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The Role of ACE Gene Rs 1799752 I/D Polymorphism and Serum ACE Activity in Recurrent Miscarriage Pathogenesis in Iraqi Women

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ABSTRACT

BACKGROUND

Recurrent miscarriage is a major health public health problem that affects one to three percentage of women of childbearing age and is diagnosed as a spontaneous miscarriage if happened more than two times and occur often to the completion of 20 weeks amenorrhea. The variation in Angiotensin-Converting Enzyme (ACE) may be have an important role in the recurrent miscarriages (RM) in Iraqi women.

OBJECTIVE

The present study aimed to investigate the role of ACE gene polymorphism and its serum level in the pathogenesis of recurrent miscarriage in Iraqi women.

MATERIALS AND METHODS:

Blood samples were collected from fifty patients with RM of unknown cause and fifty apparently healthy women (control). DNA was extracted for genotyping and serum samples were obtained to determine the concentration of ACE activity. ACE gene polymorphisms were determined by using Polymerase Chine Reaction –High Resolution Melting (PCR-HRM), while, serum level of ACE were determined by using Enzyme-Linked Immunosorbent Assay (ELISA).

RESULTS

The research data indicated that genotype II was accompanied by a lower risk of recurrent miscarriage, while there is no relation was obtained of allele frequency among the recurrent miscarriage cases. Moreover, it's showed that there is a significant differences were noted between RM patients and controls as related with serum ACE level. High ACE activity was noted in the RM patients carrying D allele in compared to healthy individuals. In all trimesters of the RM incidence was higher in the ID genotype carriers and 70% of RM patients were in the first trimesters.

CONCLUSION

These results concluded that the ACE gene polymorphism at rs179952 have a role in the RM pathogenesis and the genotype II may serve as a protective factor against RM, it's also showed that there is a low serum ACE levels which might be a new marker for recurrent miscarriage risk in Iraqi women. The results also showed that there is a strong association between the rs1799752 genetic polymorphism in the ACE gene and the increased risk of miscarriage, especially in pregnant women carrying the ID genotype.

Keywords: *Recurrent miscarriage, ACE gene polymorphism, ACE Enzyme.*

1. INTRODUCTION

Recurrent miscarriage (RM) is one of the main public health problems that affects only 1% to 3% of pregnant women of reproductive age, before twenty weeks of gestation considered spontaneous abortion [1] and it has been found that a high percentage of women suffer from this painful condition, the MR rate becomes higher, under the age of 35 years ; it ranges from 12% to 15%, and over the age of 40 years, it is 25% [2].

It is a multifactorial condition and there are many different causes that associated with the RM such as the genetic factors, chromosomal abnormalities, also different defects including, uterine anomalies, autoimmune diseases, lifestyle, and maternal pathogens, but exact causes of it still remains unclear [3], as well as insufficient hormone metabolism [4]. Renin-Angiotensin System (RAS) is a complex multi-organ endocrine (hormone) and physiological system involved in an important multi-regulatory system of the body, including peptides and enzymes, The system can be found in many different types of tissues including the myocardium, vascular smooth muscle, kidney with essential roles in regulation of various organs [5]

The contributions to the RAS including, angiotensin I, renin, angiotensin converting enzyme (ACE), angiotensinogen (ATG), , angiotensin II and angiotensin receptors (AGTR); the classical pathway of RAS starts with decrease in renal perfusion pressure when juxtaglomerular cells (JGA) in the kidney convert the proenzyme prorenin into active forms and release it in the blood as renin into the blood or plasma [6]. The renin activates angiotensinogen which is 453 amino acid glycoprotein produced mainly via the liver through proteolytic cleavage to produce the decapeptide angiotensin I which is released by the liver. Angiotensinogen is having a central role in cardiovascular disease. In recent works, there has been concern over the relationship between ACE gene and hypertension, others tried to reveal the effect of increased mentioned above ACE on pathological processes of tissues and organs possessing ACE enzyme, Angiotensin I is eventually hydrolyzed to angiotensin II by the angiotensin converting enzyme (ACE) located at the vascular endothelial surface of lung and kidneys [7].

