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IMMUNOLOGICAL PROFILE IN DIFFERENT GROUPS OF END STAGE RENAL DISEASE

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Abstract

Background and aim of study: End Stage Renal Disease (ESRD) is a worldwide problem in which patients are in hemo-dialysis and/or awaiting for kidney transplantation. However, the actual mechanism (s) of ESRD pathogenesis is ill-defined. The aim of this study is to investigate the role of certain immunological markers in the pathogenesis of ESRD.
Materials and methods: Sixty eight blood samples were collected from hospitalized ESRD patients with different etiology (hypertensive, diabetics, hypertensive + diabetics, and small size kidney). Twenty healthy volunteers as control group was enrolled in the study. Serum IL-10, IL-17, MCP-1 and TGF-β were estimated in all subjects.
Result: A significant elevation in the serum IL-10, MCP-1 and IL-17 mean concentration in all ESRD patients groups. TGF-β mean concentration exhibited decreasing level in the hypertensive, hypertensive + diabetes and small size kidney groups and a slight elevation in the diabetes group. The IL-10: IL-17 ratio expressed elevation in all ESRD patients groups.
Conclusion: There is a progress of inflammatory reactions in all ESRD patients groups in which IL-17 and MCP-1 are playing major roles. TGF-β1 is not played its anticipated pro-fibrotic role and anti-inflammatory function in the studied group. The ratio of IL-10: IL-17 point out a slight shifting of the immunosuppressive reaction over the inflammatory reaction in all ESRD patients groups.

Key words: ESRD, IL-10, IL-17, MCP-1, TGF-β1.

Introduction

End-stage renal disease (ESRD) is a devastating medical, social and economic problem in the community which needs dedicated supervision and health care and is fatal unless treated properly [1]. Interleukin-10 effectively down regulates pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, and also reduces the production of chemotactic factors, such as IL-8 [2]. Interleukin-10 increases in response to inflammation and in healthy individuals it is predominantly cleared by the kidneys. Consequently IL-10 is frequently found to be higher in kidney diseases than healthy populations, and it has anti-inflammatory benefits [2].T_H17 cells and IL-17 play an important role in host immunity, with the capacity of protection against extracellular pathogens and fungi, whereas they are also important drivers of autoimmune disease, and have inflammatory properties [3]. Th17 cytokines can promote renal inflammation in part by increasing TNF-α expression and up-regulating chemokines that lead to the invasion of immune cells into the kidneys [4].
Transforming Growth Factor-β-1 (TGF-β1) is a pro-fibrogenic cytokine that likely plays an important role in the process of chronic kidney disease progression [5]. At glomerular level, TGF-β1 mainly contributes to glomerular filtration barrier alteration, fibrosis and sclerosis, which reduce the

filtration surface and finally cause glomerular collapse [6]. One of the actions of TGF-β1 is to induce the accumulation of monocytes and stimulation of fibroblasts by increasing the expression of the monocyte Chemo-attractant protein-1 (MCP-1/CCL2) [7]. In the renal diseases, chemokines play a pivotal role in directing migration and adhesion of infiltrating cells to an inflammatory lesion [8]. The aim of this study is to investigate the immunological profile of three inflammatory markers (IL-17, MCP-1 and TGF-β) and one anti-inflammatory marker (IL-10) in the context of four causal groups of ESRD patients.

Materials and methods

Sixty eight patients out of the 154 ESRD under dialysis or awaiting for kidney transplantation patients were selected according to their disease etiology plus 20 apparently healthy control subjects were enrolled in this study. All participants had signed a written consent for their acceptance to donate blood samples for the research. The ESRD patients were classified according to their etiology into four groups; hypertensive (HP), diabetics (D), hypertensive plus diabetics (HP + D), and small size kidney and others (SSK and others include all other causes for ESRD other than HP, D and HP + D including traumatic, inflammatory, malignancy, congenital, and idiopathic). All patients were hospitalized in Al-Karama and Baghdad educational hospitals in Baghdad city. The period of the study extended for the period from September 2014 to February 2015. Blood samples were taken from all subjects and their sera were subjected to serological analysis investigating the serum levels of four immunological markers (MCP-1, IL-17, IL-10, and TGF-β) by ELISA kits (CUSABIO, China for IL-10, IL-17, MCP-1 and DEMEDITEC, Germany for TGF-β). The age range of all study subjects were 30 – 70 years with a duration of dialysis of at least two years.

