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# Tumor Necrosis Factor-Alpha Gene Polymorphism in diabetic patients with Respiratory Failure Undergoing Intensive Care Treatment

**Mahaboob Vali Shaik [1*], Munni Shaik [1], John Basha Shaik [2], G Jayaram [3], Babulal S [4], Nissi Molli[5], Swarna Deepak Kuragayala[6]**

1. Department of Research, Nimra Institute of Medical Sciences, Vijayawada, India.
2. Department of Biochemistry, MNR Medical College & MNR University, Sangareddy.
3. Department of Physiology, Nimra Institute of Medical Sciences, Vijayawada, India.
4. Department of Pulmonology, Nimra Institute of Medical Sciences, Vijayawada, India
5. Student, International National Indian School, Al Jubail, Saudi Arabia.
6. Department of Critical Care Medicine, Apollo Health City, Jubilee Hills, Hyderabad, Telangana 500033. India.

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*** Corresponding author:**

Mahaboob Vali Shaik, Ph.D.

**Address:** Department of Research, Nimra Institute of Medical Sciences, Vijayawada-521456, Andhra Pradesh, India.

**E-mail:** valishaik31@gmail.com

**Abstract**

**Introduction:** Reactive oxygen species (ROS) levels increase in diabetes mellitus due to excessive glucose oxidation. The inflammatory response in cells triggers the activation of proinflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-1, interleukin (IL)-6, and interleukin (IL)-18. Variations in the promoter region of the TNF-α gene can influence both the susceptibility to and severity of the disease. This research examines the correlation between TNF-α gene polymorphisms and the development of respiratory distress in patients with diabetes who are undergoing treatment in an intensive care unit.

**Materials and Methods:** A total of 100 participants were involved in the study, comprising 50 individuals diagnosed with diabetes and an additional 50 non-diabetic individuals who served as a control group. The nested T-ARMS PCR assay was employed to determine the genotypes associated with the TNF-α T>C single nucleotide polymorphisms (SNPs). Randomly selected samples underwent sequencing to validate the PCR findings, which revealed distinct genotypes for the TNF-α SNPs. Patient data were collected, and laboratory variables were assessed. The information was subsequently entered into SPSS Version 26.

**Results:** The presence of the heterozygous C/T genotype emerged as a notable risk factor, exhibiting an odds ratio (OR) of 5.87 (65% CI, 1.12 - 27.8) with a p-value of 0.03. In contrast, the homozygous TT genotype did not demonstrate statistical significance, presenting an OR of 1.87 (95% CI, 0.59 - 7.33) and a p-value of 0.25. These findings are derived from the genotyping of the TNF-α (rs1799964) C/T single nucleotide polymorphism (SNP) in a cohort comprising patients experiencing respiratory distress and a control group.

**Conclusion:** The association between the emergence of respiratory diseases and the TNF-α (rs1799964) T/C polymorphism has been established. This specific genetic variant may represent a potential risk factor for the development of respiratory distress and could also function as a prognostic indicator for patients with diabetes who are undergoing treatment in intensive care units.

**Keywords:** intensive care unit, Tumor necrosis factor alpha, respiratory distress, single nucleotide polymorphism.

## 1. Introduction

In 60% of patients diagnosed with diabetes mellitus, abnormalities in pulmonary function have been observed [1,2]. A study indicated that individuals with undiagnosed diabetes (4.6%) and those with insulin-dependent diabetes (4.5%) exhibited a higher incidence of reintubation compared to their nondiabetic counterparts (1.8%; $p < 0.01$). Within the intensive care unit setting, patients with undiagnosed diabetes (9.9%) and insulin-dependent diabetes (8.6%) were more likely to require mechanical ventilation for durations exceeding twenty-four hours than nondiabetic patients (4.8%; $p < 0.01$) [3].

Chronic inflammation is believed to play a significant role in the pathophysiology of respiratory diseases, particularly in individuals suffering from chronic obstructive pulmonary disease (COPD). Research has demonstrated that patients with COPD exhibit increased levels of tumor necrosis factor-alpha (TNF-α) in both sputum and serum, indicating its involvement in the systemic and localized inflammatory processes associated with the condition [4,5].

As a pro-inflammatory cytokine, TNF-α is integral to the inflammatory response. The gene encoding TNF-α is located on chromosome 6 (6p21.3) [6]. Multiple studies have shown elevated concentrations of TNF-α in the sputum, bronchoalveolar lavage fluid, and bronchial biopsies of individuals with COPD, underscoring its significant role in the disease's progression [7,8,9].

