Serum Immunoglobulins and the Complements C3 and C4 Levels in the Patients with Chronic Renal Failure

Hussein Kadhem Abdul Hussein, Intidhar Sahib and Naba'a Ali Hashim
Department of Chemistry, College of Science, University of Karbala
Karbala, Iraq.
Correspondence to: Dr. Hussein Kadhem Abdul Hussein, College of Science,
E-mail: headm2000@yahoo.com

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Abstract

Chronic renal failure (CRF) often accompanied by different biochemical disorders. The study of the possible changes in the serum component in CRF is very important issue. In many glomerular diseases, the complements and immunoglobulins are deposited in renal tissues. This work studied the possible changes in the serum concentration of complements C3 and C4 and immunoglobulins A, G and M in patients with CRF

Twenty one patients with chronic renal failure and twenty six healthy persons were participated in this work. The serum levels of IgG, IgA and IgM and *C3* and C4 in both groups were quantitatively evaluated using immunodiffusion plates.

There is a significant increase at ($p<0.05$) in serum C3 and no significant difference in serum C4 in CRF patients as compared with healthy control group. The results showed no significant changes in the concentration of immunoglobulins in CRF patients in comparing with healthy control at ($p<0.05$).

There is a slight immunological disturbance in the complements and immunoglobulins. Serum C3 only showed a significant increase in CRF patients. The results discussed according to the restricted protein diet and to the treatment of previous infections.

Keywords: IgG, IgA, IgM, C3, C4, Immunoglobulins, Complements, Chronic renal failure, and immunodiffusion.

الخلاصة

العجز الكلوي المزمن يترافق مع تغيرات كيموحياتية. دراسة التغيرات المحتملة في مكونات الدم تعتبر من المواضيع المهمة. في الكثير من الإمراض الكلوية لوحظ وجود تجمع للمتممات والكلوبيولينات المناعية. في هذا البحث تمت دراسة تراكيز للمتممات C4 و C3 والكلوبيولينات المناعية IgG, IgA, IgM لدى مرضى العجز الكلوي المزمن.

استُخدم في هذه الدراسة21 شخصا مصابا بالعجز الكلوي المزمن وُاخذ 26 شخصا سليما من المرض لغرض المقارنة. تم تقدير المتممات C4 و C3 والكلوبيولينات المناعية IgG, IgA, IgM باستخدام طريقة الانتشار المناعي لدى مرضى العجز الكلوي المزمن ومقارنتها بمجموعة السيطرة.

أظهرت النتائج ارتفاعا معنويا ($p<0.05$) في تركيز المتمم C3 بينما لايوجد اختلاف معنوي ($p<0.05$) في تركيز C4 في مصول مرضى العجز الكلوي المزمن مقارنة بمجموعة السيطرة. لا يوجد فرق معنوي في تراكيز الكلوبيولينات المناعية IgG, IgA, IgM بين مرضى العجز الكلوي المزمن و مجموعة السيطرة

يوجد تغير طفيف في تراكيز المتممات C4 و C3 والكلوبيولينات المناعية IgG, IgA, IgM لدى مرضى العجز الكلوي المزمن. حيث أن تركيز المتمم C3 فقط قد اظهر ارتفاعا معنويا عند هؤلاء المرضى. نوقشت النتائج من خلال قلة تناول البروتين لدى المرضى وكذلك نتيجة للمعالجات السابقة للالتهابات عند المرضى.

مفاتيح الكلمات: المتممات،C4, C3 الكلوبيولينات المناعية، IgG, IgA, IgM العجز الكلوي المزمن.

Introduction

Chronic renal failure (CRF) is a pathophysiologic process with multiple etiologies, resulting in the inexorable attrition of nephron number and function and frequently leading to an irreversible deterioration in renal function [1] [2]. It has been estimated that at least 6% of the adult U.S. population have chronic renal damage [3]. Acute Renal failure (ARF) complicates approximately 5% of hospital admissions and up to 30 % of admissions to intensive care units [3]. Chronic renal failure often accompanied by hypertension, proteinuria or anemia [4]. Because of the wide spread effects of renal failure, symptoms and signs may develop related to almost every body system. Patients may present with complaints which are not obviously renal in origin, such as tiredness or breathlessness. In the end-stage renal failure, patients appeared ill and anemic [3]. Cardiovascular abnormalities, hematological abnormalities, infection, gastrointestinal disorders, neuropathy, and myopathy may also present [5].

