**Fc Receptor-Like Gene Expression in Renal Transplantation Patients**

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**Abstract**

**Background:** It has been well-documented that the Fc receptor-like (FCRL) molecule contributes to the pathogenesis of certain autoimmune disorders. FCRL molecules belong to the immunoglobulin superfamily produced by B cells. Also, these molecules induce activating or inhibitory signals of B cells. According to this information and also considering the critical role of immune reactions in organ transplantation, the following experiment was performed to analyze the gene expression level of FCRLs in peripheral blood mononuclear cells of kidney transplant recipients. **Materials and Methods:** Blood samples were obtained from 32 renal transplant patients on days 1, 3, and 7 post-transplantations. Patients were divided into two groups according to the presence or absence of rejection. Also, 24 age-matched healthy subjects were enrolled as control group. After total RNA extraction from peripheral blood mononuclear cells (PBMC) and cDNA synthesis, the gene expression levels of FCRL1, FCRL2, and FCRL4 in each group were measured by real-time polymerase chain reaction. **Results:** Our results showed that FCRL1 expression levels in kidney transplant patients were significantly less than healthy controls. The overall FCRL2 expression level was not significantly different between them. However, at days 1 and 7, following transplantation in the non-rejected group FCRL2 level was significantly higher than the control group. Comparing the FCRL4 gene expression levels of both groups with healthy controls showed a significant decrease in the third and seventh days post-transplantation. **Conclusion:** It can be concluded that mononuclear cells, mainly B cells, have an essential role to play in kidney transplantation. [GMJ.2020;9:e1730] DOI:10.31661/gmj.v9i0.1730

**Keywords:** Fc Receptor-Like Molecules; Kidney Transplantation; Peripheral Blood Mononuclear Cells

**Introduction**

Nowadays, renal transplantation has become a well-accepted therapy for patients with end-stage renal disease [1–4]. After solid organ transplantation, the production of donor-specific antibodies (DSAs) increases and causes rejection [5]. B lymphocytes have a major contribution to the balance of transplant rejection [6]. B cells are considered to...
increase the humoral immune response because of their potential for antibodies production [7]. Although antibody-mediated rejection (ABMR) is the major reason for allograft loss [8, 9], B cells can promote allograft rejection as an antigen-presenting cell (APC) or through the production of DSAs [10]. However, the function of B cells is affected by numerous molecules with different properties. Some of these molecules have been recognized to be capable of increased responsiveness of the immune system. Fc receptor-like (FCRL) molecules are an important family with alternative names, including IFGP, SPAP, FCRH, and IRTA [11]. FCRL molecules are related to the Fc receptor (FCR) gene family by structural, genomic organizational, and chromosomal position [12]. In human beings, the FCRL family includes eight genes located on chromosome 1q 21-23. FCRLs 1-6 transmembrane glycoproteins consists of Ig-like domains immune receptor tyrosine-based inhibitory motifs (ITIM) and/or tyrosine-based activation motifs (ITAM) [11, 13, 14]. However, phylogenetic of FCR and FCRL 1–5 molecules to be of five, unlike subtypes [12, 15]. FCRLs 1-5 are expressed mainly by the B cell lineage. Today, the expression of FCRL molecules has been studied in some malignancies and infections [16, 17]. Association of FCRL3, with autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, and Graves’ disease (GD), has been reported recently [18]. The reports are limited to the expression profile of the FCRL family in renal transplantation. We attempted to investigate the expression patterns of FCRL1, FCRL2, and FCRL4 molecules at the mRNA level in peripheral blood mononuclear cells (PBMC) derived from renal transplanted patients.

Materials and Methods

Patients and Control Subjects
Three EDTA-treated blood samples were taken from the patients at first, third, and seventh days post-transplantation. PBMCs were isolated by Ficoll (lymphodex, Germany) density gradient centrifugation. PBMCs were separated from EDTA-treated blood samples and subsequently stored at -80°C until all the samples were collected. Blood samples were divided into two groups, including rejection (6 patients) and non-rejection (26 patients). Furthermore, the present study used 24 healthy subjects as a control group. The control group’s age and sex were matched with the healthy controls to compare the expression levels of FCRL. The control group displayed no autoimmune diseases. The Ethical Committee of Shiraz University of Medical Sciences approved the present study (ethical code:12593).

