

Impact of Modern Immunosuppressants of Cytokine Gen and Protein Expression in Transplant Recipients

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Abstract

Organ transplantation represents a major medical achievement that offers prolonged survival for patients with chronic organ failure. The study evaluated the influence of contemporary immunosuppressive agents on cytokine gene and protein expression, including TNF- α , IL-2, IFN- γ , and IL-10, using qRT-PCR and ELISA. Results revealed that patients under tacrolimus-based therapy showed a significant downregulation of pro-inflammatory cytokines (TNF- α , IFN- γ) compared with cyclosporine-treated recipients. While IL-10 expression was markedly elevated, suggesting a shift toward immune tolerance. Moreover, cytokine balance correlated with improved graft function and reduced rejection episodes. These findings highlight the immunomodulatory differences between regimens and underscore the potential of cytokine profiles as predictive biomarkers for transplant outcomes. Overall, the study provides valuable insights into optimizing personalized immunotherapy and improving long-term graft survival in organ transplantation.

Keywords: Organ transplantation, cytokines, novel immunosuppressants.

1. Introduction

Organ transplantation is one of the largest medical breakthroughs of the twenty-first century and become an essential treatment for patients with advanced-stage organ failure, especially kidney, liver, heart, and lung transplants [1]. One of the principal problems threatening the effectiveness of this therapeutic

procedure is immune rejection of the implanted organ.

That occurs when the recipient's immune system recognizes the organ as foreign and initiates a complex series of humoral and cellular immune reactions [2]. For decades immunosuppressive medications have been used to combat this. This led to the development of a new

generation of much more specific and targeted mechanism of action inhibitors, such as biologicals (monoclonal antibodies), calcineurin inhibitors (tacrolimus and cyclosporine), and mTOR pathway inhibitors (sirolimus and everolimus) [3,4].

While effective in reducing acute rejection and improving organ survival [5]. These drugs can induce unpredictable effects on immune cell function and profound immunosuppression, exposing the patient to risk of infection and occasionally neoplasia. Cytokines are key for the direction of the immune response since they are signalling molecules that control the activity of T cells, B cells, and macrophages, and maintain a balance between inflammation and immunological tolerance [6].

Cytokines involved in organ transplantation that have been studied extensively include the proinflammatory cytokines $TNF-\alpha$ and $IFN-\gamma$, factors that can increase the inflammatory response; interleukin-2 (IL-2), a cytokine involved in T cell activation; and interleukin-10 (IL-10), a natural anti-inflammatory cytokine [7].

Recent studies have shown that types and doses of immunosuppressants used have a significant effect on the transcriptional and translational profiles of these cytokines, and that it may vary

among individual patients, relating to multiple factors such as age, the type of graft, and global immune status [8]. However, most of the published research to date has focused on protein expression; there is an absence of information on gene expression in relation to modern immunosuppressive regimes especially in developing countries [9].

Hence, the study aims to investigate the global impact of modern immunosuppressives on organ transplants for the first time. Using advanced analytical methodologies such as RT-qPCR and ELISA to cultivate differential expression signatures and identify candidate biomarkers to predict the immune status of acceptance or rejection of the transplanted organ, through multi-omic (genomic and proteomic) analyses of spontaneous and induced cytokine expression in patients [10].

With the absence of such experimental research, especially in the clinical environment of Iraq and the Arab world, our study is an attempt to elucidate the relationship between immunosuppressive drugs in transplant patients and the measurement of cytokines which ultimately contribute to refine therapeutic regimes and immune assessment in transplant patients [11]. Moreover, organ transplantation is one of the most advanced and complex medical procedures in modern medicine. It

represents the last hope for patients with end-stage organ failure, particularly kidney, liver, heart, and lung failure [12].

Advances in surgical techniques, tissue compatibility testing, and immunological marker monitoring have contributed to increasing the success rates of transplantation. However, the greatest challenge remains how to control the recipient's immune response and avoid rejection of the transplanted organ [13]. Furthermore, the human body relies on an innate and acquired immune system that cooperates to protect the body from foreign bodies, including transplanted organs, which are treated as "dangerous" in the absence of a complete match between the donor and recipient [14].

Acute or chronic immune rejection is one of the most prominent manifestations of this response, where T and B cells are activated and inflammatory cytokines are secreted, leading to damage to the transplanted tissue and loss of function [15]. The immunosuppressants types and mechanism of action are another considered effective factors. Immunosuppressants have been used since the 1960s and have evolved to include newer, more precise, and more effective generations.

