sICAM-1 and sVCAM-1 Levels in Normotensive and Hypertensive Patients with End Stage Renal Disease on Hemodialysis

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ABSTRACT

Background: End stage renal disease (ESRD) is now considered a prototypical situation of chronic inflammatory state. Inflammation is thought to contribute to initiation and aggravation of atherosclerosis through a process predominantly mediated by adhesion molecules. The expression of soluble Intercellular cell adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) on the cell surface is upregulated by inflammation. The aims of this study were to measure sICAM-1 and sVCAM-1 in sera of normotensive and hypertensive CRF patients under hemodialysis (HD) and compare them with healthy controls. Also, the effect of dialyzer on these molecules was studied in a single uncomplicated HD session.

Methods: We evaluated 40 patients with CRF under treatment with regular HD, they were classified into two groups: Group I: 20 hypertensive patients (12 males and 8 females) aged 30±8.2 years, Group II: 20 normotensive patients (11 males and 9 females) aged 34.0±16.8 years. Ten healthy volunteers constituted group III (5 males and 5 females), aged 33.0±10.2 years. Blood samples were taken from all patients 4 times, at start and at the end of HD session and from the inlet and exit lines of the dialyzer, 5ml of blood were collected from controls. sICAM-1 and sVCAM-1 were measured by ELISA.

Results: We found that there was no significant difference in sICAM-1 and sVCAM-1 between group I and group II before and after the dialyzer (p >0.05). There was a highly significant difference in sVCAM-1 between patients and controls (p<0.001), while there was no significant difference between patients of group I and group II (p>0.05), we found no statistically significant difference in sICAM-1 in all studied groups (p>0.05). Conclusions: No influence of HD membrane on the levels of sICAM-1 and sVCAM-1, the elevated sVCAM-1 compared with controls, in patients on maintenance HD, may activate immunocompetent cells. The presence of hypertension doesn't add impact to sICAM-1 and sVCAM-1 levels in CRF patients.

INTRODUCTION

CRF and its treatment are associated with complex impairment of the immune system. Infections in CRF patients may result from the insufficient migration of immunocompetent cells, possibly leading to the impaired defence against pathogens[1]. Uremia in ESRD is an atherogenic condition and, although the exact mechanisms are not well understood, inflammation has been incriminated.[2]

ICAM-1 and VCAM-1 are two members of the immunoglobulin gene superfamily that play important, but different roles in the adhesion of leukocytes to the vascular endothelium.[3]

Cytokine activation dramatically up-regulates their expression on the cell surface where they support the interaction of leukocytes and endothelial cells. In addition to being expressed on the cell surface, soluble forms of adhesion molecules have been detected in circulating blood and have been shown to retain their functional ability. Although little is known of the processes that govern the shedding and clearance of these molecules, their concentrations in serum or plasma can be readily determined[4].

A number of clinical investigators have measured levels of s-ICAM-1 and/or s-VCAM-1 as potential biomarkers for endothelial dysfunction and early atherosclerosis[5].

HD–related complications are at least in part attributed to contact between the blood and the artificial surface of the dialyzer. This leads to the activation of both cellular and plasmatic components. The expression of ICAM-1 and VCAM-1 change during hemodialysis sessions, thus serving as markers of biocompatibility[6].

Hypertension is the single greatest cause of mortality in the world. Chronic Kidney disease (CKD) patients, particularly those with ESRD, are at much higher risk of cardiovascular disease than the general population. Cardiovascular disease is by far the leading cause of morbidity and mortality.
in dialysis patients, accounting for almost 40% of hospitalizations and almost 50% of deaths \[5\].

The pathologic effects of hypertension such as endothelial dysfunction and disturbed balance between vasoconstrictive and vasorelaxing factors result in changes in blood vessels including fibromuscular hyperplasia of vessel walls and accelerated arteriosclerosis which is a leading cause to renal failure \[5\].

However, investigation of soluble adhesion molecules shed proteolytically from cells into the circulation has given contradictory results in HD patients \[1,2,6,7&8\].

It is still not clear whether the type of dialyzer or a single dialysis session influences the concentrations of soluble adhesion molecules in these patients.

In this study, we evaluated the serum levels soluble adhesion molecules (sVCAM-1 and sICAM-1) in patients on maintenance HD.

Our aim was to analyze whether a single dialysis session might influence adhesion molecule concentrations, thus modifying the immunological profile of patients on chronic HD.