Results:

The mean concentration of IL-10 was significantly increased above normal level for all ESRD patients groups. The above normal frequency of IL-10 was ranging from 55.6% in SSK and others group to 68.8 in HP and D groups with total of 63.2 (Tables 1 and 2).

The mean concentrations of MCP-1 in all groups of ESRD patients (except HP+D group) were significantly elevated above normal serum concentration (57.73, 97.41, 55.52 and 59.42 pg/ml for HP, D, SSK groups and Total respectively) compared to 3.36-27.80 pg/ml in control group (Table 1). These results were confirmed in Table 2 which demonstrated that the highest percent of above normal MCP-1 serum concentration was noticed in group D (43.8%) compared to 27.8%, 6.3% and 22.2% in HP, HP+D and SSK groups respectively.

For IL-17 all ESRD patients groups, as it is shown in Table 1, exhibited a significant increased mean concentration above normal with variable degrees ranging from 10.8±12.038 in SSK group to 53.8±78.846 in HP group in comparison with the control normal range concentration (3.29-8.94 pg/ml). Table 2 expressed an increased above normal frequency level of IL-17 in HP and D groups (50.0% and 56.3% respectively) and a decreases below normal frequency of IL-17 in HP+D and SSK and others group (22.2% and 45.6% respectively) with a total 55.6% of above normal frequency.

The current study reported a mean concentration of TGF-β; 68.34, 77.94, 48.05 and 56.30 pg/ml for HP, D, HP+D and SSK ESRD patients groups respectively with a total of 62.64 pg/ml compared to 73.97-95.85 pg/ml in healthy control group (Table 1). Such results indicate that there was a decreased concentration of this factor in most ESRD patients groups except group D of diabetic nephropathy (77.94 pg/ml) which was within the normal range of this factor in healthy control group. Table 2 confirmed these results in which the above normal frequency of TGF-β was from 50.0% to 81.3 for the four ESRD patients groups with a total percent of 63.2%.

The balance between Th17/Treg cells plays an important role in the inflammation in the adaptive immune system. Table 10 shows the IL-10: IL-17 ratio in different ESRD groups and control. In control that represents the healthy normal individuals, the mean concentration of both interleukins was low and the ratio was 1: 1.65. In ESRD patients groups, the mean concentration exhibited a significant elevation in all groups ranging between 32.65 pg/ml in SSK group to 60.77 pg/ml in HP group for IL-10, and between 10.83 pg/ml in SSK group to 53.78 pg/ml in HP group for IL-17. The IL-10: IL-17 ratio was shifting in favor of the IL-10 in all ESRD patients groups (1: 0.88, 1: 98, 1: 36, and 1: 33 in HP, D, HP+D, and SSK groups respectively).

Table (1): Mean concentration of immunological markers among study groups of ESRD patients.

ESRD Groups	Statistics	Immunological Markers			
		IL-10	**MCP-1**	**IL-17**	**TGF-B**
HP *n*= 18	**Range**	.00-290.3	1.53-165.1	1.22-296.8	10.9-138.5
	Mean± SD	57.8±43.143	55.7±38.423	50.8±38.846	68.3±36.735
	t-test	3.10	4.27	2.89	7.89
	P-value	***P<0.01***	***P<0.01***	***P<0.01***	***P<0.01***
D *n*= 16	**Range**	.38-356.9	14.2-385.5	2.24-436.9	17.4-151.4
	Mean± SD	45.0±31.016	95.4±63.363	50.1±42.120	77.9±37.360
	t-test	2.21	4.17	1.83	8.35
	P-value	***P<0.05***	***P<0.01***	***P>0.05***	***P<0.01***
HP+D *n*= 16	**Range**	4.3-290.5	2.45-81.4	1.3-92.8	1.2-86.3
	Mean± SD	42.7±26.140	27.7±20.120	15.9±12.376	48.0±26.403
	t-test	2.49	5.51	3.03	7.28
	P-value	***P<0.05***	***P<0.01***	***P<0.01***	***P<0.01***
SSK *n*=18	**Range**	.01-296.7	.98-214.4	1.4-48.9	9.3-145.9
	Mean± SD	28.5±23.869	54.5±42.515	9.4±7.038	54.3±27.854
	t-test	2.04	4.49	3.82	7.27
	P-value	***P<0.05***	***P<0.01***	***P<0.01***	***P<0.01***
Total *n*= 68	**Range**	.00-356.9	.98-385.5	1.22-436.9	1.2-151.4
	Mean± SD	41.5±32.776	51.4±34.557	31.3±28.980	60.3±30.678
Range of normal value (healthy controls) *n*=20		0.67-11.64 g/ml	3.36-27.8 pg/ml	3.29-8.94 pg/ml	73.97-95.85 pg/ml

