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Evaluation of Interleukin-21, 23 and 27 mRNA Expression and Protein Level in Liver Transplant Patients

Afsoon Afshari[1,2], Ramin Yaghobi[1], Negar Azarpira[1], Bita Gerami Zadeh[1], Seyed Ali Malek Hosseini[1], Mojtaba Darbouy[2], and Mohammad Hossein Karimi[1]

[1] Shiraz Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2] Department of Molecular Genetics, Science and Research, Islamic Azad University, Shiraz, Iran

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ABSTRACT

Cytokines have prominent roles in activating of different T cells and shifting the immune response, in this study the role of three cytokines (*IL-21*, IL-23 and *IL-27*) is investigated in the liver transplant rejection.

Three EDTA-treated blood samples were collected from each liver transplanted patient in 1st, 4th and 7th day of post-transplantation. The expression level of the mentioned cytokines was determined using real-time PCR for all samples. Also, the serum levels of cytokines were determined using ELISA tests.

In acute rejection (AR) group (51 patients), mRNA expression pattern of *IL-21*and *IL-23* showed a steady increase, but this pattern was converse for *IL-27*. Our results in non-acute rejection (non-AR) group (54 patients) showed an elevation in day 4 and then a decrease in day 7 for *IL-21* and *IL-23* genes. This pattern was converse again for *IL-27* gene. In comparison between the two groups, in all 3 sampling times the mean of mRNA expression level of *IL-21* and *IL-23*, showed an increase in AR group which this increase was significant for *IL-21* in the 3rd (p=0.007) and for *IL-23* in 2nd (p=0.048) and 3rd (p=0.049) sampling time, but the pattern of mRNA expression for *IL-27* was contrary to the results of *IL-21* and *IL-23*. Furthermore, ELISA technique also, showed the serum level changes the same as cytokines.

In this study *IL-21* and *IL-23* showed pro-inflammatory properties in the liver transplant rejected patients. Also, *IL-27* having different expression pattern, showed anti-inflammatory behavior which needs more considerations in future.

Keywords: Graft rejection; Interleukin-21; Interleukin-23; Interleukin-27; Liver; Transplantation

INTRODUCTION

Different circumstances and diseases can make liver mal-functions and finally cause liver failures. The orthotopic liver transplantation (OLT) is considered a final therapeutic goal for liver failures.[1] Although the

Corresponding Author: Mohammad Hossein Karimi, PhD; Shiraz Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Tel/Fax: (+98 71) 3647 3954, E-mail karimimh@sums.ac.ir

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liver has immune privilege in comparison to other transplanted organs, still more than 30% of the grafted livers are convicted to graft dysfunction and finally cause graft loss.[2] Then long term of the graft survival is due to managing the destructive processes that can cause liver transplant rejection (LTR) after activating the recipient's immune system.[3] Acute rejection is a phenomenon that can cause early graft loss after liver transplantation. The role of cytokines in liver transplant acute rejection is irrefragable. Now, many pro-inflammatory cytokines are detected in relevant to different kinds of transplanted organ acute rejections.[1] Recent studies have been introduced cytokines that have regulatory effects during inflammatory responses like *IL-21*. *IL-21* is a T cell derived cytokine which is related to both adaptive and innate immune responses.[4] *IL-21* is able to activate CD8+ T cells and NK cells[5] and it is also believed that the proliferation effects of *IL-21* on the CD8+ T cells are strong.[6] Some studies propose that *IL-21* can act as the interface or mediator between innate and adaptive immunity.[5,6] By considering the importance of *IL-21* in allogeneic responses, researchers believe that it can be another critical cytokine in the organ transplantation.[7] Researchers showed that *IL-21/IL-21R* system is involved in the acute renal rejection[8] and Baan et al proved the detectable up-regulation of *IL-21* mRNA and protein in the biopsies of human heart transplantation at the time of acute rejection.[7]

