Original paper

A Study of Some Immunological Markers in Patients with Chronic Kidney Disease in Kerbala

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Abstract

Background: Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years.Immune status of patients with CKD represents a fruitful area of research world-wide. Many hypotheses were mounted in this regards and most of them based on levels of certain immunological markers.
Aim: To evaluate the serum levels of some immunologic markers in patients with CKD and to predict their correlation with the disease severity.
Study design and objective: This is a cross sectional survey study. It was performed during December, 2013 through April, 2014at Al-Hussein Medical City, Holly Kerbala.
Method: A total of 60 doctor-diagnosed patients with chronic kidney diseases for different causes and in variable stages of the disease. Their gender distribution and age range were (39 women; 30-62 years and 21 men; 33-70 years). Other 20 healthy persons were chosen as control group. An informed consent was taken from all the participants submitted to the study. Age, gender and presence of diabetes or hypertension were recorded. Then, 3-ml venous blood sample was taken from each participant and sera were isolated via centrifuge. The serum levels of the following markers were determined using ELISA; haptoglobin, alpha1 anti-trypsin, immunoglobulin-A (IgA), IgG, IgM, alpha-2-macroglobulin (A2M) and the complement proteins C3 and C4.
Results: Serum levels of all studied immunologic markers were significantly lower in patients with CKD than those in the control subjects, p value < 0.05. An exclusion is the non significant rise in serum level of haptoglobin and alpha-2-macroglobulinnoticed in CKD stages other than ESRD.In addition, serum levels ofhaptoglobin, A2M, IgA and IgM are significantly lower in ESRD than other stages of CKD (p value < 0.05), while levels of C3 and C4 are significantly lower in moderate CKD than other stages of renal impairment included in this study (p value < 0.05).
Discussion: It is well known that the CKD patients suffer significant immune compromization. Our data revealed down regulation of certain immunological parameters in their sera such as alpha1 anti-trypsin, immunoglobulin-A (IgA), IgG, IgM, and the complement proteins C3 and C4.This could explain the susceptibility to infection and abnormally delayed wound healing accompanying CKD.
Conclusion: It was concluded that serum levels of all studied immunologic markers, excepthaptoglobin and alpha-2-macroglobulin,were significantly lower in patients with CKD than those in the healthysubjects. Furthermore, this is not associated with the presence or absence of diabetes and/or hypertension. This finding could, in part, prove that the immune deficiency status in these patients is purely due to their impaired renal functions.
Key words: immunologic markers, chronic kidney disease.

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Introduction

Chronic kidney disease resulted in 735,000 deaths all over the world in 2010. This figure represented relatively rapid "jump" when compared with 400,000 deaths in 1990 [(1)]. Often, CKD is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with hypertension or diabetes mellitus and those with a positive family history of CKD. From lab point of view, CKD is identified by testing for a serum creatinine level. Higher levels of creatinine indicate a lower glomerular filtration rate and thus a decreased capability of the kidneys to get rid of waste products. Recent professional guidelines classify the severity of chronic kidney disease in five stages, with stage 1 being the mildest and usually causing few symptoms and stage 5 being a severe illness with poor life expectancy if untreated. Stage 5 CKD is often called end stage renal disease (ESRD)[(2)].

The three most common causes of CKD are diabetes mellitus, hypertension, and glomerulonephritis.The clinical course and outcome of these disorders are extremely variable and difficult to predict. The clinical trajectories range from a benign and spontaneously remitting condition to a symptomatic and rapidly progressive disease[(3)].

It is noticeable that CKD is one of the common causes of immune deficiency state as revealed by data from the evidence-based studies in this field. Various immunologic parameters were widely covered by such studies in association with different levels of renal impairment disorders. These studied markers belong to varying arms of the immune response incuding cytokines, immunoglobulin classes, complement components, acute phase reactants, inflammatory mediators and others[(3-10)].

