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Transforming Growth Factor-β1 Levels and rs1800471 Variant in Gram-Negative Sepsis: A Case–Control Study

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P A P E R I N F O

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A b s t r a c t

Background: Sepsis continues to be a primary cause of morbidity and mortality around the world, yet early diagnostic tools are currently lacking. transforming growth factor-β1(TGF- β1) is an immunoregulatory cytokine with a role in modulating inflammation, and polymorphism within the *TGF- β1* gene might lead to variation in host response during infection .

Objective: This study aimed to explore the relationship between *TGF- β1* rs1800471 (C>T) polymorphism and risk of gram-negative sepsis and examined serum TGF-β1 as a diagnostic biomarker.

Methods: A Case–control + repeated measures within cases study was carried out in Imam Hussein Medical City (Karbala, Iraq) during the period from December 2024 to April 2025. A total of 204 subjects (104 ones with sepsis and 100 healthy controls) were recruited. Culture of blood was carried out using BacT/ALERT® FA Plus and then followed by identification with Vitek 2 Compact., in addition to SOFA Score. Serum TGF-β1 levels were detected using sandwich ELISA. Genotype of rs1800471 was determined by allele-specific PCR. Statistical analysis was conducted with SPSS v23 and GraphPad Prism 8. They used odds ratio (OR) and receiver operating characteristic (ROC) curve .

Results: The patients with sepsis demonstrated a decreased serum TGF-β1 level compared to the controls; however, this difference was not significant (p=0.19). The distribution of rs1800471 genotypes was significantly different between cases and controls (p=0.0148). The CT genotype was also significantly associated with sepsis development (OR=11.38, 95% CI=2.29–56.55, p=0.001), whereas the TT genotype had a stronger association (OR=14.10, 95% CI=1.63–122.18, p=0.008). ROC analysis further revealed a poor diagnostic value for the rs1800471 polymorphism (AUC = 0.630, p = 0.004) as well as no diagnostic potential for serum TGF-β1 levels (AUC = 0.50, p = 0.936).

Conclusion: The *TGF-β1* rs1800471 polymorphism is a risk factor for gram-negative sepsis. However, its diagnostic value is insufficient. Serum TGF-β1 is insufficient as an independent diagnostic biomarker. Subsequent diagnostic algorithms should combine genetic markers with clinical and laboratory measurements

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1. INTRODUCTION

Gram-negative infections are one of the major clinical problems related to antimicrobial resistance and lack of effective therapeutics (Pogorzelska et al.,2020). These pathogens are among the most commonly responsible for bloodstream infections and sepsis, especially in hospitalized or immunosuppressed patients. *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are typical gram-negative bacteria that cause sepsis (Guclu et al.,2013). Sepsis is characterized by life-threatening organ dysfunction caused by a dysregulated host response to infection. It has high mortality worldwide and is still a public health problem (Wada et al., 2018; Popescu et al.,2022). In the setting of critical illness, despite progress in critical care, early diagnosis remains challenging because appropriate gold-standard test is still lacking. Clinical findings including fever, leukocytosis, tachycardia or hypotension are nonspecific (Rudd et al.,2020; Nunnally et al., 2021).

TGF-β1 is a pleiotropically acting cytokine involved in the regulation of immune responses, and tissue repair and remodeling. It is produced by macrophages, neutrophils and damaged tissue and its main function is anti-inflammatory response with suppression of immune cell activation and proliferation. There is also the potential that genetic variation in TGF-β1 may modulate cytokine production and influence risk for inflammatory disorders. The single nucleotide polymorphism rs1800471 (C>T) has been suggested to influence TGF-β1 expression and was also associated with different inflammatory responses (Laskin et al.,2011; Bae et al., 2014). This study was designed to analyze the association between TGF-β1 rs1800471 polymorphism and risk for gram-negative sepsis, and, whether serum TGF-β1 levels can be a diagnostic biomarker for identification and early prediction of sepsis.

2.Methods

2.1. Study design and setting

Case–control + repeated measures within cases study from December 2024 to April 2025 at Imam Hussein Medical City, Iraq.

2.2. Study population

We enrolled 104 sepsis patients and 100 healthy controls, with a total of 204 individuals. Males and females aged over 20-80 years were enrolled in the study. Three time-points of samples at day 0, day 3, and day 6 in hospital were obtained from sepsis patients.

