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Assessment of Estrogen Receptor Gene 1 PvuΠ Polymorphism and Its Relation to Male Infertility

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ABSTRACT

Background: Estrogen, which is usually associated with women, is important for a variety of physiological activities in men, involving metabolism of bone, testicular, cardiovascular, & sexual function.

Aim and objective: This study aimed to assess the correlation among estrogen receptor gene 1 Pavol Ivanyi (Pvu) Π polymorphism & male infertility.

Patients and methods: This Cross-sectional comparative research was performed on 92 Egyptian married men who were divided into 2 groups.

Results: There was a positive association among single nucleotide polymorphisms (SNPs) results & motility, vitality, and abnormal sperm forms in the fertile group (p<0.001, <0.001 & p= 0.008 respectively), but no significant alteration among SNP results and count or volume (p=0.416 & 0.087 respectively). There was a positive association among SNP result & sperm count (p=0.006), while there was no significant variance among SNP result & semen volume, total sperm motility, vitality and abnormal forms in infertile group (p= 0.512, 0.070, 0.145 & 0.676 respectively).

Conclusion: The distributions of Pvull genotype were not significantly various among infertile & fertile groups. However, there was positive correlation between SNP result and motility, vitality and abnormal sperm forms in fertile group. While in infertile group there was positive association among SNP result & sperm count where total sperm count (TSC) genotype was higher than total testosterone (TT) and clomiphene citrate (CC) genotypes.

Keywords: Estrogen receptor gene 1, Men, Infertility.

INTRODUCTION

The classification of infertility as a public health concern is maintained by the World Health Organization [(1)]. Infertility is defined by the World Health Organization & the American Society for Reproductive Medicine as the inability of a couple to conceive naturally following twelve months of consistent sexual activity without the use of contraceptives [(2)]. Infertility affects ten to fifteen percent of couples, with male factors accounting for half of the cases [(3)].

Estrogen, which is usually associated with women, is important for a variety of physiological activities in male, involving metabolism of bone, testicular, cardiovascular and sexual functions [(4)] and is present in semen in higher concentrations than in female serum [(5)]. The principal source of circulating estradiol in males is adipocytes' peripheral aromatization of circulating testosterone [(6)].

Estrogen functions via estrogen receptors (ERs), which are nuclear receptor proteins comprising a DNA-binding domain & an estrogen-binding domain [(7)]. The purpose of this research endeavor was to evaluate the association among infertility in men & the estrogen receptor gene 1 PvuΠ polymorphism.

PATIENTS AND METHODS

This was a cross-sectional comparative research performed on 92 Egyptian married men. They were grouped into infertile patients (n=46) & normal fertile men (n=46) in the Andrology Unit, Department of Dermatology, Venerology & Andrology, Suez Canal University Hospital and Cleopatra IVF centre in Ismailia (Relevant Private Sector).

Inclusion criteria: Male patients aged 20 – 60 years, sexually active patients suffering from infertility of more than 1 year and diagnosed by history and abnormal semen analysis.

Exclusion criteria: Hypogonadism, hormonal therapy, varicocele and history of acute urogenital infection.

Control group criteria: Male subjects aged 20 – 60 years and fertile men who were able to conceive within a year and had normozoospermic parameters.

Sample size calculation: The study population were enrolled using simple random sample technique of selection. For determining the sample size, the following formula was utilized:

$$n = \left[\frac{Z_{\alpha/2} + Z_{\beta}}{P_1 - P_2}\right]^2 (p_1 q_1 + p_2 q_2)$$

Dawson & Trapp [(8)].

Where:

n = sample size. Zα/2 = 1.96 (The critical value that separates the central ninety five percent of the Z distribution from the five percent in the tail). Zβ =

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0.84 (The critical value that separates the lesser twenty percent of the Z distribution from the upper 80%).
P1 = Prevalence / proportion of CC genotypes in the study population = 29.82% [9]. P2 = Prevalence / proportion of TT/TC genotypes in the study population = 70.18% [9].
q = 1-p.
So, by calculation, the sample size was 46 male infertile patients and other 46 age-matched healthy control subjects.

