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Impact of serum IL 10 on prediction of early allograft rejection in liver transplantation

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Abstract

Tissue transplantation is the preferred treatment for end organ failure such as heart, lung, kidney, and liver. The immune system recognizes the transplant as non self if the donor and recipient are not genetically identical. Multiple cytokines are involved in this process; however, little is known about their predictive role in rejection. Interleukin 10 (IL-10) which exhibits anti-inflammatory activity could be used as early predictor of acute rejection. The current study intended to determine any potential relationship between acute allograft rejection and blood IL-10 levels in liver transplant (LT) recipients. This study included 45 patients with cirrhotic liver diseases planned for transplantation. Patients were followed up for 2 months and then divided into two groups: patients who developed early acute rejection and those who did not develop rejection (as controls). Of the study patients, 38 (84.4%) patients did not develop rejection and 7 (15.6%) patients developed rejection. The levels of IL-10 did not change during rejection of the LT. In conclusion, the findings of the current study indicated no relation of IL-10 levels during LT rejection.

Keywords: breast cancer, HPV, Treg

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Introduction

During episodes of acute rejection, patients may be asymptomatic, or may describe general malaise or discomfort in the upper quadrant. The diagnosis should be considered in liver transplant recipient's patient with rising serum transaminase levels, particularly if this is accompanied by sub-therapeutic blood levels of immunosuppressive agents. A liver biopsy is mandatory to confirm the diagnosis. This procedure is often uncomfortable. Moreover, rejection symptoms are often discovered too late. Physicians have been trying to find a biomarker that identifies rejection without having to do a biopsy.

Several lines of evidence have demonstrated that genetic delivery of interleukin-10 (IL-10) to

allografts leads to improved graft acceptance in animal heart[1] or liver transplantation[2] models. However, part of the interest in this cytokine as an anti-inflammatory therapy has decreased due to the modest successes of the initial clinical trials employing recombinant IL-10. The subject has been revitalized by new research indicating that regulatory T cells and even T-helper 1 T cells may produce IL-10, demonstrating the cytokine's potency to modulate immune responses. Research into how the IL-10 gene is expressed in diverse cell types may result in the development of novel therapies to boost or decrease IL-10 production. So, in this study we tested the value of IL 10 as a marker for prediction of early graft rejection.

Patients and Methods

This was a prospective case-control study carried out in the Organ Transplantation unit, Faculty of Medicine, Ain Shams University specialized hospital on 45 patients with cirrhotic liver diseases planned for transplantation. Patients were given standard immunosuppressant regimens.

They were followed up for two months after which they were divided into two groups. Group 1, included patients who developed early acute rejection of liver transplantation, defined by clinical symptoms as deterioration of present manifestations of liver disease or development of new manifestations of liver disease. Then liver biopsy was done to confirm rejection. Group 2 included patents who did not develop rejection (as controls). Thus, the inclusion criteria were adult liver transplant recipients from living donors.

All members of the study were subjected to full medical history taking, clinical examination, and determination of ABO blood groups. The Model End Stage Liver Disease (MELD) score was performed for assessing severity of chronic liver disease.

Laboratory investigation included complete blood count, kidney, and liver function tests. Blood urea nitrogen (BUN) and creatinine (Cr), and serum aspartate aminotransferase (AST), alanine transaminase (ALT), International normalized ratio (INR), albumin (Alb), total and direct bilirubin were determined by an enzymatic colorimetric assay using an autoanalyzer (Beckman coulter AU480 Auto Analyzer), according to the manufacturer's instructions. Data for complete blood count (CBC), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver biopsy and pelvic-abdominal ultrasound, were obtained from hospital records.

Also, estimation of serum IL-10 was performed just before transplantation, and then at weekly intervals (day 0, 7, 14, 28) for one month after adult liver transplantation. Serum concentration of IL-10 was measured by enzyme immunosorbent assay (ELISA) commercial kits (Cat.No：E0102Hu Shanghai Korain Biotech CO. LTD), according to the manufacturer's instructions. Briefly, standards and patient's serum samples were added to their respective wells, (pre-coated with IL-10 monoclonal antibody in the kits). After incubation, anti IL10 antibodies labeled with biotin were added to unite with streptavidin-horseradish peroxidase (HRP). Unbound enzymes were removed after incubation and washing. Substrate was added. After an incubation period, a stopping solution was added. The absorbance (OD) of each well was measured at 450 nm by an ELISA reader (cat number: 51119000, Multiskan FC by Thermofisher, USA). According to standards' concentrations and the corresponding OD values, the standard curve was plotted, and IL-10 values of the patient's samples were extrapolated from the curve.