Authors in this field have left a wide area of controversy regarding the nature and exact cause of immunodeficiency state accompanying the condition of chronic renal impairment. Some studies regarded CKD as a chronic inflammatory condition with over expression of inflammatory markers such as interferon- (IFN-) gamma and tumor necrosis factor (TNF). Macdougall (2004) investigated the ability of anti-inflammatory cytokine therapy to improve poor treatment outcomes in patients with chronic renal failure. He suggested that these patients have chronic immune system activation which is reflected by abnormally raised level of pro- and anti-inflammatory cytokines and that patients who respond well to erythropoietin treatment exhibit fairly normal expression of these cytokines. Patients who persistently fail to respond, however, express abnormally raised levels of pro-inflammatory cytokines, TNF-alpha and IFN-gamma. Paradoxically, these patients also express abnormally high levels of anti-inflammatory cytokines, IL-10 and IL-13. Although anti-inflammatory in nature, these cytokines might also affect erythropoiesis[(11)].

On the other hand, many authors concentrate on the fact that CKD being associated with pure deficiency status in most if not all immune elements and this contributes to the higher susceptibility to infectious disorders and delayed wound healing due to impaired inflammatory response.

This study has investigated the association between levels of certain immunologic markers with varying degrees of CKD.In addition to their association with patients' age, gender and presence of diabetes and/or hypertension.

Patients and method

Sixty patients (39 women; 30-62 years and 21 men; 33-70 years)who were doctor-diagnosed with varying stages of CKD for less than 2 years attending the nephrology unit in Al-Hussein Medical City were recruited to participate in this study during the period from December, 2013 through

April, 2014. Other 20 healthy persons were chosen as control group. The selected patients were stratified according to the severity of CKD determined by serum creatinine into five stages, with stage 1 being the mildest and stage 5 being end stage renal disease (ESRD)[2]. Patients' age, gender and presence of diabetes or hypertension were recorded. Blood specimens were collected from patients and 20 healthy unrelated controls after taking informed consent from each participant.Sera were separated via centrifuge. The serum levels of creatinine, haptoglobin, alpha-1-antitrypsin, immunoglobulin-A (IgA), IgG, IgM, alpha-2-macroglobulin (A2M) and the complement proteins C3 and C4 determined by using ELISA technique.

Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed using the software package, graph pad, prism version 6.0 for Windows. Differences between the groups were compared using unpaired *t* test for normally distributed variables. To evaluate the relation between variables, Pearson's correlation coefficient in normally distributed variables was used. A *p* value less than 0.05 was considered as significant.

Results

Immunological investigations analyzed in this study were the serum levels of haptoglobin, alpha-1-antitrypsin, IgA, IgG, IgM, alpha-2-macroglobulin and the complement proteins C3 and C4. All these markers (except haptoglobin and alpha-2-macroglobulin) were shown to be significantly lowered in CKD patients compared to healthy control subjects. However, there was non-significantly high serum levels of IgA and IgG in case of moderate and severe CKD (Table 1).Meanwhile, serum levels of C3 and C4 were shown to be significantly lower in moderate CKD than other stages of renal impairment included in this study, *p* value < 0.05 (Table 1). It was noticeable that serum levels of haptoglobin and alpha-2-macroglobulin show some non significant increase in stages ofCKD other than ESRD compared to the healthy subjects. However, these two markers are significantly lower in ESRD than other stages of renal impairment and the control group included in this study, *p* value < 0.05 (Table 1). Furthermore, there was no significant association of any of the studied immunologic markers with patients' age, gender or presence of diabetes or hypertension (Tables 2, 3, 4 and 5).

Discussion

Our data revealed significantly reduced serum levels of IgG, IgM and IgA and this could represent part of the general immunodeficiency status in patients with CKD as it is evident in many other studies in this field [12,13,14]. It was estimated that ESRD is associated with B-cell lymphopenia and suggested that one of the major causes of this disturbance is an increased susceptibility to the death of B-cells by apoptosis[13]. This lymphopenia might contribute to the reduced expression of immunoglobulins in these patients. However, our data are inconsistent with that from a previous study which has documented that serum IgG isotypes, and both IgM and IgA production are normal in dialysis patients [15].

In the current study, serum levels of C3 and C4 were also shown to be lowered in CKD patients especially in those with moderate degree of renal impairment. This finding goes with that of many researchers who found depressed complement system activity with deficiency of varying components in patients with CKD [5,9,16,17,18]. Because the complement system is the main mediator of innate immunity and contributes to the recognition, opsonization, and lysis of microorganisms, therefore, these patients are immunocompromised, susceptible to

bacterial infections, and in a state of chronic inflammation[5].