IL-23 is another cytokine with pro-inflammatory effects.[9] It is known that *IL-23* stimulates memory T cell population rather than naïve T cells in a specific manner, and is produced from activated macrophages and dendritic cells (DCs).[10,11] This cytokine is detected also as the developer of Th17 cells that specifically produce *IL-17* pro-inflammatory cytokine.[10] *IL-23* is also important in some of inflammatory auto-immune diseases.[10,12,13] Blocking this cytokine in some diseases like EAE has been shown to be useful for preventing from induction of EAE.[13,14] One study proposed that genetic variation in *IL-23/Th17* pathway is important in the stem cell transplantation outcome.[15] Another study showed that the serum protein level of *IL-23/IL-17* increases significantly in the hepatic allograft rejection.[16] Skin rejection model also demonstrated a prominent role for *IL-23* among mice.[17]

IL-27 belongs to *IL-6/ IL-12* super family of cytokines which is believed to have pro- and anti-inflammatory properties and also has an additional role as the key regulator of immune responses to infection. *IL-27* is produced in response to stimulation and has effects on preliminary responses of T cells and upon activation increases the proliferation and effector functions of human CD+8 T cells. *IL-27* is the cause of Th1 response which renders to the expression of *T-bet* and *IFN-γ* by naïve T cells.[18,19] As an anti-inflammatory cytokine, it is shown that *IL-27* has down-regulating function on the effector Th cells, especially Th17 differentiation as well as Th1 and Th2 subset of T cells and also APCs. In addition, *IL-27* has pro-inflammatory effects on CD8+ T cells.[20] In inflammatory diseases, the elevate rate of *IL-27* is observed in mice and human.[18] The role of *IL-27* in auto-immune diseases is also detected.[19,21] The role of *IL-27* in transplantation is merely studied; in one study, the increase of *IL-27* was observed in experimental models of cardiac allograft, which the author believed to have a role in the tolerance mechanism.[22]

Although researches on the role and function of inflammatory cytokines such as *IL-21*, *IL-23* and *IL-27* is a promising area in auto-immune diseases[23] further studies would help for detecting the exact kinetic of cytokine production in post-transplant times, so in this study we focused on the expression pattern and plasma level of these cytokines in rejected and non-rejected liver transplanted patients.

MATERIALS AND METHODS

Patients

In this retrospective study, a total number of 130 patients were participated from Namazi Hospital, Shiraz Iran (2011–2013). These patients were recruited based on inclusion criteria including first time liver transplantation and regimen of the immunosuppressive drugs, which were composed of calcineurin inhibitors (CI) and was the same in dosage for patients of each study groups. Moreover, we imposed exclusion criteria to our sample population which are consisted of if a patient had re-transplantation, and/or if got a combined liver/kidney transplantation. We also excluded those patients who were deceased before the completion of the sample collection or had missing data points. Furthermore, patients with several viral infections like cytomegalovirus (CMV) and polyoma BK virus were ruled out of the project. Patients receiving high doses of corticosteroids and pulse therapy were excluded either. The remainder 105 patients were divided into two

groups after finishing the analyses; non-AR group, composed of 54 patients without any history of acute rejection during the course of this study and AR group including 51 patients who experienced acute rejection. The condition of acute rejection of each patient were confirmed by an expert pathologist according to the level of liver serum enzymes, bilirubin level (in non-biliary problems), and histologic features of the liver biopsies and clinical and biochemical responses to steroids with high-dose, according to Banff criteria.[24] Cross match is done for all liver transplant recipients and donors.

This study was conducted according to the guidance provided by Helsinki Declaration and was approved by the Ethical committee at the Shiraz University of Medical Sciences. Accordingly, the patients were informed and signed the consent form prior to participating in this study. According to the rules of Transplant ward of Namazi Hospital, all of the transplanted patients receive organs from cadaver.

Immunosuppressive Drugs and Transplant Details

The regimen of the immunosuppressive drugs was a routine one, which was composed of tacrolimus or cyclosporine with mycophenolate mofetil. The drug dosage for patients was the same, varying just by the body mass. HLA typing was not done in this center for liver transplant patients. ABO blood compatibility was checked.

Sampling Procedure

In the 1st week after transplantation, 3 EDTA-treated blood samples were collected from each patient in day 1st, 4th and 7th post-transplantation. As the rejection starts in the first hours after transplantation, these days selected for sampling. Also, other studies done by other researches showed that in the mentioned sampling timing course, the changes in kinetic of cytokines are more detectable. Using Ficol gradient (Nycomed, Zurich, Switzerland), serum and buffy coat were separated and serum kept in -80°C for further studies.

