Original Article
Pseudo-hypoalbuminemia in peritoneal dialysis patients

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Abstract: Introduction: Oxidative stress in peritoneal dialysis (PD) patients produces molecular modifications of serum albumin that disturb its biological functions and interfere with its detection by the commonly used bromocresol green (BCG) assay. This study aimed to evaluate the role of oxidized serum albumin (OSA) in hypoalbuminemic PD patients. Methods: Twenty four PD stable patients with serum albumin levels measured by BCG assay (SACBCG) ≤ 3.0 g/dl enrolled in the study. Serum albumin, OSA, oncotic pressure, hydration status, lean tissue index (LTI), normalized protein equivalent of total nitrogen appearance (nPNA) and high sensitivity C-reactive protein (hsCRP) were determined. OSA was assessed by a specific albumin detection index (ADI). ADI was defined as the ratio between the readout of the non-oxidized serum albumin measured by the BCG assay (SACBCG) to the total (non-oxidized and oxidized) serum albumin concentration in the fraction that determined by absorbance at 280 nm (OD280) (TSACOD280):

\[ \text{ADI} = \frac{\text{SAC}_{BCG}}{\text{TSAC}_{OD280}} \]

When the SACBCG decreased, as a result of the oxidation of serum albumin, the ADI will be < 1. When low serum albumin levels was determined by the BCG assay (SACBCG) in hypoalbuminemic PD patients with ADI < 1, it was usually refers to “pseudo” hypoalbuminemia because it is not includes the OSA fraction. To establish a diagnosis of true hypoalbuminemia, the total serum albumin including the non-oxidized and oxidized fractions (TSACOD280) should be determined. Participants were assigned to two groups according to their ADI (< 0.5 or ≥ 0.5). Results: Both study groups were comparable in age, BMI, gender, presence of diabetes, PD modality, peritoneal membrane characteristics, Kt/v, residual renal function (RRF), PD vintage and serum albumin levels. All participants had ADI < 1.0 and, therefore, had “pseudo” hypoalbuminemia. Mean ADI was 0.53±0.12 in all patients, 0.43±0.01 in patients with ADI < 0.5 and 0.62±0.03 in patients with ADI ≥ 0.5 (P < 0.001). Extracellular/Intracellular water ratio (E/I ratio) was lower in patients with ADI < 0.5 than in patients with ADI ≥ 0.5 (P = 0.002). Oncotic pressure and hsCRP were higher in patients with ADI < 0.5 than in patients with ADI ≥ 0.5 (P = 0.024, P = 0.032, respectively). nPNA, RRF and LTI were similar in both groups. Conclusions: “Pseudo” hypoalbuminemia, results from the presence of undetectable OSA fraction by the BCG assay, seems to be a very common finding and may mislead medical staff decisions in PD patients. Although OSA may contribute to better oncotic pressure and hydration status of these patients, its pathogenic ability to accelerate atherosclerosis should be kept in mind.

Keywords: Peritoneal dialysis, hypoalbuminemia, oxidized serum albumin

Introduction

Hypoalbuminemia is a well known and important problem in peritoneal dialysis (PD) patients and is considered one of the strongest predictors for morbidity and mortality in this population [1]. Although, traditionally, hypoalbuminemia defined as serum albumin levels < 3.5 g/dl, about two thirds of PD patients have a serum albumin of < 3.8 g/dl [2]. The goal should be to maintain a normal plasma albumin concentration (> 4.0 g/dl). Several factors may contribute to the development of hypoalbuminemia among PD patients including inadequate dialysis, a feeling of fullness resulting from the presence of dialysate in the abdomen that dulls the appetite, slow gastric emptying, hyperglycemia-induced anorexia and protein loss into the peritoneal cavity (often as high as 8 g/day) and urine [3-6]. Hypoalbuminemia is prevalent in clinical states associated with chronic inflammation and oxidative stress including PD. Oxidative stress in dialysis patients produces molecular modifications of serum albumin such as carbonylation [7, 8], formation of advanced glyoxidation end products [9] and advanced...
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Serum albumin oxidation disturbs its biological functions due to conformational alterations such as increased negative charge and exposure of hydrophobic regions, decreased antioxidant activity and drug binding [8, 10-12]. To our best knowledge, the consequences of such modifications on serum albumin levels measured by the commonly used clinical assay bromocresol green (BCG) in PD patients has not yet been investigated. This study aimed to evaluate the role of oxidized serum albumin (OSA) in hypoalbuminemic PD patients.