The study of the possible changes in the serum component in CRF is very important issue. The biochemical changes in CRF include water, electrolytes, endocrine and metabolic disorders and acid-base disturbances. In CRF ammonia generation is decreased leading to decrease amount of buffer excreted lead to metabolic acidosis lead to extra renal buffering mechanism[4]. Proteins, immunoglobulin G (IgG), C3, and C4 were measured in other renal disorders; C3 and C4 levels were normal in acute tubular interstitial nephritis [6].

Infection due to decreased in both humeral and cellular immunity, impaired inflammatory reaction, leukocytes dysfunction and increased exposure to pathogenic bacteria and viruses by hemodialysis or blood transfusion (Like: staphisepsis, hepatitis) [5]. Restriction of dietary protein leads to decrease blood urea nitrogen and this decreases the symptom [3] [5].

The changes in the serum concentration of some complements and immunoglobulin in some patients may be due to the erythropoietin drug (epoetin) that used in the therapy of anemia in maintenance hemodialysis patients. In one research, scientists [7] noticed that the administration of epoetin to maintenance patients under hemodialysis not only treats the anemia but also results in favorable changes in immune system. Epoetin is probably not only hemopoietic factor but also an immunomodulatory cytokine [7].

Parathyroid hormone (PTH) has an adverse effect on the immune system and may cause immunologic disorders in patients with chronic renal failure. The serum levels of C3,_C4 IgG, IgA and IgM showed significant increase until 12 months after parathyroidectomy. From these results it can be concluded that parathyroidectomy showed beneficial effects on humoral immunological markers. The effects are probably due to the remarkable PTH reduction and partly improved nutritional state after parathyroidectomy [8].

The aim of this work is to obtain the levels of serum C3, C4, IgG, IgA and IgM in CRF patients depending on many observations. Intravenous Immunoglobulins have been advocated as efficacious for some disorders [9]. Another observation is that IgA nephropathy (IgA-N) is considered the most common glomerular disease in the world and leads to renal failure in a substantial number

of patients. Although many studies have looked at the pathogenesis of the disease, many points need to be clarified, including the mechanism of complement activation-mannose-binding lectin or mannose binding protein, initiates activation of the complement cascade (lectin pathway). The lectin pathway of complement activation evidently contributes to the development of glomerular injury in a significant number of cases with IgA-N. In addition, these findings will add insight to the pathogenesis of IgA-N ، including its relation to infection. [10]

In many glomerular diseases, the complements and immunoglobulins are deposited in renal tissues. By immunofluorescence, the average diameter of tubules in immunotactoid glomerulopathy predominantly capillary wall, thick, ribbon-like glomerular deposits contained IgG, IgM, kappa and lambda light chains of equal intensity, C3, C4 and fibrin related antigens. Mild to moderate glomerular cell proliferation associated with nodular sclerosis has been assumed to be causally related to immunotactoid deposits [11].

Materials and methods

Twenty one male patients with chronic renal failure and twenty six healthy male persons were participated in this work. These cases were collected from Al-Hussein hospital at Karbala city. All patients were examined by senior doctor, and there are no other disorders recorded. Venous blood samples were collected from patients before taking any medications. Sera were separated and stored at (-20ْ C) until analysis.

After placing 5 ml of serum on each cavity on plates IgG, IgA and IgM and *C3* and C4 levels of both groups were quantitatively studied with immunodiffusion plates (Biomaghreb). Serum samples were incubated on plates for 72 hours at room temperature. At the end of this period the diameter of precipitation was measured and converted to mg/dl units. Normal values of the plates used are as follows:
IgG: (710-1520 mg/dl), IgA: (90-310 mg/dl), IgM: (40-250 mg/dl), *C3*: (84-193 mg/dl), *C4*: (20-40 mg/dl). The student's t-test used to assess the comparisons between groups.