RNA Isolation and Reverse Transcription

The separated buffy coats used for cell counting and from each sample 2x106 cells collected for RNA isolation. Total RNA extracted from the buffy coats by using RNX Plus (Cinnagen, Iran) reagent. For checking the purification of extracted RNA, its purity (A260/280) was measured using Nanodrop (Thermofisher Scientific, USA). Also, the quality of extracted RNA was checked by running on 1% agarose gel.

For cDNA synthesis, extracted RNAs (1 µg) were converted to cDNA by adding random hexamer (1 µL, 0.2 µg), dNTP (1 µL, 10mM) and incubated the mix at 65°C for 7 min and then put on ice for 2 min. In the next step, M-MULV reverse transcriptase (RT) enzyme (1 µL, 200U), RT buffer (2 µL, 10x) and RNase inhibitor (1.3 µL, 60U) were mixed and added to the first mix. The new whole mix incubated for 90 minutes at 45°C and then 5 minutes at 85°C (enzymes were purchased from Vivantis Company, Malaysia). Also, all of RNA samples were treated with DNase (Thermo Fisher Scientific, Waltham, MA, USA) before changing to cDNA. For this reason, 1 µL for each µg of RNA were treated with DNase.

Quantitative Real-Time Polymerase Chain Reaction

The expression level of *IL-27*, *IL-21* and *IL-23* of transcripts were determined by using step-one plus real-time PCR (ABI, USA) for all the samples. For choosing internal control both *GAPDH* and *β-actin* were checked but *β-actin* was chosen because of having minor fluctuations, which this result was concluded during evaluating between *β-actin* and *GAPDH* transcripts using clinical and normal samples. Also, in set-up procedure standard curves were used to choose the study analysis mode, and for eliminating analytical mistakes and certifying the results of real-time PCR results Livak ($2^{-\Delta\Delta CT}$) method was chose.

Primers for *IL-21*, *-23* and *-27* genes and also, *β-actin*, as an internal control were designed using Oligos and DNASTAR softwares (Table 1). The PCR mix was composed of SYBR Green Premix (10 µL of Ex taq, Takara, Japan), Rox reference dye (0.2 µL), forward and reverse primers (10 pM) and template (2 µL of synthesized cDNA) for each reaction. Nuclease free water also added to this reaction up to reaching to 20 µL total volume. The program for thermo-cycling was: One cycle 95°C for 5 min, followed by 40 cycles of 95°C for 30 seconds, 65°C for 20 seconds. For each reaction, the melt curve was analyzed to confirm the specificity of reaction.

Measuring Cytokines Protein Levels

The plasma of all samples was collected and stored at -80°c for further analysis and the protein level of all

Table 1. Primer sequences used for Real-time analysis of IL-21, IL-23, IL-27 and internal control genes for studying expression level in liver transplanted patients.

Gene	Gene ID	Primer sequence	Product length
IL-21	NM-021803.2	F:5'TCCAGTCCTGGCAACATGGAGA R:5'GGCGATCTTGACCTTGGGAGC	97 bp
IL-23	NM-016584.2	F:5'AGTGGAAGTGGGCAGAGATTC R:5'CAGCAGCAACAGCAGCATTAC	115 bp
IL-27	NM-14565.3	F:5'GCACTGGGCAGCGCCTTACA R:5'TCCCGCACGGCCCGAGATAA	110 bp
B-actin	NM-001199954.1	F:5'GGCGGCACCACCATGTACCC R:5'GACGATGGAGGGGCCCGACT	203 bp

samples was measured using ELISA kits (for *IL-21* and *IL-23* Mabtech, Sweden and for *IL-27* Bioassay technology kit, Korea, were used according to the manufacturer protocol).

Statistical Analysis

All data were analyzed in SPSS v.21 software (SPSS, Chicago, IL, USA). For determining the expression level of each gene, comparison within groups and also between understudied groups was performed, non-parametric tests (Mann-Whitney tests, two independent tests and k independent tests) for studying the expression levels of genes ΔCT and $2^{-\Delta\Delta CT}$ (Livak method) were also used. Also, $p<0.05$ was considered statistically significant. Histograms are drown based on ΔCT and fold changes.