METHODS

All patients were subjected to the following: Physical examination. General examination: The development of secondary sex characters, body fat distribution, breast examination for gynecomastia, body hair distribution, signs of systemic disorders and abdominal scars. **Local genital examination:** The scrotum, penis, epididymis, testes, vas deferens, spermatic cord, & prostate were among the structures examined. Any scrotal discolouration, testicular asymmetry, and the position and size of the penile meatus opening were all recorded [10].

Investigations

Semen analysis: Semen samples were collected in a private room after sexual abstinence, with detailed instructions for collection and analysis. The samples were ejaculated into a sterilised glass container, kept between 20°C and 37°C to prevent temperature variations. The man's name, identification number, and collection date were recorded. The specimen was then placed in an incubator for analysis. Results were compared to **WHO's** standard values for semen analysis.

Reproductive hormones estimation:

Serum was separated from blood samples with an ELISA technique in order to measure prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (T), & estradiol (E2).

Genotyping of PvuΠ Polymorphism in ESR1 Gene: The genotyping for the Pvu polymorphism in the ESR1 gene was carried out utilizing the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method described by **Safarinejad *et al.* [11],** with the following major steps: a) Genomic DNA extraction from frozen whole blood samples, b) Amplification of ESR1 gene using thermal cycler PCR, c) Digestion of the product by PvuII restriction enzyme, d) Visualization and detection of digested products using gel electrophoresis.

Interpretation: After the fragments were separated, we looked at the gel to determine what sizes of bands were there. The DNA fragments illuminated when a gel was stained with a DNA-binding dye and exposed to UV light, allowing us to observe the DNA present at various positions along the length of the gel. We calculated the estimated size of bands in a sample by comparing them to a DNA ladder.

Ethical considerations: All participants signed informed written consent forms (Signature or fingerprints of participants). In case of illiterate participants, a witness signature is required. The consent contained: A clear and simple explanation of the study's goal for the general public. All information were deemed private and not be shared with anybody outside of the study. The study's results were announced to all participants. Participants were permitted to withdraw from the study at any time and without reason if they so desired. There is no financial recompense for the patients' time and effort. The study adhered to the Code of Ethics of the World Medical Association, specifically the Declaration of Helsinki, for research involving human subjects.

Statistical analysis

After being confirmed and coded by the researcher, the data were examined utilizing IBM-Statistical Package for Social Sciences (IBM-SPSS/PC/VER 21) analysis software. The distributional differences in the frequency of several risk variables between the two groups were compared utilizing the Chi square test. For normally distributed data, the mean variations in continuous variables between the two groups were tested utilizing the Student t-test. For non-parametric data, the median variations in continuous variables between the two groups were tested utilizing the Mann-Whitney test. For normally distributed data, a one-way ANOVA was computed to examine mean variations in continuous variables across groups. For non-parametric data, a Kruskall-Wallis test was computed to examine median variations in continuous variables between groups. The association between the most prevalent risk variables and the illness state was evaluated utilizing Spearman's Rank association Coefficient. Test findings were deemed significant when the p-value was ≤ 0.05.

RESULTS

There was significant variance among studied groups as regards semen volume, count, total motility, vitality, and abnormal forms ($p < 0.05$), while there wasn't significant variance among the studied groups regarding pus cells in semen p=0.054 (Table 1).