2.3. Inclusion and exclusion criteria

Inclusion criteria included admission with confirmed clinical diagnosis of sepsis by specialist physicians, laboratory evidence of gram-negative bacterial infection and SOFA Score. Chronic inflammatory diseases, autoimmune disorders, cancer, thyroid disease, systemic immune diseases, or growth of gram-positive bacteria were excluded

2.4. Collection of samples and identification of bacteria

Aseptic venous blood samples were obtained. Samples were separated into whole blood for microbiological and molecular analysis and serum for immunological assays. Blood cultures performed with BacT/ALERT® FA Plus bottles and identification using the Vitek 2 Compact system. For both sepsis and control patients, blood cultures were taken to exclude bloodstream infection in the control group and to confirm sepsis-relatedness of the participants.

2.5. Serum TGF-β1 measurement

Citing a commercial sandwich ELISA kit (BT Lab), TGF–β1 concentrations in serum were measured according to the manufacturer's instructions.

2.6. DNA extraction and genotyping

Genomic DNA was extracted from whole blood using a popular DNA extraction kit (Favorgen). The genotyping of TGF–β1 rs1800471 polymorphism was performed by allele-specific PCR. Primers were designed on Primer3Plus forward A (5′-GCAGCGGTAGCAGCAGCG-3′), forward B (5′-AGCAGCGGTAGCAGCAGCA-3′) and reverse (5′-TCCGTGGGATACTGAGACAC-3′). The PCR amplifications were conducted in 50 µL reactions containing 4 µL DNA template, 25 µL of 2× PCR master mix (SMOBIO), and primers (final concentration of 0.2 µM). Thermal cycling included initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 62°C for 40 s, and extension at 72°C for 45 s, and final extension at 72°C for 10 min. The PCR products were examined by 1.5% agarose gel electrophoresis running alongside 100 bp DNA ladder.

2.7. Ethics Approval and Consent to Participate

This research received permission from the Ethics Committee of the Ministry of Health, Karbala Health Department, Imam Hussein Medical City (no. 2023269 in 21/12/2023) Each participant in this investigation signed a letter of consent.

2.8. Statistical analysis

Statistical analyses were executed by SPSS v23 and GraphPad Prism v8. The values were checked for normality with the Shapiro–Wilk test. Serum

TGF-β1 level was evaluated using linear mixed-effects model, and the association between TGF-β1 rs1800471 polymorphism and risk of sepsis was evaluated by logistic regression (OR, 95% CI). The accuracy of the diagnostic performance was assessed by ROC curve analysis (AUC). $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Bacterial isolation from blood patients

In patients with sepsis, 108 distinct bacterial isolates were identified via blood culture from total blood samples (104 from sepsis patients and 100 from control). Blood culture bottles and the Bact/Alert - Vitek 2 apparatus have enhanced bacterial isolation and diagnosis. The results in Figure (1) illustrate the bacterial pathogens that cause sepsis obtained via blood culture which are gram - negative bacteria. Among gram negative *Escherichia coli* 30(27.778%), *Klebsiella pneumoniae* 22(20.370%), *Acinetobacter baumannii* 18(16.667%), *Burkholderia cepacia* 10(9.259%), *Salmonella typhi* 8(7.407%), *Pseudomonas aeruginosa* 6 (5.556%), *Proteus mirabilis* 6 (5.556%), *Citrobacter spp* 2(1.85%), *Enterobacter cloacae* 2(1.85%), *Serratia marcescens* 2(1.85%), and *Stenotrophomonas* 2 (1.85%).

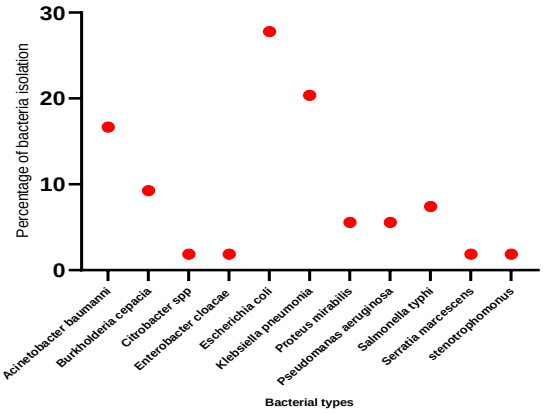

Figure 1. Frequency of gram-negative bacterial isolates obtained from blood cultures, according to the study population.