RESULTS

Descriptive Characteristics

The patients participating in the study were composed of non-AR group with the mean of 37.5±18.14 ranging 1-74 years, and AR group with the mean of 35.5±11.25 ranging 2-69 years. Non-AR patients also were composed of 37 (68.5%) male and 17 (31.5%) female and AR patients were composed of 36 (70.5%) male and 15 (29.5%) female. The underline diseases distribution is shown in Table 2 which reveals that among viral reasons for liver failure, Hepatitis B Virus positive patients are more susceptible ones (near 42%) and also among other causes, primary sclerosing cholangitis (PSC; near 38%) is a critical reason for liver failure.

Table 2. Distribution of the underlying diseases in acute rejected (AR) and non-AR liver transplant patients

	Non-AR patients		AR patients	
	Male n (%)	Female n (%)	Male n (%)	Female n (%)
Cryptogenic	5(9.2)	3(5.5)	9(17.6)	1(1.97)
Hepatitis B Virus+	13(24)	2(3.7)	6(11.78)	1(1.97)
Hepatitis C Virus +	2(3.7)	0(0)	0(0)	2(3.92)
Primary sclerosing cholangitis	4(7.4)	4(7.4)	9(17.6)	3(5.85)
Autoimmune hepatitis	4(7.4)	5(9.2)	4(7.85)	4(7.85)
Wilson disease	1(1.85)	1(1.85)	3(5.85)	2(3.92)
Hypertyrosinemia	1(1.85)	1(1.85)	0(0)	0(0)
Biliary atresia	3(5.5)	0(0)	0(0)	0(0)
Other diseases	4(7.4)	1(1.85)	5(9.9)	2(3.92)
Total	37(68.3)	17(31.7)	36(70.6)	15(29.4)

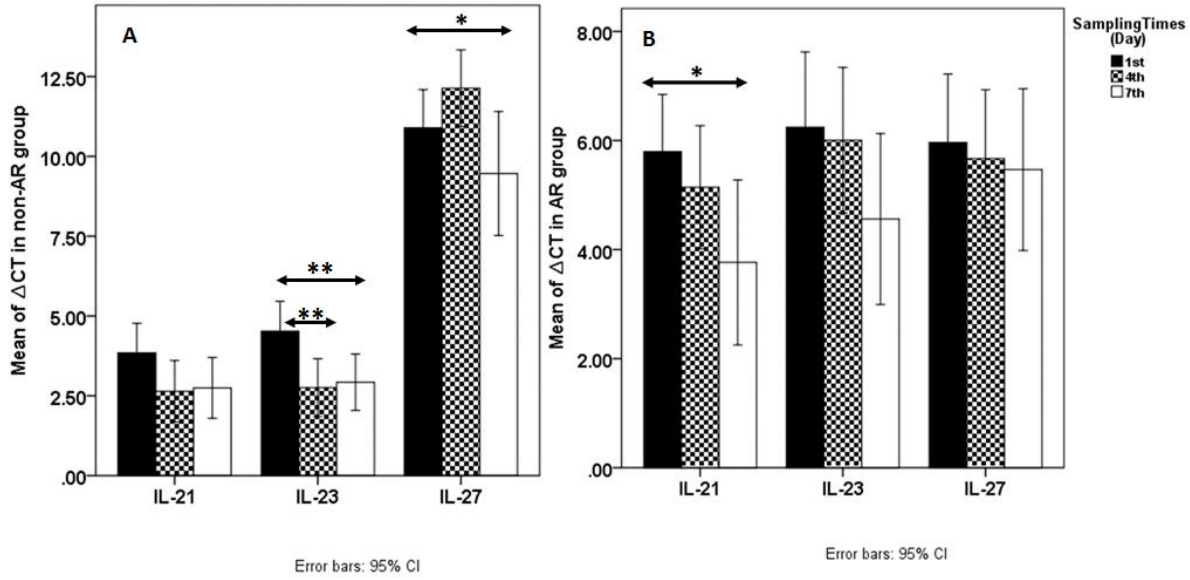

Figure 1. The pattern of *IL-21*, *IL-23* and *IL-27* mRNA expression level in the non-acute rejected (AR) (A) and AR (B) liver transplant patients; the expression level is measured based on ΔCT which is converse of expression level. Also, $p<0.05$= *, $p<0.01$= **, and $p<0.001$= *.**

IL-21, *IL-23* and *IL-27* Gene Expression Level in Non-AR and AR Groups of Patients

In Figure 1A, the pattern of mRNA expression level in *IL-21* and *IL-23* genes showed an up regulation and then down regulation in non-AR group of patients, post-transplantation, but this pattern was quite different in *IL-27* gene analysis, which showed a down regulation in day 4 of sampling time. Also, the significant *p* value level between each group in each studied cytokine is mentioned in the Figure.