Table (1): Comparison among the two studied groups according to semen analysis

Semen Analysis	Fertile group	Infertile group	U	P
Volume (ml)	**(n = 46)**	**(n = 46)**		
Mean ± SD.	3.20 ± 0.98	2.52 ± 1.16	695.0*	0.004*
Count (x10^6/ml)	**(n = 46)**	**(n = 41)**		
Mean ± SD.	71.5 ± 35.35	7.32 ± 4.38	0.0*	<0.001*
Total Motility (%)	**(n = 46)**	**(n = 41)**		
Mean ± SD.	71.2 ± 13.47	24.67 ± 14.92	0.0*	<0.001*
Vitality	**(n = 46)**	**(n = 41)**		
Mean ± SD.	83.91 ± 9.66	38.62 ± 13.05	0.0*	<0.001*
Abnormal forms (%)	**(n = 46)**	**(n = 41)**		
Mean ± SD.	61.89 ± 8.33	81.46 ± 10.26	157.0*	<0.001*
Pus cells (per HPF)	**(n = 46)**	**(n = 46)**		
Mean ± SD.	1.59 ± 1.24	1.08 ± 1.0	811.5*	0.054

U: Mann Whitney test t: Student t-test P: p value for comparing among the studied groups *: Statistically significant at p ≤ 0.05

There were no significant variations in Pvull genotype distributions among fertile & infertile groups (P = 0.332). The genotype frequencies were CC: 23.9%, TC: 58.7% and TT: 17.4% for fertile men, while the genetic frequencies were CC: 30.4%, TC: 43.5%, and TT: 26.1% for infertile subjects (Table 2).

Table (2): Comparison among the 2 studied groups corresponding to molecular analysis of PvuII polymorphism

Molecular analysis	Fertile group (n = 46)		Infertile group (n = 46)		χ^2	p	OR	95% C.I.
	No.	%	No.	%				
SNP Result								
C/C®	11	23.9	14	30.4	2.203	0.332	1.000	–
T/C	27	58.7	20	43.5			0.582	0.22 – 1.55
T/T	8	17.4	12	26.1			1.179	0.36 – 3.89
Allele								
C®	49	53.3	48	52.2	0.022	0.883	1.000	–
T	43	46.7	44	47.8			1.045	0.59 – 1.86

χ^2: Chi square test OR: Odds ratio CI: Confidence interval

There was no significant distinction among SNP results regarding age, history of smoking or FSH level in fertile group p= 0.350, 0.715, 0.460 respectively (Table 3).

Table (3): Relation between SNP Result & variance parameters in fertile group (n= 46)

	SNP Result						Test of Sig.	p
	C/C (n= 11)		T/C (n= 27)		T/T (n= 8)			
	No.	%	No.	%	No.	%		
Age (years)								
Mean ± SD.	31.91 ± 7.52		32.44 ± 7.01		36.50 ± 8.80		F= 1.077	0.350
Smoking								
Non smoker	4	36.4	14	51.9	4	50.0	χ^2= 0.826	0.715
Smoker	7	63.6	13	48.1	4	50.0		
FSH (IU/l)								
Mean ± SD.	5.45 ± 2.17		6.07 ± 2.70		4.61 ± 1.55		H= 1.554	0.460

F: F for ANOVA test H: H for Kruskal Wallis test P: p value for association between different categories

There was a positive association among SNP results and motility, vitality, and abnormal sperm forms in the fertile group (p<0.001, <0.001 & p= 0.008 respectively), but no significant variance among SNP results and count & volume (p=0.416 & 0.087) (Table 4).

Table (4): Relation among SNP result & semen analysis in fertile group (n= 46)

Semen Analysis	SNP Result			Test of Sig.	p
	C/C (n= 11)	T/C (n= 27)	T/T (n= 8)		
Volume (ml)					
Mean ± SD.	2.87 ± 0.92	3.37 ± 0.98	3.06 ± 1.07	H= 1.754	0.416
Count (×10⁶/ml)					
Mean ± SD.	59.82 ± 48.75	76.81 ± 31.72	69.63 ± 23.96	H= 4.893	0.087
Total Motility (%)					
Mean ± SD.	57.27 ± 4.67	80.56 ± 8.59	58.75 ± 6.41	F= 50.919*	<0.001*
Vitality (%)					
Mean ± SD.	75.90 ± 6.64	89.07 ± 7.47	77.50 ± 8.86	H= 18.90*	<0.001*
Abnormal forms (%)					
Mean ± SD.	67.27 ± 6.07	58.89 ± 7.76	64.63 ± 9.01	H= 9.570*	0.008*