3.2. SOFA Score for a Patient with sepsis

Organ dysfunction was estimated using the SOFA score of coagulation (platelets) and renal system (creatinine/urea). The mean partial SOFA score was 7.88 ± 1.37, largely influenced by low thrombocytopenia (90.25 ± 90.65 $\times 10^3/\mu L$) and severe renal impairment (creatinine: 6.88 ± 0.27). All the patients fulfilled the criteria of sepsis (SOFA ≥2), thus diagnosing 100% positive cases for sepsis, as shown in Table 1:

TABLE 1: SOFA Score for a Patient with sepsis

SOFA Component	Clinical / Laboratory Parameter	(Mean ± SD)	SOFA Score Contribution
Coagulation	Platelet count ($\times 10^3/\mu L$)	90.25 ± 90.65	2.08 ± 1.38
Renal	Creatinine	6.88 ± 0.27	4.0 ± 0.0
Renal (supporting)	Urea	227.65 ± 47.94	Included in renal dysfunction
Total Partial SOFA Score	Sum of coagulation + renal	—	7.88 ± 1.37
Final SOFA Category	Overall SOFA classification	4	Severe organ dysfunction
Sepsis Status	Partial SOFA ≥ 2	100.00%	Confirmed sepsis

3.3. Comparison of transforming growth factor-β1 concentration between the study groups

In all patients, the serum TGF-β1 was not significantly lower in the follow up sample collection group than in the healthy control group, with a mean+SD of (0.72± 0.021; 0.921± 0.043 and 0.812± 0.022 ng/ml) and in the healthy individual group (0.95 ± 0.2 ng/ml). Mean value of serum TGF-β1 concentration in sepsis group was lower than healthy individuals but the difference was not statistically significant ($p = 0.19$). as shown in Figure 2. This figure shows the importance of TGF-β1 as a factor for increasing the severity of infection.

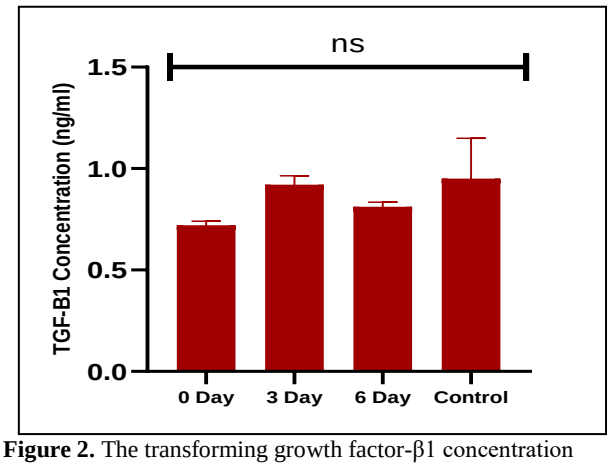

Figure 2. The transforming growth factor-β1 concentration

3.4. Comparison of *TGF-β1* gene (rs1800471) genotype frequencies between the study groups

TABLE 1 displays the genotype frequency distributions for the two groups according to the Hardy-Weinberg equilibrium. When the sepsis group was compared with the control group, the experimental results revealed a correlation between the expected and observed frequency distributions of the different TGF-β1 genotypes (p=0.0148 and p=0.9787, respectively).

TABLE 1. Hardy-Weinberg equilibrium-based genotype frequency distribution by group.

Genotype *TGF-β1* rs1800471	Sepsis *n* = 104		Control *n* = 100	
	Observed	Expected	Observed	Expected
CC	60	51.24	94	95.92
CT	26	43.52	4	2.08
TT	18	9.62	2	2
χ^2	16.854		0.084	
p	0.0148*		0.9787	

(χ^2)=Chi square; (*n*)=cases ; (*)= significant at p < 0.05

The frequency of the *TGF-β1* genotype (rs1800471) was compared between the sepsis and control groups as shown in **Figure 3**.