**MATERIAL & METHODS**

This study was conducted on 40 patients with CRF, under hemodialysis sessions in Nephrology Unit – Benha University Hospital, during the period between March 2010 to July 2010. They were classified into two groups, Group I (consisted of 20 hypertensive patients, they were 12 males and 8 females, main age 30.5 years, range 23.5 to 48.5, main duration of dialysis 8.6 years, range 1.5 to 9 years) and Group II (consisted of 20 normotensive patients, they were 11 males and 9 females, main age 34.0 years, range 28.0 to 51.0, main duration of dialysis 7.5 years, range 1.7 to 11 years). The control group: Group III (consisted of 10 healthy volunteers from the relatives of the patients. None of the subjects studied had clinical or laboratory evidence of active infections, malignancies, liver disease, or any inflammatory conditions; they were not taking any antibiotics or immunosuppressive medications at least one month before sampling. All control subjects had normal urinalysis and renal function tests.

To all patients HD sessions (4–5 hours) were performed three times a week through a–v fistulas using bicarbonate dialysate. The membrane area was between 1.0 m² and 1.6 m²; the dialyzers (Hemoflow F 60S1, Fresenius, Germany) were not reused.

All patients were on a stable anticoagulation regimen using low–molecular–weight heparin. All of them took erythropoietin twice a week.

Blood samples were drawn from the efferent line of the first–use dialyzer before starting an uncomplicated HD session, blood was drawn simultaneously from the entrance and the exit line of the dialyzer in order to determine the dialyzer clearance for the adhesion molecules. Posttreatment blood samples were drawn using the “slow flow/stop pump technique,” which minimizes sample dilution with recirculated blood \[2\], and at the end of HD session.

In the controls the blood was drawn from a peripheral vein. Samples were centrifuged at 4°C at 2000 × g for 10 minutes and then the serum samples were stored at −20°C until assay.

**Detection of sICAM-1 and sVCAM-1 by ELISA:**

Serum concentrations of sICAM-1 and sVCAM-1, were evaluated by quantitative sandwich ELISA kits (R and D Systems, Co. Ltd., Shanghai PRC 200050., China). Each sample was measured in duplicate and the arithmetic mean was considered as a final result. 100 ul of sICAM-1 or sVCAM-1 conjugated to horseradish peroxidase were added. Standards (recombinant human sVCAM-1 and sICAM-1) and 20 fold diluted serum samples were transferred to 96-well microplates coated with mouse monoclonal antibodies to human sICAM-1 sVCAM-1, respectively. The wells were first incubated with a sheep monoclonal antibody to recombinant human antigen, then with the appropriate substrate (tetramethylbenzidine). The reaction was stopped with 2 N sulfuric acid solution, then the absorbance was measured at 450nm. Results were calculated by reference to standard curves. Limits of detection were as follows: sICAM-1 0.35 ng/ml and sVCAM-1 0.35 ng/ml.

Results of sICAM-1 and sVCAM-1 measurements were corrected according to hemoconcentration at the time of blood collection (t). The percent change of plasma volume reduction (%) was used as the correction factor (Fc).

If hematocrit values at the time t (Ht) as well as before the beginning of the hemodialysis session (H0) are measured the correction factor Fc can be derived from the equation described by van Beaumont \[9\]:

\[
Fc = \frac{H0 (1_Ht)}{Ht (1_H0)}
\]
Statistical Methods

Collected data were analyzed statistically using the software Sigma plot, version 11.0, build 11.0.0.77, 2008 (Sigma stat Incorporation, Germany). Statistical mean (M), standard deviation (SD±) and standard error of the mean (SE±) were used for descriptive analysis. One way ANOVA on RANKS was used for multiple group comparisons while t test was used for two group comparisons. Correlation statistics were done by Spearman Rank Order method.

RESULTS

The results of the present study are shown in the following tables:

Table (1): Clinical and laboratory data of the studied groups:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.5±8.2</td>
<td>34.0±16.8</td>
<td>33.0±10.2</td>
</tr>
<tr>
<td>Males</td>
<td>12</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Time on dialysis (years)</td>
<td>8.6±5.1</td>
<td>7.5±4.0</td>
<td>-</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>159±15.0</td>
<td>110±8.1</td>
<td>100±18.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>98.8±14.3</td>
<td>75±5.5</td>
<td>72±9.5</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>9.5±0.45</td>
<td>9.4±0.63</td>
<td>13.4±2.6</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>25±3.1</td>
<td>26±2.5</td>
<td>40.2±3.4</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>124±12.7</td>
<td>129±10.4</td>
<td>31±8.2</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>13.9±3.1</td>
<td>13.4±4.9</td>
<td>0.75±0.34</td>
</tr>
</tbody>
</table>