Results

The results of the concentrations of serum immunoglobulin A, G, and M in chronic renal failure patients as compared with control group are presented in Figure (1). The results showed no significant changes in the concentration of immunoglobulins in CRF patients in comparing with healthy control at ($p<0.05$). However, Table (1) revealed that there are different results out of cut-off values (mean±2standard deviation) of these immunoglobulins. The percentages of patients who have less than cut-off value for IgA, IgG, and IgM were 15.3%, 5.3%, and 15.8% respectively. While the percentages of patients who have higher than cut-off value for IgA, IgG, and IgM were 15.8%, 15.3%, and 10.5% respectively.

Figure (2) showed the serum complements C3 and C4 in CRF patients as compared with healthy control group. There is significant increase in serum C3 and no significant difference in serum C4 in CRF patients as compared with healthy control group. The percentages of patients who have less than cut-off value for C3 and C4 were 10% and 15.8% respectively. While the percentages of patients who have higher than cut-off value for C3 and C4 were 21.1% and 15.8% respectively.

Attempts were carried out to find any correlation between serum urea and these immunoglobulins and complements. The correlation coefficients (r) of these relationships are recorded in Table (2).

Discussion

The results of this work can be explained mainly according to the possible deposition of these immunoglobulins and complements in different locations within the kidney tissues as noticed in different researches [11-14]. The deposits containing IgG, IgM, IgA, C1q, C4, and C3) were located primarily in the mesangium of proliferative glomerulonephritis [15]. In a previous work, Immunofluorescence studies of renal biopsy in renal disease patients showed: IgG (85 %), IgA (36%), IgM (90 %), C3 (90 %), C1q (47 %), and C4 (33 %) deposits, mainly located in subendothelial position [16].

In an immunohistological study for the intra-extracapillary proliferative glomerulonephritis (IEKPGN), the deposition results of (IgG, A, M), complement C3, C4) are positive in 53% of cases, thus secondary trapping of components of humoral immune reaction may be involved [12]. Another confirmation obtained from the observation of children with moderate chronic renal failure. In those patients, serum concentrations of albumin, total protein, transferrin, IgG, IgA, IgM, C3 and C4 and blood lymphocyte counts were within normal limits [17].

The increases in C3 protein in this work are unique and disagreed with other works [18, 13]. In one study, the patients with epidemic renal disease of unknown etiology had normal or low C3 and C4 levels, in about 40% of the cases. The clinical course varies from benign to rapidly progressive renal failure. Biopsies usually reflect an immune-complex mediated mesangiopathic glomerulonephritis with IgA, IgG, IgM, and C3 deposites [19]. The reason may be due to the difference in the race of patients, sample size, medication taken, in addition to the fact that our inpatients were having restricted diets with low protein contents. However, our finding indicated the importance of C3 complement in CRF patients which already found to be a strong prognostic factor in other renal disorders such as diffuse proliferative lupus glomerulonephritis [18].

Table (1) indicated that some (but not all) patients had a change in their immunoglobulins and complement levels. These patients must be experience many other biochemical, histological, and immunological tests to obtain the exact reason about the change in the serum immunoglobulins and complement levels.In a large skill study, amongst 125 patients with mesangiocapillary glomerulonephritis, eleven had a consistently low plasma concentration of C3; only three, however, had an initial low plasma concentration of C4, which rose and then remained normal in two. Tests for the C3 nephritic factor were positive in thirteen patients, and plasma C1q was normal in 8 out of 11 cases invested. Ten out of twelve (seven of them with low plasma C3 (showed C3 deposition by immunofluorescence in the glomerular tuft [20].

Parameters of humoral immunity were studied in 18 patients with chronic renal failure undergoing hemodialysis. IgG, IgA and IgM serum Levels presented no differences compared with healthy donors. High immunoglobulins levels were found in 40 patients with chronic renal failure and conservative treatment. Complement components C1q, C4 and C9 were normal, but C3 and C3A were significantly low prior to dialysis. During hemodialysis, the complement system showed activation by the alternative pathway. The circulating immune complexes (CIC) were also high. These results suggested a certain humoral immune alteration during chronic Hemodialysis [21].