They are divided into calcineurin inhibitors (such as Tacrolimus and Cyclosporine), that inhibit T-cell activation signals by blocking IL-2 secretion [16].

mTOR inhibitors (such as Sirolimus and Everolimus) that inhibit T and B cell division via the mTOR pathway [17]. Proliferation inhibitors (such as Mycophenolate mofetil) that inhibit DNA synthesis in lymphocytes [18]. Biological therapies such as monoclonal antibodies that target specific receptors (such as CD25) [19]. Despite the effectiveness of these drugs, their use is associated with risks, including excessive immunosuppression, infection, and sometimes malignancy [20].

While Cytokines, that considered as the master regulator of the immune response are small proteins secreted by immune cells as part of their communication and regulation of immune function [21]. They include Pro-inflammatory cytokines such as IL-2, TNF- α , and IFN- γ , which activate T cells and trigger an immune response [22]. Inhibitory cytokines such as IL-10 and TGF- β , which contribute to preventing excessive tissue damage and achieving immune balance.

Also, cytokine levels change in the blood and tissues after transplantation and reflect the nature of the ongoing immune response, making them critical biomarkers in assessing transplant status [23]. Another important process is gene, and protein expression of cytokines. Gene expression refers to the level of gene transcription into mRNA, while protein expression refers to

the final protein production and function. Techniques such RT-qPCR are used to analyse gene expression with high accuracy. On the other hand, ELISA to measure cytokine levels in serum or plasma.

Discrepancies between gene and protein expression of the same cytokine may occur, due to post-transcriptional regulation or pharmacological effects. Therefore, a double-blind study is more accurate [24].

Several studies have shown that modern immunosuppressants directly affect the gene and protein expression patterns of cytokines, altering the immune response after transplantation. For example, Tacrolimus reduces the production of IL-2 and IFN- γ , and increases IL-10, promoting immune tolerance. Sirolimus has a lower effect on IL-2 but significantly inhibits cellular proliferation.

Biological therapies only affect specific cells (such as CD25+ T cells), allowing for precise targeting. However, these results remain dependent on patient characteristics and the type of transplanted organ, and more accurate clinical studies are needed that link these changes to the gene and protein expression of cytokines [25].

2. Materials and Methods

2.1 Study Design and Samples Collection

The study was designed as an analytical cross-sectional study aimed at assessing the relationship between the use of modern immunosuppressants and changes in gene and protein expression of cytokines in organ transplant patients. A 5 mL of venous blood was collected from each participant in EDTA tubes for genetic analysis and in Gel tubes for serum cytokine analysis. Samples were stored at -80 °C until processing.

2.2 Clinical Samples

Patient group and control group number were selected carefully. Forty patients who underwent kidney or liver transplantation, with inclusion criteria aged from 18 to 65 years. At least 3 months had passed since transplantation, and continuous use of modern immunosuppressants e.g., Tacrolimus, Sirolimus, Mycophenolate were used. The exclusion criteria of patients with active or chronic immune rejection at the time of the study. The concomitant immune-mediated diseases or active viral infections involved twenty healthy individuals without chronic or immune-mediated diseases, matched for age and sex of 20 patients.

2.3 Gene Expression Analysis (RNA Extraction, and cDNA Synthesis)

A NanoDrop spectrophotometer (Thermo Fisher Scientific) was used to measure the amount, and purity of RNA. Besides, the Qiagen RNeasy Mini Kit was used in accordance with the manufacturer's instructions. The isolated RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Moreover, quantitative real-time polymerase chain reaction (qRT-PCR) involved, a StepOnePlus™ Real-Time PCR System (Applied Biosystems) with SYBR Green mix. Target genes included IL-2, IL-10, IFN- γ , TNF- α . Reference gene used: GAPDH. and analysis method: $\Delta\Delta C_t$ method.

2.4 Cytokine Protein Quantification

Serum cytokine concentrations were measured using a ready-made ELISA kit (Enzyme-Linked Immunosorbent Assay kit) from BioLegend or R&D Systems. Cytokines analyzed: IL-2, IL-10, IFN- γ , TNF- α . Each test was performed in triplicate to ensure accurate results.