Table (2): Laboratory data before and after hemodialysis session among the studied patients:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Before dialysis</th>
<th>Group I After dialysis</th>
<th>P</th>
<th>Group II Before dialysis</th>
<th>Group II After dialysis</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>25±3.1</td>
<td>28±1.9</td>
<td>&lt;0.05</td>
<td>S</td>
<td>26±2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>129.7±6</td>
<td>60±6.1</td>
<td>&lt;0.001</td>
<td>HS</td>
<td>120±11.9</td>
<td>66±8.3</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>14.8±3.8</td>
<td>7.0±1.8</td>
<td>&lt;0.001</td>
<td>HS</td>
<td>13.6±4.1</td>
<td>6.7±1.4</td>
</tr>
<tr>
<td>sICAM-1 (pg/ml)</td>
<td>238±51</td>
<td>243±40</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>246±62</td>
<td>268±</td>
</tr>
<tr>
<td>sVCAM-1 (pg/ml)</td>
<td>1562±380</td>
<td>172±310</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>1865±492</td>
<td>1894±</td>
</tr>
</tbody>
</table>

S = significant        HS= highly significant        NS= non significant

Table (3): sICAM-1 and sVCAM-1 before and after the dialyzer in the studied patients:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Before dialysis</th>
<th>Group I After dialysis</th>
<th>p</th>
<th>Group II Before dialysis</th>
<th>Group II After dialysis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1 (pg/ml)</td>
<td>243±40</td>
<td>235±31</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>246±62</td>
<td>251±54</td>
</tr>
<tr>
<td>sVCAM-1 (pg/ml)</td>
<td>172±310</td>
<td>1696±458</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>1894±510</td>
<td>1850±492</td>
</tr>
</tbody>
</table>

Table (4): Basal levels of sICAM-1 and sVCAM-1 in the studied groups:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>IVII</th>
<th>IVIII</th>
<th>p</th>
<th>IVII</th>
<th>IVIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1 (pg/ml)</td>
<td>238±51</td>
<td>246±62</td>
<td>231.3±40.7</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>sVCAM-1 (pg/ml)</td>
<td>1562±380</td>
<td>1865±492</td>
<td>239.6±57.6</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>
DISCUSSION

All patients with CRF included in this study was was treated with hemodialysis, the efficacy of dialysis sessions was shown in table (2) where there was a high significant clearance of plasma creatinine and blood urea.

The present study showed that there was no significant differences in sICAM-1 and sVCAM-1 before and after the passage of the dialyzer (table 3).

Our results were in agreement with Liacopoulos et al.[2] who found that the concentrations of sICAM-1 and sVCAM-1 were the same when measured before and after the passage of the dialyzer, whatever the type of the dialyzer (biocompatible high-flux or bioincompatible low-flux).

The present study showed no significant differences, in sICAM-1 and sVCAM-1 before and after dialysis (table 2)

Therefore, there was no important clearance of these molecules during the HD procedure that could result in alterations of their levels to contradict those of immune activation and cytokine secretion after the contact between leucocytes and HD membrane. Also, might be explained by the possibility that the preceding endothelium activation induced by uremia itself reaches a level that makes further expression of the adhesion molecules impossible.

In previous studies decreased,[10] stable,[11] or increased[12] levels of sICAM-1 and sVCAM-1 were found after HD in comparison to predialysis values. These contradictory findings may have two causes: The unavailability of standard commercial ELISA kits in the early studies and the fact that in many studies the results were not corrected for the hemoconcentration caused by ultrafiltration during the HD session, or were corrected inaccurately.

In this study all results were corrected according to van Beaumont’s equation,[9] which accurately overcomes the influence of hemoconcentration on the concentration of a substance in the serum, taking into account the discrepancy in proportional changes between hematocrit and plasma volume.

On the other hand Musial et al.[11] studied soluble adhesion molecules in children and young adults on chronic HD, including sICAM-1 and sVCAM-1 and concluded that their concentrations were differ according to the type of the dialyzer as following: A single polysulfone (PS) session had no impact on adhesion molecules, whereas a vitamin E-modified cellulose (VE) session increased the level of sVCAM-1 while in cuprophane (CU ) patients, sVCAM-1 and sICAM-1 concentrations rose after HD.

Other results were obtained by Mrowka et al.[13]. Rabb et al.[14] observed a significant decrease of sVCAM-1 levels after a 3-h HD session on new CU dialyzers and suggested the adsorption of adhesin onto the membrane as a possible mechanism.

Possible detachment of bound adhesion cannot be excluded, that is why adsorption of adhesion molecules may be less pronounced.

In agreement with most previous studies[1,2,9,12,13,15,16] the results presented here confirmed the elevated levels of sVCAM-1 in the serum of patients undergoing chronic HD.