In AR group, all three genes showed a steady increase (Figure.1B) in mRNA expression level during sampling time post-transplantation. Also, the significant *p* value level between each group in each studied cytokine is mentioned in the Figure.

Comparing *IL-21*, *IL-23* and *IL-27* Gene Expression in Non-AR and AR Liver Transplant Patients

The expression level of *IL-21* is compared between non-AR and AR groups of patients (Figure 2A). In the 1st and 2nd sampling times the mRNA level of *IL-21* showed an increase in AR group ($p>0.05$) but this increase is significant in the 3rd sampling time (day 7, $p=0.007$, 95% CI=0.00-0.037).

The expression level of *IL-23* is compared between non-AR and AR groups of patients. In all sampling times, the mRNA level of *IL-23* showed an increase in AR group but this increase was significant in the 2nd (day 4, $p=0.048$, 95% CI=0.028-0.163) and 3rd (day 7, $p=0.049$, 95%CI=0.00-0.096) sampling time and this increase was not significant in the 1st (day 1, $p=0.848$, 95% CI=0.769-0.927; Figure 2B).

The expression level of *IL-27* is compared between non-AR and AR groups of patients. In the 1st sampling time, the *IL-27* mRNA expression level is increased in AR patients ($p=0.09$, 95% CI= 0.034-0.146), in the 2nd sampling time, it reached almost the same level in both studied groups ($p=0.55$, 95% CI= 0.452-0.648) and in the 3rd one, the *IL-27* mRNA expression level is decreased in AR patients compared to non-AR ones ($p=0.36$, 95% CI= 0.226-0.454). But these mRNA expression level changes were not statistically significant in none of the comparisons; Figure 2C.

Comparing *IL-21*, *IL-23* and *IL-27* Plasma Level in Non-AR and AR Liver Transplant Patients

The plasma level of *IL-21* cytokine protein was estimated by ELISA technique. Significant increases were detected in all of the sampling days especially in day 4 ($p=0.005$; 95% CI= -0.1 to -0.02) and day 7 sampling time ($p=0.03$; 95% CI= -0.07 to -0.005, also day1 was: $p=0.41$; 95% CI= -0.05 to 0.02) in AR liver transplanted patients comparing to non-AR ones. These results were also in accordance with the estimated mRNA level (Figure 3A).

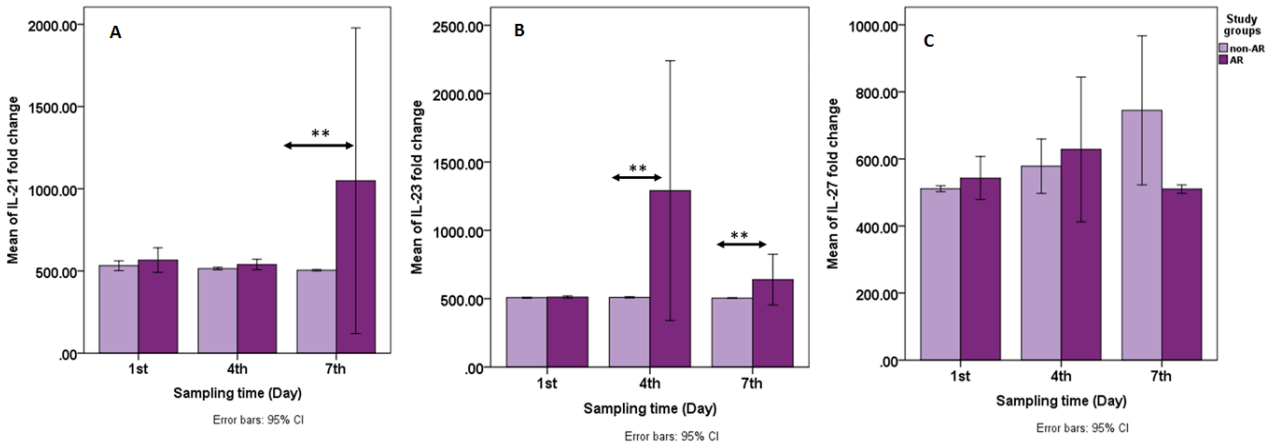

Figure 2. Comparing the fold change between non-acute rejected (AR) and AR rejected patients; (A): *IL-21*, (B): *IL-23*, and (C): *IL-27* fold change. In this Figure, the fold changes are calculated using $2^{-\Delta\Delta CT}$. Also, $p<0.05$= *, $p<0.01$= **, and $p<0.001$=*.**