Statistical Analysis

Data were analyzed using STATA intercooled version 14.2. (Stata Corp LLC, College Station, TX, USA). Quantitative data were represented as mean and standard deviation. The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using independent t-test while with non-parametric distribution were done by using the Mann-Whitney test. Follow up for more than two paired samples were done using repeated measures ANOVA for parametric data and

followed by post hoc analysis using the Bonferoni test. For non-parametric data, the follow up was done by Friedman test followed by post hoc analysis using Wilcoxon Rank test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at $p < 0.05$.

Results

Demographic and clinical characteristics of all participants

The present study included 45 patients. Of these, 38 (84.4%) patients did not develop rejection. They included 27 males and 11 females. The other 7 (15.6%) patients developed rejection. They were 5 males and 2 females. There was a significant decrease in the mean age (± SD) of the cases with rejection (ranged 18-62 years) than the group who did not develop rejection (ranged 18-63) (p=0.027). The main etiology of liver disease was hepatitis C virus (HCV) (57.9%), hepatocellular carcinoma (HCC) (47.4%), Bilharziasis (18.4%), autoimmune hepatitis (AIH) (13.2%) then hepatitis B virus HBV and cryptogenic cause (10.5%), (Table 1).

Table 1. Demographic data and characteristics of the studied patients.

		Acute rejection		*p*-value
		Negative No. = 38	Positive No. = 7	
Sex	Female	11 (28.9%)	2 (28.6%)	NS
	Male	27 (71.1%)	5 (71.4%)	
Age	Mean ± SD	51.50 ± 10.44	39.86 ± 20.40	0.027
	Range	18 – 62	18 – 63	
ABO grouping	A positive	18 (47.4%)	3 (42.9%)	NS
	B positive	10 (26.3%)	2 (28.6%)	
	AB positive	1 (2.6%)	1 (14.3%)	
	O positive	9 (23.7%)	1 (14.3%)	
Etiology	HBV	4 (10.5%)	0 (0.0%)	NS
	HCV	22 (57.9%)	2 (28.6%)	NS
	HCC	18 (47.4%)	2 (28.6%)	NS
	AIH	5 (13.2%)	4 (57.1%)	0.008
	Cryptogenic	4 (10.5%)	1 (14.3%)	NS
	Bilharzial cirrhosis	7 (18.4%)	0 (0.0%)	NS
MELD score	Mean ± SD	14.26 ± 4.60	15.14 ± 5.84	NS
	Range	7 – 25	8 – 23	

HBV: hepatitis B virus, HCV: hepatitis C virus, HCC: hepatocellular carcinoma, AIH: autoimmune hepatitis, MELD: Model End Stage Liver Disease. * $P > 0.05$ is not significant (NS).

Table 2 shows a statistically significant increase in serum level of ALT among cases with acute rejection compared to those without rejection. While there was no difference in the level of AST, Alb, INR, total and direct bilirubin, and IL-10 at day 0.

Table 2. Follow up of the laboratory data among the two studied groups at day 0.

Day 0		Acute rejection		*p*-value
		Negative No. = 38	Positive No. = 7	
AST	Median (IQR)	40.5 (31 – 66)	60 (23 – 80)	NS≠
	Range	17 – 111 U/L	23 – 81 U/L	
ALT	Median (IQR)	33 (18 – 44)	51 (40 – 99)	0.017≠
	Range	9 – 116 U/L	21 – 104 U/L	
Alb	Mean ± SD	2.67 ± 0.63	2.84 ± 0.73	NS•
	Range	1.4 – 3.7 g/dl	1.8 – 3.4 g/dl	
INR	Mean ± SD	1.56 ± 0.70	1.60 ± 0.35	NS•
	Range	1 – 4.2	1.1 – 2.1	
T. bilirubin	Median (IQR)	2 (1 – 4)	3.2 (1 – 6.3)	NS≠
	Range	0.3 – 16.3 mg/dl	1 – 7.4 mg/dl	
D. bilirubin	Median (IQR)	0.8 (0.3 – 1.7)	1.5 (0.2 – 5.2)	NS≠
	Range	0.1 – 9.2 mg/dl	0.2 – 5.8 mg/dl	
IL-10	Median (IQR)	60 (50 – 110)	70 (60 – 80)	NS≠
	Range	10 – 400 pg/mL	50 – 100 pg/Ml	

AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio, IL-10: Interleukin 10, *P* > 0.05 is not significant (NS), •: Independent t-test; ≠: Mann-Whitney test.

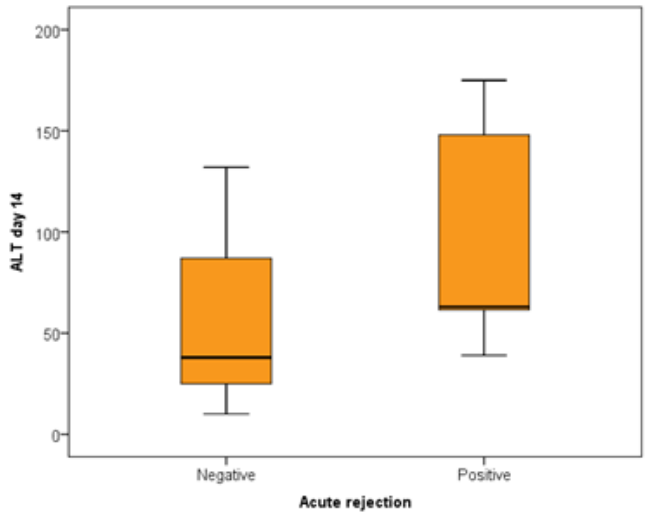

Figure 1. Box plot showing significant difference in the median alanine aminotransferase (ALT) level between cases with and without acute rejection at day 14.

Table 3 shows a statistically significant increase in serum level of ALT and AST among cases with acute rejection compared to those without rejection. While there was no difference in the level of Alb, INR, T bilirubin, D bilirubin, IL-10 on day 28.

Table 3. Comparison between follow up laboratory data among the two studied groups on day 28.

Day 28		Acute rejection		*p*-value
		Negative No. = 38	Positive No. = 7	
AST	Median (IQR)	26 (18 – 44)	280 (65 – 428)	<0.0001≠
	Range	1 – 325 U/L	44 – 428 U/L	
ALT	Median (IQR)	35.5 (25 – 70)	320 (70 – 553)	0.001≠
	Range	20 – 323 U/L	50 – 553 U/L	

Table 3. Continued.

Day 28		Acute rejection		*p*-value
		Negative No. = 38	Positive No. = 7	
Alb	Mean ± SD	2.91 ± 0.48	2.99 ± 0.54	NS•
	Range	2 – 4 g/dl	2 – 3.8 g/dl	
INR	Mean ± SD	1.11 ± 0.23	1.09 ± 0.14	NS•
	Range	0.8 – 1.9	1 – 1.4	
T. bilirubin	Median (IQR)	0.8 (0.7 – 1.2)	0.9 (0.8 – 9)	NS≠
	Range	0.2 – 9 mg/dl	0.8 – 10.6 mg/dl	
D. bilirubin	Median (IQR)	0.5 (0.2 – 0.8)	0.4 (0.4 – 4)	NS≠
	Range	0.1 – 5.2 mg/dl	0.4 – 5.2 mg/dl	
IL-10	Median (IQR)	360 (250 – 460)	400 (400 – 400)	NS≠
	Range	164 – 700 pg/mL	350 – 400 pg/Ml	

AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio, IL-10: Interleukin 10, * *P* > 0.05 is not significant (NS). •: Independent t-test; ≠: Mann-Whitney test.