Table 1. Mean serum levels of some immunologic markers in 60 patients with CKD compared to 20 control subjects measured by ELISA.

	Hapto. mcg/dl	**A1A** ng/dl	**IgA** mg/dl	**IgG** mg/dl	**IgM** mg/dl	**A2M** mg/dl	**C4** mcg/dl	**C3** mcg/dl
Control group								
Mean	128.26	177.5	278.6	958.7	148	138.6	37.5	129
SD	21.24	27.97	82.17	89.25	30.8	29.76	5.91	9.39
Mild CKD Scr<2mmol/dl								
Mean	189.6	176.6	248.3	853.1	120	155.2	31.8	125
SD	84.35	29.12	48.72	431.3	66.8	63.28	19	67.1
Moderate CKD Scr2.1-4.9 mmol/dl								
Mean	110.77	150.8	301.2	1416	118	194	18.9	70.8
SD	34.334	57.12	191.7	768.3	42.1	111	5.25	30.8
Sever CKD Scr 5-9.9 mmol/dl								
Mean	158.38	188.1	320.7	1212	118	158.5	34.2	83.9
SD	94.833	80.51	183.2	479	57.3	56.03	17.3	33.5
ESRD Scr>10 mmol/dl								
Mean	40.06	132.9	165.4	813	90.5	109.2	29	66.2
SD	23.958	50.34	106	353.2	30.5	33.74	3.89	22.7
p value	0.126	0.024*	0.016*	0.022*	0.019*	0.377	0.019*	0.02*

*CKD: chronic kidney disease, ELISA: enzyme-linked immunosorbant assay, Hapto: haptoglobin, A1A: alpha-1 antitrepsin, Ig: immunoglobulin, A2M: alpha-2 macroglobulin, C3 and C4: complement components number 3 and 4, Scr: serum creatinine, SD: standard deviation, ESRD: end stage renal disease, *: significant.*

Table 2. Mean serum levels of some immunologic markers according to age distribution in 60 patients with CKD measured by ELISA.

AGE GROUPS	**Hapto.** mcg/dl	**A1A** ng/dl	**IgA** mg/dl	**IgG** mg/dl	**IgM** mg/dl	**A2M** mg/dl	**C4** mcg/dl	**C3** mcg/dl
30-39 years								
Mean	91.04	157.37	253.14	1043.7	100.7	147.77	32.59	96.82
SD	12.33	16.97	42.67	83.75	43.18	44.06	7.76	7.32
40-49 years								
Mean	69.88	161.92	268.23	1028.71	110.9	153.33	24.89	89.12
SD	7.7	19.73	28.92	43.63	46.22	45.49	11.4	17.51
50-59 years								
Mean	100.47	132.48	271.32	1262	128.4	170.29	25.34	75.19
SD	14.034	41.41	51.43	76.3	52.61	56.11	6.72	21.08
60-69 years								
Mean	78.38	201.61	203.47	1127	110.2	131.98	27.12	78.23
SD	14.33	56.01	62.12	74.7	37.13	37.63	10.13	16.98
Correlation coefficient *"r"*	0.23	0.11	0.21	0.27	0.19	0.27	0.19	0.22

We observed that serum haptoglobin level isnon significantly increased in CKD other than ESRD and significantly reduced in patients with ESRD.The role of haptoglobin in CKD was found to be significant in term of susceptibility to the disease and response to the treatment [10,19,20]. Our finding isconsistent with that of previous study in which the author found a decreased plasma haptoglobin

level and regarded the latter as a cardiovascular-protective protein, and this could explain the accelerated atherosclerosis process accompanying hemodialysis in ESRD patients [10].

Table 3. Mean serum levels of some immunologic markers according to gender distribution in 60 patients with CKD measured by ELISA.

CKD Patients	**Hapto.** mcg/dl	**A1A** ng/dl	**IgA** mg/dl	**IgG** mg/dl	**IgM** mg/dl	**A2M** mg/dl	**C4** mcg/dl	**C3** mcg/dl
Males n=21	76.24	141.09	249.94	971.29	117.17	129.18	32.34	80.15
Mean								
SD	15.94	22.07	22.21	43.11	23.66	27.13	8.26	6.99
Females n=39								
Mean	97.91	165.26	258.79	1044.88	112.9	143.02	26.76	74.27
SD	13.97	32.12	31.67	29.46	37.18	29.97	10.04	12.28
Correlation coefficient *"r"*	0.17	0.25	0.31	0.3	0.26	0.15	0.2	0.32

Table 4. Mean serum levels of some immunologic markers according to presence or absence of diabetes mellitus in 60 patients with CKD measured by ELISA.

