13 (26)	

* Chi square ** Fisher exact test

3.2. The Obstetrical Characteristics of the Study Sample.

Table 3.2 reveals that most of the pregnant women who had recurrent miscarriage did not do the investigation of the previous recurrent miscarriage 37(74%) and were normal previous U/S 46(92%), and a large proportion of both groups are overweight 26(52%), multi para 26 (52%), 21 (42%), and taking folic acid 48(96%), 50(100%), respectively. In addition, abortion has a significant association

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with parity *P*-Value ≤ 0.001, while did no association with each BMI and supplement taking.

Table 3.2: Pregnant women's Obstetrical Characteristics and their association with recurrent pregnancy loss.

		miscarriage		
		Control F (%)	Recurrent miscarriage 3 and more F (%)	*P*- Value
Investigation of the previous recurrent miscarriage	Yes No	0 (0) 50 (100)	13 (26) 37 (74)	
Previous U/S	Normal Presence of deformity	50 (100) 0 (0)	46 (92) 4 (8)	
BMI	Underweight (BMI below 18.5) Healthy weight (BMI 18.5 to 24.9) Overweight (BMI 25 to 29.9) Obese (BMI 30 or more)	0 (0) 10 (20) 26 (52) 14 (28)	1 (2) 7 (14) 22 (44) 20 (40)	0.381**
Parity	Para (0) Primi para Multi para (2-4) Grand multi para (=> 5)	0 (0) 24 (48) 26 (52) 0 (0)	14 (28) 14 (28) 21 (42) 1 (2)	≤ 0.001**
Supplement Taking	Folic acid Vitamin D Multivitamin	48 (96) 12 (24) 17 (34)	50 (100) 12 (24) 16 (32)	0.968*

* Chi square ** Fisher exact test

3.3 Women's Past History Variables of Study Sample.

This table presents that most of the pregnant women in both groups had no either chronic disease 43 (86%), 39 (78%), surgical history 27 (54%), 17 (34%), family history of thrombophilia 47 (94%), 49 (98%), Social behavior history 40 (80), 41 (82), and stress 43 (86), 41 (82). At the same time, recurrent miscarriage has a highly significant association with the pregnant women past surgical history *P*-Value ≤ 0.001 while no association with rest.

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Table 3.3: Pregnant Women's Past History Variables and their Association with Recurrent Pregnancy Loss

		miscarriage		
		Control F (%)	Recurrent miscarriage 3 and more F (%)	*P*- Value
Past medical history	HTN	4 (8)	3 (6)	0.389**
	DM	3 (6)	7 (14)	
	Autoimmune disease	0 (0)	1 (2)	
	No Chronic diseases	43 (86)	39 (78)	
Past surgical history	No Surgical History	27 (54)	17 (34)	≤ 0.001**
	CS	22 (44)	12 (24)	
	D&C	0 (0)	25 (50)	
	Laparoscope	1 (2)	9 (18)	
	Previous operation	0 (0)	1 (2)	
	Hysteroscope	1 (2)	3 (6)	
Family history	Thrombophilia	3 (6)	1 (2)	0.617**
	None	47 (94)	49 (98)	
Social behavior history	Hadn't	40 (80)	41 (82)	0.399**
	Smoking	8 (16)	7 (14)	
	Alcohol	2 (4)	0 (0)	
	Caffeine	0 (0)	2 (4)	
Psychological history	Stress	7 (14)	8 (16)	0.786**
	No stress	43 (86)	41 (82)	
	Under treatment	0 (0)	1 (2)	

* Chi square ** Fisher exact test

3.4. The Association of Miscarriage with each (PAI-1) and (ACE) Genes in the Study Sample.

Table 3.4 displays that the history of recurrent miscarriage has a highly significant association with Plasminogen activator inhibitor l, but no association with Angiotensin-Converting Enzyme genes.

Table (3.4): The Association of Miscarriage with each of Plasminogen Activator Inhibitor l (PAI-l) 4G/5G and Angiotensin- Converting Enzyme genes (ACE) 287bp insertion/deletion (I/D).

Thrombophilic Genes (ACE, PAI-1)		Miscarriage		
		Control F (%)	Recurrent miscarriage 3 and more F (%)	*P*- Value
Plasminogen activator inhibitor l (PAI-l) 4G/5G	Normal	6 (12)	8 (16)	0.009*
	Homozygote	33 (66)	18 (36)	
	Heterozygote	11 (22)	24 (48)	

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Angiotensin-Converting Enzyme genes (ACE) 287 bp insertion/deletion (I/D)	Normal	8 (16)	3 (6)	0.275*
	Homozygote	24 (48)	26 (52)	
	Heterozygote	18 (36)	21 (42)	

* Chi-square

Discussion

Successful embryo implantation depends on regulating coagulation and fibrinolysis to prevent excessive fibrin accumulation in placental vessels and intervillous spaces. Research into ACE I/D and PAI-1 polymorphisms has explored their potential links to recurrent miscarriage. The ACE I/D polymorphism, associated with conditions like stroke and preeclampsia, impacts miscarriage risk by influencing thrombotic events. The D allele is linked to higher ACE levels, increasing thrombotic risk [21, 22, 23].

Elevated PAI-1 levels, influenced by the ACE I/D polymorphism, also correlate with miscarriage risk [24, 25]. Despite numerous studies, results have been inconsistent.

This study found a significant association between recurrent miscarriage and PAI-1 but not ACE genes, aligning with Aarabi et al. [26], who found the 4G/4G genotype of PAI-1 significantly linked to higher miscarriage risk. This contrasts with Fazelnia et al. [23], who reported a significant association between the DD genotype of the ACE I/D polymorphism and recurrent miscarriage in northern Iran. Similar results were noted in a meta-analysis by Zhan Huong et al. (2017), which suggested that the PAI-1 4G/5G polymorphism increases miscarriage susceptibility in various populations [27].

Conversely, studies from Poland (Kurzawińska et al., 2016) . and Sudan found no significant association between PAI-1 polymorphisms and miscarriage, potentially due to geographical differences [21,28].

The current study also found a significant association between miscarriage and parity (P-value ≤ 0.001), differing from Bigdeli et al. (2018), who found no significant relationship between gravidity, parity, and miscarriage [29]. Regarding anthropometric characteristics, no link was found between BMI or supplement use and miscarriage, consistent with Bigdeli et al. (2018) . However, a study in Karachi, Pakistan, found higher BMI associated with increased miscarriage risk [30]. While smoking is a recognized risk factor for miscarriage, this study did not find a significant association, diverging from research in Sofia, Poland, and the Middle East, which consistently linked smoking to increased miscarriage risk [31, 32, 33].

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