Research Article

Serum Levels of Intercellular Adhesion Molecules, Vascular Cell Adhesion Molecules, and C - reactive Protein in Chronic Renal Failure Patients Receiving Haemodialysis with Different Etiology

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ABSTRACT

The objective of present study was to verify the changes of serum levels of ICAM-1 and VCAM-1 in Chronic Renal Failure (CRF) Patients undergoing haemodialysis. Sixty five of haemodialysis patients were enrolled in this study and compared with forty apparently healthy subjects. Thirty from these patients were followed up to six months for serial assessment of these biomarkers as well. The results indicated significant elevations of ICAM-1 and VCAM-1 serum levels when compared with the control group ($P < 0.001$). Also the statistical analysis showed a significant difference in serum level of ICAM-1 and VCAM-1 within different etiology of hemodialysis patients ($P < 0.001$). Moreover there were significant differences in 4th reading of ICAM-1 and VCAM-1 and a significant difference in 3rd reading of VCAM-1 when compared to first reading of ICAM-1 and VCAM-1 serum levels through the period of serial assessment ($P < 0.05$). Further, a significant negative correlation was observed for ICAM-1 and VCAM-1 serum levels with the glomerular filtration rate GFR of patients and a significant positive correlation was demonstrated for the level of ICAM-1 and VCAM-1 with the duration of dialysis ($P < 0.05$). The results pointed out that ICAM-1 and VCAM-1 levels are involved in the pathophysiology of the disease and they were useful in the prediction of the disease earlier to the clinical manifestations of atherosclerotic and cardiovascular disease. In addition the measurements of these biomarkers were significantly correlated with etiology of CKD, duration of dialysis and GFR in haemodialysis patients.

Keywords: Chronic Renal Failure, Haemodialysis, ICAM-1, VCAM-1.

INTRODUCTION

Chronic renal failure (CRF) is defined as a glomerular filteration rate less than 60 ml/min./1.72 m^{2}for at least 3 months duration, it is usually the end result of conditions such as diabetes mellitus, hypertension, primary glomerular nephritis, autoimmune disease, obstructive uropathy, polycystic kidney disease, renal artery stenosis, infection, tubular dysfunction and the use of nephrotoxic drugs. The most important biochemical evidents in CRF are increase in serum urea and creatinine concentration, Other findings include: metabolic acidosis , and fixed urine osmolality[1]. End Stage Renal Disease ESRD include patients treated by dialysis or transplantation, irrespective of the level of GFR[2]. With the improvement in conservative management and dialysis, the life span of patients with chronic renal failure CRF has been increased. As the patient's survival has approached the 10 years, there is an increasing indication that accelerated atherosclerosis may remain a major unresolved problem threatening the longevity of CRF patients[3]. Infections and cardiovascular diseases remain the leading causes of complications and death in end stage renal disease patients[4]. The clinical course of patients with chronic renal failure CRF is conditioned by a series of immunological and inflammatory alterations which, in turn, lead to cardiovascular diseases[5]. These processes are reflected in the changing expression of the membrane-bound receptors of adhesion molecules and the altered release of endothelial factors, which are believed to play a significant role in cardiovascular morbidity and mortality[6].

Increased plasma levels of cellular adhesion molecules CAMs have been shown to be predictors of all cause mortality in individuals with chronic renal failure and patients with end-stage renal disease receiving haemodialysis[7]. Elevated concentrations of CAMs are found in persons with chronic kidney disease and reduced glomerular filtration[8]. In end-stage renal disease ESRD patients, chronic inflammation may be associated with endothelial dysfunction[9]. Increased levels of soluble adhesion molecules and cytokines have previously been reported in patients with CRF, both on conservative treatment and on hemodialysis HD, but the influence of dialysis membranes on their secretion as well as their pathological and clinical implications remains largely unknown[9].

PATIENTS AND METHODS

Sixty five patients of CKD (29 females, 36 males) were enrolled in this study. Their ages range from 20-70 years with mean of age (38.43 ± 17.65 year). Thirty, (12 females, 18 males) from these patients were followed up to six months for four readings (zero reading at 1st month and the others at 3rd month, at 5th month and 7th month respectively) for serial assessment for estimation of serum levels of ICAM-1 and VCAM-1 in haemodialysis patients. Patients with Acute renal failure, HBs Ag positive and Nephrotic syndrome were excluded from the current

study while the control group was consisted of forty subjects who were free from signs and symptoms of renal diseases, lipid metabolism disorders, diabetes mellitus, and hypertension. 18 of them were females and 22 were males. Their ages range from 25 to 65 years with mean of age (33.23 ± 14.75 year).