Table 4 shows statistically significant increase in serum level of ALT, T. bilirubin and, D. bilirubin between cases with rejection compared to those without acute rejection. As regards liver biopsy there was statistically significant increase in mild and moderate acute cellular rejection between cases with rejection compared to those without acute rejection (Figure 2). While there was no difference in biliary stricture, recurrent HCV and cholangitis after 2 months follow up.

Table 4. Follow up of the laboratory data among the two studied groups after 2 months.

After 2 months		Acute rejection		*p*-value
		Negative No. = 38	Positive No. = 7	
ALT	Median (IQR)	30 (20 – 43)	330 (180 – 400)	<0.0001≠
	Range	17 – 280 U/L	108 – 480 U/L	
T. bilirubin	Median (IQR)	1 (0.8 – 1.2)	3 (2 – 8)	<0.0001≠
	Range	0.4 – 8 mg/dl	2 – 8.5 mg/dl	
D. bilirubin	Median (IQR)	0.3 (0.2 – 0.5)	1 (0.8 – 3)	0.002≠
	Range	0.1 – 4.5 mg/dl	0.5 – 3.3 mg/dl	
Liver biopsy	Negative	33 (86.8%)	0 (0.0%)	<0.0001*
	Mild acute cellular rejection	0 (0.0%)	4 (57.1%)	<0.0001*
	Moderate late cellular rejection	0 (0.0%)	3 (42.9%)	<0.0001*
	Biliary stricture	2 (5.3%)	0 (0.0%)	NS*
	Recurrent HCV	2 (5.3%)	0 (0.0%)	NS*
	Cholangitis	1 (2.6%)	0 (0.0%)	NS*

ALT: alanine transaminase, HCV: hepatitis C virus, *P* > 0.05 is not significant (NS). *: Chi-square test; ≠: Mann-Whitney test.

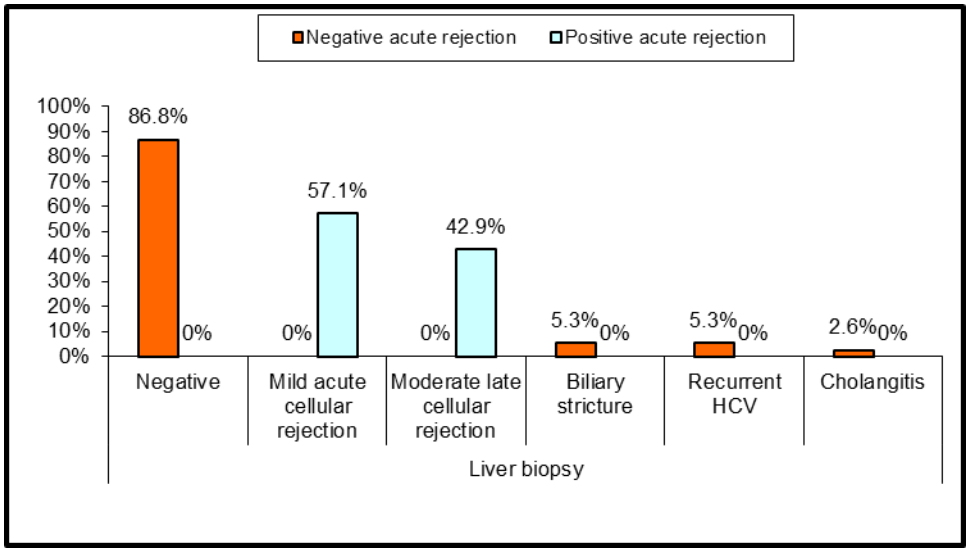

Figure 2. Bar chart showing the difference in liver biopsy findings between study cases with and without acute rejection.

Data in Table 5, show significant correlation between IL-10 and age, MELD score, CRP, albumin, total and direct bilirubin of all cases and with no acute rejection at day 0.

Table 5. Correlation of serum IL-10 level with data of the studied parameters at day 0.