High levels of sVCAM-1 do not simply mirror activation, but can probably be attributed to the decreased renal clearance or catabolism of this molecule in chronic renal failure (CRF). This assumption can be based on a previous study in patients with CRF (predialysis stage) who showed a positive relation between the levels of sVCAM-1 with serum creatinine.[7] However, it also seems probable that increased expression of VCAM-1 can be due to an activation of the vascular endothelium induced by either uremia or the HD treatment, a condition well documented in many studies.[17,18]

We found that the levels of sICAM-1 did not elevated in CRF patients compared with controls (table 4), our results were in agreement with Liacopoulos et al.[2] , Mrowka et al.[13] and Musial et al.[15].

Other studies showed increased levels of sICAM-1[1,7,10,11].

These discrepancies may result from the susceptibility of the immune system of a patient to blood-membrane contact, generating cell hyporeactivity due to chronic stimulation.

sICAM-1 is also a marker of endothelial activation that could be more intense in adults on HD than in young patients investigated in some of these studies.

In the present study we found that there was no statistically significant differences in sICAM-1 and sVCAM-1 between patients with CRF with and without hypertension (table 4).

These results are in agreement with Liakopoulos et al.[2] who studied the plasma levels of sICAM-1 and sVCAM-1 in 35 patients with ESRD on hemodialysis, they were 12 normotensives ,23 hypertensives, 8 diabetics and 27 non diabetics , and concluded that the levels of sICAM-1 and sVCAM-1 did not differ among the various patient subgroups and were equal in diabetics and non-diabetics.
hypertensives and normotensives, and patients
dialyzed with biocompatible (high-flux) and
bioincompatible (low-flux) membranes.

We also found that the levels of sICAM-1
and sVCAM-1 in hypertensive CRF patients
were significantly elevated than in controls
(table 4) The same results were obtained by
Liakopoulos et al.[3], also increased levels of
sICAM-1 in hypertensive patients were
observed in[5,19,20,21,22] while elevated sVCAM-
1 in hypertension were also observed in[19-23].

Shalia et al.[5] reported that premenopausal
hypertensive women demonstrated notable
increase of sICAM-1 while postmenopausal
hypertensive women demonstrated
nonsignificant similar rise in sVCAM-1 as
compared to respective healthy women

Also, Claudia et al.[24] in multiple linear
regression models controlling for age, case-
control status, and other cardiac risk factors,
SBP was independently associated with
increased levels of sICAM-1

Conclusion
From this study it can be concluded that no
influence of HD membrane on the levels of
sICAM-1 and sVCAM-1 and also supports the
findings of previous works about elevated
sVCAM-1 compared with controls, in patients
on maintenance HD , that may illustrate
deficiency and activation of immunocompetent
cells and impairment of the adhesion cascade,
which may be partially responsible for the
dysregulation of the immune response in HD
patients . The presence of hypertension doesn't
add impact to sICAM-1 and sVCAM-1 levels
in CRF patients . The functional role of these
adhesion molecules in uremia , relations to
infections ,relationship to dialyzer membrane
surfaces and their impact on morbidity and
mortality in CRF patients need further
evaluation.

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مستوى الجزيئات الملتصقة الذاتية في مرضى الفشل الكلوي المزمن تحت الاستصفاء الدموي

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قسم الميكروبيولوجيا والمناعة الطبية - قسم أمراض الباطنة العامة

كلية طب بنها – ج: ع

تم جراء البحث على 40 مرضي من المرضى المترددين على وحدة الاستصفاء الكلوي الدموي للعلاج من الفشل الكلوي المزمن وقد تم تقسيمهم إلى مجموعتين

المجموعة الأولى: تشمل 20 مريضاً يعانون من ضغط الدم المرتفع (130 جرّال و 8 سيادات)

المجموعة الثانية: تشمل 10 مريضاً يعانون من ضغط الدم المرتفع (110 جرّال و 6 سيادات)

المجموعة الثالثة: تشمل 10 مريضاً يعانون من ارتفاع ضغط الدم، وللمرضى الذين يعانون من ارتفاع ضغط الدم، يتم الكشف عن ارتفاع ضغط الدم في حالة حساسية للنفايات و الاختلاطات الميدانية. وقد تم اجراء البحث وبعد جلسة الاستصفاء الدموي، وبعد مرشح الاستصفاء الدموي، وانخفاض الذبابة في المجموعتين.

وهذا ساعدنا على التعرف على البلاستات المختلفة بالأطوار – و ودأ ليد诊断 مختلف في مستوى هذا التجربة قبل وبعد المرشح الكلوي. ولاحظ التفاصيل المختلفة أيضاً بين المجموعتين الأولى والثانية، أما بالنسبة للجريان في الجريان الثاني، فقد تختلف بين كل المجموعتين و وانخفض عدد حساب الجزيئات الذاتية في مرضى المجموعتين الأولى والثانية.