Five milliliters of venous blood samples were collected from each patient after an over night fasting before the haemodialysis session was started. A slow aspiration of the venous blood sample via the syringe was carried out; the samples were dropped into clean disposable tubes, left at room temperature for 30 minutes for clot formation and then centrifuged for 20 minutes at 3000 xg. The sera were separated in a disposable tube and stored at -80 °C for estimation of biomarkers later. Similarly blood samples were taken from the control group by vein puncture and sera subjected to processing exactly as that for patients. Serum levels of ICAM-1 and VCAM-1 were measured by enzyme linked immuno-sorbent assay (ELISA).

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for windows. Results are expressed as mean ± standard deviation, t – test used to estimate differences in each parameter between groups, linear regression analysis also used to study the relations between different parameters and Anova to compare differences in parameter among different groups, accepted significant was $P < 0.05$.

RESULTS

Table 1: Characteristics of patients included in the study

Variable	Mean ± SD
Age, years	38.43 ± 17.65
Duration of dialysis, months	25.39 ± 16.45
GFR, ml/min	13.11 ± 5.6
Body mass index, kg/m^2	22.37 ± 4.17
Blood urea, mmol/L	28.6 ± 5.8
Serum creatinine, µmol/L	701.8 ± 136.3
Hb, g/L	97.7 ± 26
Numbers of session / week	2±1.2

The evaluation of the data indicated that the enrolled patients were distributed according to different trends. They were distributed according to the age, sex, BMI, cause of disease, GFR and duration of dialysis. The characteristics of the enrolled CRF patients are mentioned in table 1 by mean and the standard deviation (Mean ± SD).

Most causes of CKD patients was found to be of glomerulonephritis (GN) etiology (18, 28%), the second most common one was hypertension (HT) (12, 18%), followed by diabetes mellitus (DM) (10, 15%) , renal stones (6, 10%), polycystic kidney (5, 7%) and chronic pylonephritis (CPN) (4, 6%), amyloidosis (2, 3%), while the rest (8, 13%) of unknown causes. A significant difference was observed in serum levels of ICAM-1 and VCAM-1 within different etiology of haemodialysis patients (Table 2).

Table 2: Serum levels of ICAM-1 and VCAM-1 in chronic renal failure patients receiving haemodialysis within different etiology.

	NO.	ICAM-1 pg/ml	VCAM-1 ng/ml
GN	**18**	1700 ± 610 **@ † ‡**	70.1±22.2 **∞ * ∞ @ ‡ * ∞ @ ‡**
HT	**12**	1849 ± 299.7 **@ † ‡**	43.5±26.3 *** @ † ‡**
DM	**10**	3065 ± 830.6 *** ∞ †**	67.8±13.5 **∞ † ‡**
Stones	**6**	1062 ± 722 *** ∞ @ ‡**	77.2±12.4 **∞**
Unknown	**8**	2871± 831 *** ∞ †**	89.5±7.2 **∞ @**
P- value		< 0.001	< 0.001

* means $p < 0.05$ significantly difference with respect to GN group.

∞ means $p < 0.05$ significantly difference with respect to HT group.

@ means $p < 0.05$ significantly difference with respect to DM group.

† means $p < 0.05$ significantly difference with respect to Stones group.

‡ means $p < 0.05$ significantly difference with respect to Unknown group.

Table 3: Serum levels of ICAM-1 and VCAM-1 in Chronic Renal Failure patients receiving haemodialysis and the control group.

Parameter	Subject	NO.	Mean ± SD	Range	P-value
ICAM-1 pg/ml	Patients Control	65 40	1966.34±850 582.5±601	236-4000 26-2500	< 0.001
VCAM-1 ng/ml	Patients Control	65 40	67.26±22.3 15.5±10.3	9.5-99.9 1.8-40.7	< 0.001

Table 4: Serial assessment of serum levels of ICAM-1 and VCAM-1 of thirty chronic renal failure patients receiving haemodialysis during six month for four readings each per two months.

	First reading	Second reading	Third reading	Fourth reading
ICAM-1	1957.29 ± 935.84	1985.927 ± 895.41	2031.637 ± 822.43	2035.927 ± 895.41*
VCAM-2	64.12 ± 16.90	69.12 ± 16.90	73.20 ± 16.17*	74.96 ± 15.53*

* means p < 0.05 significantly difference with respect to the first reading

Thirty patients were followed up to six months for four readings each per two months for serial assessment of serum levels estimation of ICAM-1 and VCAM-1 in haemodialysis patients, they were 10 from each of glumerulonephritis, hypertension and diabetes mellitus group patients (Table 4).