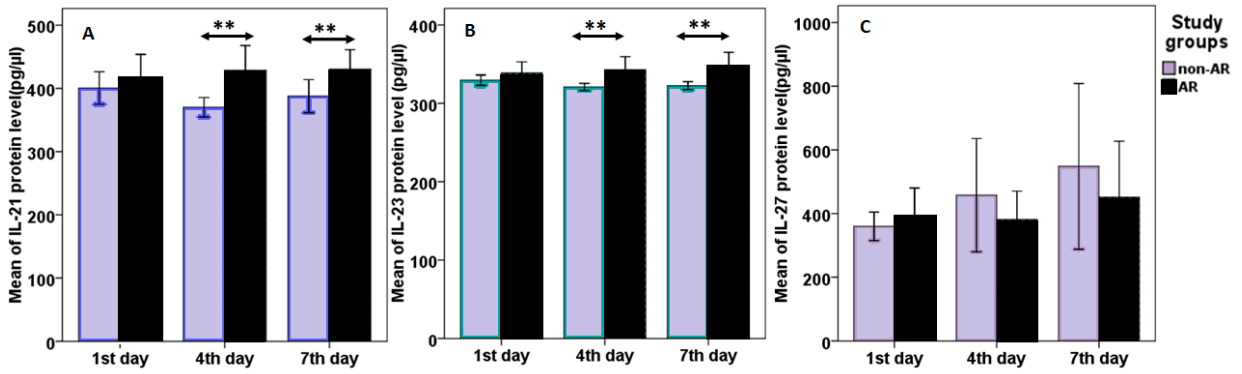

Figure 3. Comparing the *IL-21* (A), *IL-23* (B), and *IL-27* (C) protein level between non-acute rejected (AR) and AR rejected patients

The plasma level of *IL-23* cytokine protein was estimated by ELISA technique. Significant increases were detected in all of sampling days especially in day 4 (p=0.008; 95% CI=-0.056 to -0.009) and day 7 sampling time (p=0.004; 95% CI=-0.063 to -0.013, also day's1 was: p=0.22; 95% CI=-0.036 to 0.008) in AR liver transplanted patients comparing to non-AR ones. These results were also in accordance with the estimated mRNA level (Figure 3B).

The plasma level of *IL-27* cytokine protein was estimated by ELISA technique. An increase was detected in the first sampling day (p=0.42; 95%CI=-0.246 to 0.109) which followed by a decrease in the 4th day (p=0.39; 95% CI=-0.212 to 0.517), and the 7th day (p=0.49; 95% CI=-0.384 to 0.77) in AR liver transplanted patients comparing to non-AR ones. These results were also in accordance with the estimated mRNA level (Figure 3C).

DISCUSSION

Cytokines are important for orchestrating of the immune responses, so researchers believe that cytokines or their receptors may be the key for the treatment of inflammatory diseases.[25] Cytokines are also common cause of changes in immune system in the liver transplantation, which may speculate them as the main player in the rejection phenomenon.[11] Considering all of these facts, we decided to evaluate the role of some cytokines like *IL-21*, *IL-23* and *IL-27* in the liver transplant patients who experienced acute rejection in comparison to non-rejected ones.

In this study, mRNA expression pattern of *IL-21* and *IL-23* genes through the sampling period was the same and showed a steady increase during acute rejection, which may be the outcome of starting the expression of pro-inflammatory cytokines during an immunologic response like acute rejection. But this pattern was converse for *IL-27* mRNA expression which is explained later. Our results in non-AR group of patients showed an elevation in day 4 (2nd sampling time) and then a decrease in day 7 (3rd sampling time) in both *IL-21* and *IL-23* genes. This pattern in these pro-inflammatory cytokine expression level may be due to the kinetic of cytokines in facing a transient inflammatory condition.[26] This pattern was again converse for *IL-27* gene.