RNA Extraction and cDNA Synthesis
TRizol reagent (Invitrogen, USA) was used to extract total RNA from the samples according to the manufacturer’s instruction. A NanoDrop spectrophotometer (Thermo Scientific, USA) was applied to evaluate the concentration of RNA (adjusted to 250 ng/μl). Subsequently, cDNA was synthesized using a cDNA synthesis kit (Takara, Japan), according to the manufacturer’s instruction. The cDNA obtained in this study was stored at -20°C until used for real-time polymerase chain reaction (PCR) experiments.

Quantitative Real-Time PCR
Real-time PCR was used to determine FCRL1, FCRL2, and FCRL4 genes in the patients and controls. The human glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was applied as a housekeeping gene or internal control. Table-1 indicates specific primers used for real-time PCR. The PCR was carried out using SYBR®Premix (Takara, Japan) with the Real-time PCR System (ABI step one plus, Applied Biosystems, USA). PCR was conducted in a final volume of 20 μl containing a 2-μl cDNA template, forward and reversed primers, SYBR Premix, ROX Reference Dye II, and dH2O. Table-1 showed the PCR cycle programs used; also, to validate specific amplification, each reaction’s melting curves were monitored. The 2−ΔΔCT formula determined the relative fold changes in FCRL gene expression of the patients and controls.

Statistical Analysis
The analysis of the collected data was conducted using the nonparametric Mann–Whitney U test. The mean ± standard error of the mean (SEM) was used to measure differenc-
es between the two groups. The Spearman correlation test was applied to evaluate the correlation between $FCRL$ gene expression levels and clinical trials. Statistical analyses were carried out using SPSS version 19 (IBM, USA). P-values less than 0.05 were considered to be significant.

**Results**

The non-rejection group included 26 patients, containing seven females (27%) and 19 males (73%), ranging from 26 to 74 years old (mean of $51.62 \pm 10.6$ years). The rejected group included six patients, containing one female (15%) and five males (85%), ranging from 27 to 69 years old (mean of $50.95 \pm 10.61$ years). Blood group O+ exhibited the most frequent ABO blood group in both patient groups. Table-2 shows patient demographics and laboratory tests conducted for each group. As shown in Table-3 and Figures-1, $FCRL1$ gene expression in days 1, 3, and 7 of both non-rejection and rejected patients differ significantly from the control group ($P=0.0001$). However, no significant differences were found in $FCRL2$ gene expression compared with the control group except that of the non-rejection group showed a significant difference in days 1 and 7 ($P=0.0001$). However, significant differences were detected in the $FCRL4$ gene expression in the non-rejection group in days 1, 3, and 7 ($P=0.0001$).

**Discussion**

$FCRL$ molecules are indicated as a receptor family wholly expressed by lymphocytes, mainly B cells that play critical regulatory roles in responses and development of B cells [19]. Signaling pathways of B cell receptors might be making the immunomodulation of their responses, autoimmune or immunodeficiency diseases [20, 21]. In the present study, the

| Table 1. List of Specific Primers Used in this Study. |
|---------------------------------------------------|
| **Genes**      | **primers** | **Primer sequences (5’-3’)** | **Amplicon size** | **Thermo cycling conditions** |
|----------------|-------------|------------------------------|-------------------|-------------------------------|
| $FCRL1$        | F           | GGTCATACTGGTGCGAGGCAC        | 157               | $95°C/30$ s, $95°C/15$ s, 40 cycles of $58°C/20$ s and $72°C/30$ s |
|                | R           | CAGATGAGGACCAGCCT            |                   |                               |
| $FCRL2$        | F           | GTATGTCAATGTGGCTCTG          | 162               | $95°C/30$ s, $95°C/15$ s, 40 cycles of $60°C/20$ s and $72°C/30$ s |
|                | R           | TCTGATTCCTCCTCAAGTGTTATG     |                   |                               |
| $FCRL4$        | F           | GTGAGGGGTTAATCGCACAAGG       | 148               | $95°C/30$ s, $95°C/15$ s, 40 cycles of $61°C/20$ s and $72°C/30$ s |
|                | R           | CTTCAGCCACGGAGGAGGAC         |                   |                               |
| GAPDH          | F           | GGAATCTAGACGCCAGTCA          | 199               | $95°C/30$ s, $95°C/15$ s, 40 cycles of $57.5°C/20$ s and $72°C/30$ s |
|                | R           | CCAGTGAGGCGAGGTGAGT          |                   |                               |