There is also other important factor that related to the possible changes in serum electrolytes that may affect the immunological factors. Serum calcium Ca^{+2} in CRF is lower than normal but rarely be symptomatic in patients with renal failure. Causes of decrease Ca^{+2} level are due to decrease intestinal absorption (corrected by active vitamin D analogues) and in late stages calcium-phosphate deposited in soft tissues lead to decrease serum Ca^{+2} [3] [5]. Serum magnesium in CRF is moderately increased [5].

The findings of this work are in accordance with the result of other renal disorders research [22] except the value of C3 complement. Serum Immunoglobulins (IgG, IgA, and IgM) and complement (C3 and C4) were normal in the mesangial glomerulonephritis patients and their relatives [22].

Immunoglobulin and complement component deposition in nonimmune disease was studied in different groups of patients. C3 and properdin were detected in 77 to 100 %of all groups; in 18 patients, C3 and properdin were present without detectable C1q and C4. Immunoglobulins, primarily IgM, and components of the classic and alternative C pathways are regularly present in hyalinizing glomeruli irrespective of the etiology of the renal failure. These observations suggest that an immune process is operative in glomerular

obsolescence regardless of the underlying etiology of the renal disease [23]. Same explanation may be used to predict our results i.e. immune processes are involved in CRF.

Complementuria is a common finding in patients with heavy proteinuria from a variety of causes, and was detected in 23 out of 34 nephrotic Subjects. The renal handling of complement appears to be largely molecular weight dependent, an inverse relationship between the sieving coefficient and molecular weight of transferrin, IgG, C3, and C4 obtaining in nephrotic patients irrespective of the nature of their glomerulopathy or degree of renal function. Furthermore, glomerular sieving of C3 and C4 was not significantly different in patients with immune glomerular injury associated with extensive glomerular complement deposition, from that in patients with non-immune glomerulopathy, suggesting that no unique mechanism exists for the transglomerular passage of complement from serum into the urine of the former group. The finding of a large increase of sieving of C3 and C4 in nephrotic patients with end-stage renal failure may indicate a failure by atrophic tubules to reabsorb and catabolize filtered complements [24]. This phenomenon is another possible cause for the changing in complement concentration in CRF patients.

Table (2) showed that there is no significant correlation (correlation coefficient $r<0.5$) between serum urea and serum complements and immunoglobulins concentrations in CRF patients. These results may be due to the fact that our patients were receiving a restricted protein diets to prevent any increase in serum urea. However, the decrease in the measured serum complement and immunoglobulins in patients with chronic renal failure receiving low protein diets was previously noticed [25]. Hence the synthesis of these proteins may be restricted. Mean levels of plasma transferrin; complement C3 and globulin Gc were lower and plasma prealbumin higher in chronic renal failure patients than in normal subjects. Plasma complement C4 and albumin were not different from normal [25]. Seven out of nine patients who tolerated the supplementation showed a significant increase in plasma transferring, prealbumin and complement C3 but not in complement C4, globulin Gc or albumin. Correlations between the percentage of essential amino acids and each of plasma transferrin, prealbumin and complement C3 and also between several of the plasma proteins further substantiate their value in the assessment of dietary intake in chronic renal failure [25].

Table (1): Percentages of the patients with higher or lower cut-off values of serum complements and immunoglobulins.