CKD Patients	**Hapto.** mcg/dl	**A1A** ng/dl	**IgA** mcg/dl	**IgG** mcg/dl	**IgM** mcg/dl	**A2M** mcg/dl	**C4** mcg/dl	**C3** mcg/dl
Diabetics n=26								
Mean	83.73	175.16	239.99	990.69	102.37	135.61	30.94	76.93
SD	13.23	18.07	24.78	23.72	22.72	21.81	7.83	8.11
Non diabetics n=34								
Mean	90.44	131.76	261.09	1064.43	127.28	137.51	29.34	78.46
SD	15.61	21.62	27.82	22.84	27.68	23.91	11.18	11.67
Correlation coefficient *"r"*	0.12	0.32	0.23	0.27	0.22	0.11	0.12	0.15

Table 5. Mean serum levels of some immunologic markers according to presence or absence of hypertension in 60 patients with CKD measured by ELISA.

CKD Patients	**Hapto.** mcg/dl	**A1A** ng/dl	**IgA** mg/dl	**IgG** mg/dl	**IgM** mg/dl	**A2M** mg/dl	**C4** mcg/dl	**C3** mcg/dl
Hypertensive n=23								
Mean	92.84	155.94	258.28	1009.47	110.80	119.94	22.98	66.24
SD	12.76	17.23	23.25	26.76	21.93	21.43	9.12	9.45
Normotensive n=37								
Mean	81.72	151.62	242.56	1044.93	116.27	150.01	39.01	88.67
SD	14.81	23.92	22.98	19.08	25.42	23.27	10.46	10.93
Correlation coefficient *"r"*	0.22	0.2	0.32	0.26	0.12	0.34	0.27	0.35

Regarding the levels of alpha-1-antitrypsin (A1A), our study revealed a significant reduction in its serum level in the studied group of CKD patients compared to the control subjects.Alpha-1-antitrypsin is a potent inhibitor of several proteolytic enzymes that inhibit neutrophil superoxide production[21]. Alpha-1-antitrypsin is related to the a therogenesis process, and to diverse ischemic cerebrovascular and cardiovascular disease risks. Furthermore, serum A1Ahas been reported to be an

important index of chronic inflammation in hemodialysis patients[6]. Exogenous administration of A1Acould confer protection against ischemic/reperfusion injury [7]. Because hemodialysis is an ischemia-reperfusion process, A1Amight serve as another therapeutic target. Other researcher found that serum A1Abut not complement C3 and C4 predicts chronic inflammation in hemodialysis patients.[6].
We observed anon significant elevation in serum level of alpha-2-macroglobulin (A2M) in patients with CKD other than ESRD. Alpha-2-macroglobulin is a major protease inhibitor in vivo. So far, several reports have been published suggesting the presence of protease inhibitors such as A2M in amyloid fibrils from dialysis-related amyloidosis patients.A relevant study demonstrated the presence of circulating A2M-beta-2 microglobulin complex in hemodialysis patients and concluded that the formation of this complexis implicated in dialysis-related amyloidosis.[22]

Conclusion

As a conclusion,there is clear evidence of immune deficiency status in patients with CKD as it was shown by the data from this study. Collectively, serum levels of all studied immunologic markers, except haptoglobin and alpha-2-macroglobulin, were significantly lower in patients with CKD than those in the healthy subjects. Meanwhile, this immune deficiency status was shown to be not associated with the presence or absence of diabetes and/or hypertension suggesting that it was purely due to CKD rather than being a secondary effect of these disorders.

Recommendations

Hoping for introducing an effective immune-based diagnostic and even therapeutic strategies, we recommend further studies to investigate the role of serum levels of different immunological markers in association with specific underlying pathologies for CKD diagnosed by using reliable histopathological assay. Secondly, the role of such markers is to be examined in other renal disorders namely nephrotic syndrome in children which represent a fruitful area of research.

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