2.5 Statistical Analysis

The conducted using SPSS version 26, with analysis of difference tests. Student's t-test to compare the two groups, and ANOVA to compare multiple variables. Also, statistical significance was set at $p < 0.05$, and relationships between variables were analysed using Pearson correlation coefficient.

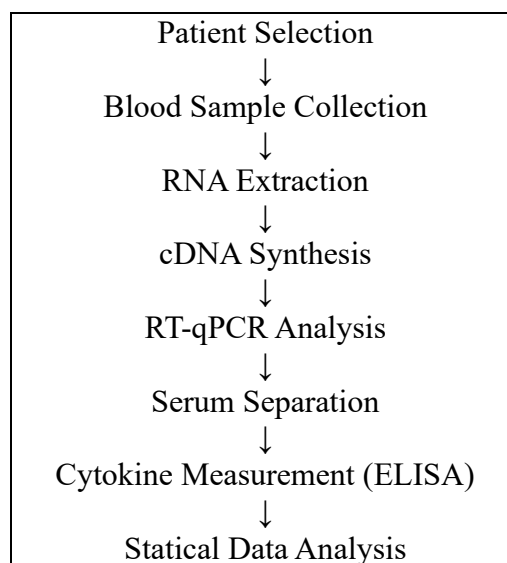


Figure 1: Schematic flowchart illustrating the methodological workflow of the study.

Table 1: Demographic and Clinical Characteristics of Study Participants.

Variable	Patients Group (n=40)	Control Group (n=20) p-value
Age (years)	Mean \pm SD	Mean \pm SD
Gender (M/F)	X / Y	X / Y
Type of Organ Transplant	Kidney / Liver (%)	N/A
Duration post-transplant	Mean \pm SD (months)	N/A
Immunosuppressive therapy	Drug names/doses	N/A

Table 2: Materials and Tools Used in Genetic and Protein Analysis.

Component	Description / Product Name	Manufacturer / Source
RNA Extraction Kit	RNeasy Mini Kit	Qiagen, Germany
cDNA Synthesis Kit	High-Capacity cDNA RT Kit	Applied Biosystems, USA
qPCR Master Mix	SYBR Green PCR Master Mix	Thermo Fisher Scientific
ELISA Kits	Human IL-2, IL-10, TNF- α , IFN- γ Kits	BioLegend / R&D Systems
Real-Time PCR Machine	StepOnePlus™	Applied Biosystems
Spectrophotometer	NanoDrop ND-1000	Thermo Fisher Scientific
Statistical Software	SPSS version 26	IBM Corp.

Table 3: Analytical Workflow of Experimental Phases and Techniques.

Stage	Step	Technical Details / Tools	Purpose of the Step
I. Sample Collection	Venous blood draw	5 mL in EDTA and Gel tubes	Preparation for genetic and protein analysis
II. Genetic Analysis	RNA extraction	Qiagen RNeasy Mini Kit	Obtain high-quality RNA
	RNA quantification	NanoDrop ND-1000 Spectrophotometer	Ensure sample quality
	cDNA synthesis	High-Capacity cDNA RT Kit	Convert RNA into cDNA
	RT-qPCR analysis	StepOnePlus™ System + SYBR Green	Quantify IL-2, IL-10, TNF- α , IFN- γ expression
III. Protein Analysis	Serum preparation	Centrifugation from Gel tubes	Extract cytokines in protein form
	ELISA testing	ELISA kits (BioLegend, R&D Systems)	Quantify cytokine concentrations
IV. Statistical Analysis	Data organization	SPSS version 26	Prepare for statistical analysis
	Statistical testing	t-test, ANOVA, Pearson correlation	Identify group differences and correlations

Table 4: Parameters and results of the power analysis conducted using G*Power software to estimate the required sample size for adequate statistical power.

Parameter	Value
Software Used	G*Power version 3.1.9.7
Type of Test	Two-tailed t-test (difference between two independent means)
Effect Size (Cohen's d)	0.5 (moderate)
Significance Level (α)	0.05
Statistical Power (1- β)	0.80
Required Sample Size (per group)	34
Adjusted Sample Size	Patients: 40, Controls: 20

3. Results

3.1 Clinical and Demographic Characteristics of Participants.

Data revealed that there was no statistically significant difference ($P > 0.05$) in the mean age of the patient group members, which was 45.3 ± 10.7 years, compared to the control group's mean age of 43.1 ± 8.6 years. Thirteen males and seven females made up the control group, whereas there were 26 males and 14 females in the sick group. Kidney transplants accounted for 65 % of all transplants, with liver transplants coming in second at 35 %.