	IgA	IgG	IgM	C3	C4
%Patients have less than (M±2SD)	**15.8**	**5.3**	**15.8**	**10.5**	**15.8**
%Patients have more than(M±2SD)	**15.8**	**15.8**	**10.5**	**21.1**	**15.8**

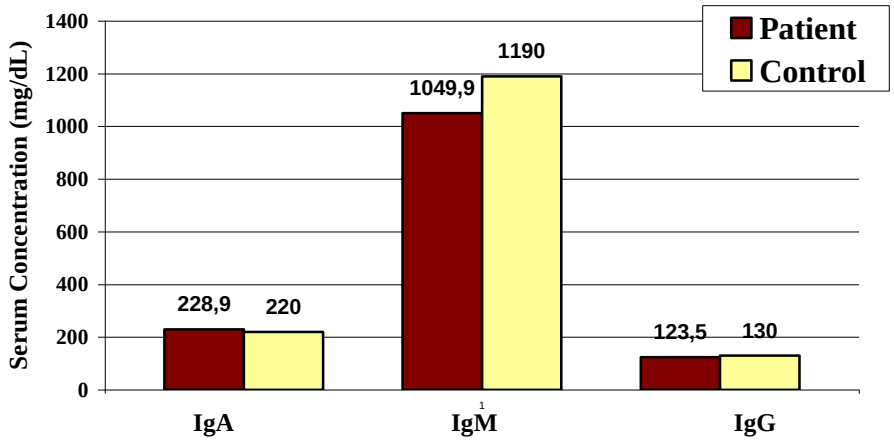

Figure (1): Serum immunoglobulins in chronic renal failure patients as compared with control group.

Table (2): Correlation coefficient values (r) of the serum urea against the value of serum complements and immunoglobulins.

Correlation	Correlation Coefficient (r)
Urea *vs.* IgA	0.15
Urea *vs.* IgG	0.20
Urea *vs.* IgM	0.37
Urea *vs.* C3	0.17
Urea *vs.* C4	0.39

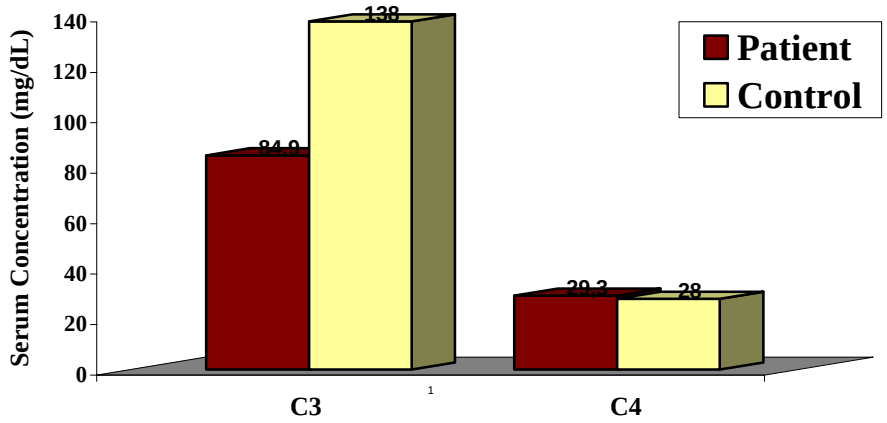

Figure (2): Serum complements C3 and C4 in CRF patients as compared with healthy control group.

References

1- Ganong W.F. (2004): "Reviw of medical physiology" 21st Edition, McGraw-Hill, N. Y.

2- Leonard G.Gomella, Steven A.Halst, (2004):" Clinician's Pocket reference" 10th Edition, McGraw-Hill, N. Y.,184; 340; 239.

3- Fauci A. S., Martin J.B., Hauser D.L., *et al* (2005): "Harrisons Principles of Internal Medicine" 16th Edition, McGraw-Hill N.Y., 1663-1644.

4- Haslett C., Chilvers E. R., Hunter J. A. , Boon N. A. (2000): "Davidson`s Principles and practice of medicine" 18 th edition, Harcourt pub. UK, 429-437.

5- Lee Goldman, J. Bennett (2000): "Clande"Cecil" 21st edition, W.B. Saunders Co. U.S.A., 567-578.

6- Nikolic V, Bogdanovic R, Ognjanovic M, Stajic N., Acute tubulointerstitial nephritis in children ***Srp Arh Celok Lek.***, 2001, **129 Suppl 1**, 7.

7- Debska-Slizien A, Rutkowski B, Manitius J, *et al*, Influence of erythropoietin on immunological system of patients with chronic renal failure ***Pol Merkuriusz Lek.***, 2003, **15(88)**, 7.

