Gene Polymorphism Of TNF-A (Rs1799964) In Aborted Women With Respiratory Disease In Al-Diwaniyah Province

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KEYWORDS
Aborted Women, TNF-A , Polymorphism (SNP), Respiratory Diseases

ABSTRACT
Background: TNF-α, a proinflammatory cytokine, plays a significant role in the etiology of various illnesses. The encoding gene is found on chromosome 6's short arm in the major histocompatibility complex class III region. Polymorphisms in the TNF-α gene promoter region are believed to impact illness susceptibility and severity. This review summarizes the research on the association between TNF-α gene and receptor polymorphisms and respiratory illness development. This study aim to determine of gene polymorphism of TNF-α in aborted women with respiratory diseases.
Methods : The study was carried out for 100 pregnant women, including 60 aborted women with and without respiratory diseases, and 40 women as healthy control group. Genotypes in TNF-α T>C SNPs were identified using self- designed nested T-ARMS PCR tests. Sequencing validated randomly chosen PCR results that represented unique genotypes in TNF-α SNPs.
Results : The results of TNF-α (rs1799964) C/T SNP genotyping between aborted women with respiratory diseases and healthy control revealed that the heterozygous C/T genotype was a significant risk factor with an OR of 6.28, while the homozygous TT genotype was non-significant (OR= 2.09).
Conclusion : The rs1799964 TNF-α polymorphisms are possible genetic risk factors of respiratory diseases and might be its predictive markers.

1. Introduction

Point mutations can result in gene polymorphisms, also known as single nucleotide polymorphisms (SNPs), which are single nucleotide variations in certain DNA sequences in the homologous interval (1). SNPs are present in more than 1% of the general population, while other mutations are found in fewer than 1% of individuals. (2).

SNPs occur in both coding and non-coding sequences. Some SNPs in coding areas may be unrelated to changes in the amino acid sequence and hence have no functional implications. SNPs in non-coding areas, on the other hand, may disrupt the nucleotide sequence excision process causing complete mRNA breakdown (3). This study aim to determine of gene polymorphism of TNF-α in aborted women with respiratory diseases.

2. Methodology

Collection of Blood Samples

Blood samples were taken from 100 samples , including 60 aborted women with and without respiratory diseaes, and 40 women as control group in Maternity and Children's Teaching Hospital in Al-Diwaniyah city. The collection period extended from October 2023 to January2024 . A 2 ml of venous blood were collected in aseptic condition technique, it were put in ethylenediamine tetraacetic acid (EDTA) tube to use for DNA exaction.

Genomic DNA Extraction

Genomic DNA was extracted from frozen blood samples using the gSYAN DNA kit (extraction kit) provided by Geneaid, Taiwan, following company instructions. The collected blood genomic DNA was tested using a Nanodrop spectrophotometer (Techne, UK) to measure the concentration of DNA (ng/µL) and check the purity of the DNA by reading the absorbance at (260/280 nm).

Tetra- ARMS-PCR Method

The ARMS-PCR reaction (tetra-primer amplification refractory mutation system- polymerase chain) is a simple and cost-effective method for genotyping single nucleotide polymorphisms. It uses four primers in a single PCR and is followed solely by gel electrophoresis. However, the optimisation stage may be time-consuming and labour intensive. Two SNPs were selected under various amplification settings. DNA extraction methods, annealing temperatures, PCR cycle protocols, reagents, and primer concentrations were all tested. The use of T-ARMS-PCR to SNPs in cytosine and guanine-rich DNA regions. The melting temperature was determined to be the most interfering component. Small reagent concentration changes, notably MgCl2, have a considerable impact on PCR. Additionally, the inner primer band must be modified. So, to balance the inner primer band, Find the owner of the weaker band and enhance it by increasing its concentration.

Table (1): The T-ARMS-PCR Primers for TLR2 rs1898830 A/G gene polymorphism with their sequence and product size.