HP= Hypertension, D= Diabetes mellitus, SSK= Small size kidney.

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Table 2: Frequency level of immunological markers among different groups of ESRD patients

Immunological markers level			ESRD patients groups				Total (n=68)	*P-value*
			HP (n=18)	D (n=16)	HP+D (n=16)	SSK& Others (n=18)		
IL-10	↓	№	6	2	1	6	15	<0.05
		%	33.3%	12.5%	6.3%	33.3%	22.1%	
	↔	№	1	3	4	2	10	--
		%	5.6%	18.8%	25.0%	11.1%	14.7%	
	↑	№	11	11	11	10	43	< 0.01
		%	61.1%	68.8%	68.8%	55.6%	63.2%	
MCP-1	↓	№	8	4	7	6	25	> 0.05
		%	44.4%	25.0%	43.8%	33.3%	36.8%	
	↔	№	5	5	8	8	26	--
		%	27.8%	31.3%	50.0%	44.4%	38.2%	
	↑	№	5	7	1	4	17	< 0.05
		%	27.8%	43.8%	6.3%	22.2%	25.0%	
IL-17	↓	№	1	2	2	9	4	>0.05
		%	6.3%	12.5%	11.1%	13.2%	22.2%	
	↔	№	7	5	12	28	4	--
		%	43.8%	31.3%	66.7%	41.2%	22.2%	
	↑	№	8	9	4	31	10	< 0.01
		%	50.0%	56.3%	22.2%	45.6%	55.6%	
TGF-B	↓	№	9	8	13	13	43	<0.01
		%	50.0%	50.0%	81.3%	72.2%	63.2%	
	↔	№	4	2	3	4	13	--
		%	22.2%	12.5%	18.8%	22.2%	19.1%	
	↑	№	5	6	0	1	12	>0.05
		%	27.8%	37.5%	.0%	5.6%	17.6%	

HP= Hypertension, D= Diabetes mellitus, SSK= Small size kidney.
↓ = Below normal, ↔ = Normal, ↑ = Above normal

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Table (3): The ratio of IL-10: IL-17 in control group and other ESRD patients groups.

Groups	IL-10 Concentration (pg/ml)	IL-17 Concentration (pg/ml)	Ratio (IL-10: IL-17)
Control	**3.2718**	**5.3847**	**1: 1.65**
HP	**60.7791**	**53.7820**	**1: 0.88**
D	**53.0056**	**52.0722**	**1: 0.98**
HP + D	**46.7022**	**16.9431**	**1: 0.36**
SSK	**32.6543**	**10.8386**	**1: 0.33**

Discussion

In humans, IL-10 is pluripotent cytokine encoded by the IL-10 gene, which is located on chromosome 1 and is primarily produced by monocytes and, to a lesser extent, Th2, mastocytes, $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells, and certain subset of activated T cells and B cells [9]. The immunomodulatory actions of T-regs are proposed to occur by releasing cytokines like TGF-β and IL-10 that can inhibit the release of Th1 cytokines. The protective role for T-regs against renal injury is supported by evidence showing that expanding the T-reg population can delay the onset of renal injury and inflammation associated with autoimmune-induced nephritis [10]. IL-10 downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages, enhances B cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway [11]. The elevation of IL-10 mean concentration in all ESRD groups in the current study might refer to an involvement of this anti-inflammatory cytokine effectively in down-regulation of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. In fact, IL-10 also function to reduce the production of chemotactic factors, such as IL-8 or CC chemokines, that may attract further leukocytes to the location of inflammatory activity [12]. The IL-10 results of the current study were in consistency with another study [13].