Day 0	IL-10					
	All study cases		Acute rejection		No acute rejection	
	r	*p*-value	r	*p*-value	r	*p*-value
Age	0.294	0.050	-0.111	NS	0.378	0.019
MELD score	-0.392	0.008	0.333	NS	-0.454	0.004
WBC	-0.011	NS	0.333	NS	-0.049	NS
Hb	0.229	NS	0.550	NS	0.181	NS
PLT	-0.263	NS	-0.110	NS	-0.316	0.054
ESR	0.179	NS	0.110	NS	0.174	NS
CRP	-0.540	0.000	-0.593	NS	-0.576	0.000
CR	0.216	NS	-0.222	NS	0.186	NS
BUN	0.123	NS	0.330	NS	0.069	NS
AST	-0.172	NS	0.330	NS	-0.170	NS
ALT	-0.192	NS	-0.145	NS	-0.194	NS
Alb	0.411	0.005	0.040	NS	0.467	0.003
INR	-0.268	NS	0.697	NS	-0.294	NS
T. bilirubin	-0.432	0.003	0.333	NS	-0.439	0.006
D. bilirubin	-0.384	0.009	0.333	NS	-0.435	0.006

MELD: Model End Stage Liver Disease, WBC: White blood cells, Hb: hemoglobin, PLT: platelet count,
ESR: erythrocyte sedimentation rate, CRP: C reactive protein, CR: creatinine, BUN: blood urea nitrogen,
AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio,
*: Spearman correlation coefficient, $P > 0.05$ is not significant (NS).

Data in Table 6 show significant correlation between IL-10 and Hb of all study cases. Also, there was a significant correlation between IL-10 and ESR, total and direct bilirubin of all study cases and cases with acute rejection at day 7. Furthermore, there was a significant correlation between IL-10 and CRP of all study cases and with group without acute rejection at day 7.

Table 6. Correlation of serum IL-10 level with data of the studied parameters on day 7.

Day 7	IL-10					
	All study cases		Acute rejection		No acute rejection	
	r	*p*-value	r	*p*-value	r	*p*-value
WBC	0.168	NS	-0.630	NS	0.318	0.052
Hb	-0.360*	0.015	-0.661	NS	-0.236	NS
PLT	-0.094	NS	-0.596	NS	-0.060	NS
ESR	0.367*	0.013	0.804*	0.029	0.206	NS
CRP	-0.342*	0.021	0.000	NS	-0.376*	0.020
CR	-0.032	NS	0.151	NS	-0.112	NS
BUN	0.130	NS	0.257	NS	0.067	NS
AST	0.213	NS	0.182	0.696	0.225	NS
ALT	0.173	NS	0.294	NS	0.124	NS
Alb	0.118	NS	-0.722	NS	0.226	NS
INR	0.102	NS	-0.094	NS	0.080	NS
T. bilirubin	0.344*	0.021	0.771*	0.043	0.234	NS
D. bilirubin	0.303*	0.043	0.778*	0.040	0.186	NS

WBC: White blood cells, Hb: hemoglobin, PLT: platelet count, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, CR: creatinine, BUN: blood urea nitrogen, AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio, $p > 0.05$ is not significant (NS), *Spearman correlation coefficient.

Data in Table 7 show significant correlation between IL-10 and Hb and PLT count (Figure 1) in the group of no acute rejection on day 14. Also, there was a significant correlation between IL-10 and CR of all study cases and with group of no acute rejection at day 14.

Table 7. Correlation of serum IL-10 level with data of the studied parameters on day 14.

Day 14	IL-10					
	All study cases		Acute rejection		No acute rejection	
	r	*p*-value	r	*p*-value	r	*p*-value
WBC	0.013	NS	-0.359	NS	0.077	NS
Hb	-0.274	NS	-0.621	NS	-0.332*	0.042
PLT	0.222	NS	-0.623	NS	0.391*	0.015
ESR	0.125	NS	0.186	NS	0.209	NS
CRP	-0.101	NS	0.311	NS	-0.127	NS
CR	-0.332*	0.026	0.402	NS	-0.355*	0.029
BUN	0.095	NS	-0.056	NS	0.133	NS
AST	0.104	NS	0.369	NS	-0.020	NS
ALT	0.027	NS	0.171	NS	-0.118	NS
Alb	0.252	NS	-0.020	NS	0.231	NS
INR	0.019	NS	-0.311	NS	0.082	NS
T. bilirubin	0.290	0.053	0.585	NS	0.197	NS
D. bilirubin	0.290	0.053	0.657	NS	0.168	NS

WBC: White blood cells, Hb: hemoglobin, PLT: platelet count, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, CR: creatinine, BUN: blood urea nitrogen, AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio, $P > 0.05$ is not significant (NS). *Spearman correlation coefficient.