The ACE aids in the blood pressure regulation and in the processes of water and salt homeostasis. This is accomplished by releasing the C-terminal His-Leu from angiotensin I that converts it to angiotensin II and increases its vasoconstrictor action. The ACE besides its s pathological and clinical application, also has some function in the organs where it is situated [8] and [9]. angiotensin I to angiotensin II transforms via the ACE, predominantly present in lung or kidney vascular endothelial cells and whose function is to cleave off two amino acid residues to produce angiotensin II [10] and [11].

The ACE gene, situated at the band 23.3 of the long arm (q) of chromosome 17, contains 26 exons and 25 introns [12]. Understanding the genetic character is very important in calculating the risk factors associated with recurrent threats of miscarriage and many studies have correlated such genes with miscarriage including Ace genes [13]. The sequence of human ACE gene is unique as it contains a genetic polymorphism which is the ACE Insertion/Deletion includes a variation of the reference sequence 1799752. It is within a region of DNA made up of 287 bp nucleotides known as Alu sequence and the insertion or deletion of the this fragment located at intron 16 spans approximately 21,597 nucleotides which the ACE gene is represented by insertion and the absence in the intron denotes deletion. [14]. This region is a part of one variant of the ACE gene; it is known as the insertion or I allele, while another variant lacks this DNA segment and is termed the deletion or D allele. Because individuals have two copies of every gene, each person can be II (two I alleles), DD (two D alleles), or ID (one copy of each allele) [15]. The expression of ACE variable is mainly estimated through ACE gene polymorphisms. Therefore, the polymorphism is associated with the concentration of the circulating enzyme. The DD pattern was found to be associated with higher levels of ACE in tissue and plasma than the homozygotes II pattern, while the heterozygotes ID pattern was related to intermediate levels [16]. The most frequent interaction of ACE gene I/D polymorphisms contributes to complications in pregnancy, infertility, and syndromes of the reproductive tract [17]. Moreover, proper correlation was found between (ACE I/D) polymorphism and infertility [18], [19]. and [20].

Other disorders which occur in individuals with this pattern include renal tubular dysgenesis, which is usually characterized by a disordered renal development before birth, inability of the kidney to form urine, anuria, and severe diastolic low blood pressure. These conditions consequently lead to a deficiency of amniotic fluid, oligohydramnios, which causes birth defects resulting in the Potter sequence. The enzymatic activity of ACE was two times higher in DD carriers than in II carriers and it is intermediate increase in ID carriers, thus it showing co-dominance among all alleles. It has been stated that ACE I/D polymorphisms might be included in different transcriptional regulation and ACE pre-mRNA splicing [22].

This proposal seeks to shed light on the ACE Insertion/Deletion genotype (rs1799752 SNP) and its association with recurrent miscarriage, along with determining serum ACE levels too. This would thereby enable the investigation of its association with RM. The findings from the study will add to our knowledge of the trimesters of gestation and may allow ways and many reasons for diagnosing new problems which will improve the outcome for women suffering from this condition.

2. MATERIALS AND METHODS

2.1 Subjects

The current study involved fifty females with idiopathic recurrent miscarriage (mean age 33.22 ±1.14) attending Khanaqin hospital, Diyala, Iraq between Jun 2023 to December 2023, along with fifty fertile females with at least 2 live labors and without history of abortion. The subjects to be excluded were patients with an autoimmune disease, infection anatomical endocrine, and metabolic disorder. In this regard, ethical approval was obtained for the study from Khanaqin general hospital. Blood samples were collected from the two study groups for DNA extraction and to obtain serum samples.

2.2 DNA Extraction and genotyping

DNA was extracted from the whole blood by using EasyPure® Blood Genomic DNA-TransGen, biotech EE121. The counting of the concentration and DNA purity per each sample was achieved by Nanodrop. For genetic analysis, the study of polymorphisms, means that HRM analysis was achieved using a Rotor gene Q Real-time CYTO PCR System by QIAGEN. The oligonucleotide primers designed by the Primer 3 plus, V4, together with their reference sequences in the National Center for Biotechnology Information database. They were utilized and lyophilized by Alpha DNA Ltd., using real time thermocycler-HRM, SNP genotyping assessed according to the protocol of the kit as shown in table (1), (2) and (3).

Table 1: The quantitative Real-Time PCR reaction components of HRM analysis for genotype.

