Diagnostic and Prognostic Values of Adhesion Molecules VCAM-1, ICAM-1 and C - reactive protein in Egyptian Patients with Chronic Kidney Disease

Makram F. Attalah, Lamiaa A. Adel, Dina E. Fahmy

Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University. Hemodialysis Unit, El Zaiton Specialized Hospital, Cairo, Egypt.

Soluble intracellular adhesion molecule-1 (ICAM-1) and soluble vascular adhesion molecule-1 (VCAM-1) are markers of endothelial dysfunction which is linked to the atherosclerotic process causing a series of complications in patients with chronic kidney disease. The aim of this study was to evaluate C-reactive protein and adhesion molecules VCAM-1, ICAM-1 in patients with predialysis, chronic renal failure (CRF), on maintenance hemodialysis (HD) and after kidney transplantation (KT). Ten patients with predialysis CRF, 20 on maintenance HD, 5 after KT and 10 subjects as a control group were included in this study. We evaluated serum levels of ICAM-1, VCAM-1 as acute phase proteins, C-reactive protein (CRP) as an inflammatory marker and body mass indices (BMI), serum albumin, cholesterol, triglyceride as nutritional indices. The mean values of serum levels of VCAM-1 and ICAM-1 were significantly higher in the three groups of patients than those of the controls (P ≤ 0.05). There was statistically significant difference between the serum levels of VCAM-1 in the HD patients versus all other groups with the highest level in the HD patients. The circulating level of CRP, there was a progressive increase from controls to KT, CRF and to HD with the highest level in HD patients. There was a statistically significant difference between CRP serum levels in the HD patients versus all other groups with the highest level in the HD patients. Regression analysis showed significant positive correlation between CRP and ICAM-1 in KT patients (r = 0.9051; P = 0.0346), in CRF patients (r = 0.7621; P = 0.0170) and in HD patients (r = 0.4449, P = 0.0493). As regards the correlation between CRP and VCAM-1, there was positive correlation in KT patients (r = 0.9006; P = 0.0370), in CRF patients (r = 0.7088; P = 0.0326) and in HD patients (r = 0.4495; P = 0.0466). Age and BMI did not statistically differ in the study groups. In conclusion, serum levels of soluble adhesion molecules sVCAM-1, sICAM-1 correlate positively with the stage of renal disease. Also their serum levels were correlated positively with CRP as an inflammatory index in renal diseases. Further studies are needed to assess use of monoclonal antibodies against adhesion molecules and CRP as targets for therapeutic intervention in chronic kidney diseases.

Cell-cell and cell-matrix interactions play vital roles in morphogenesis, inflammation, thrombosis, wound healing, immune surveillance, growth and metastasis. A number of cell surface receptors, so-called cell adhesion molecules (ICAMs) mediate these events in various cell types (Arnaout, 1993). Activation of some adhesion molecules mediates endothelial dysfunction which plays pivotal roles in the initiation, progression and propagation of the atherosclerotic process (Hansson, 2005). Adhesion of circulating leukocytes to the endothelial cells and subsequent transendothelial migration to sites of inflammation is suggested as an important step in the initiation and aggravation of atherosclerotic lesions (Ross, 1999). This process is predominantly mediated by cellular adhesion molecules ICAM-1 and VCAM-1, which are expressed on the endothelial membrane in response to several inflammatory stimuli (Blankenberg et al., 2003). The expression process is induced by pro-inflammatory cytokines, which are present at increased levels in the uraemic circulation (Suliman et al., 2006). Serum levels of soluble intercellular and vascular adhesion molecules (ICAM-1, VCAM-1) are reported to be increased in conservatively treated renal patients and kidney transplanted patients with acute or chronic rejection.
Diagnostic and prognostic values of VCAM-1, ICAM-1 and CRP in Egyptian patients with chronic kidney disease

(Kurkijarvi et al., 2001). C-reactive protein (CRP) is a nonglycosylated protein produced by human hepatocytes and its synthesis is regulated by different cytokines (Vaccaro et al., 2007). In the last years CRP has gained a lot of attention in the general population especially with regard to its link with atherosclerosis, several studies suggest that CRP may be useful as a parameter in predicting future cardiovascular events in both the general population and in patients with end stage renal disease (Van der Sande et al., 2006). Tumor necrosis factor alpha (TNFα), a pleiotropic cytokine, plays important inflammatory roles in renal diseases, the proinflammatory and immunoregulatory roles for TNFα are strikingly illustrated in patients on anti-TNFα medications. These treatments are greatly beneficial in certain inflammatory diseases. The indication for anti-TNFα treatments in renal inflammatory diseases is still under discussion (Xydakis et al., 2008).