Data in Table 8, show significant correlation between IL-10 and white blood cell (WBC) count of all study cases and significant correlation with the group of no acute rejection at day 28. While it shows significant correlation between IL-10 and CRP of all study cases and the group of

no acute rejection at day 28. Also, there was a significant correlation between IL-10 and CR of all study cases and with the group of no acute rejection on day 28. On other hand, it shows significant correlation between IL-10 and ALT and Alb of all study cases at day 28.

Table 8. Correlation of serum IL-10 level with data of the studied parameters on day 28.

Day 28	IL-10					
	All study cases		Acute rejection		No acute rejection	
	r	*p*-value	r	*p*-value	r	*p*-value
WBC	0.375*	0.012	-0.133	NS	0.455**	0.004
Hb	0.097	NS	-0.133	NS	0.063	NS
PLT	0.264	NS	-0.135	NS	0.310	NS
ESR	0.032	NS	0.393	NS	0.017	NS
CRP	-0.455**	0.002	0.135	NS	-0.499**	0.001
CR	-0.373*	0.013	0.707	NS	-0.361*	0.026
BUN	-0.213	NS	-0.399	NS	-0.254	NS
AST	0.275	NS	-0.133	NS	0.260	NS
ALT	0.299*	0.048	0.000	NS	0.289	NS
Alb	0.315*	0.037	0.399	NS	0.297	NS
INR	0.077	NS	0.548	NS	0.080	NS
T. bilirubin	0.075	NS	-0.405	NS	0.124	NS
D. bilirubin	-0.013	NS	-0.465	NS	0.043	NS

WBC: White blood cells, Hb: hemoglobin, PLT: platelet count, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, CR: creatinine, BUN: blood urea nitrogen, AST: serum aspartate aminotransferase, ALT: alanine transaminase, Alb: albumin, INR: International normalized ratio, $P > 0.05$ is not significant (NS). Spearman correlation coefficient.

Table 9 shows a significant correlation between the level of serum IL-10 and HCV infected patients from day 0, and significant correlation between IL-10 and patients with HBV at day 7 and HCC at day 7 and 14. On the other hand, it shows a significant correlation with Bilharziasis on day 7.

Table 9. Correlation of serum IL-10 level with the etiology of rejected liver transplantation in patients.

		IL-10							
		Day 0		Day 7		Day 14		Day 28	
		Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
HBV	No	70 (50 – 100)	NS	150 (100 – 170)	0.009	250 (160 – 320)	0.054	360 (275 – 400)	0.053
	Yes	230 (60 – 400)		365 (250 – 480)		450 (300 – 600)		500 (400 – 600)	
HCV	No	60 (50 – 60)	0.000	170 (110 – 260)	NS	300 (300 – 320)	NS	380 (305 – 400)	NS
	Yes	100 (70 – 140)		140 (100 – 155)		200 (160 – 325)		370 (250 – 550)	
HCC	No	60 (50 – 100)	NS	155 (140 – 270)	0.006	300 (300 – 400)	0.013	365 (330 – 400)	NS
	Yes	90 (60 – 110)		140 (100 – 155)		200 (150 – 275)		385 (250 – 505)	

Table 9. Continued.

		IL-10							
		Day 0		Day 7		Day 14		Day 28	
		Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value	Median (IQR)	*p*-value
AIH	No	70 (55 – 110)	NS	150 (120 – 250)	NS	225 (157.5 – 365)	NS	375 (250 – 425)	NS
	Yes	60 (50 – 80)		150 (110 – 170)		300 (300 – 310)		375 (330 – 550)	
Cryptogenic	No	80 (50 – 110)	NS	150 (100 – 250)	NS	300 (170 – 320)	NS	380 (310 – 460)	NS
	Yes	60 (60 – 60)		155 (155 – 260)		160 (155 – 400)		170 (165 – 400)	
Bilharziasis	No	75 (60 – 100)	NS	150 (110 – 170)	0.030	275 (160 – 320)	NS	370 (250 – 400)	NS
	Yes	50 (10 – 400)		400 (100 – 480)		500 (120 – 600)		380 (320 – 600)	

HBV: hepatitis B virus, HCV: hepatitis C virus, HCC: hepatocellular carcinoma, AIH: autoimmune hepatitis, $p > 0.05$ is not significant (NS). Spearman correlation coefficient.