T-ARMS-PCR Primer	**Sequence (5'-3')**	**Product size**
Forward inner primer (C allele):	AAGCA AAGGAGAAGCTGAGAATA C	**183bp**
Reverse inner primer (T allele)	AGACCCTGACTTTTCCTGCA	**207bp**
Forward outer primer	TGTGTCTGGGAGTGAGAACTTC	**346bp**
Reverse outer primer	**CATACTCGACTTCCATAGCCCT**	

Statistical analysis :

Social sciences statistical software (SPSS) version 26 and Microsoft Office Excel 2010 were used for data collection, summarization, analysis, and presentation. The standard deviation and range of numerical values were displayed. If the variable had a regular distribution, any two groups' mean differences may be compared using an independent sample t-test.

The association between any two category variables was examined using the chi-square test. The thresholds for significance were established at 0.01 or less for the level of significance and 0.05 or less for the P-value.

3. Results and discussion

The TNF-α distribution of rs1799964 polymorphism was detected by T-ARMS-PCR technique. Figure (1):

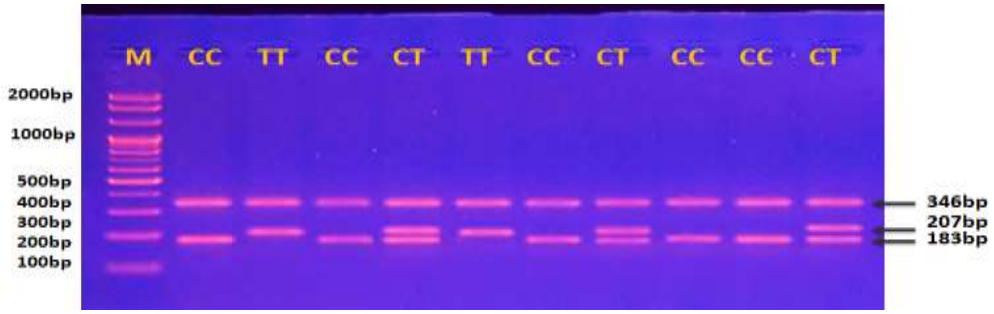

Figure (**1**): Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of TNF-α-1031 C/T(rs1799964) gene polymorphism in some patients samples. Where M: marker (2000-100bp). The lane (CC) wild type homozygote were showed only C allele at 207bp T-ARMS-PCR product. The lane (TT) mutant type homozygote were showed only T allele at 183bp T-ARMS-PCR product , whereas the (CT) heterozygote were showed as both C and T allele at 207bp and 183bp T-ARMS-PCR product . The outer internal control were observed at 346bp T-ARMS-PCR product.

Table (2) displays the genotype and allele frequency comparison of TNF-α(rs1799964) C/T SNP between patients and healthy controls. In terms of genotype frequency, there was a non-significant difference ($p < 0.05$) in the genotype frequency distribution between the patient and control groups. The homozygous TT genotype was found to be a non-significant risk factor (OR= 2.52) by risk analysis. This indicates that compared to patients with other genotypes, those with homozygous TT genotype are around three times more likely to acquire illness. In terms of the allele analysis, P=0.128 indicated that there was no significant difference between the patient and control groups.

Table (2): *TNF-α (rs1799964) C/T* POLY genotype frequency in patients and healthy

TNF-α (rs1799964)	**Patients *n* = 60**	**Control *n* = 40**		***P***	**OR**	**95% CI**
Genotype frequency						
TT	14 (23.3%)	4 (10.0 %)		0.128	2.52	**0.74-8.56**
C/T	10 (16.7%)	10 (25.0%)		0.527	0.72	**0.26-1.98**
CC	36 (60.0 %)	26 (65.0 %)		**Reference**		
Allele frequency						
T	38 (31.7%)	18 (22.5%)	0.15 7	1.59		**0.84-3.05**
C	**82 (68.3%)**	**62 (77.5%)**		**Reference**		

n: number of cases; ¥: Chi-square test; **NS**: not significant at $P > 0.05$.

The frequency distribution of genotypes varied significantly (p>0.05) between the with and without respiratory diseases in terms of genotype frequency **Table(3)**. The heterozygous C/T genotype was a significant risk factor with an OR of 6.28, but the homozygous TT genotype was a non-significant risk factor (OR= 2.09), according to risk analysis. Accordingly, compared to patients with other genotypes, those who have a homozygous TT genotype are around twice as likely to experience a respiratory illness. In terms of allele analysis, there was a significant difference (P=0.048) between women who had respiratory disorder and those who did not .