8- Yasunaga C, Nakamoto M, Matsuo K, *et al*, Effects of a parathyroidectomy on the immune system and nutritional condition in chronic dialysis patients with secondary hyperparathyroidism ***Am.J.Surg.***, 1999, **178(4)**, 6.

9- Vazquez Garcia MJ, Olalde Carmona R, Juarez JA, *et al*, Treatment of lupus crisis with IgIV**.** ***Rev Alerg Mex.***, 1998, **45(6)**, 6.

10- Endo M, Ohi H, Ohsawa I, *et al*, Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy. ***Nephrol Dial Transplant.***, 1998, **13(8)**, 90.

11- Ferluga D, Hvala A, Vizjak A, *et al*, Immunotactoid glomerulopathy with unusually thick extracellular microtubules and nodular glomerulosclerosis in a diabetic patient. ***Pathol Res Pract.***, 1995, **191(6)**, 96.

12- Rumpelt HJ, Clinical pathology of the glomerulus--from phenomenon to entity; the extracapillary lesion. ***Verh Dtsch Ges Pathol,*** 1989, **73**, 83.

13- Hall RP, Stachura I, Cason J, *et al*, IgA-containing circulating immune complexes in patients with IgA nephropathy ***Am J Med.,*** 1983**, 74(1)**, 56.

14- Laurent B, Gonthier R, Genin C, *et al*, Circulating immune complexes, detection in human glomerulonephritis by the PEG-EDTA technique (author's transl). ***Pathol Biol (Paris),*** 1982, **30(3)**, 6.

15- Jones E, Magil A., Nonsystemic mesangiopathic glomerulonephritis with "full house " immunofluorescence. ***Am J Clin Pathol.***, 1982, **78(1)**, 29.

16- Tarantino A, De Vecchi A, Montagnino G, *et al*, Renal disease in essential mixed cryoglobulinaemia. Long-term follow-up of 44 patients. ***Q J Med*** , 1981, **50(197)**, 1.

17- Orejas G, Santos F, Malaga S, *et al*, Nutritional status of children with moderate chronic renal failure. ***Pediatr Nephrol.,*** 1995, **9(1)**, 52.

18- Magil AB, Ballon HS, Chan V, *et al*, Diffuse proliferative lupus glomerulonephritis Determination of prognostic significance of clinical, laboratory and pathologic factors**.** ***Medicine (Baltimore)***, 1984, **63(4)**, 20.

19- Hoy WE, Megill DM, Hughson MD ., Epidemic renal disease of unknown etiology in the Zuni Indians**.** ***Am J Kidney Dis.***, 1987, **9(6)**, 96.

20- Vargas R, Thomson KJ, Wilson D, *et al*, Mesangiocapillary glomerulonephritis with dense

"deposits" in the basement membranes of the kidney. ***Clin.Nephrol***., 1976, **5(2)**, 73.

21- Rus HG, Cristea A, Gherman M, *et al*, Immunologic alterations associated with chronic hemodialysis. ***Med Interne***., 1983, **21(4)**, 95.

22- Gutierrez Millet V, Navas Palacios JJ, Ortega Ruano R, *et al*, Familial and hereditary mesangial glomerulonephritis with IgA deposits author's transl. ***Med Clin (Barc),***1981, **Jan 10; 76(1)**, 1.

23- Velosa J, Miller K, Michael AF. , Immunopathology of the end-stage kidney. Immunoglobulin and complement component deposition in nonimmune disease ***Am J Pathol.***, 1976, **84(1)**, 62.

24- Klajman A, Avital A, Myers BD. , Renal handling of the third (C3) and fourth (C4) components of the complement system in the nephrotic syndrome. ***Nephron***, 1976, **16(5)**, 43.

25- Young GA, Keogh JB, Parsons FM., Plasma amino acids and protein levels in chronic renal failure and changes caused by oral supplements of essential amino acids. ***Clin Chim Acta.***, 1975, **61(2)**, 13.