Reaction Components	**Volume (µl)**
2x-TransStart®Tip- Green qPCR Super Mix	10
Nuclease free water	4
Forward – primer	1
Reverse – primer	1
DNA	4
Final volume	20µl

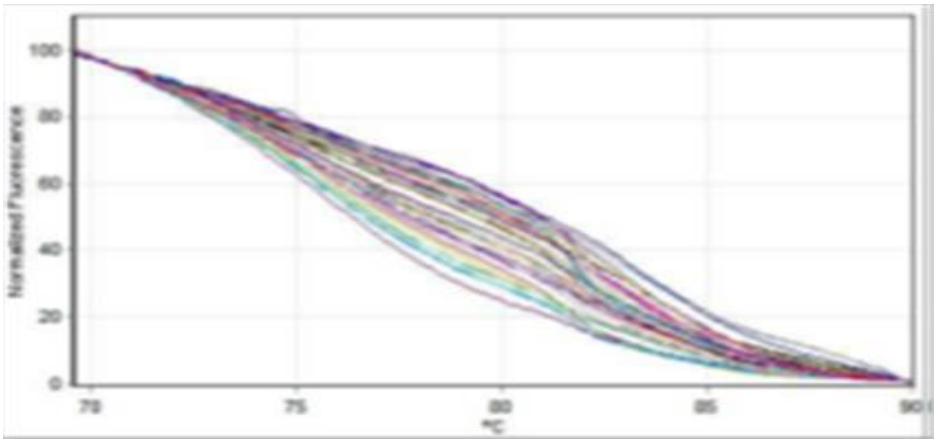
Table 2: The thermal profile of the Real Time PCR-HRM Technique for ACE rs 1799752.

Step	**Temp.(°C)**	**Time (sec.)**	**NO. Cycle**
Enzyme Activation	94	30	1
Denaturation	94	5	40
Annealing	60	15	
Extension	72	20	
HRM	60 - 95	0.1 degree for 2 sec.	

Table 3: ACE I/D gene rs 1799752 Primers .

SNP	**Primer Direction**	**Primer size bp**	**Product size bp**	**Tm °C**
ACE rs 1799752	F-´5GGGACTCTGTAAGCCACTGC 3´	20	128	59
	R- ´5GCTTGTAAGGGGAGCTCAGA 3´			

The figure (1) and (2) showed the diagrams curve of the real time PCR-HRM for ACE genotype in the control and the patients.



Cycle number

Figure 1: PCR-HRM curve of ACE genotype in the patients.

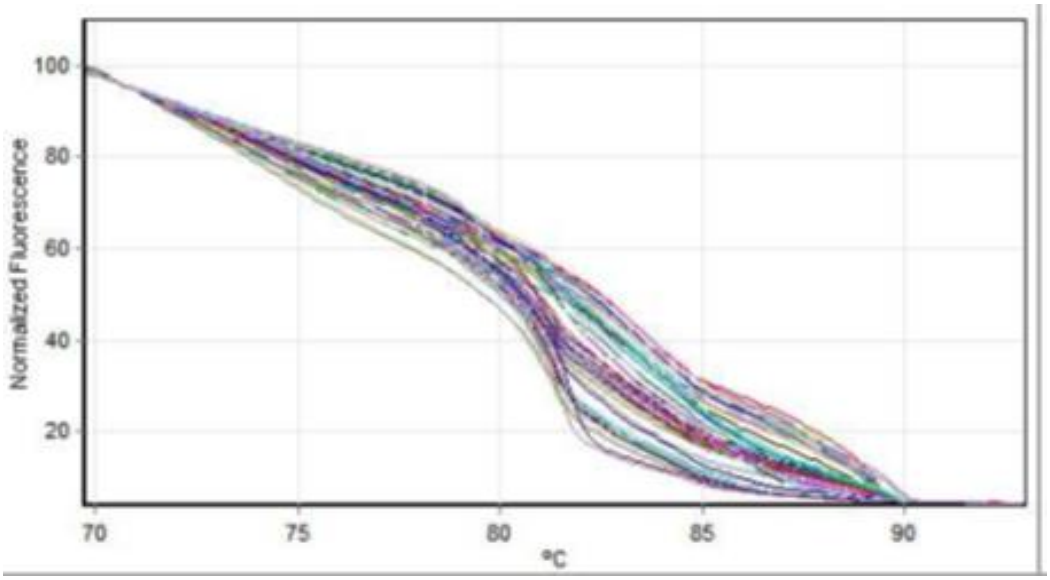


Figure 2: PCR-HRM curve of ACE genotype in the control.

