Serum Visfatin Levels in Pediatric Hemodialysis Patients: Association with Circulating HDL-Cholesterol

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Background: Visfatin has recently been defined as an adipokine. Increased circulating visfatin may be associated with both endothelial damage and increased mortality in end-stage renal disease (ESRD). HDL cholesterol is an independent, strong inverse predictor of cardiovascular events. The aim of this study was to assess serum visfatin levels in hemodialysis (HD) patients in correlation with parameters of lipid metabolism as well as markers of inflammation. Methods: A total of 26 HD children (mean age 11.66 ± 4.01 years) and 15 healthy age- and sex-matched controls (HC) (mean age 9.0 ± 2.16 years) were studied. Serum visfatin levels as well as parameters of lipoprotein profile and high-sensitivity C-reactive protein (hs-CRP) were measured in all studied groups. Results: Serum visfatin levels were significantly increased in HD children compared to the healthy control group (p = 0.006). Within the HD group, serum visfatin levels were positively correlated with hs-CRP (p = 0.04) and serum triglycerides (p = 0.02) but inversely correlated with HDL cholesterol (p = 0.01). Conclusion: Visfatin is a beneficial indicator of lipid profile in HD patients. Reduced circulating HDL cholesterol may hint at an increased probability of cardiovascular events in HD patients with elevated serum visfatin concentration. Visfatin may reflect the inflammatory status in HD patients. J Clin Basic Cardiol 2010; 13 (online): 19-22.

Key words: visfatin, HDL cholesterol, end-stage renal disease, hemodialysis, children

Visfatin (MW52Kda) was defined by Fukuhara et al [1] as a visceral adipose-derived secretory adipokine that mimics the properties of the pre-B-cell colony-enhancing factor (PBEF), which stimulates interleukin 7 (IL-7) and B-cell precursors. It is known to activate 3 distinct activities to cellular energetics and innate immunity. First, within the cell, visfatin functions as a nicotinamide phosphoribosyl transferase, the rate-limiting step in a salvage pathway of nicotinamide adenine dinucleotide (NAD) biosynthesis. Visfatin regulates NAD-positive dependent reactions and promotes maturation of vascular smooth muscle cells [2]. Second, as an extracellular cytokine visfatin induces cellular expression of inflammatory cytokines such as tumor necrosis factor α (TNF-α), IL-1β and IL-6 [3]. Finally, visfatin may afford insulin-mimetic actions, although whether visfatin binds to the insulin receptor remains controversial [4].

Dialysis patients are suggested to have several adipokines increased (adiponectin, leptin, IL-6) and TNF-α, particularly due to low renal clearance [5]. This group of patients may be expected to have higher visfatin levels along with other adipokines. Moreover, there is increasing evidence that hyper-visfatinemia is associated with impaired endothelial function and higher mortality in patients with end-stage renal disease (ESRD) [6]. However, clear associations between visfatin and parameters of lipid metabolism have not been established.

In this study, enzymatically active visfatin in the serum of healthy controls (HC) and maintenance hemodialysis (HD) children was measured using the well-characterized ELISA. The ELISA predominantly detects dimeric visfatin, which is the enzymatically active form [7]. The aim of this study was to assess serum visfatin levels in HD patients in correlation with parameters of lipid metabolism as well as markers of inflammation.

Subjects and Methods

26 children with ESRD undergoing hemodialysis at the hemodialysis unit of the Center of Pediatric Nephrology and Transplantation (CPNT), Children’s Hospital, Cairo University, were investigated for this study. The study was conducted from March 2009 to September 2009. Inclusion criteria included children on regular HD treatment for not less than 4 months, using bicarbonate dialysate and free from any apparent acute illness. Patients with clinical signs or laboratory findings of generalized inflammatory disease, end-stage malignancies, or acute medical events were excluded. For all patients polysulfone dialyzer, bicarbonate dialysate using a blood flow rate of 80–150 ml/min, and the dialysate flow rate of 500 ml/min were employed. Each subject was dialyzed 3 times per week using polysulfone membranes. Their underlying renal disorders were due to anatomic causes (n = 4; 15.38 %), glomerular disease (n = 4; 15.38 %), hereditary causes (n = 8; 30.78 %), and unknown causes (n = 10; 38.46 %).

A total of 16 (61.54 %) patients received calcium channel blockers, 12 (46.15 %) patients received angiotensin-converting enzyme (ACE) inhibitors and 4 (15.38 %) patients received betablockers as antihypertensive drugs. Further medications were phosphate-binding agents (n = 26; 100 %), Act Vit D (n = 26; 100 %), folate acid (n = 26; 100 %), recombinant human erythropoietin (n = 26; 100 %), and intravenous iron (n = 12; 46.15 %).