In figure 3, association between TGF-β1 rs1800471 genotypes and the risk of sepsis Binary logistic regression analysis was used to examine the associations between TGF-β1 rs1800471 and susceptibility to sepsis. The collectors of discharges confirmed that the frequency of TT genotype (mutant homozygote) was found to be significantly higher in sepsis patients compared to controls and it was shown to confer a substantially high risk of developing sepsis (OR = 14.10; 95% CI: 1.63–122.18, p = 0.008). In addition, the CT genotype (heterozygous mutant) was observed to exert a significant effect on the risk of sepsis presentation in comparison with CC genotype as reference, demonstrating significantly higher odds for sepsis (OR = 11.38, 95% CI: 2.29–56.55; p = 0.001). Overall, the results indicated that individuals harboring the T allele (CT + TT) are significantly more susceptible to sepsis and suggesting that the TGF- β1 rs1800471 polymorphism may be a candidate genetic risk factor for sepsis development (p < 0.05). as demonstrated by the results in **Figure 4.**

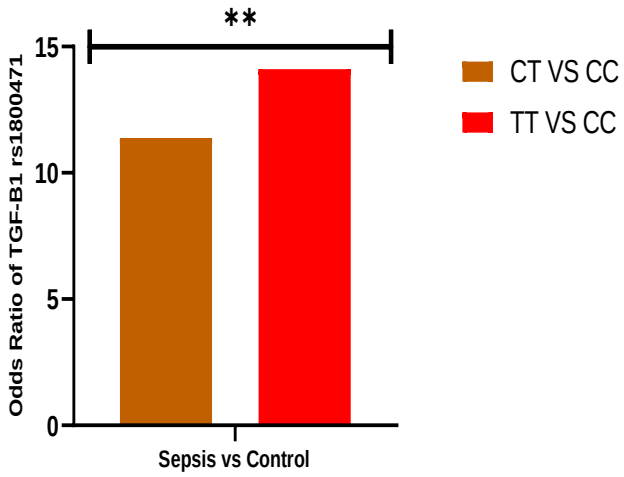

Figure 3. The *transforming growth factor-β1* Gene (rs1800471) genotype frequencies.

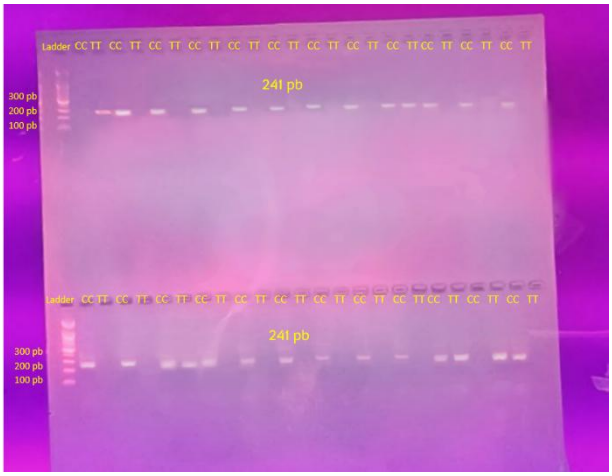

Figure 4. Ethidium bromide-stained agarose gel illustrating the allele-specific primer PCR products for the *transforming growth factor-β1* Gene (rs1800471) polymorphism (241 bp).

3.5. The performance of the ROC curve for *transforming growth factor-β1* biomarkers in relation to sepsis prediction

The TGF rs1800471 C>T polymorphism shows little diagnostic value, even though it is statistically significant (AUC = 0.630, p = 0.004), which means that it only slightly separates groups. It might help with risk stratification a little, but because it isn't very accurate, it can't be used as a valid diagnostic sign by itself. On the other hand, serum TGF-β1 is not helpful for diagnosis (AUC = 0.50, p = 0.963), which means it is no better than chance, as shown in figure 5.