*: Statistically significant at p ≤ 0.05

There were no significant variations among SNP results and smoking, age, complaint and FSH in infertile group (p= 0.081, 0.893, 0.904 & 0.895 respectively) (Table 5).

Table (5): Relation among SNP Result & distinct parameters in infertile group (n= 46)

	SNP Result						Test of Sig.	p
	C/C (n= 14)		T/C (n= 20)		T/T (n= 12)			
	No.	%	No.	%	No.	%		
Smoking								
Non smoker	6	42.9	16	80.0	7	58.3	χ^2= 5.031	0.081
Smoker	8	57.1	4	20.0	5	41.7		
Age (years)								
Mean ± SD.	31.42 ± 6.80		30.90 ± 5.05		30.33 ± 5.99		F= 0.112	0.893
Complaint								
Primary infertility	11	78.6	15	75.0	10	83.3	χ^2= 0.361	0.904
Secondary infertility	3	21.4	5	25.0	2	16.7		
FSH (IU/l)								
Mean ± SD.	7.26 ± 1.03		5.72 ± 1.48		5.40 ± 1.59		H= 0.223	0.895

MC: Monte Carlo

There was a positive association among SNP result & sperm count (p=0.006), while there was no significant variance among SNP result and semen volume, total sperm motility, vitality and abnormal forms in infertile group (p= 0.512, 0.070, 0.145 & 0.676 respectively (Table 6).

Table (6): Relation among SNP Result & semen Analysis in infertile group (n= 46)

Semen Analysis	SNP Result			Test of Sig.	p
	C/C (n= 14)	T/C (n= 20)	T/T (n= 12)		
Volume (ml)					
Mean ± SD.	2.49 ± 1.31	2.70 ± 1.04	2.23 ± 1.22	H= 1.337	0.512
Count (×10⁶/ml)					
Mean ± SD.	4.80 ± 3.67	9.75 ± 3.64	6.73 ± 4.57	H= 10.109*	0.006*
Total Motility (%)					
Mean ± SD.	18.93 ± 12.58	30.25 ± 15.26	22.08 ± 14.69	F= 2.827	0.070
Vitality (%)					
Mean ± SD.	30.35 ± 15.86	38.0 ± 18.80	30.0 ± 18.46	H= 3.858	0.145
Abnormal forms (%)					
Mean ± SD.	82.14 ± 9.14	70.0 ± 31.12	67.50 ± 33.41	H= 0.782	0.676

H: H for Kruskal Wallis test P: p value for association among different categories *: Statistically significant at p ≤ 0.05.

DISCUSSION

Estrogen receptors were implicated in the physical actions of estrogens (ERs). Two subtypes of ER have been identified: ERα & ERβ. The ESR1 gene on chromosome 6q25 encodes ERα, which is a 595-amino-acid protein, while the ESR2 gene on chromosome 14q22-24 encodes ERβ, which is a 530-amino-acid protein [(12)]. Several polymorphic locations in the ERα gene locus have been discovered through genetic screening [(13)]. Two polymorphisms in the ERα intron 1 have been extensively examined. These polymorphisms are the T/C transition (rs2234693) and the G/A transition (rs9340799) within fifty base pairs downstream of the former [(14)].

PvuII and XbaI are the single nucleotide polymorphisms (SNPs) in ESR1 that have been investigated the most frequently. PvuII is also known as IVS1-397 T/C or rs2234693, while XbaI is also known as IVS1-351 A/G or rs9340799. When it comes to the former, the T and C alleles are commonly stated as the p and P alleles, and when it comes to the latter, they are frequently described as the x and X alleles, respectively. There is a correlation between the ESR1 XbaI polymorphism and azoospermia or idiopathic severe oligospermia in men [(15)].