Fifteen healthy, age-, gender- and Body Mass Index (BMI-) matched children (9 [60 %] male, 6 [40 %] female [average age 9.00 ± 2.16 years], range 7–12 years) were recruited from the pediatric clinic of National Research Centre to serve as controls. Written consent was obtained from the parents of each patient. All patients were subjected to a full history-taking and thorough clinical examination.
Blood Sample Collection

A peripheral blood sample was obtained prior to hemodialysis sessions from the venous part of the arteriovenous fistula using a specially selected disposable plastic syringe. The specimen was transferred to a plastic tube and then immediately centrifuged was done for 10 min at 5000 rpm at 4 °C. The centrifuged serum was transferred into sterile tubes. All samples were stored in a refrigerator at 4 °C up to the time of analysis.

Biochemical Tests

Pre- and post-dialysis kidney function tests and serum albumin estimations were determined by standard laboratory methods. Estimation of plasma concentration of total cholesterol, triglyceride (TG), and HDL-cholesterol were done using the AU400 Olympus, an automated clinical chemistry analyzer (Olympus America Inc, Centre Valley, PA, USA).

Determination of High-Sensitivity CRP

Determination of high-sensitivity CRP (hs-CRP) in serum was performed by solid-phase chemiluminescent immunometric assay (IMMULITE / IMMULITE 1000, supplied by Siemens Medical Solution Diagnostics, Eschborn, Germany) [8].

Determination of Visfatin in Human Serum

Determination of visfatin in human serum by means of Alpco immunoassays. This kit is an enzyme-linked immunosorbent assay. A monoclonal antibody specific for human visfatin has been pre-coated onto a 96-well microplate. Standards and samples were pipetted into the wells and any visfatin present was bound by immobilized antibody. Bound visfatin was captured by purified anti-human visfatin polyclonal antibody. HRP-conjugated anti-rabbit IgG was added. After washing, a substrate solution was added. The colors develop in proportion to the bound visfatin quantity. The color development was stopped and the intensity of color was measured (RayBiotech, Inc, USA) [1, 7].

Statistical Analysis

The SPSS (Statistical Package for Social Science) program, version 11.0, was used for analysis of data. Data were summarized as mean ± SD, range, or percentage. Comparisons of continuous variables between the 2 groups were performed by independent-samples t test where appropriate. Pearson's correlation was performed to predict the association between the serum concentration of visfatin and other individual variables. Multiple linear regression analysis with backward method was performed to determine the contribution of various factors as independents or covariates to visfatin as the dependent variable. P < 0.05 was considered significant.

Results

Clinical and biochemical data of the studied groups are summarized in Table 1. The concentration of blood urea nitrogen, serum TG, and hsCRP levels were significantly elevated in HD patients when compared to the HC. On the other hand, serum albumin and HDL-cholesterol concentrations were significantly lower in HD patients when compared to the HC.

The serum visfatin concentration was significantly higher in HD patients (63 ± 10.78 ng/ml) than in HC (50.14 ± 5.93 ng/ml) (p = 0.006; Fig. 1).

Positive correlations were observed between serum visfatin levels and both serum TG and hsCRP (r = 0.57; p = 0.009 and r = 0.40; p = 0.04, respectively). However, no correlations were found between visfatin and other parameters (Table 2).

On correlating the visfatin values to numerical variables by multiple linear regression analysis, HDL-cholesterol and TG were variables that were independently associated with elevated visfatin values. An inverse correlation was revealed between serum visfatin levels and HDL cholesterol (β = -0.76; p < 0.01). However, there was a positive correlation between serum visfatin levels and serum TG in HD patients (β = 0.63; p < 0.02) (Table 3).

Discussion

Extracellular visfatin is a cytokine predominantly secreted by adipocytes [9]. In the last 2 years, conflicting results about circulating visfatin concentrations in humans have been reported. In vitro studies with visfatin initially led to the as-
sumption that it had insulin-mimetic effects and increased glucose consumption in the peripheral tissue, thus increasing insulin sensitivity [10]. However, more recent in vivo and in vitro studies have suggested that visfatin may be related to insulin-resistance, endothelial dysfunction, and increased inflammation [11, 12]. Despite these findings it should be noted that comprehensive results did not exist about the pathophysiological role of visfatin [13].