The aim of the present study was to evaluate important inflammatory mediators, which take part in pathogenesis of chronic kidney disease; such as C-reactive protein and adhesion molecules VCAM-1, ICAM-1 in patients with predialysis, chronic renal failure (CRF), on maintenance hemodialysis (HD) and after kidney transplantation (KT) and to correlate them with some indices of inflammation and nutrition.

Patients and Methods

During April 2010 to March 2011, this study was conducted on 35 patients from the nephrology department in El-Zaitoun Specialized Hospital in Cairo and 10 controls (Group I). An informed consent was taken from patients or patient's guardians. Full details about the study, its benefits and investigations were explained to them. The collected data and samples were kept in confidentiality and patient's privacy was considered. The 35 patients included five patients after kidney transplantation (KT). All KT patients were treated with steroids and other immunosuppressive drugs, periods past transplantation ranged from 2 to 9 months; 10 patients with predialysis chronic renal failure (CRF), in the CRF group the disease was attributed to glomerulonephritis (n = 6), tubulointerstitial nephritis (n = 3) and polycystic kidney (n = 1); 20 patients on maintained hemodialysis (HD) with F6-HPC dialyzers three times a week; non residual renal function was evident in all HD patients. Study exclusion criteria were active systemic infection, inflammatory diseases, malignancy, diabetes mellitus, history of atherosclerosis and patients receiving antibiotics or corticosteroids (except for transplanted patients) or non-steroidal anti-inflammatory drugs.

Blood samples (5ml) were collected from all patients and controls. In HD patients the samples were collected immediately before the beginning of HD session directly from arteriovenous fistula. In CRF and KT patients blood samples were taken during their visit to outpatient clinic. The blood was allowed to clot at room temperature for 2hours; the serum was then obtained by centrifugation at 1,500xg for 6min. A serum aliquot was kept frozen liquated after separation at -20°C for ICAM-1 and VCAM-1 measurement while the remaining serum was used to determine serum levels of creatinine (Photometric Colorimetric Test for Kinetic measurement, Human Gesellschaft for Biochemical and Diagnostic mbH, Germany), CRP (Avitex CRP latex test, Omega Diagnostics LTD, Scotland, United Kingdom), albumin, cholesterol and triglycerides (automatically by Beckman Coulter Synchron CX9 PorA, U.S.A).

sICAM-1 and sVCAM Enzyme Linked Immunosorbent Assay: Serum concentration of sICAM-1 and sVCAM-1 were determined by enzyme-linked immunosorbent assay (ELISA) using Ray Bio® Human ICAM-1 and VCAM-1 ELISA Kit [RayBiotech, Inc. 3607 Parkway Lane, Suite 200, Norcross GA 30092, United States]. Assay's sensitivity was 3.6 and 1.1 ng/mL for ICAM-1 and VCAM-1 respectively. This assay employs an antibody specific for human ICAM-1 or VCAM-1 coated on a 96-wells plate. Standard and samples was pipette into the wells; ICAM-1 or VCAM if present in a sample bind to the wells through immobilized antibody. Following washing of wells, biotinylated anti human ICAM-1 and VCAM-1 antibody is added. After washing away unbound biotinylated antibody, substrate solution is added to the wells and color develops in proportion to the amount of ICAM-1 or VCAM-1 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Mean absorbance for each of set of duplicate standards was calculated. A standard curve OD was plot on Log-Log paper with standard concentration on the X-axis and absorbance on the Y-axis. The best fit line was drawn
through the standard 5 points. Using these curves, the concentrations of both patients groups and control group were calculated.