Discussion

This study tried to evaluate the possible association between serum IL10 level in liver transplant patients and acute allograft rejection. We investigated serum levels of IL-10 just before transplantation then weekly (day 0, 7, 14, 28) for one month after adult liver transplantation to evaluate the possibility of using it as an early predictive biomarker of acute rejection.

The present study included 45 patients, patients were followed up for 2 months after which they were further divided into two groups: rejection group and non-rejection group (controls), matched in age, gender, and types of operation. All patients were given calcineurin inhibitors (tacrolimus and cyclosporin A in the control and rejection groups) as a part of standard immunosuppressant regimens.[3]

The age range was 18-62 years of studied group who developed rejection and significantly decreased (18-63 years) than the group of non-rejection. This agreed with findings of a study, demonstrated that the rate of acute rejection episodes was lower in older patients,[4] supporting the hypothesis that they are less immunologically active than young recipients.

The main etiology of liver disease was HCV (57.9%), HCC (47.4%), Bilharziasis (18.4%), AIH (13.2%) then HBV and cryptogenic cause (10.5%). On days 0, 7, 14, and 28, the CBC, ESR, CRP, kidney function tests, AST, ALT, liver function tests, INR, and IL-10 were equivalent in both groups (rejection and non-rejection). There was no statistically significant difference between the two groups on day 0, with the exception of ALT, which was consistent with the definition of early acute rejection provided by Karasu et al., 2004,[5] who defined it as an increase in liver enzymes absent vascular or biliary issues.

On day 7 there were no significant differences in all parameters between two groups, while on day 14 found the same finding as day 0 as regard significance of ALT between two groups. On day 28 there was a significant difference in both ALT and AST between two groups.

In the current investigation, we found that the median level of serial IL-10 increased across all cases, particularly in the acute rejection group, going from 70 at day 0 to 400 at day 28 without any significant correlation, which may be related to a decline in the number of rejection groups. Furthermore, a prior work by Warlé et al., 2003,[6] demonstrated the correlation between graft tolerance and Th2 cytokines such IL-10. Thus, it may be concluded that patients with an IL-10 genotype associated

with low IL-10 production are more vulnerable to rejection, whereas those with an IL-10 genotype associated with high production are primarily non-rejectors.

After 2 months of follow up there was a significant increase in serum level of ALT, total and direct bilirubin between cases with rejection compared to those without acute rejection. As regards liver biopsy there was significantly increase in mild and moderate acute cellular rejection between cases with rejection compared to those without acute rejection while there was no difference in biliary stricture, recurrent HCV, and cholangitis.

As regard correlation between IL-10 and age, MELD score, hemoglobin, WBC, PLT, ESR, CRP, serum creatinine, BUN, AST, ALT, albumin, INR, total and direct bilirubin, the current study showed significant correlation between IL-10 and age, MELD score, CRP, albumin, total and direct bilirubin of all study cases and with group without acute rejection at day 0.

Our finding of correlation between serum IL-10 levels and MELD score agreed with the finding of a study that included 64 patients with stable cirrhosis and found higher IL-10 levels in those with Child-Pugh C and MELD score.[7] This same study demonstrated a decrease in human leukocyte antigen-D related (HLA)-DR expression in patients with advanced cirrhosis, possibly mediated by high IL-10 levels in response to endotoxemia.[7] These findings, characteristic of ‘‘immune paralysis” in patients with cirrhosis, may explain the relationship between higher IL-10 levels and the poor prognosis observed in this study.

