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Analysis of Interleukin-17 mRNA Level in the Urinary Cells of Kidney Transplant Recipients with Stable Function

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ABSTRACT

Introduction: Kidney transplantation supports patients with end-stage kidney diseases. Many factors control the allograft function in kidney transplant recipients. Interleukin-17 (IL-17) can be used as a non-invasive diagnostic biomarker of rejection. The aim of this study was to evaluate the expression of IL-17 mRNA in urinary cells of kidney transplant recipients with stable function.

Material and methods: A total of 40 renal transplant recipients who were admitted for surgery and 30 healthy controls were enrolled in the study. From each patient, 30 mL urine samples were collected in 50 mL tubes on days 3 and 5 after renal transplantation; also, 30 mL urine samples were obtained from controls. Quantitative Real-Time PCR (qRT-PCR) technique was used for analysis of IL-17 mRNA level in the tested groups; $2^{-\Delta\Delta CT}$ method was performed for determining the relative gene expression between tested groups.

Results: The mRNA expression mean ± SE of fold in patients and controls were 3.58±1.61 fold and 2.85±1.37 fold, respectively. The mRNA expression mean of IL-17 (fold) was not statistically different in tested groups (P-value = 0.63).

Conclusions: In kidney transplant recipients, urinary IL-17 expression provides informative data in relation to the allograft function regardless of allograft pathology.

Keywords: interleukin-17, kidney transplant recipients, urinary cells.

INTRODUCTION

Kidney transplantation is the last therapeutic method to support patients with complicated end-stage kidney diseases (1). Several factors complicate the allograft function in renal transplant recipients. Occurrence of rejection is rising after cellular interactions in the immune system. T cells have a critical role in allograft recognition (2). Under cytokine cascade, naive T cells differentiate into multiple subtypes of T helper (Th) cells. Th1 cells yield inflammatory cytokines (such as IL-1β, IL-2, IFN-γ, IL-6, and

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TNF-α), but Th2 cells produce anti-inflammatory cytokines (such as IL-4, IL-10, IL-11, and IL-13) (2). Th17 cells secrete IL-17, IL-17F, IL-6, IL-22 and TNF-α and also trigger a particular inflammatory response in a wide range of diseases, e.g., asthma, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis and also in tumorigenesis and transplant rejection (3-6). Many investigations have implied an important role for IL-17 in allograft rejection. Overall, production and secretion of IL-17 were found in patients with acute rejection after liver transplantation (7-9). Also, IL-17 is expressed in human and experimental renal allograft rejection (10-12). IL-17 is not produced in normal kidneys but it is found in transplants undergoing rejection. IL-17 mRNA within urinary sediment could be undertaken as a possible noninvasive predictive biomarker for subclinical kidney allograft rejection (11). Li et al (2011) indicated that Th17 cells play a key role in acute kidney allograft rejection, and IL-17 can be used as a diagnostic biomarker of acute kidney rejection (13). In humans, there is a relationship between shorter kidney allograft function and the presence of Th17 cells (14, 15). The presence of Th1 and Th17 cells in the blood of kidney transplant patients has been associated with delayed graft function or acute rejection (16). In humans, IL-17 gene expression has not been studied in kidney allograft outcome.

The aim of this study was to evaluate the association between IL-17 gene expression and graft function in renal transplant patients. ❑

MATERIALS AND METHODS

This study was approved by the Ethical Committee of Urmia University of Medical Sciences (Ir.umsu.rec.1394.148). During the study period, 40 renal transplant recipients who were admitted for surgery and 30 healthy controls were enrolled in the study. This study was carried out at Imam Khomeini Hospital, Urmia University of Medical Sciences (Urmia, Iran). In the Kidney Transplantation Center, urine samples (30 mL) were collected in 50 mL tubes from each patient, on days 3 and 5 after renal transplantation, as well as from each healthy subject in the control group. RNA extraction was started within 24 h after sample collection using RNX plus (Cinnagen, Iran), and RNA samples were preserved in -80°C until cDNA synthesis was performed.

Graft function was evaluated by an expert renal transplantation team based on the accepted criteria such as increased serum levels of serum creatinine level. Routine immunosuppression regimen consisting of cyclosporine was administered. Donors were selected on the basis of HLA matching. The purity of RNA extracts was tested via measuring absorption at 260 nm and 280 nm in a biophotometer (Eppendorf AG, Germany), and a 260A/280A ratio greater than 1.8 was considered as acceptable. The Thermo Scientific RevertAid First Strand cDNA Synthesis Kit was used for cDNA synthesis from 2 µL of each sample. Thermo scientific SYBER Green/ROX qPCR Master Mix (2X) and 2 µL template cDNA were used for Real time PCR in the iQ™5 Multicolor Real-Time PCR Detection System (BIO-RAD). In our samples, IL-17 mRNA expression was evaluated using two sets of primers including F: 5'-tct ggg agg caa agt gcc gc-3' and R: 5'-ggg cag tgt gga ggc tcc ct-3' for IL-17 and F: 5'-ggc ggc acc acc atg tac cc-3' and β-actin R: 5'- gac gat gga ggg gcc cga ct-3' for β-actin (internal control). The program includes: 95 °C for 5 min; 40 cycles: 95 °C for 30 s and 65 °C for 20 s (9). All analyses were carried out in duplicate reactions. The $2^{-\Delta\Delta CT}$ method was performed for determining the relative gene expression between tested groups (17). Data are reported as the fold ± SE normalized to β actin as endogenous reference.