Researchers believe that the precise role of *IL-21* in immune system is not completely defined.[5] Besides, it should be mentioned that *IL-21* is produced by Th17 cells, which their possible role has been mentioned in acute rejection.[1] Reversely, *IL-21* also induce *IL-17* production.[26,27] Th17 cells and its important cytokine (*IL-17*) was mentioned as an important factor in human inflammatory and auto-immune diseases before, and it is known that in the early stages of auto-immune diseases, inflammatory responses can activate memory T cells to secrete *IL-17* and *IL-21*.[27] Pro-inflammatory effects of *IL-21* is due to its role in the activation of CD8+ cells as well as NK cells and promote their cytotoxicity and also their potentiality to convert CD4+T cells into Th17 cells, which are involved in transplant rejection.[8] A research has been demonstrated that blocking of *IL-21* production and its signaling pathway can be considered as a new strategy for the treatment of immune responses and also may help in the prevention of graft rejection occurrence.[7]

In comparison between the two groups (non-AR and AR), in all 3 sampling times the mean of mRNA expression level of *IL-21* showed an increase in the AR group, and this increase was statistically significant in the 3rd sampling time for *IL-21*. Furthermore, comparing the ELISA results between groups showed up regulation in the rejected group of patients, which was statistically significant in 2nd and 3rd sampling times.

The results of our study can be confirmed by other researches which showed that *IL-21* and its receptor system are important in renal allograft acute rejection by mentioning that *IL-21* promote CD8+ T lymphocytes effector function which these cells are one of critical factors in the grafted tissue destruction. Also, the authors believed that during the process of allograft rejection about 70% of highly activated monocytes are recruited to the graft blood vessels and by expressing their pro-inflammatory cytokines facilitate acute rejection.[7,8] Putting together, as *IL-21* role is obvious in the activation of several immune cells in many immune responses like infection, auto-immune diseases, tumor invasion and finally transplant rejection, it is critical to investigate its role in immune system responses of liver transplanted patients.[28]

IL-23 is another cytokine which can produce inflammation and also guide Th17 cells toward the destruction of inflamed tissues.[29] Like *IL-17*, *IL-23* has an influence on the immune cells and induces them for the production of specific cytokines.[30-32] So that, the role of this cytokine in activation of Th17 in acute transplant rejection is one of the important issues to be investigated.[15] Studies have shown the role of *IL-23* in auto-immune diseases and cancer.[33-35] In the other hand, *IL-23* is an inducer for *IL-17* producing T cells which themselves are important in transplant rejection.[1,35] Fabrega showed the protein production of *IL-17/IL-23* molecules increase in the liver transplant acute rejection.[11] Very limited researches are available which focus on the role of *IL-23* in transplantation, Yoshida et al showed the substantial effects of *IL-23/IL-17* in the induction of rejection in the lung transplantation.[36] The role of *IL-23*/Th17 in the result of T cell-dependent allogeneic stem cell transplantation is reported by Carvalho et al and it was shown that *IL-23*/Th17 pathway can affect the incidence of acute GVHD in these patients.[15] When all these data are contemplating together, it can show a whole picture of *IL-23* notability in transplantation immunity and its relation to other pro-inflammatory cytokines like *IL-17*. Thus, our results about *IL-23* in liver transplanted patients are in accordance with other results achieved by other researchers.[11,15,17] The expression level of this cytokine in both groups was the same as *IL-21* pattern and by showing more significant differences in comparison with the expression level of this cytokine between the two mentioned groups (non-AR and AR), it may be the result of more changes in the expression level of this cytokine in comparison to *IL-21* cytokine at the time of inflammation specially in the liver transplantation. Furthermore, comparing the ELISA results between groups showed up regulation in the rejected group of patients, which was statistically

significant in 2nd and 3rd sampling times.