In our study, the link between IL-10 and acute phase reactants, such as CRP and platelets, highlights the significance of IL-10 as an anti-inflammatory cytokine that can control hepatic damage in vivo that is mediated by T cells and macrophages. The study by Platz et al., 1996,[8] observed that the liver also produces IL-10. Additionally, the stress axis is crucial in controlling the in vivo expression of IL-10.[9] Catecholamines are released when the central nervous system is inflamed or when endotoxemia or bacteremia indirectly activates the stress axis, which increases the production of IL-10 in macrophages, especially in the liver.[9] According to additional evidence, IL-10 shields the liver from damage caused by hepatic ischemia/reperfusion by preventing nuclear factor kappa B (NF-κB) activation and the consequent production of pro-inflammatory mediators.[10] Treatment with IL-10 has been demonstrated to be helpful in the context of liver transplantation, increasing allograft survival.[11]

Biber et al., 2002[12] showed that IL-12 and IL-10 levels before the transplant may be sensitive indicators of the success of the allograft after the transplant. Due to low levels of IL-10 in the serum, which may serve as a sign of relative immunosuppression, enhanced immunological reactivity may be predicted. They observed that recipients of cadaveric renal allografts who later experienced biopsy-proven acute rejection had higher levels of pre-transplant IL-12 and IL-10 than those who did not. This is because the host response to an allograft involves both the innate and adaptive immune responses.

Among our patients the pre-transplantation level of IL-10 (week 0) in the rejected group were low similar to findings of a previous study[12] but without significant correlation with the other non-rejected group.

We identified a significant link between the level of IL-10 and HCV-infected patients at day 0 when we looked at the correlation of IL-10 with the etiology of rejected patients. The study by Taylor et al., 2006,[13] showed that serum IL-10 levels were considerably higher in chronic HCV patients, and IL-10 may be linked to hepato-carcinogenesis with immune surveillance suppression. During the follow-up period, another study showed increased spontaneous IL-10 production by peripheral blood mononuclear cells in patients with liver cirrhosis and a decrease in this production during interferon treatment.[14]

Additionally, another study by Barrat et al, 2003,[15] showed that individuals with a strong Th1 response during acute HCV infection can clear the virus, but individuals with a Th2 response (high levels of IL-10) progress towards chronicity.[15] This was clarified by Shin et al., 2003,[16] who observed that in experimental models of liver cirrhosis, IL-10 can display antifibrotic characteristics. In vivo treatment of

IL-10 to HCV-infected individuals has been hypothesized to alter the intrahepatic immunologic balance away from Th1 cytokine predominance, hence exerting its anti-inflammatory and subsequent antifibrotic effects.[17] According to another research study, IL-10 long-term therapy increases HCV viral levels while reducing hepatic fibrosis and inflammatory activity.[18]

We also demonstrated significant correlation between IL-10 and patients with HBV at day 7 which agreed with a previous study, demonstrated that elevated level of plasma IL-10 participated in the progression from acute HBV infection to chronic hepatitis B.[19]

On days 7 and 14, there was a significant connection between IL-10 and HCC. The study by Stephen et al., 2012,[20] showed that liver function tests, tumor stage, and HCC therapy had no effect on the predictive value of IL-10. Since some preclinical investigations have shown that HCC cell lines secrete IL-10, the IL-10 level is theoretically a good indicator of tumor burden. However, Chau et al. (2000), on the other hand showed significant correlation with Bilharziasis at day 7.[21]

Regarding IL-10, similar to our current result, some investigations reported that IL-10 levels rise right before a rejection episode,[22] while others claimed that IL-10 levels remain stable while the liver transplant is being rejected.[23] Furthermore, earlier research studies demonstrated the connection between graft tolerance and Th2 cytokines such as IL-10.[24, 25] We cannot confirm any of them, but it may be inferred that individuals with an IL-10 genotype that corresponds to low IL-10 production are more prone to rejection than those with a genotype that corresponds to high production.

Finally, the current study showed an increase in the median level of serial IL-10 among study patients but failed to demonstrate any significant relation of IL-10 levels during rejection of the liver transplant.

Author Contributions

AO, ME; idea of the research. MAE, EEA, MM; Data collection, data analysis, discussion, references. RM; methodology. RS; reviewing and writing of the paper, revision of all chapters.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 202/2018).

Informed consent

Before being included in the study, each patient signed a written informed consent form to agree to participate in the study.

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