Statistical Analysis

The Kolmogorov-Smirnov, t-student test, Mann-Whitney U tests were performed for data analysis by Statistical Package for Social Sciences (SPSS) 22 software. Statistically significant level was set at a P value less than 0.05. ❑

RESULTS

Clinical findings among patients enrolled in the study are summarized in Table 1. Forty patients – 21 (52.5%) males and 19 (47.5%) females – who met the inclusion criteria were evaluated. None of them had drug toxicity. All patients had a body mass index (BMI) of 23.78±4.65 kg/m². Creatinine level was 1.5±0.42 mg/dL in males and 1.01±0.21 mg/dL in females (p < 0.001). In this study, patients with high blood pressure had a high level of creatinine (1.39±0.42 vs
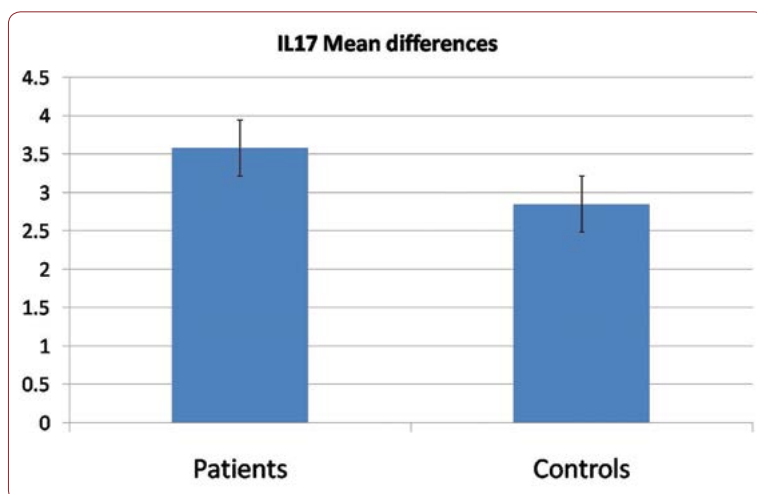
FIGURE 1. Expression analysis of IL-17 mRNA in the studied groups implies that mean of IL-17 (fold) was not statistically different.

Parameters	Frequency (%)
Polycystic Kidney Disease	5 (12.5%)
Diabetic nephropathy	5 (12.5%)
Blood pressure	28 (70%)
Renal stones	8 (20%)
Reflux after kidney transplantation	7 (17.5%)
Infection in patients with kidney transplantation	9 (22.5%)
Chronic glomerulonephritis	2 (5%)
FEV_1/prediction	84.87±17.51

TABLE 1. Characteristics of subjects

0.93±0.18) (P=0.007). The Mean ± SE of fold in patients and controls was 3.58±1.61 fold and 2.85±1.37 fold, respectively. The mRNA expression mean of IL-17 (fold) was not statistically different in the tested groups (P-value=0.63). A low expression of Interleukin-17 mRNA in urinary cells from kidney transplant recipients with stable function was found.

DISCUSSION

IL-17 promotes from production and secretion of pro-inflammatory cytokines (TNF-α, MCP-1 and MIP-1)18 and expression of CD80, CD40 and MHC class II antigens (19). Human IL-7 induces tissue damage and inflammation (20). IL-17 gene expression has been evaluated in several human diseases such as asthma (21), inflammatory bowel disease (22), Vitiligo (23), cancer (24), silicosis (25) and organ transplantation (9). The involvement of

IL-17 in rejection of kidney graft has also been investigated. Some documents hypothesize that the presence of IL-17 mRNA in urine samples could be considered as a predictive biomarker for rejection of kidney allograft in human and experimental models (11, 12, 16). IL-17 and intragraft IL-17 inhibition appears to be useful for delaying allograft rejection (27, 28). Some reports showed that the expression of IL-17 increased from post-transplant day 3 and was strongest at post-transplant day 5; therefore, it could be a predictive parameter in acute renal allograft rejection in rats (29). The role of IL-17 expression was not well studied in human renal allograft rejection. In this serial study of IL-17 mRNA expression, we found that the level of IL-17 mRNA expression was higher in patients but differences were not statistically significant on post-transplant days 3 and 5 (P-value >0.05). Recently, several urine biomarkers have been studied in renal transplant recipients (30, 31). Post-transplant monitoring of the clinical status of kidney transplants is critical for observation of allograft function. An alteration in several functional factors suggests the occurrence of rejection, but allograft biopsy as an invasive method is required for confirmation (11, 13, 31). RNA based biomarkers of urinary cells and peripheral blood cells changes primarily compared with serum creatinine levels in patients with kidney transplantation. A molecular non-invasive biomarker provides an important tool for predictive and prognostic information regarding allograft function. Urine biomarkers of renal allograft rejection include NKG2D mRNA (32), Tim-3 and IFN-γ (33), FOXP3 mRNA (34), and CD103 mRNA (35). Many research centers investigate the clinical value of molecular biomarkers in human disorders (for example, cytokines genes expression profiling in allograft transplantation). Urine and peripheral blood mononuclear cells are readily accessible for genes expression profiling.

This study showed that IL-17 gene expression profiling may be helpful in the selection of immunosuppressive therapy in the management of renal transplantation. The present study has some limitations, including small number of tested patients, lack of cases with acute/chronic rejection, poor quality of histopathology data, and immune monitoring assay after renal transplantation. Finally, our study did not compare results of patients serially after post-transplant day 5. Follow-up studies are necessary to evaluate the level of IL-17 mRNA expression after post-transplant day 5. ❑

CONCLUSION

In this serial study of IL-17 mRNA expression, the level of IL-17 mRNA expression was not statistically higher on post-transplant days 3 and 5 in patients with stable graft function. So, there is a correlation between the absence of IL-17 and the stable allograft function. It can be concluded that, in kidney transplant recipients, urinary IL-17 expression provides informative data in relation to allograft function, regardless of allograft pathology. ❑

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