The analysis of our data revealed no statistically significant variance (P = 0.332) in the distributions of Pvull genotypes among the fertile & infertile groups. The genotype frequencies were CC: 23.9%, TC: 58.7% and TT: 17.4% for fertile men, while the genetic frequencies were CC: 30.4%, TC: 43.5% and TT: 26.1% for infertile subjects, and the observed genotype frequencies were consistent with Hardy-Weinberg for SNP Result.

In their meta-analysis conducted in Iran, **Mobasseri *et al.*** [(16)] found a strong protective correlation among ER-PvuII & male infertility in the homozygote model (p = 0.042). A similar link was discovered in the asthenozoospermia subgroup (p = 0.025). In the heterozygote co-dominant model, the ER-PvuII polymorphism was likewise correlated with a lower incidence of men infertility, according to meta-analysis (p = 0.042). In the heterozygote co-dominant model, research with sample sizes of less than 400 participants also indicated a significant connection (p = 0.023). The bioinformatics results suggested that the ER-PvuII polymorphism could have a big impact. **Liaqat *et al.*** [(17)] found that statistically there was no significant variance observed in the frequencies of the T and C alleles between cases & controls with regard to the PvuII polymorphism (p=0.76013). Infertile individuals had a higher prevalence of the heterozygous TC PvuII genotype, which was strongly associated with an increased risk of infertility compared to the homozygous TT (p=0.00003) & uncommon homozygous CC (p=0.00005) genotypes among cases & controls respectively. The presence of rare homozygous CC genotypes and homozygous TT (p=0.84154) did not correlate significantly with infertility risk in any of the patient. A significant association was observed between individuals carrying the heterozygous genotype TC & the probability to have infertility when combined with patients carrying the uncommon homozygous genotype CC (p=0.00158).

ESR1 PvuII variant C allele carriers were also correlated with a lower possibility of men infertility (CT vs. TT, OR = 0.78, Ninety-five percent confidence interval (CI): 0.62–0.98), according to **Ge *et al.*** [(18)]. Further ethnic subgroup studies revealed that the ESR1 PvuII polymorphism is associated with a lower possibility in Asian populations & an enhanced risk in Caucasian populations. **Safarinejad *et al.*** [(11)] found that the examined SNPs had a statistically significant effect on the three main sperm parameters (motility concentration & morphology) [(11)].

Also, in this study the sperm count in ER-α PvuII TC genotype (9.75 ± 3.64) was higher than that found in ER-α PvuII TT genotype (6.73 ± 4.57), and ER-α Pvull CC genotype (4.80 ± 3.67), while there was no significant relation between different genotypes and semen volume, total sperm motility, vitality and abnormal forms in infertile group. **Lazaros *et al.*** [(9)] demonstrated a link between ER polymorphisms & sperm motility & concentration, highlighting the gene's importance in spermatogenesis & sperm quality.

LIMITATIONS

Our research was limited to the Egyptian population & hence cannot be generalized to other ethnic or racial groups. We still don't have accurate explanation of causative correlation for those particular polymorphisms. We didn't have enough power to figure out interactions or conduct subgroup analysis.

CONCLUSION

The distributions of Pvull genotype were not significantly variance among infertile & fertile groups. However, there was positive correlation between SNP result and motility, vitality and abnormal sperm forms in fertile group. While in infertile group there was positive correlation among SNP result & sperm count where TC genotype was higher than TT and CC genotypes.

DECLARATIONS

- **Consent for publication:** I certify that each author granted permission for the work to be submitted.
- **Funding:** No fund
- **Availability of data and material:** Available.
- **Conflicts of interest:** No conflicts of interest.
- **Competing interests:** None.

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