2.3 Determination of ACE activity.

The determination is based on sandwich-type ELISA technology for determining the level of ACE in human blood serum. This technology is based on the specific interaction between antibodies and ACE antigen. The wells of the ELISA plate are preliminarily coated with ACE-specific antibodies. When the sample or standards are added to the wells, ACE present in them will be bound by the coated antibodies. Another antibody coupled with horseradish peroxidase was then added, binding to the ACE-I antibody complex. The HRP enzyme reacts with TMB to form a colored reaction-a blue product that changes to yellow on the addition of stop solution. The intensity of the yellow color developed would be directly proportional to the concentration of ACE present in the sample and measured by spectrophotometry at 450 nm.

2.4 Statistics

The SAS program was utilized (Statistical Analysis System, SAS 2018); to identify the impact of different groups, patients, and control in study parameters. The t-test and LSD test was utilized for significant comparison between means. The Chi-square test was used for a significant comparison between percentages. 0.05 and 0.01 probability.

3. RESULTS

3.1. Recurrent Miscarriage

In the present study, 50 apparently healthy subjects and 50 patients with recurrent miscarriage were used. According to the age, no significant differences were noted between the two groups: they were 34.08±0.65 versus 33.22±1.14 years old, respectively.

Table (4) shows distribution of results according to the percentage of cases of recurrent miscarriage in three trimesters of gestation among the patients.

Table 4: Distribution of sample study according to No. RM with Trimester :1st , 2nd and 3rd in patients group.

Variable		Summation	Percentage (%)
No. Recurrent Miscarriage		146	100%
Trimesters	1st Tri.	107	73.29
	2nd Tri.	18	12.33
	3rd Tri.	22	15.7
P-value	---	0.0001 **	
** (P≤0.01).			

As shown in table (4), the percentage of recurrent miscarriage cases was significantly (P<0.01) higher in the first trimester of gestation than those of second trimester and third trimester (73.29% versus 12.33% and 15.7%) respectively.

Table (5) :Genotype distribution and allele frequency of ACE I/D gene(rs 1799752) in the patients and control groups.

Genotype/ ACE rs 1799752	Control No.(%)	Patient No(%)	Chi-Square(χ^2)	P-value	O.R. (C.I)
II	24 (48.00%)	11 (22.00%)	4.828 *	0.028	1.207 (0.87-2.18)
ID	21 (42.00%)	31 (62.00%)	1.923 NS	0.165	0.571 (0.32-1.29)
DD	5 (10.00%)	8 (16.00%)	0.692 NS	0.405	0.394 (0.24-1.07)
Total	**50**	**50**	--	--	--
I	69 (0.69)	53 (0.53)	2.098 NS	0.147	0.736 (0.38-1.18)
D	31 (0.31)	47 (0.47)	3.282 NS	0.070	0.962 (0.42-1.84)
*** (P≤0.05), NS: Non-Significant.**					

Our results showed that a consequence of miscarriage in Iraqi women (rs1799752 SNP) in the ACE gene, the incidence with II was significantly lower in women with recurrent miscarriage than in healthy individuals (22% versus 48%, respectively) represent as a protective factor. This result is that there is at least one copy of the allele that can determine the risk of recurrent miscarriage.

3.2. Effects of rs 1799752 on serum ACE activity.

The results of serum ACE enzyme levels according to the age was presented in table (6).

Table 6: Comparison between patients and control groups in serum ACE Enzyme concentration according to the age.

Group	Mean ±SE	
	Age (year)	ACE conc. (mg/dl)
Control	34.08 ±0.65	0.824 ±0.06
Patients	33.22 ±1.14	0.977 ±0.10
T0-test	2.589 NS	0.244 NS
P-value	0.513	0.215
NS: Non-Significant.		

As shown from the table (6), no significant differences were noted between apparently healthy subjects and RM patients as related with serum ACE enzyme concentrations. Table (7) shows the relationship between the genotype of the ACE I/D gene (rs1799752) and ACE enzyme concentration in the RM patient and control groups.

Table (7) : Relationship between genotype of ACE I/D gene (rs1799752) and ACE Enzyme concentration in patients and control groups.