**Statistical Analysis**

The data were analyzed statistically using the software statistical package for social science (SPSS) version 14 (SPSS Corp., Chicago, Illinois, USA). The data obtained were expressed as descriptive statistics (mean ± standard deviation). Statistics included ANOVA test, Tukey-Kramer Multiple Comparisons Test and Pearson correlation for correlating categorical parameters. \( P < 0.05 \) was considered significant.

**Results**

Demographic and biochemical data of the four groups of patients and controls included in the study are reported in Table (1). ANOVA demonstrated significant difference for all variables except for age and BMI. Total cholesterol was elevated in CRF more than other patients while serum creatinine was elevated in both CRF and HD more than KT.

**Table 1. Demographic and biochemical data of the four groups of subjects included in the study.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Control</th>
<th>Kidney transplantation (KT)</th>
<th>Chronic renal failure (CRF)</th>
<th>Maintained hemodialysis (HD)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>59.1 ± 14.6</td>
<td>52.2 ± 7.9</td>
<td>58 ± 9.5</td>
<td>54.9 ± 15.3</td>
<td>NS</td>
</tr>
<tr>
<td>S. creatinine</td>
<td>0.9 ± 0.2</td>
<td>2.2 ± 0.6</td>
<td>8.6 ± 4.5</td>
<td>8.7 ± 2.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>242.4 ± 59.1</td>
<td>238.6 ± 35.5</td>
<td>300.2 ± 52.9</td>
<td>190.1 ± 41.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>4.6 ± 0.8</td>
<td>3.7 ± 0.2</td>
<td>3.5 ± 0.9</td>
<td>3.8 ± 0.6</td>
<td>0.0025</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>249.1 ± 67.9</td>
<td>262.2 ± 66.4</td>
<td>371.3 ± 58.3</td>
<td>206.2 ± 102.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>71.4 ± 11.9</td>
<td>73.0 ± 8.8</td>
<td>70.3 ± 11.9</td>
<td>82.8 ± 18.8</td>
<td>NS</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>9.2 ± 2.1</td>
<td>17.8 ± 3.5</td>
<td>24.1 ± 5.3</td>
<td>28.8 ± 3.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>9.0 ± 2.2</td>
<td>21.6 ± 5.8</td>
<td>21.1 ± 5.8</td>
<td>25.9 ± 4.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>3.4 ± 1.0</td>
<td>12.8 ± 1.6</td>
<td>23.6 ± 3.8</td>
<td>45.9 ± 9.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\( P < 0.05 \) is significant. NS= not significant

Circulating levels of VCAM-1 in the four groups were shown in Figure 1. The mean values of the three groups of patients were significantly higher than those of the controls (\( P \leq 0.05 \)). The highest values of VCAM-1 were in patients undergoing hemodialysis.

![Figure 1. Serum level of VCAM-1 in the four groups (\( P \leq 0.05 \)).](image-url)
We compared serum levels of VCAM-1 in each group versus all other groups using Tukey-Kramer Multiple Comparisons Test. There was a statistically significant difference between serum levels of VCAM-1 in the controls versus all other groups with the lowest level in the controls. Statistically significant higher serum levels of VCAM-1 was observed in the HD patients versus all other groups. Also, there was statistically significant difference in serum levels of VCAM-1 in KT patients versus CRF patients.

With respect to circulating level of ICAM-1, they were similar to that of VCAM-1 levels as shown in Figure 2. The mean values of the three groups of patients were significantly higher than those of the controls ($P \leq 0.05$). The highest values were in hemodialysis patient's group.

Serum levels of ICAM-1 were significantly lower in the control group than in patient’s groups; highest levels were observed in HD patients. No significant difference was detected regarding the serum levels of ICAM-1 among different patient’s groups; HD, KT and CRF patients.

The circulating levels of CRP in the four groups studied are shown in Figure 3. There was a progressive increase from controls to KT, CRF and to HD with the highest level in HD patients.