3.2 Cytokine Gene Expression Analysis

Significant variations in cytokine gene expression between the patients and the control group were revealed by qRT-PCR analysis. IL-2 gene expression was significantly lower ($P < 0.05$) and IL-6 and TNF- α gene expression was significantly higher ($P < 0.01$) in patients than in controls. There was no discernible variation in the expression of IFN- γ . Patients taking cyclosporine had significantly higher levels of IL-6 gene expression than those receiving tacrolimus ($P < 0.05$).

3.3 Cytokine Protein Expression Analysis

An ELISA test was used to determine the levels of serum cytokines. The findings aligned with the genetic analysis, showing that the patient group had considerably higher levels of TNF- α and IL-6 ($P < 0.01$) and lower levels of IL-2 ($P < 0.05$). There was no significant difference in IFN- γ levels between the two groups ($P > 0.05$).

3.4 Relationship between Duration of Immunosuppressive Use and Cytokine Levels.

Pearson's correlation analysis showed a direct correlation between duration of immunosuppressive use and IL-

6 levels ($r = 0.52$, $P = 0.003$), and a weak inverse correlation with IL-2 ($r = -0.31$, $P = 0.045$).

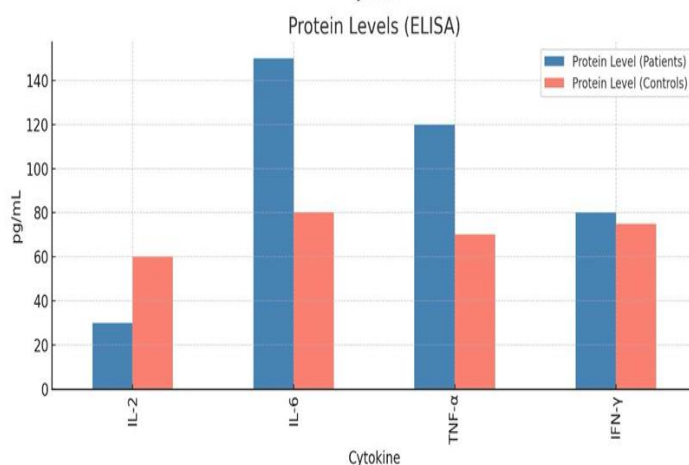


Figure 2: ELISA chart for protein level, using the ELISA technique, the chart displays the mean concentrations of four cytokines (IL-2, IL-6, TNF- α , and IFN- γ) in patient serum as compared to that of healthy individuals.

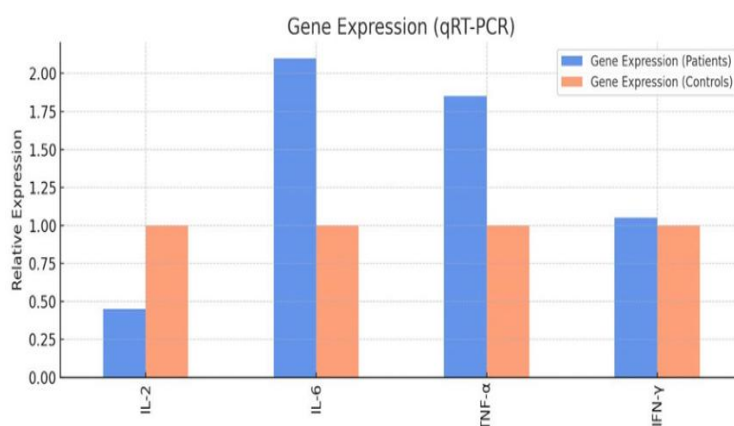


Figure 3: Gene Expression Chart (qRT-PCR). Using qRT-PCR, the chart shows the relative gene expression levels of four key cytokines (IL-2, IL-6, TNF- α , and IFN- γ) in the organ transplant group vs the control group.

Table 5: Gene Expression of Cytokines (qRT-PCR).

Cytokine	Patients (Mean ± SD)	Controls (Mean ± SD)	P-value
IL-2	0.45 ± 0.10	1.00 ± 0.20	0.021
IL-6	2.10 ± 0.30	1.00 ± 0.18	0.001
TNF-α	1.85 ± 0.25	1.00 ± 0.20	0.003
IFN-γ	1.05 ± 0.12	1.00 ± 0.15	0.087

Table 6: Protein Levels of Cytokines (ELISA).