| Table 2. Demographic and Laboratory Indexes of Kidney Transplant Recipients with and without Graft Rejection. |
|-------------------------------------------------------------|
| **Patient characteristics** | **Patients without rejection** | **Patients with rejection** |
| Age (years), mean±SEM                                      | $51.62 \pm 10.60$               | $50.95 \pm 10.61$               |
| Sex, n(%)                                                   |                                   |                               |
| Female                                                      | 7 (27)                            | 1 (15)                         |
| Male                                                        | 19 (73)                           | 5 (85)                         |
| Blood group, n(%)                                          |                                   |                               |
| A positive                                                  | 8 (30.8)                          | 1 (16.7)                       |
| B positive                                                  | 5 (19.2)                          | 2 (33.2)                       |
| AB positive A positive                                      | 1 (15.4)                          | 0 (0)                          |
| O positive                                                  | 8 (30.8)                          | 2 (33.3)                       |
| O negative                                                  | 1 (3.8)                           | 1 (16.7)                       |
expression levels **FCRL1** gene were assessed using real-time PCR in PBMCs derived from kidney transplant rejected and non-rejection patients and compared with the control group. Additionally, we focused mainly on **FCRL1**, **FCRL2**, and **FCRL4** because of the presence of two and three ITAMs in the cytoplasmic region of **FCRL1** and **FCRL4** them enhancer and inhibitory receptors, respectively [19]. **FCRL2** has two ITIMs and a cytoplasmic domain that it may have two function receptors. On the other hand, a mutational investigation recommended that the B cell response parameter has a negative immunomodulatory function of **FCRL2** [20]. The expression levels of **FCRLs** genes have been studied in autoimmune diseases, such as Hashimoto’s thyroiditis (HT), GD, and RA [22-24]. The present study investigated **FCRL1**, **FCRL2**, and **FCRL4** genes expression in patients with
kidney transplanted. As our results showed, there were significant differences in the FCRL1 gene expression in both rejecting and non-rejecting groups. However, FCRL2 gene expression showed no significant alteration except for the non-rejecting group showing a significant difference; furthermore, a significant difference was found in the expression level of the FCRL4 gene in the non-rejecting group on days 1, 3, and 7 with compare to the control group. In some studies, it has been demonstrated that the FCRL1 gene expression levels expression in patients with multiple sclerosis, lupus anticoagulants, arteritis, and von Willebrand disease are higher than that of healthy subjects [21]. This finding suggested that FCRL1 might play a critical role in kidney transplant pathogenesis or allograft rejection. In a previous study, two other autoimmune disorders, HT and GD, showed a significant decrease in the FCRL1 gene expression level but a considerable increase in the FCRL2 and FCRL4 genes expression with compare to the corresponding healthy controls [22]. FCRL4 was expressed in significantly lower levels in patients with kidney transplantation than those of the control. Yeo et al. [18] reported the involvement of FCRL4 in RA. Besides, they introduced a new subset of B cells capable of expressing FCRL4 with a different pro-inflammatory and bone destructive cytokine pattern in the rheumatoid synovium. Accumulating evidence indicated that this subset of B cells is a pathogenic B cell subset in kidney reject transplanted. Although FCRL2 and FCRL4 are most likely expressed by memory B cells, FCRL4 is expressed mainly on a unique subset of memory B cells identified by the IgD-CD27-phenotype [23, 24]. FCRL2 expression has been suggested to be a negative regulator for B cell [20]; therefore, its higher expression could be a compensatory mechanism to decrease B cell function. However, further studies are required to determine the FCRL signaling pathways and find its relation to rejection or non-rejection of kidney transplantation.

Conclusion

Previous findings and our results demonstrated the potential roles of FCRL molecules in graft survival. FCRL1, FCRL2, and FCRL4 are suggested to be critical elements in the graft’s immunological processes. It can be concluded that mononuclear cells, mainly B cells, play important and effective roles in kidney transplantation through the FCRL pathway.

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Conflict of Interest

The authors declare no potential conflicts of interest.

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