By comparing the serum levels of CRP in each group versus all other groups. There was a statistically significant difference between the serum level of CRP in the control group versus both CRF patients and HD patients. On the other hand, no statistically significant difference between the serum levels of CRP in the control group versus KT patients was identified. However, there was a statistically significant difference between the serum levels of CRP in the HD patients versus all other groups with the highest level of CRP observed in the HD patients. Also, there was a statistically significant difference between the serum levels of CRP in the KT patients versus CRF patients.
Regression analysis showed a significant positive correlation between CRP and ICAM-1 in KT patients \( (r = 0.9051; P = 0.0346) \), in CRF patients \( (r = 0.7621; P = 0.0170) \) and in HD patients \( (r = 0.4449, P = 0.0493) \). As regards the correlation between CRP and VCAM-1, there was positive correlation in KT patients \( (r = 0.9006; P = 0.0370) \), in CRF patients \( (r = 0.7088; P = 0.0326) \) and in HD patients \( (r = 0.4498; P = 0.0466) \). No significant correlation was found between circulating adhesion molecules and all other variables except for a positive correlation between level of VCAM-1 and serum creatinine in CRF patients.

Discussion

Chronic kidney disease is a worldwide growing problem in public health. It is a risk factor for complications in patients with acute coronary syndrome; diabetes, hypertension, as well as the rapid development of atherosclerosis are responsible for higher prevalence of cardiovascular diseases in patients with chronic kidney disease.

Inflammatory process of unknown aetiology belongs to the so-called non-traditional risk factors in development of cardiovascular system diseases. It is thought that this process is responsible for adverse remodeling of atherosclerosis plaque and its instability which causes plaque rupture and as a result a coronary syndrome occurrence. Important inflammatory mediators, which take part in pathogenesis of acute coronary syndrome and
chronic kidney diseases, are acute phase proteins such as: C-reactive protein, adhesion molecules VCAM-1, ICAM-1, selectins, plasma amyloid A, metalloproteinases, interleukins-1 and -6, tumor necrosis factor-α and vascular endothelial growth factor (Owczarek et al., 2011).

Endothelial cells have important roles in inflammatory responses, emigration of leukocytes from blood is dependent on their ability to roll along endothelial cell surfaces and subsequently adhere to endothelial cell surfaces. Inflammatory mediators and cytokines induce chemokine secretion from endothelial cells and other vascular cells and increase their expression of cell-surface adhesion molecules, such as ICAM-1, VCAM-1, integrins, and selectins. Chemokines are chemotactic toward leukocytes and toward sites of inflammation and tissue injury. The movement of leukocytes through endothelial junctions into the extravascular space is highly orchestrated through various interactions with different adhesion molecules on endothelial cells (Vanderslice & Woodside., 2006).

Plasma levels of adhesion molecules ICAM-1 and VCAM-1 are markers of endothelial dysfunction and also risk factors for cardiovascular diseases (Xydakis et al., 2008). The main processes in the development of atherosclerosis are the recruitment of circulating leukocytes to the vascular endothelium and their migration into subendothelial space. The main mediators for this process are selectin that mediates the initial rolling of leukocytes along the endothelium while VCAM-1 and ICAM-1 have the effective role in the attachment of leukocytes (Blann & McCollum, 1994). Expression of VCAM-1 and ICAM-1 has been demonstrated in human plaques (Davies et al., 1993). In mice models, the expression of VCAM-1 was identified on activated endothelium and vascular smooth muscle cells in the course of atherosclerosis and is strongly associated with accumulation of leukocytes in the intima (Bro et al., 2004).

The result of this study showed that the mean values of ICAM-1, VCAM-1 in the three groups of patients (kidney transplantation KT, chronic renal failure CRF and hemodialysis HD) were significantly higher than those of the controls. This goes in agreement with the results of Musical et al., 2005 who reported an increase in the level of ICAM-1, VCAM-1 in adult and young patients with CRF or on maintenance HD.