Genotype/ ACE rs 1799752	Mean ±SE of Pt. ACE Enzyme conc. (mg/dl)	
	Control group	Patients group
II	0.596 ±0.19a	0.588 ±0.08 b
ID	0.998 ±0.13a	0.929 ±0.09 ab
DD	1.069 ±0.16a	1.033 ±0.12 a
LSD	0.602 NS	0.329 *
P-value	0.276	0.0490
Means having with the different letters in same column differed significantly, * (P≤0.05).		

No statistically significant differences in mean ACE enzyme concentration were observed between the different genotypes (II, ID, and DD) in the control group ($P > 0.05$). However, in the patient group, the mean ACE enzyme concentration was significantly higher in individuals carrying the DD genotype compared to those carrying the II genotype ($P < 0.05$). There were no significant differences between the other genotypes in the patient group. Comparison between serum ACE enzyme concentration in the patient's women with RM versus control, revealed a significant decrease ($P \leq 0.05$) in the DD genotype carriers, the mean was (0.588 ±0.08 and 0.696 ±0.19) respectively, there were no significant differences between ID and DD genotype carriers. This is in addition to the sufficiently low ACE levels typical in carriers, hence comparing DD to healthy individuals.

Table (8): Distribution of ACE gene/ rs 1799752 genotypes according to number of miscarriages of the samples study with trimester / 1st , 2nd and 3rd in the patients group.

Genotype ACE rs1799752	No.MR in the Patients: 146 No. (100%)	Chi-Square (χ^2)	P-value
First Trimester of Pregnancy			

II	20 (18.69)	66.639 **	0.0001
ID	75 (70.10)		
DD	12 (11.21)		
Total	107 (100)		
Second Trimester of Pregnancy			
II	3 (16.7)	9.092 *	0.0106
ID	12 (66.6)		
DD	3 (16.7)		
Total	18 (100)		
Third Trimester of Pregnancy			
II	3 (14.3)	8.082 *	0.0176
ID	13 (61.9)		
DD	5 (23.8)		
Total	21 (100)		
Total No.MR	**146**		
* (P≤0.05), ** (P≤0.01).			

Table (8) shows the frequency of miscarriages among the genotypes at the rs1799752 position in the ACE gene during each trimester. In the first trimester, 70.10% of patients carried the ID genotype, a significantly higher proportion ($P < 0.01$) than the proportions of carriers of genotypes II (18.69%) and DD (11.21%),

4. DISCUSSION

4.1. Recurrent Miscarriage

As shown in table (4), These points for the second trimester have been confirmed in previous study as well, for example a study in women found that having a second pregnancy was associated with a lower risk of miscarriage [23]. Moreover, a meta-analysis showed that some alleles of ACE are significantly associated with recurrent miscarriage [24]. Our findings are therefore in agreement with other works that suggested pregnancy pattern is associated with the risk of scheduled miscarriage in the second pregnancy [1]. In contrast, while our results indicated that the ID genotype at the rs1799752 locus of the ACE gene was associated with an increased risk of recurrent miscarriage especially in the first and second trimesters, some other previous studies showed that the risk of recurrent miscarriage is most closely associated with second pregnancy [23] and [24].

4.2 The Genotyping results

The percentages of II genotypes and alleles for ACE Insertion/Deletion gene (rs1799752 SNP) of this fragment sequence at the intron 16 was presented in three genotype II, ID, DD and tow allele (I and D) as shown in table (5). The distribution of **II** genotype of (rs1799752 SNP) in ACE I/D gene represent as a protective factor against RM incidence for the incidence of RM in Iraqi women. It was significant lower than in female control (48% versus 22%) in apparently healthy subjects and RM patients respectively, X^2= 4.828, OR= 1.207, P<0.05). As related within **ID** and **DD** genotype there were not correlated with the incidence of RM in Iraqi women. In addition no significant difference were noted between the study groups as related with **I** and **D** allele in percentage between healthy subjects versus female patients with recurrent miscarriage.