In addition to that the linear regression analysis demonstrated a significant negative correlations were observed for ICAM-1 (r = 0.34, p < 0.007) and VCAM-1 (r = 0.33, p < 0.008) levels with the GFR of patients. Further, a significant positive correlations for the level of ICAM-1 (r = 0.38, p < 0.02) and VCAM-1 (r = 0.37, p < 0.003) with the duration of dialysis of patients. (Figure 1,2,3 and 4 respectively).

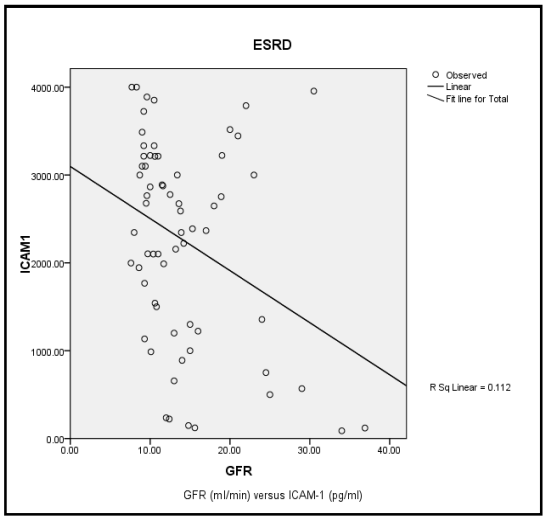

Figure 1: The correlation of GFR with ICAM-1 in chronic renal failure patients receiving haemodialysis.

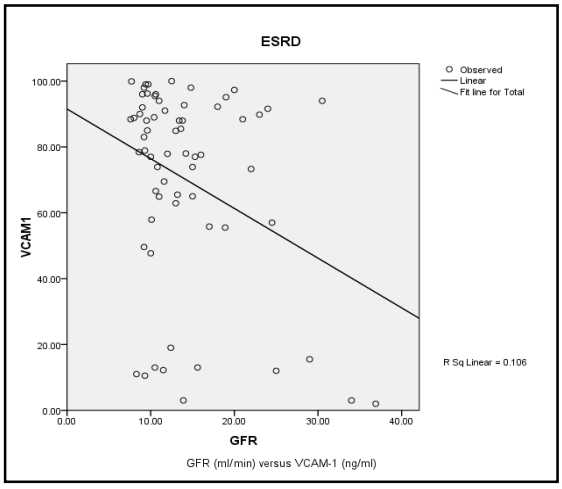

Figure 2: The correlation of GFR with VCAM-1 in chronic renal failure patients receiving haemodialysis.

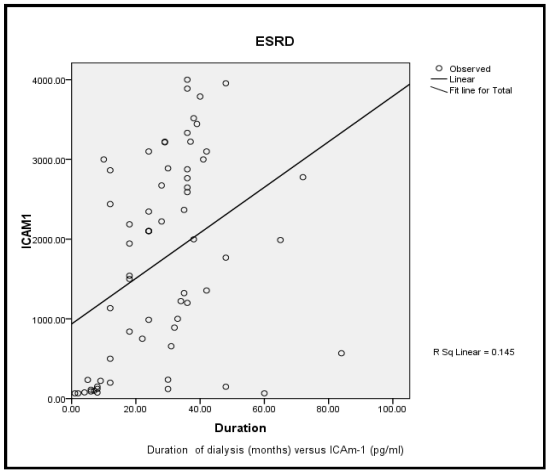

Figure 3: The correlation of duration of dialysis with ICAM-1 in chronic renal failure patients receiving haemodialysis.

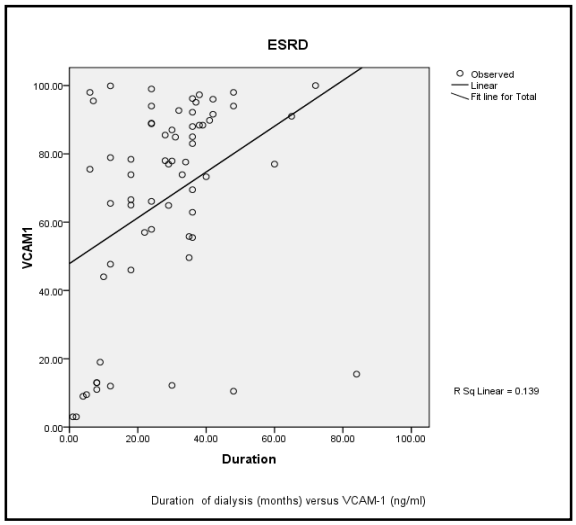

Figure 4: The correlation of duration of dialysis with VCAM-1 in chronic renal failure patients receiving haemodialysis.