The highest values of ICAM-1 and VCAM-1 were found in hemodialysis group, this is in accordance with the results of Vaccaro et al., 2007 and this may suggest that the kidney plays an important role in their catabolism. This may be true particularly with VCAM-1 as there is direct correlation between VCAM-1 and serum creatinine in CRF patients and HD patients but this is not true in KT, may be due to the relatively small number of patients included in these two groups. The lower levels of these molecules in predialysis patients (CRF) than hemodialysis patients (HD) indicate that HD may influence their synthesis and release.

CRP is an acute phase protein (APP) whose synthesis in the liver is regulated by different cytokines. Base line levels of CRP in apparently healthy individuals are an independent risk factor for cardiovascular events including myocardial infarction and peripheral artery diseases. In HD patients plasma levels of CRP are strong predictors for mortality in subsequent years (Tripepi et al., 2005).

In this study there was a progressive increase in the level of CRP from controls to KT, CRF and to HD with the highest level in HD patients. The level of CRP is a strong index of inflammation; there is evidence that inflammation is an integral part of the process of atherosclerosis. In end stage renal diseases
there is chronic and low grade inflammation. The presence of highest level of CRP in HD patients goes in agreement with that of Musical et al., 2005 this may be due to that, in hemodialysis there is a combination of an impaired immune response related to the uremic state and persistent immune/inflammatory response (blood membrane contact, water quality, bioincompatible membrane, vascular access) resulting in persistent immune system stimulation, low grade systemic inflammation and altered cytokine balance. In our study there was also positive correlation between serum level of adhesion molecules and that of CRP, This go in agreement with the result of Vaccaro et al., 2007. So serum level of adhesion molecules increased with the increasing severity of the disease as CRP is an index of inflammation and severity of renal diseases.

These advances in studying and understanding the correlations between adhesion molecules and CRP give a promise to discover a novel class of anti-inflammatory and anti-adhesions drugs which will likely be effective in many clinical situations facing the nephrologists that lead to a new regimen of treatment for patients with chronic renal diseases.

Aikawa & Libby (2004), stated that VCAM-1 is found in very low levels on the cell surface of resting endothelial cells and other vascular cells, such as smooth muscle cells and fibroblasts. IL-1 and TNFα increase expression of VCAM-1, P-selectin, and other cell adhesion molecules on the vascular endothelial cells, which leads to leukocyte adhesion to the activated endothelium. Furthermore, VCAM-1 expression is induced by oxidized low-density lipoproteins under atherogenic conditions.

Overexpression of VCAM-1 by atherosclerotic lesions plays an important role in their progression toward vulnerable plaques, which may erode and rupture. Akhtar et al., (2010) developed microparticles of iron oxide (MPIO) composed of iron particles with a diameter of ~4.5 µm. MPIO targeted with anti-VCAM-1 monoclonal antibody (mAb) M/K-2.7 (VCAM-MPIO) was developed as a non-invasive agent for VCAM-1 expression in vascular endothelial cells during different stages of inflammation in atherosclerosis and renal ischemia.

Also in experimental animal models McAteer et al., (2008) found that Dual-ligand microparticles of iron oxide (MPIO) conjugated to monoclonal antibodies against vascular cell adhesion molecule-1 (VCAM-MPIO) or P-selectin (P-selectin-MPIO), were bound to endothelium over atherosclerosis in vivo, under flow conditions. MPIO may provide a functional magnetic resonance imaging (MRI) probe for detecting endothelial-specific markers in a range of vascular pathologies.

In conclusion, serum levels of soluble adhesion molecules correlate positively with the stage of renal disease, suggesting that the involvement of the adhesion molecules in the pathogenesis of endothelial dysfunction in atherosclerotic process which is linked to series of complications in chronic kidney diseases. Also Serum level of soluble adhesion molecules were correlated positively with CRP as an inflammatory index in renal diseases. Those results support investigating chronic renal diseases, especially inflammatory ones; for sICAM-1, sICAM-1 and CRP. Further studies assessing the use of monoclonal antibodies against adhesion molecules and CRP as target for therapeutic intervention may be of value in chronic kidney diseases.

References
1. Diagnostic and prognostic values of VCAM-1, ICAM-1 and CRP in Egyptian patients with chronic kidney disease