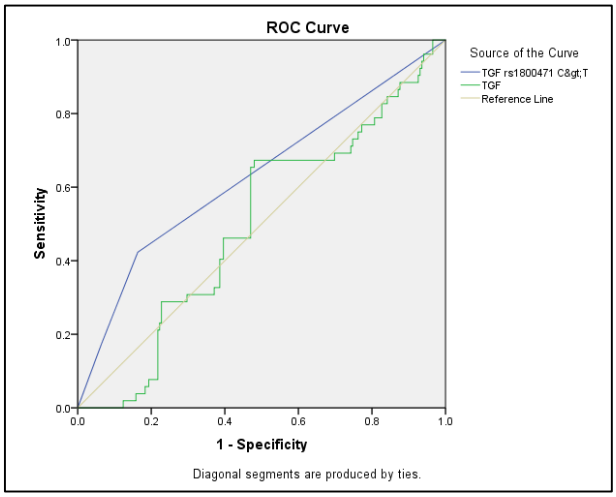

Figure 5. ROC curve of serum level and SNP of *TGF-β1* rs1800471 in sepsis groups

4. Discussion:

Sepsis is still a leading cause of morbidity and mortality globally, while infections caused by gram negative bacteria are an important clinical challenge due to the propensity for antimicrobial resistance and fast evolution of the disease (Vincent et al.,2009; Singer et al., 2016). Early detection and risk stratification are thus critical to better clinical outcomes with decreased mortality (Popescu et al.,2022). In our study, Escherichia coli and Klebsiella pneumoniae were the most commonly isolated pathogens, consistent with previous studies that cited these as two of the top three gram-negative bacteria responsible for bloodstream infections and sepsis (Rudd et al.,2020; Gourd and Nikitas, 2020).

In present study, serum TGF-β1 was lower in patients with sepsis than that in controls, but without statistical significance ($p = 0.19$). This could be attributed to the dynamic and intricate nature of immune response seen in sepsis where cytokine profiles oscillate due to disease stage/time as well as immune state such as early hyperinflammatory response and subsequent net-anti-inflammatory compensation (Bergmann et al.,2021). Furthermore, biological variation in both severity of infection and timing of patient specimen collection could result in inconsistent circulating cytokine levels making serum TGF-β1 an unreliable single-diagnostic biomarker (Knapp et al., 1998; Papic et al., 2023).

Nevertheless, the *TGF-β1* rs1800471 (C>T) polymorphism was found to be associated with the risk of gram-negative sepsis. The prevalence of sepsis appeared to be dramatically elevated among both individuals with CT and TT genotypes, indicating that this variant could work as a genetic risk factor. There is a biological basis for this association as *TGF-β1* polymorphisms have been reported to affect cytokine expression and immunoregulation (Zheng et al.,2021; Juarez et al., 2021). The T allele was reported to be associated with diminished production of TGF-β1, leading to dysregulation of regulation mechanisms and the excess activation of inflammation in association with uncontrolled immune responses and organ damage during severe infection (Pietruczuk et al.,2019; Juarez et al., 2021).

Although statistically significant, ROC analysis suggested a very modest diagnostic performance of rs1800471 individually (AUC = 0.630) and thus little clinical utility as a single diagnostic marker. This difference indicates that genetic polymorphisms might play a role as susceptibility modifier more than direct diagnostic biomarker and may have clinical significance of predictive value of convenience when combining with clinical scores such SOFA and other laboratory biomarkers (Sun and Zhou et al., 2024). Additionally, serum TGF-β1 had no diagnostic capability (AUC = 0.50), further suggesting that it is not a valid independent factor to identify early sepsis in these patients.

This study has several limitations. First, it was performed in a single center which limits the applicability of the results to other populations. Second, the sample size, especially in TT genotype, was relatively small and hence this may have resulted in wider confidence interval and less statistical power. Third, cytokines were not serially determined and measurements at certain time points are a significant limitation, since dynamic changes in the levels of cytokines during sepsis can occur and may change even within the same clinical course (Bergmann et al., 2021). Other inflammatory mediators and genetic polymorphisms were not tested. Consequently, further research needs to include high quality multi-center cohorts and the analysis of combination panels that include genetic polymorphisms as well as clinical and laboratory factors in order to improve diagnostic/prognostic qualities (Sun and Zhou et al., 2024).

Conclusion: A significant correlation was demonstrated between the *TGF-β1* rs1800471 (C>T) polymorphism and the increased risk of gram-negative sepsis. The plasma TGF-β1 concentration was slightly lower in patients with sepsis than in healthy controls. However, the difference did not reach statistical significance and revealed minimal diagnostic power. ROC analysis further confirmed that rs1800471 all showed modest performances, while serum TGF-β1 was not a candidate diagnostic factor, indicating that these indicators were unsatisfactory for stand-alone diagnosis."

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Authors Contributions

All authors were equally contributed.

Conflict of interest: none

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