DISCUSSION

The current study was evaluated serum levels of the two soluble adhesion molecules ICAM-1 and VCAM-1 in patients with end-stage renal disease ESRD on maintenance hemodialysis. It showed a significant increase of ICAM-1 and VCAM-2 in haemodialysis patients when compared with the control those reported previously. Elevated concentrations of CAMs are found in persons with chronic kidney disease and reduced glomerular filtration[11] but the influence of the HD membranes on their secretion, as well as their pathophysiological implications, remains largely unknown

so that serum levels of ICAM-1, and VCAM-1 are increased in HD patients but the exact mechanisms responsible for these alterations are yet to be fully elucidated. Increased levels of adhesion molecules are associated with inflammation, dyslipidemia, and cardiovascular events[12]. Patients with CRF and on maintenance haemodialysis have altered concentrations of soluble adhesion molecules, resulting from either inadequate clearance or disturbed synthesis and release also patients with chronic renal failure CRF present with an impaired immune response through leukocyte migration[13]. ESRD is now considered a prototypical situation of chronic inflammatory state[14]. Adhesion molecules are upregulated in chronic inflammatory states[15] and this upregulation is currently considered as an expression of endothelial dysfunction[16], so during haemodialysis, the expression of different adhesion molecules changes serving as markers of biocompatibility of dialysis membranes which have different effects on the concentration of adhesion molecules and their association with leukocytes and pro-inflammatory cytokines[17]. An effective immune response depends on leukocytes migration to the site of inflammation. The membrane bound forms of selectins and molecules of the immunoglobulin superfamily, such as ICAM-1 and VCAM-1, take part in a process of leukocyte migration called the "adhesion cascade"[18]. However, investigation of soluble adhesion molecules shed proteolytically from cells into the circulation has given contradictory results in haemodialysis patients[19]. Also the elevation of serum levels of ICAM-1 and VCAM-1 in the current results may be attributed to decreased elimination by the impaired kidney as the kidney plays an important role in their catabolism[20]. Various humoral and cellular inflammatory reaction cascades can be triggered and can explain elevated post dialysis levels of ICAM-1 and VCAM-1 in current results[11]. Another explanation for elevation of the adhesion molecules is the effect of ultrafiltration which leads to hemo-concentration of these adhesion molecules[21]. Also the higher serum levels of ICAM-1 and VCAM-1 in haemodialysis patient are due to the effect of dialysis membranes which is less with the use of high-flux filters, so the present information indicates that adhesion molecules mediate leukocyte dysfunctions caused by haemodialysis, therefore the effects of different dialysis membranes on leukocyte adhesion receptors should be taken into account as an important index of biocompatibility[21]. Elevated levels of these adhesion molecules are associated with neutrophils activation[17]. Also uremic patient on chronic hemodialysis have also clinical evidence of impaired lymphocyte function, these defects in immunity result, in part, from the uremic state or as a consequence of therapy. Hemodialysis is associated with complement activation, upregulated expression of granulocyte cell surface, and release of pro-inflammatory cytokines[22]. In addition to that leukocyte membrane interactions have been implicated as being responsible for some of the clinical problems observed in HD patients, such as dialysis related hypoxia and, delayed rate of recovery from acute renal failure which mediated, in part, by leukocyte adhesion molecules[23], so that the haemodialysis causes a profound, transient neutropenia whose possible mechanisms include leukocyte aggregation and adhesion to endothelia[24]. Many researchers Francesco[21], Robert[22] and Sawires[17] were reported that the level of ICAM-1 and VCAM-2 in haemodialysis patients increased when compared with the control group.

On the other hand the current results were contradictory with Musial[18,19] about the levels of these adhesion molecules in haemodialysis patients whose supported that some of these adhesion molecules were not significantly elevated attributed that to bounding of these adhesion molecules to dialysis membranes and may be due to single dialysis session rather than continuous dialysis studies.