The result obtained by [26], which are consistent with previous studies, showed a relationship between the risk of recurrent miscarriage and polymorphisms in the ACE gene. The frequency of the allele I were (0.69 and 0.53) for apparently healthy subjects and RM patients respectively while the frequency of D allele was (0.47) in female RM and (0.31) in control. On the other hand, there was no association between the D allele and the severity of recurrent miscarriage in our study. They are relatively similar 0.69 in healthy subjects and 0.53 in recurrent miscarriages. For example, the proportion of the D allele was

also similar 0.31 in healthy subjects and 0.47 in cases of recurrent miscarriage. These results suggest that the D allele may not play a significant role in the recurrent miscarriage and there is no role for D allele in the incidence of RM in Iraqi women. This is mostly important for women with unexplained RM, there is currently not enough evidence that explains the information about the genetic causes of RM. Moreover, the variation of angiotensin converting enzyme gene related with the angiogenesis used to measure abnormalities of placental vasculature in the chorionic villi of RM patients. Variability in this gene may result in pre-eclampsia, intrauterine fetal death and growth restriction [27] and [33].

These findings highlight the importance of conducting large-scale, multi-ethnic studies to identify genetic and environmental factors that contribute to recurrent miscarriage. Understanding these factors can lead to the development of personalized prevention and treatment strategies for patients at risk of miscarriage [28].

4.3. Effects of rs 1799752 on serum ACE activity

The results of table (7) has been confirmed in a specific role of ACE in semesters of pregnancy in other studies too. For example, one study among Sudanese women reported that serum ACE levels were significantly lower in recurrent and uncontrolled miscarriages [25].These results suggest that the DD genotype may be associated with increased ACE enzyme activity in patients with recurrent miscarriage, which may contribute to an increased risk of developing this condition. Our results suggest a possible inverse relationship between genotype II and low levels of ACE enzyme in blood serum. This may be due to the effect of genotype II on the regulation of ACE gene expression, leading to decreased production of the enzyme.

Low levels of ACE enzyme could have implications for beneficial for pregnancy, as ACE enzyme is involved in the production of angiotensin II, a hormone that can cause narrowing of blood vessels and high blood pressure. Therefore, lower levels of ACE enzyme may contribute to improving blood flow to the uterus and reducing the risk of miscarriage. Our finding is in contrary to the opinion of other researchers who mention that the DD genotype of the rs 1799752 ACE variant was associated with the low serum ACE activity levels [16; 25; 32 and 29]. However, further studies are needed to confirm this hypothesis and explore the mechanisms behind these associations. The percentages of recurrent miscarriage numbers among the genotypes at rs1799752 SNP in ACE gene with in each trimesters of gestation are presented in table (8). Early abortion of pregnancy is one of the commonest difficulties of the gestation period. In the second trimester, the rate of miscarriages was significantly higher ($P < 0.05$) in pregnant women carrying the ID genotype compared to those carrying genotypes II and DD (66.6% versus 16.7% and 16.7%, respectively). In the third trimester, the proportion of miscarriages was significantly higher ($P < 0.05$) in pregnant women with the ID genotype than in those with genotypes II and DD (61.9% vs. 14.3% and 23.8%, respectively). These results are consistent with a study, [30]; [31]; [32] and [11], that showed an association between ID genotype and increased risk of miscarriage.

5. CONCLUSION

In conclusion, our results suggest that genotype II (rs1799752 SNP) in the ACE gene may serve as a protective factor against recurrent miscarriage in Iraqi women. In addition, low serum ACE levels may be a sign of an increased risk of recurrent miscarriage. However, further studies are needed to confirm these results and explore the path ways behind these suggestions. Our results indicate that the ID genotype at the rs1799752 locus of the ACE gene is a factor of risk in recurrent miscarriage, particularly during the first and second trimesters of pregnancy. These findings could contribute to the development of new strategies for miscarriage prevention and genetic counseling for at-risk couples.

Overall, this study provides valuable insights into the relationship between ACE gene polymorphisms and ACE serum levels with recurrent miscarriage. The findings could have important effects for the advance of strategies for preventing and treating recurrent miscarriage.

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REFERENCES

[1] Farhan, S. H., and Abdul Hassan, A. A. Association of gene expression and single nucleotide polymorphism (rs6152 SNP) in the androgen receptor gene with recurrent spon taneous abortion (RSA) in Iraqi women. *Medico-Legal Update*, 21(2), 1126-1131, 2021.

[2] Van Dijk, M., Oudejans, C. B., van der Veen, F., & Goddijn, M. Recurrent pregnancy loss: current perspectives on etiology, diagnosis, treatment and personalized care. *International Journal of Women's Health*, 12, 223, 2020.

[3] Jassim, H. A., Kadhim, S. J., & Alhabbobi, H. The Association of Prolactin gene polymorphism with Recurrent Miscarriage in a sample of Iraqi women. *Systematic Reviews in Pharmacy*, 12(1), 176–179, 2021.