The present study demonstrated that visfatin is significantly increased in patients undergoing HD when compared to the controls. This finding is in accordance with recent studies [5, 14, 15]. The mechanism behind increased visfatin levels may be due to the reduction in the renal clearance of visfatin as other adipocytokines. The present study showed a reduction of plasma HDL-cholesterol at concomitantly increased serum visfatin concentration. HDL-cholesterol is an independent, strong inverse predictor of cardiovascular events [16]. Also the inverse correlation between visfatin and circulating HDL-cholesterol levels in HD patients may provide an explanation for the increased rate of cardiovascular events in HD patients with high visfatin levels. This result is in accordance with the results of Niisuen et al [15].

In patients with chronic renal failure, elevated visfatin levels predict increased mortality most often caused by cardiovascular events [5, 17]. Patients with atherosclerosis showed an increase in visfatin concentration [18]. An association between hyper-visfatinemia and endothelial dysfunction has been described in patients with uremia [6]. Nevertheless, visfatin itself may protect cells. Thus, visfatin activity has a critical impact on energy availability, function, and survival of cells [19]. Direct cardioprotective effects of visfatin in mice have been previously described [20]. Therefore, the adverse effects associated with elevated visfatin levels may be caused mainly by secondary factors, for example increased resistin or decreased HDL-cholesterol. It is unlikely that the protective characteristics of visfatin are sufficient to balance these factors.

As regards the association between visfatin and other parameters of lipid metabolism, we observed a positive correlation between serum TG concentration and serum visfatin in HD patients. The relationship between visfatin and lipid profiles may be explained in the light of the cytosolic function of visfatin as a nicotinamide phosphoribosyl transferase (NAMPT). The change of HDL-cholesterol correlated significantly with the change of NAD concentration in total blood cells [21]. On the other hand, Kato et al [22] reported no association between visfatin and serum cholesterol or triglyceride levels.

In this study, the increased serum hsCRP concentration provides evidence for inflammation, possibly accompanied by energy consumption [15]. Inflammation and energy wasting, however, both major causes of mortality in HD patients, may be promoted directly by visfatin [17]. Visfatin itself has pro-inflammatory properties [11, 23].

In our study, visfatin was positively correlated with high-sensitivity CRP, confirming a close association of visfatin with inflammation consistent with Kato et al [22]. However, Nünsten et al [15] showed no correlation with visfatin despite increased CRP. Increased visfatin gene expression and protein secretion promote both cell function and survival under conditions of hypoxia, poor nutrient supply, or enhanced energy demand, for example because of inflammation [24].

Our sample provides reliable data on enzymatically active visfatin in children on HD using a well-characterized ELISA. Serum visfatin levels are significantly elevated in HD patients when compared to healthy individuals. The most significant result is that visfatin is inversely correlated with HDL-cholesterol. Thus, visfatin is a beneficial indicator of lipid profile and may be an independent predictor of cardiovascular events in HD patients with elevated serum visfatin concentration. An independent association between visfatin level and hsCRP concentration suggests that visfatin may reflect the inflammatory status in HD patients, which is one of the major causes of mortality in these patients. Furthermore, mechanistic studies in this direction may help understand the physiological role of circulating visfatin.

### References:


### Table 2. Pearson’s correlations between visfatin levels and individual parameters in HD patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.41</td>
<td>0.07</td>
</tr>
<tr>
<td>Duration of dialysis</td>
<td>0.23</td>
<td>ns</td>
</tr>
<tr>
<td>BMI</td>
<td>0.21</td>
<td>ns</td>
</tr>
<tr>
<td>Kt/V</td>
<td>-0.37</td>
<td>ns</td>
</tr>
<tr>
<td>Pre-dialysis urea</td>
<td>0.24</td>
<td>ns</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.25</td>
<td>ns</td>
</tr>
<tr>
<td>Total serum cholesterol</td>
<td>0.12</td>
<td>ns</td>
</tr>
<tr>
<td>Serum triglyceride</td>
<td>0.57</td>
<td>0.009**</td>
</tr>
<tr>
<td>Serum HDL-cholesterol</td>
<td>-0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Serum hsCRP</td>
<td>0.40</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; BMI: Body Mass Index; Kt/V: adequacy of dialysis; hsCRP: high-sensitivity C-reactive protein

### Table 3. Multiple linear regression analysis comparing the correlation of visfatin level with individual variables in serum of HD patients

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visfatin</td>
<td>0.63</td>
<td>0.02*</td>
</tr>
<tr>
<td>TG</td>
<td>-0.76</td>
<td>0.01*</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.19</td>
<td>ns</td>
</tr>
<tr>
<td>hsCRP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; TG: triglyceride; hsCRP: high-sensitivity C-reactive protein

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