A variety of studies have explored the association between TNF-α polymorphisms and the risk of developing COPD, with particular emphasis on the TNF-α +489 G/A polymorphism, which has been implicated in the onset of the disease. Previous research has also established a link between the TNF-α gene polymorphism (-308G/A) and susceptibility to tuberculosis [10].

Significant associations between the TNF-α gene polymorphism (-308G/A) and the prevalence of type 2 diabetes mellitus (T2DM) have been documented in numerous previous studies [11]. However, there is a lack of published research addressing gene polymorphisms in T2DM patients who are experiencing respiratory distress. The present investigation seeks to explore the distribution of TNF-α gene polymorphism among T2DM patients undergoing treatment in intensive care units (ICUs), building upon the insights gained from earlier research. Consequently, this study will concentrate on the TNF-α gene polymorphism and its potential role in the onset of respiratory distress in individuals with diabetes [12].

## 2. Materials and Methods

### Inclusion & Exclusion Criteria

A total of 100 patients treated in the intensive care unit (ICU) for various underlying conditions were included in the study, comprising 50 individuals with diabetes and 50 without. Patients with a prior history of treatments that influenced their TNF-α levels or those diagnosed with cancer were excluded from the study.

### Obtaining Blood Samples

A comprehensive clinical examination was conducted, during which standard measurements and a fundamental diabetes profile were obtained. Blood samples were collected from all 100 participants, comprising 50 individuals diagnosed with diabetes and 50 non-diabetic subjects serving as the control group. For the purpose of DNA extraction, 5 mL of venous blood was collected and transferred into a tube containing ethylenediamine tetraacetic acid (EDTA).

### Genomic DNA isolation

Genomic DNA was isolated from blood samples in accordance with the instructions provided in the DNA extraction kit. The extraction of mononuclear cells (MNCs) from these blood samples was accomplished utilizing the HISTOPAQUE density gradient centrifugation technique. Subsequently, aliquots of purified DNA were obtained from the peripheral blood mononuclear cells through the application of the QIAmp DNA micro kit (Qiagen, Hilden, Germany). The quality and concentration of the extracted genomic DNA were assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA), which measured absorbance at wavelengths of 260 and 280 nm to evaluate both the DNA content (ng/µL) and its purity.

### Tetra- ARMS-PCR procedure

In the ARMS-PCR procedure, four primers are used within a single PCR reaction, which is subsequently analyzed through gel electrophoresis. Two single nucleotide polymorphisms (SNPs) were selected under varying amplification conditions. T-ARMS-PCR is utilized to detect SNPs located in regions of DNA that are rich in cytosine and guanine. The most significant factor affecting the process was identified as the melting temperature. Additionally, PCR is

notably sensitive to minor fluctuations in reagent concentrations, especially magnesium chloride (MgCl2). An adjustment was made to the inner primer band. Consequently, it is essential to determine the source of the weaker band and enhance its concentration to achieve equilibrium with the inner primer band. The DNA bands were visualized and documented using a gel documentation system (Syngene, US).

**Table 1**. The T-ARMS-PCR Primers for TLR2 rs1898830 A/G gene polymorphism

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

### Statistical analysis

The chi-square test was employed to examine the association between pairs of categorical variables. The distribution of numerical values was presented along with the corresponding standard deviation. In cases where the variable exhibited a normal distribution, an independent sample t-test was applied to assess the mean differences between two groups. Data analysis was conducted using Version 26 of the Statistical Package for the Social Sciences (SPSS). The significance levels were established at a p-value of 0.05 or lower, and a significance level of 0.01 or lower.

## 3. Results

The fundamental clinical and laboratory parameters of the patients in both the case and control groups showed variability in their measurements [Table 1]. Regarding genotype frequency, there was no significant difference in the distribution of genotypes between the patient and control groups. Risk assessment indicated that the homozygous TT genotype did not represent a significant risk factor (OR= 2.16). This finding suggests that individuals with the homozygous TT genotype are approximately three times more likely to experience adverse outcomes compared to those with alternative genotypes. The allele analysis, with a p-value of 0.082, indicates that there is no significant difference between the case and control groups [Table 2].