In this study, another cytokine which was measured among the same patients, is *IL-27*. *IL-27* and *IL-23* are related to the same superfamily of cytokines; it is believed that their function is related to each other but they have distinct patterns for the regulation of inflammation.[35] This cytokine is recognized to have pro- as well as anti-inflammatory effects on immune responses and is also important in the regulation of responses to infections.[25] *IL-27* is known to be elevated in several human and murine model auto-immune diseases.[18,19,21] This cytokine is also important in defense of host against viral infections such as Hepatitis C.[18] The property of *IL-27* which in one hand is able to activate inflammatory immune responses and in the other hand can suppress immune responses has made a dilemma in deciding about its role in any related diseases.[37]

It is believed that pro-inflammatory properties of *IL-27* is due to the fact that *IL-27* increases the production of IFN-γ by CD4+ T cells which have *IL-27* receptor on their surface, which finally, renders to the activation of the genes that are capable in TH1 cells polarization and responses through activation of STAT1 and then T-bet transcription factors, but this chain of reactions results in down-regulation of *IL-27*. It is believed that, because the pro-inflammatory role of *IL-27* is detected mostly as a result of parasitic or bacterial infection till now, this property of *IL-27* seems to become as a secondary attitude than its suppressor effects.[38]

As an anti-inflammatory cytokine, it can suppress Th2 and Th17 differentiation. This property partly is due to its ability to skew T helper cells polarization. Also, *IL-27* can apply its anti-inflammatory effects, directly or indirectly by suppression of inflammatory cytokines such as *IL-17*, *IL-21*, *IL-23* and *IL-16* which previously mentioned to be important in transplant rejection.[1,37] To confirm this augment, Moon et al in 2013 did a research, which based on their claim, was the first study that demonstrates the anti-inflammatory property of *IL-27* by regulating Th17 and Treg cells simultaneously that can contribute to having anti-arthritic effects.[39] The pattern of mRNA expression for *IL-27* was different from *IL-21* and *IL-23* in our study. The expression pattern of this gene showed an increase in the AR group in the 1st sampling time and then became somehow the same in the 2nd sampling time and finally demonstrates a decrease in the AR group of patients in the 3rd sampling time comparing to the non-AR group. So, it seems that *IL-27* is acting like an anti-inflammatory cytokine. In confirming out results, a study which was done by Le Texier et al on the immune-regulatory function of *IL-27* in experimental models of cardiac transplantation, up-regulation of *TGFβ1/IL-27* was reported which can be concluded that these molecules may have a role in tolerance mechanisms. This study group has proposed that over-expression of *IL-27* together with immune suppression may be considered as a new therapeutic strategy for the treatment of rejection in transplants.[22] Also, ELISA results of *IL-27* comparing between the two groups, disclosed a different pattern from *IL-21* and *IL-23*. In the 1st sampling *IL-27* was increased in the rejected group of patients, but it had a decrease criterion during the 2nd and 3rd sampling times.

The result of *IL-27* mRNA expression pattern in this study is in accordance with the studies made by Le Texier et al.,[22] and showed the expression pattern converse to the two other investigated cytokines (*IL-21* and *IL-23*) specially in non-AR group of patients. In these patients, *IL-27* expression pattern may be the result of its anti-inflammatory effects in an inflammatory situation. But the expression pattern of this cytokine in AR group of patients showed a steady increase in the expression level during the sampling time which may be the result of the huge changes in the immune system in the time of responding to acute rejection. Also in comparing *IL-27* expression pattern between the non-AR and the AR groups of patients, it obviously showed a converse pattern comparing to the other two pro-inflammatory cytokines (*IL-21* and *IL-23*). Only by accepting this fact that the anti-inflammatory effects of this cytokine are more potent than its pro-inflammatory effects in transplant situation, these data can explain the role of *IL-27* in helping the immune system for accepting or at least controlling the acute rejection.

It seems that studying cytokines is important due to their ability to identifying the type of immune response which is created by T cells. T cells are critical in the acceptance or rejection of liver. The expression pattern of *IL-21* and *IL-23* in this study confirms their pro-inflammatory roles, therefore, they increase in the rejected group of patients as the day of rejection increases during the 1st week post-transplantation. *IL-27* act as a pro- and anti-inflammatory cytokine, which its expression pattern in the current study certifies its

anti-inflammatory nature, but the role of this cytokine in transplantation is merely studied and accepting this study as a primary screen, to declare and confirm the importance of these studied cytokines in acute liver rejection further completed studies are needed. Also, this study had some limitations which should be surmounted in further studies, some of our samples missed due to decease of patients before finishing the sampling time. More samples and extended sampling time can give more precise results.

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