The results of the current study showed that diabetic patient (group D) had expressed the highest mean concentration of serum MCP-1 (97.41 pg/ml) and the highest frequency of above MCP-1 normal level (43.8%) in comparison with all other ESRD groups (Tables 1 and 2). Another study demonstrated that serum MCP-1 was significantly elevated in patients with microalbuminuria and poor glycemic control compared to normoalbuminuric diabetic patients, and controls, respectively. These observations suggested that MCP-1 might involve in the pathogenesis of diabetic nephropathy [14]. In one more study, in all subjects, there were significant correlations between the urinary levels of albumin, serum creatinine and serum MCP-1. These findings suggested that monocyte activation is implicated in the development and progression of DN. Determination of MCP-1 level in blood serum may have important diagnostic implications [15]. Classically activated macrophages release inflammatory cytokines, promote oxidative stress, and the development of renal fibrosis [16].

Interleukin-17, a cytokine with multiple inflammatory and haemopoietic effects including release of pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) [17], up-regulation of adhesion and MHC molecules and recruitment of monocytes and neutrophils. A number of studies have proposed a role for IL-17 in the pathogenesis of certain experimental animal models and human immune-mediated

diseases. Th17 cells and IL-17 play an important role in host immunity, with the capacity of protection against extracellular pathogens and fungi, whereas they are also important drivers of autoimmune disease, and have inflammatory properties. Th17 cytokines can promote renal inflammation in part by increasing TNF-α expression and up-regulating chemokines that lead to the invasion of immune cells into the kidneys [4]. The current study demonstrated an increase in the serum IL-17 level in all ESRD studied groups (although the elevation was sharper in the HP and D groups than HP+D and SSK) which was in consistency with a Chinese study [18].

The results of TGF-β indicate that there was a decreased concentration of this factor in most ESRD patients groups except group D of diabetic nephropathy (77.94 pg/ml) which was within the normal range of this factor. Other study concluded that TGF-beta1 was significantly reduced in hemodialysis patients, in particular in those with severe cardiovascular disease [19]. In an Indian study, similar results to the current study concerning TGF-β1 was also found. TGF-β1-mediated renal fibrosis is a common pathology implicated in this form of kidney disease. In the Indian study, circulating protein and mRNA levels of TGF-β1 cytokine were investigated among ESRD patients and respective controls from North India. Mean TGF-β1 protein levels were 2.7-fold lower in ESRD patients as compared to normal controls. Additionally, TGF-β1 mRNA transcripts of this cytokine were also significantly lower in the diseased population compared to controls. These results imply that TGF-β1 has not played its anticipated pro-fibrotic role and anti-inflammatory function in the studied population [20].

The ratio between different cytokines in different diseases was partially investigated in some recent literatures. In one study, it was stated an increased number of Th17 and Th1 cells among the ESRD patients in comparison to healthy controls. In the same study, ESRD patients with renal transplantation and had acute antibody mediated acute rejection, acute rejection and chronic rejection displayed a greater number of Th17, Th1 and Th17/Th1 cells as well as a high level of serum IL-2, IFN-γ, TNF-α and IL-17. However, there was a lower level of Treg cells and IL-10 compared to transplant stable patients [21].

In the current study, both IL-10 and IL-17 were elevated, with variable degrees, in all ESRD patients' groups subjected to hemodialysis or awaiting for renal transplantation (Table 3). In our opinion, such unique cytokine profile indicate that both mechanisms of inflammatory and immunosuppressive reactions were in progress during the advanced stages of renal injuries whatever the cause is, but with slight dominance of the IL-10 over IL-17 as it is indicated by the ratio of IL-10: IL-17 which point out a slight shifting of the immunosuppressive reaction over the inflammatory reaction. However this study could not elucidate the effect and amplitude of such profile on the progress and prognosis of the ESRD patients and whether further activation of the CD4+CD25+Foxp3 (Treg) cells or manipulation of the serum or renal IL-10 level (by passive IL-10 injection or genetic IL-10 activation) would improve the kidney functions in such patients.

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