**Table 2.** Basic characteristics and laboratory variables of diabetic cases and control individuals

| Demographics | Controls (Mean±SD) | Cases (Mean±SD) | p-value |
|---|---|---|---|
| Age (years) | 42.5±16.5 | 41.65±17.5 | 0.09 |
| Gender | | | |
| Male | 28 | 25 | 0.22 |
| Female | 22 | 25 | |
| BMI (kg/m$^2$) | 24.04±2.03 | 24.34±1.11 | 0.1 |
| HTN | | | |
| Yes | 10 | 18 | 0.2 |
| No | 40 | 32 | |

| | | | |
|---|---|---|---|
| Treatment of DM | | | |
| Oral | - | 28 | - |
| Insulin | - | 14 | - |
| Both | - | 8 | - |
| Duration of diabetes (years) | - | 10.1±10.08 | - |
| SBP | 115.8±10.5 | 121.5±15.5 | 0.0001 |
| DBP | 77.9±12.5 | 89.6±16.9 | 0.0001 |
| Fasting blood sugar (mg/dl) | 98.5±10.5 | 125.6±15.5 | 0.025 |
| Total cholesterol (mg/dl) | 185.6±20.8 | 250.5±50.5 | 0.0001 |
| Triglycerides (mg/dl) | 100.7±19.5 | 245.31±52.5 | 0.0001 |
| HDL-C (mg/dl) | 49.6±7.5 | 43.18±10.5 | 0.0001 |
| LDL-C (mg/dl) | 52.5±12.5 | 135.5±40.8 | 0.0001 |
| VLDL-C (mg/dl) | 21.3±11.6 | 49.9±11.2 | 0.0001 |
| HbA1c (%) | 5.49±0.6 | 8.6±2.6 | 0.0002 |

p value is considered as significant when p ≤ 0.05

**Table 3.** Genotype and allele frequency comparison of TNF-α(rs1799964) C/T SNP between patients and controls

| TNF-α (rs1799964) | Patients n = 50 | Control n = 50 | P value | OR | 95% CI |
|---|---|---|---|---|---|
| Genotype frequency | | | | | |
| TT | 11 (22.0%) | 5 (10.0 %) | 0.098 | 2.16 | 0.58-7.12 |
| C/T | 8 (16.0%) | 13 (26.0%) | 0.457 | 0.66 | 0.26-1.88 |
| CC | 31 (62.0%) | 32 (65.0%) | Reference | | |
| Allele frequency | | | | | |
| T | 32 (32.0%) | 23 (23.0%) | 0.082 | 1.46 | 0.69-2.78 |
| C | 68 (68.0%) | 77 (77.0%) | Reference | | |

p value is considered as significant when p ≤ 0.05

**Table 4.** TNF-α (rs1799964) C/T POLY genotype frequency in patients with/without respiratory distress

| TNF-α (rs1799964) | Positive n = 25 | Negative n = 32 | P value | OR | 95% CI |
|---|---|---|---|---|---|
| Genotype frequency | | | | | |
| TT | 6 (24.0%) | 7 (21.87%) | 0.25 | 1.87 | 0.59-7.33 |
| C/T | 6 (24.0%) | 3 (9.37%) | 0.03 | 5.87 | 1.12 -27.8 |

| | | | | | |
|---|---|---|---|---|---|
| CC | 13 (52.0 %) | 22 (68.75%) | Reference | | |
| Allele frequency | | | | | |
| T | 38 (38.0%) | 14 (23.3%) | 0.025 | 2.09 | 0.85-4.56 |
| C | 62 (62.0%) | 46 (76.7%) | | Reference | |

p value is considered as significant when $p \leq 0.05$

Risk analysis indicates that the homozygous TT genotype does not present a significant risk factor (OR=1.87), whereas the heterozygous C/T genotype was identified as a significant risk factor (OR=5.87). Consequently, individuals possessing the homozygous TT genotype are approximately twice as likely to experience respiratory issues compared to those with alternative genotypes. Furthermore, a significant difference (P=0.025) was observed in allele analysis between patients experiencing respiratory distress and those without. Additionally, genotype frequency analysis revealed marked differences between diabetic patients with respiratory conditions and those without.

## 4. Discussion

The present study indicates that the patient cohort is susceptible to respiratory failure due to the prevalence of genetic variants in TNF-α. Specifically, polymorphisms such as -308G/A, -850T/C, -238G/A, -1031T/C, and -863A/C were analyzed in individuals experiencing respiratory failure, with functional data corroborating the dysfunction of the TNF-α cytokine. Numerous research groups have recently investigated the significance of TNF-α polymorphisms, proposing a robust correlation with the onset of respiratory conditions. Consequently, the findings of this research provide a clear replication and reinforce the established link between respiratory failure and TNF-α. Several well-documented single nucleotide polymorphisms (SNPs) exist within TNF-α [13].