Table (3) *TNF-α (rs1799964) C/T* POLY genotype frequency in patients with/without respiratory diseases

TNF-α (rs1799964)	**Positive *n* = 30**	**Negative *n* = 30**	***P***	**OR**	**95% CI**
Genotype frequency					
TT	8 (26.7%)	6 (20.0 %)	0.243	2.09	**0.59-7.33**
C/T	8 (26.7%)	2 (6.7%)	0.021*	6.28	**1.16 -34.1**
CC	14 (46.6 %)	22 (73.3 %)	**Reference**		

Allele frequency					
T	24 (40.0%)	14 (23.3%)	0.048*	2.19	**0.99-4.82**
C	**36 (60.0%)**	**46 (76.7%)**		**Reference**	

n: number of cases; ¥: Chi-square test; **NS**: not significant at $P > 0.05$.

Discussion

The current study reveals that frequent genetic variations within TNF-α put the Iraqi population at risk for abortion. Prior research examined the functions of TNF-α polymorphisms -308G/A, -850T/C, -238G/A, -1031T/C, and -863A/C in women who had abortions; these findings were corroborated by functional evidence demonstrating the TNF-α cytokine's malfunction. (4). Within a brief period of time, multiple research teams have investigated the significance of TNF-α polymorphisms and proposed a robust correlation with the onset of disease. (5), Therefore, the findings of this investigation provide a unique replication and reinforce the known link between TNF- α and abortion. TNF-α contains a number of well-known SNPs. Therefore, Tetra-ARMS-PCR analysis was used to assess the genetic connections between TNF-α (rs1799964) and abortion risk in order to determine whether genetic variants in TNF-α (rs1799964) may be used as a potential genetic marker to predict the likelihood of an abortion. Unexplained abortion rates in Saudi females have been found to be associated with the TNF-α gene promoter's -308 G/A polymorphism. (6)

For this study, the TNF-α (rs1799964) T/C polymorphism gene and allele frequencies were measured in 60 patients who had abortions and 40 healthy controls . Overall, there are statistically non-significant differences in the genotype frequencies of TNF-α (rs1799964) C/ T between the patients who had abortions and the healthy controls. However, the current findings showed that a higher risk of abortion is linked to the TNF-α (rs1799964) T/C polymorphism. These outcomes align with study which demonstrated that a substantial risk gene for abortion was identified as TNF-α (rs1799964) C/T (7). According to another study, the case group had greater frequencies of the TT genotype and T allele of TNF-α (rs1799964) C/T than the control group did. Both genotypes were also shown to be higher in the case group than in the control group (5).

The frequencies of the CC, CT, and TT genotypes were 36, 10, 14 and 26, 10 and 4, among abortion cases and control, respectively. The *TNF-α (rs1799964) C/T* polymorphism was non- significant associated with a high risk of abortion (TT vs CC: OR = 2.52; 95% CI = 0.74-8.56). The TT genotype was present more 14 (23.3%), among patients women with abortion which indicates a high carrier rate in present study group for this allele.

The comparison of genotypes and allele frequencies concerning *TNF-α (rs1799964) C/T* SNP between patients and control groups . Regarding the genotype frequency there was non-significant difference between patients and control groups ($p > 0.05$).

On the other hand, the present results showed non-significant association between the *TNF-α (rs1799964) C/T* variant T allele and a higher abortion risk (OR= 1.59; 95% CI 0.84-3.05). The odds ratio and 95% confidence interval were used to assess the frequencies of alleles in each polymorphism. Abortion and the incidence of TNF-α polymorphism were strongly significantly correlated ($p<0.001$) ,(5).

Another research reported that variations in the promoter region can affect the production of TNF-α (8). According to (9), TNF-α polymorphism has been linked to elevated levels of TNF-α and has the potential to induce abortion. Further research indicates that TNF-α single nucleotide gene polymorphisms have been linked to changed TNF-α release, which may be the pathophysiology of

abortion.(10)

4. Conclusion and future scope

The rs1799964 TNF-α polymorphisms are possible genetic risk factors of respiratory dieases and might be its predictive markers.

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