REFERENCES

1. Brutis C., Edward R., David E. TIETZ Textbook of Clinical Chemistry and Metabolic Medicine, 2006, 1689-1725.
2. Crook M., Clinical Chemistry and Metabolic Medicine. London, Seventh edition, 2012, chapter 13, p 198-201.
3. Linder A., Charra B., Sherrard D.: Accelerated atherosclerosis in prolonged maintenance haemodialysis. New Engl. J. Med.; 290, 1998, 697-706.
4. Muniz-Junqueira M., Lopes C, Cassia A. Acute and chronic influence of haemodialysis according to the membrane used on phagocytic function of neutrophils and monocytes and pro-inflammatory cytokines production in chronic renal failure patients. Life Sciences, 77, 2005, 3141–3155.
5. Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger R: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. Lancet; 358, 2001, 2113–2117.
6. Stenvinkel P, Lindholm B, Heimburger M, Heimburger O: Elevated serum levels of soluble adhesion molecules predict death in pre-dialysis patients: Association with malnutrition, inflammation, and cardiovascular disease. Nephrol Dial Transplant, 15, 2000, 1624–1630.
7. Kim S., Choi D, Ko Y, Kim J, Han S, Kim B : Relation of Homocysteinemia to Contrast-Induced Nephropathy in Patients Undergoing Percutaneous Coronary Intervention. Am J Cardiol, 77, 2011, 3244-3250.
8. Ling W, Zhaohui N, Ben H, Leyi G, Jianping L, Huili D : Urinary CRP and NGAL as early predictive biomarkers in contrast-induced nephropathy, Nephron ClinPract, 35, 2008, 1079-1083.
9. Berger A, Edelsberg J, Inglese GW, Bhattacharyya S, Oster G. Cost comparison of peritoneal dialysis versus haemodialysis in end-stage renal disease. Am J Manag Care, 158, 2009, 509-18.
10. Li P, Szeto C, Piraino B, Bernardini J, Figueiredo A, Gupta A, Johnson D, Kuijper J, Lye W, Salzer W, Schaefer F, Struijk D. Peritoneal dialysis-related infections recommendations: Perit Dial Int, 304, 2010 update, 393-423.

11. Utsumi K, Mitsuhiko K, Akio H, Kae U, Yuko K, Effects of selective LDL apheresis on plasma concentrations of ICAM-1, VCAM-1 and P-selectin in diabetic patients with arteriosclerosis obliterans and receiving maintenance haemodialysis, Clinica Chimica Acta, 377, 2007, 198–200.

12. Zohny S, Abd El-Fattah M., Evaluation of circulating vascular endothelial growth factor and soluble adhesion molecules as reliable predictors of native arteriovenous fistula thrombosis in chronic hemodialysis patients. Clinical Biochemistry, 41, 2008, 1175–1180.

13. Papayianni A, Alexopoulos E, Giamalis P, Gionanlis L, Belechri M, Koukoudis P, Memmos D .Circulating levels of ICAM-1, VCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. Nephrol Dial Transplant, 173, 2002, 435-41.

14. Zoccali C, Mallamaci F, Tripepi G: Inflammation and atherosclerosis in end stage renal disease. Blood Purif, 21, 2003, 29–36.

15. Mackiewicz A, Speroff T, Ganapathi K, Kushner I: Effects of cytokine combinations on acute phase protein production in two human hepatoma cell lines. J Immunol, 146, 2005, 3032–3037.

16. Pober J, Cotran R: Cytokines and endothelial cell biology. Physiol Rev, 70, 2005, 427–451.

17. Sawires H,1 Waleed A, Mona F, High-flux and Low-flux Dialysis Membranes and Levels of Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 in Children With Chronic Kidney Failure Iranian Journal of Kidney Diseases, 6, 2012, 320-325.

18. Musial K, Zwolinska D, Jonkisz D, Berny U, Szprynger K, Szczepanska M. Serum VCAM-1, ICAM-1 and L-selectin levels in children and young adults with chronic renal failure. PediatrNephrol. 20, 2005, 52-5.

19. Buraczynska M, Wojciech Z, Iwona B, The intercellular adhesion molecule-1 ICAM-1 gene polymorphism K469E in end-stage renal disease patients with cardiovascular disease, Human Immunology, 73, 2012, 824–828.

20. Musial K, Zwolinska D, Szprynger K, Szczepanska M. Soluble Adhesion Molecules during a Single Dialysis Session in Children and Young Adults on Chronic Haemodialysis. AdvClinExp Med. 17, 2008, 183-9.

21. Francesco V., Giuseppe M., Santina C., Maurizio S., Lydia G., Circulating Levels of Adhesion Molecules in Chronic Kidney Disease Correlate with the Stage of Renal Disease and with C-Reactive Protein, Archives of Medical Research, 38, 2007, 534–538.

22. Robert A., Edimara S., Daniel R., John D., Targeted complement inhibition as a promising strategy for preventing inflammatory complications in haemodialysis, Immunobiology, 217, 2012, 1097–1105.

23. Hojs R., Robert E., Biomarkers in Haemodialysis Patients Advances in Clinical Chemistry, 57, 2012, 29–56.

24. Malyszko J., Mechanism of endothelial dysfunction in chronic kidney disease, Clinical Chimical Acta, 411, 2010, 1412–1420.

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