To determine whether genetic variants in TNF-α (rs1799964) could serve as potential genetic markers for predicting the risk of respiratory failure, Tetra-ARMS-PCR analysis was utilized to explore the genetic associations between TNF-α (rs1799964) and the likelihood of respiratory distress. The -308 G/A polymorphism in the TNF-α gene promoter has been associated with unexplained cases of respiratory failure among Saudi females [14]. In this investigation, the TNF-α (rs1799964) T/C polymorphism gene and allele frequencies were evaluated in a sample of 50 patients with respiratory distress and 50 controls. The genotype frequencies of TNF-α (rs1799964) C/T in both groups exhibited statistically insignificant differences. Nevertheless, the current study's findings suggest that the TNF-α (rs1799964) T/C polymorphism is associated with an elevated risk of respiratory distress, aligning with previous research that identified TNF-α (rs1799964) C/T as a significant risk factor for respiratory failure. [15].

In an alternative investigation, the T allele of TNF-α (rs1799964) C/T and the TT genotype were found to be more prevalent in the case group compared to the control group. Furthermore, the case group exhibited elevated levels of both genotypes relative to the control group. Among individuals experiencing respiratory distress and those in the control group, the observed frequencies of the CC, CT, and TT genotypes were 31, 8, 11, and 32, 13, and 5, respectively. The increased risk of respiratory failure did not show a significant association with the TNF-α (rs1799964) C/T polymorphism (TT versus CC: OR = 2.16; 95% CI = 0.58-7.12). Within the respiratory distress cohort, the TT genotype was notably more common, with a prevalence of 11 (22.0%), suggesting a high carrier rate for this allele in the studied population.

The analysis of genotype and allele frequencies between the patient and control groups concerning the TNF-α (rs1799964) C/T SNP indicated no significant differences. Nevertheless, the current results demonstrated a non-significant trend toward an increased risk of respiratory failure and the T allele of the TNF-α (rs1799964) C/T variation (OR= 1.46; 95% CI 0.69-2.78). The allele frequencies for each polymorphism were assessed using odds ratios and 95% confidence intervals, revealing a significant correlation ($p<0.001$) between the prevalence of TNF-α polymorphism and respiratory failure [16].

Research indicates that variations in the promoter region can influence TNF-α production. Additionally, a study suggests that a polymorphism in TNF-α is associated with elevated TNF-α levels, potentially

contributing to respiratory failure. The pathophysiology underlying respiratory distress may be connected to single nucleotide gene polymorphisms in TNF-α, which have been associated with altered TNF-α release. Furthermore, a study by Matenat et al. found that the A/A genotype was present in control groups but absent in those with pulmonary tuberculosis and extra-pulmonary tuberculosis infections [17].

Previous studies indicate that individuals with type 2 diabetes exhibit a higher prevalence of G/G genotypes compared to G/A genotypes, while those possessing A/A genotypes demonstrate lower levels relative to the other groups. TNF-α, a pro-inflammatory cytokine, plays a significant role in the pathogenesis of chronic obstructive pulmonary disease (COPD). The resultant airway inflammation facilitates rapid neutrophil infiltration, promotes neutrophil adhesion, leads to the dissociation of elastic peripheral cells, and enhances proteolytic enzyme activity, all of which are critical factors in the progression of emphysema [18,19].

Moreover, TNF-α may stimulate airway smooth muscle cells to release endothelin-1, resulting in smooth muscle contraction and cellular proliferation, which ultimately contributes to airway remodeling. Additionally, TNF-α exacerbates lung tissue damage by promoting the production of interleukin-6 (IL-6) and interleukin-8 (IL-8) by bronchial epithelial cells and alveolar macrophages. Our current research suggests that the TNF-α (rs1799964) T/C polymorphism is associated with a heightened risk of respiratory distress [20]. Furthermore, another study has shown that patients with acute COPD harboring TNF-α-308 and TNF-α-489 gene polymorphisms tend to have a poorer prognosis and more severe disease manifestations. These findings enhance the understanding of respiratory diseases, providing a theoretical framework for their prevention, assessment, and the development of personalized treatment strategies.

## 5. Conclusion

In summary, the association between the TNF-α (rs1799964) T/C polymorphism and the onset of respiratory diseases has been established. Consequently, in patients with diabetes undergoing treatment, the rs1799964 TNF-α polymorphism may serve as a predictive marker for respiratory distress and could represent a potential genetic risk factor for such conditions.

## Ethical Considerations

### Compliance with ethical guidelines

The research has received approval from the Institutional Ethics Committee (No. NIMS/IEC/2023/RC). Ethical clearance was granted by the committee, and informed consent was secured from the participants or their guardians. The study adhered to the principles outlined in the Declaration of Helsinki.

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### Author's contributions

All authors equally contributed to preparing this article.

### Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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