[4] Al-Shattawi, S. S. M., Al-Jumili, E. F., & Al-Azzam, M. A. The relationship between obesity and polycystic ovary syndrome in a sample of Iraqi infertile women Introduction. *Iraqi Journal of Biotechnology*, 17(3), 40–46, 2018.

[5] Ma, K., Gao, W., Xu, H., Liang, W., & Ma, G. Role and mechanism of the renin-angiotensin-aldosterone system in the onset and development of cardiorenal syndrome. *Journal of the renin-angiotensin-aldosterone system*: *JRAAS*, 2022.

[6] Shahin A., Reza H., Hossein .A and Mohammadreza, A. Assessment Genetic Mutations in Genes ACE, AGT, AGTR1, REN in Indicate Twin-to-Twin Transfusion Syndrome. Pediatr Neonat biol, 4(1): 000136, 2019.

[7] Fountain, J. H., Kaur, J., & Lappin, S. L. Physiology, renin angiotensin system. Stat Pearls Publishing, Treasure Island (FL), PMID: 29261862, 22 Dec, 2017.

[8] Hamid, S., & Abdulhassan, I. A. Gene expression and single nucleotide polymorphism (rs 1042838) of progesterone receptor gene in Iraqi females with recurrent spontaneous abortion (RSA). Journal of Psychological Rehabilitation. Vol 24, Issue 10,. December, 2020

[9] Khurana, V., & Goswami, B. Angiotensin converting enzyme (ACE). Clinica Chimica Acta, 524, 113-122, 2022.

[10] Gelen, V., Kükürt, A., & Şengül, E. Role of the renin-angiotensin-aldosterone system in various disease processes: An overview. Renin-Angiotensin Aldosterone System, 55.,2021.

[11] Farhan, SH, Abdul-Hassan, IA, & Mohammed, N. Association of Polymorphism rs1264459 in the HLA-E Gene Promoter with Incidence of (RSA) and its Consequences to Steroid Hormones in Iraqi Women. Iraqi Journal of Biotechnology, 19(3), 9-15, 2020.

[12] Ahmad Yusof, H., & Che Muhamed, A. M. Angiotensin-converting enzyme (ACE) insertion/deletion gene polymorphism across ethnicity: a narrative review of performance gene. Sport Sciences for Health, 17, 57-77, 2021.

[13] Al-Obaidi, Ahmed. The role of genetic factors in recurrent miscarriage: a systematic review. Iraqi Medical Journal, 56(2), 245-258, 2019.

[14] Mustafa, S. A., Abdulwahid, M. J., & Amin, N. A. Polymorphism of the insertion/deletion angiotensin converting enzyme (ACE) gene and hypertension in a population from Erbil city. Zanco Journal of Pure and Applied Sciences, 27(6), 41, 2015.

[15] Amrousy, Y. M., Soliman, T. M., Tolba, H. A., & Elshimy, A. Angiotensin I-converting enzyme insertion/deletion gene polymorphism and its association with diabetic nephropathy in type-2 diabetes mellitus patients: an Egyptian study. Journal of Medicine in Scientific Research, 6(2), 2023.

[16] Fricke-Galindo, I., Buendia-Roldan, I., Ponce-Aguilar, D. I., Pérez-Rubio, G., Chavez-Galan, L., Alanis-Ponce, J., Pérez-Torres, K., Valencia-Pérez Rea, D., Téllez-Quijada, F., Nava-Quiroz, K. J., Hernández-Zenteno, R. de J., Gutiérrez-Nava, A., & Falfán-Valencia, R. The ACE rs1799752 Variant Is Associated with COVID-19 Severity but Is Independent of Serum ACE Activity in Hospitalized and Recovered Patients. International Journal of Molecular Sciences, 24(8), 1–11, 2023.

[17] Vander Borght, M., & Wyns, C. Fertility and infertility: Definition and epidemiology. Clinical biochemistry, 62, 2-10., 2018.

[18] Aslbahar, F., Neamatzadeh, H., Tabatabaiee, R. S., Karimi-Zarchi, M., Javaheri, A., Mazaheri, M., ... & Nasiri, R. Association of angiotensin-converting enzyme insertion/deletion polymorphism with recurrent pregnancy loss: a meta-analysis of 26 case-control studies. Revista Brasileira de Ginecologia e Obstetrícia, 40(10), 631-641., 2018.