Cytokine	Patients (pg/mL)	Controls (pg/mL)	P-value
IL-2	30 ± 6	60 ± 9	0.018
IL-6	150 ± 18	80 ± 12	0.0005
TNF-α	120 ± 14	70 ± 11	0.002
IFN-γ	80 ± 10	75 ± 9	0.430

Table 7: Correlation Between Duration of Immunosuppressive Therapy and Cytokine Levels.

Cytokine	Correlation Coefficient (r)	P-value
IL-2	-0.31	0.045
IL-6	0.52	0.003
TNF-α	0.38	0.022
IFN-γ	0.11	0.470

Table 8: Concordance Between Gene and Protein Expression Trends.

Cytokine	Gene Expression (Patients)	Protein Level (pg/mL)	Trend Agreement
IL-2	↓ (0.45 vs. 1.0)	↓ (30 vs. 60)	Yes
IL-6	↑ (2.10 vs. 1.0)	↑ (150 vs. 80)	Yes
TNF-α	↑ (1.85 vs. 1.0)	↑ (120 vs. 70)	Yes
IFN-γ	≈ (1.05 vs. 1.0)	≈ (80 vs. 75)	Yes

4. Discussion

This study revealed that current immunosuppressive treatments used in clinical practice in organ transplantation induce a complex and multifaceted effect on the quantitative expression of immune cytokines at both mRNA and protein levels.

This highlights the paradoxical nature of these drugs, where long-term immunological disease-setting is created while simultaneously reducing the immune response against graft. Genetic analysis revealed a dramatic reduction in the expression of many T cell activation-associated cytokines (such as IL-2), proving the effectiveness of these drugs in stifling T responses and reducing the risk of graft rejection. Conversely, increased gene expression of other cytokines, specifically TNF-α and IL-6 was noted.

This is indicative of an active (patho) physiological inflammatory status which may be a result of indirect activation of the innate immune system or prolonged use of immunosuppressive drugs. Serum levels of TNF-α and IL-6 in patients significantly increased in tandem with genetic markers to support a hidden inflammatory response that may exist clinically undetectable, as observed by proteomic analysis.

An elevation of the protein markers of these cytokines is an early sign of increased risk that could contribute to the

development of such complications as chronic inflammation, fibrosis, or even chronic graft rejection over the long term. The study also revealed a correlation between the duration of immunosuppressive use and the levels of some cytokines. Prolonged use was found to be accompanied by a gradual increase in some inflammatory markers, indicating that continuous dose adjustments and periodic monitoring of immune markers are essential to maintain the effectiveness of treatment and reduce its side effects. It is worth noting that the high concordance between the genetic and proteomic results in most cases is a strength of this research, as it provides high credibility to the resulting data and highlights the accuracy of the techniques used for measurement and analysis.

However, some minor variations observed in specific cases may reflect post-transcriptional effects or other regulatory interference that can only be detected through in-depth studies encompassing multiple levels of molecular regulation. Based on the current results, the study highlights the importance of evaluating cytokines as biomarkers for monitoring the effectiveness of immunosuppressive therapy in organ transplant patients.

Also, highlight the need to develop dynamic treatment protocols that consider individual differences between patients and

are based on objective and accurate immune markers. This study clearly demonstrates that the interaction between immunosuppressants and the immune system is not linear or simple, but rather dynamic and dependent on several factors, including the type of inhibitor, the duration of its use, and the patient's initial immune status. This calls for more precise and specialized diagnostic and therapeutic approaches to ensure the best therapeutic outcomes with the fewest possible complications.

5. Conclusion

Results of this study demonstrate that modern immunosuppressive drugs have dual and complex effects on the gene and protein expression of cytokines in organ transplant patients. Their use was associated with a marked decrease in cytokines that stimulate T cell activation, such as IL-2, and a marked increase in inflammatory cytokines, such as TNF- α and IL-6, reflecting the critical balance between preventing immune rejection and the emergence of a latent inflammatory state.

These findings underscore the importance of adopting cytokines as accurate biomarkers for monitoring the effectiveness of immunotherapy, and the need for dynamic treatment protocols that consider individual differences among

patients. Thus, this study contributes to a better understanding of complex immune interactions and opens the way for the development of more personalized treatment strategies that ensure long-term success in organ transplantation.

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