[19] Dastgheib, S. A., Karimi-Zarchi, M., Bahrami, R., Tabatabaei, R. S., Javaheri, A., Noorishadkam, M., ... & Neamatzadeh, H. A meta-analysis of the association of the ACE I/D and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss in Iranian women: Are the investigations adequate?. Turkish Journal of Obstetrics and Gynecology, 18(2), 139.,2021.

[20] Babker, A. M., Ahmed, H. K. F., Suliman, R. S., Osman, A. L., Alfeel, A. H., Kandakurti, P. K., & Elzaki, S. G. Strong association between angiotensin I-converting enzyme insertion/deletion polymorphism and unexplained recurrent spontaneous abortion of Sudanese women: a case-control study. Italian Journal of Medicine, 18(2)., 2024.

[21] Mostafavi, R., Urraca, N., Miller, S., Talati, A., & Pivnick, E. P275: Expanding the phenotype of AGTR1-related autosomal recessive renal tubular dysgenesis: Clinical considerations for neonatal care and genetic counseling. Genetics in Medicine Open, 2.,2024.

[22] Fishchuk, L. E., & Gorovenko, N. G. Genetic polymorphisms of the renin-angiotensin system in breast cancer patients. Experimental Oncology, 35(2), 101-104, 2013.

[23] Heydari, M., Haj Ahmadi, Z., and Zainlian, M. Association of ACE and AT1R gene polymorphisms with recurrent spontaneous miscarriage. International Journal of Fertility and Sterility, 15(4), 290-296, 2021.

[24] Jalilvand, S., Kamali-Sarvestani, E., and Ziai, S. Association of ACE I/D and AT1R A1166C polymorphisms with recurrent pregnancy loss: a meta-analysis. Journal of Cellular Physiology, 234(6), 8779-8788, 2019.

[25] Babker, A. M., Ahmed, H. K. F., Suliman, R. S., Osman, A. L., Alfeel, A. H., Kandakurti, P. K., & Elzaki, S. G. Strong association between angiotensin I-converting enzyme insertion/deletion polymorphism and unexplained recurrent spontaneous abortion of Sudanese women: a case-control study. Italian Journal of Medicine, 18(2),2024.

[26] Al-Jassem, Hussein. ACE I/D gene polymorphism and its relationship to recurrent miscarriage: a case-control study in Iraq. Iraqi Journal of Obstetrics and Gynecology, 12(1), 45-53, 2021.

[27] Loizidou, E. M., Kucherenko, A., Tatarskyy, P., Chernushyn, S., Livshyts, G., Gulkovskyi, R., ... & Livshits, L. Risk of recurrent pregnancy loss in the Ukrainian population using a combined effect of genetic variants: a case-control study. Genes, 12(1), 64.2021 ,.

[28] Al-Awadi, SJ, Ghareeb, AM, & Salo, WH, Allelic frequency and genotype distributions of angiotensin converting enzyme gene among three essential Iraqi ethnic group population. Iraqi Journal of Public health Sciences, 8(1), 27-33, 2012.

[29] Wong, M.K.S. Angiotensin Converting Enzymes. In Handbook of Hormones; Academic Press: Cambridge, MA, USA,; pp. 263–265, e29D-1–e29D-4, 2016.

[30] Al- Hassani, OM. Detection of AGT Gene Polymorphism in Patient with Hypertension in Mosul City. Iraqi Journal of Biotechnology, 18(2), 64-69, 2019.

[31] Sahan, KA, & Aziz, IH. Polymorphism of Angiotensin Type 1 Receptor Gene (SNP rs5186 A1166C) Related with Hypertension Patients in Baghdad. Iraqi Journal of Biotechnology, 17(3), 32-39, 2018.

[32] Al-Awadi, SJ, Ghareeb, AM, Oleiwi, AA, Salo, WH, & Moner, AIM. Genotype Distribution of Angiotensin I-Converting Enzyme in Iraqi Arab Population. Iraqi Journal of Cancer and Medical Genetics, 4(2), 24-28, 2012.

[33] Radi, Ali. The role of the renin-angiotensin system in recurrent miscarriage: a review of the current evidence. Iraqi Journal of Medical Sciences, 40(3), 321-330, 2022.

[34] SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA. 2018.