Materials and methods

Study population

Twenty four stable patients on maintenance PD for a period of more than 12 months and with serum albumin levels < 3.5 g/dl, measured by BCG assay, were enrolled in the study. The study was approved by the Medical Ethics Committee of Galilee Medical Center, Naharyia, Israel (No. NHR-0108-13) and written informed consent was obtained from all participants. Any evidence of infection, malignancy, liver disturbances, gastrointestinal diseases, and severe hyperparathyroidism were considered exclusion criteria. After rendering full medical history and undergoing physical examination, blood samples were drown for the determination of non-oxidized serum albumin concentrations by the BCG assay (SAC$_{BCG}$) according to the Aeroset chemical analyzer instructions (Abbott Laboratories, USA) and the total (non-oxidized and oxidized) serum albumin concentration in the fraction was determined by absorbance at 280 nm (OD$_{280}$) (TSAC$_{OD280}$) [13]. A specific albumin detection index (ADI) was used to assess the presence of OSA (method details are shown below) [14, 15]. ADI is defined as the ratio between the readout of the SAC$_{BCG}$ to the TSAC$_{OD280}$ in the fraction (ADI = SAC$_{BCG}$/TSAC$_{OD280}$). When the fractionated albumin shows similar concentrations of SAC$_{BCG}$ and TSAC$_{OD280}$ then the ADI will be 1. When the SAC$_{BCG}$ decreased, as a result of the oxidation of serum albumin, the ADI will be < 1. In other words, ADI < 1 indicates the presence of oxidized fraction of serum albumin that is undetectable by the BCG assay. When low serum albumin levels determined by the BCG assay alone (SAC$_{BCG}$) in hypoalbuminemic PD patients with ADI < 1, it is usually refers to “pseudo” hypoalbuminemia because it is not includes the OSA fraction. To establish a diagnosis of true hypoalbuminemia the total serum albumin including the non-oxidized and oxidized fractions (TSAC$_{OD280}$) should be determined. Without assessing the OSA fraction, it impossible to confirm that the hypoalbuminemia is true. Hence, true hypoalbuminemia determined when the total serum albumin decreases, including the non-oxidized and oxidized fractions (TSAC$_{OD280}$). Participants were assigned to two study groups according to their ADI: group A, included 12 patients with ADI < 0.5 and group B, included 12 patients with ADI ≥ 0.5. Group A patients were with higher OSA levels as it reflected by their lower ADI, and Group B patients were with lower OSA levels as it reflected by their relatively higher ADI.

The serum samples were also used in determining the oncotic pressure, high sensitivity C-reactive protein (hsCRP) and the normalized protein equivalent of total nitrogen appearance (nPNA).

Whole-body bioimpedance spectroscopy (BIS) technique (method details are shown on below) was used for accurate assessment of the hydration status and nutritional status [16, 17].

Determination of ADI in sera

Albumin was purified from sera using Cibacron Blue 3GA Agarose (CB3GA). CB3GA was inserted into a SigmaPrep spin column and washed four times by additions of 10 mM Tris pH 8.0 and short centrifugations (8,000 g, 10 s). Serum (150 μl) was added to the washed CB3GA, incubated at r.t. for 10 min and centrifuged (12,000 g, 1 min). This step was repeated. The twice depleted serum was discarded and elution buffer (150 μl, 10 mM Tris pH 8.0 and 1.5 M NaCl) was added to the column. After 10 min incubation the albumin samples were eluted by centrifugation (12,000 g, 1 min) into a new collection tube. To deplete immunoglobulin contaminations, 100 μl of the eluted albumin sample were incubated (with shaking) for 3 hrs at 4°C with 20 μl (washed and diluted into 900 μl H$_2$O) of protein A/G Ultralink Resin (Thermo Scientific, USA). The resin bound immunoglobulins were discarded by centrifugation. The supernatant was concentrated by SPEEDVAC centrifugation (approximately 3 hours) [14, 15].
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Hydration status assessment

Hydration status was assessed by whole-body BIS technique using Fresenius Medical Care Body Composition Monitor (BCM) device, Bad Homburg, Germany [16]. This method is considered a practical, safe, simple, repetitive, reliable, accurate and non-invasive technique. Regarding the differences in body size and hydration status, hydration status index extracellular to intracellular water ratio (E/I ratio) was used as an independent and comparable indicator of the hydration status.

Nutritional status assessment

Nutritional status was determined using two measures of protein malnutrition: nPNA and LTI. Whole-body BIS technique was used to evaluate the lean tissue mass (LTM) and lean tissue index (LTI). LTI, is a height normalized expression of LTM: LTI = LTM/(height)$^2$. Under-nutrition was defined as having a LTI below the 10th percentile of an age and gender specific reference population derived from BCM measurements of 1000 healthy adult subjects aged 18-75 (http://www.bcm-fresenius.com/23.htm).

Oncotic pressure measurements in sera

Oncotic pressure was measured in sera using a colloid osmometer (Wescor, Logan, USA), which operates using a membrane with a cutoff of 30 kDa; thus the measured oncotic pressure relates only to proteins with a molecular mass > 30 kDa [14].

Statistical analysis

Statistical analysis was carried out using SPSS (IBM SPSS Statistics version 21, Armonk, NY, USA) software. P < 0.05 was considered to be significant. Continuous variables are reported as means and standard deviations, and categorical variables as frequencies and percentages. The Wilcoxon rank sum test was used to compare the differences between the study groups, including age, body mass index (BMI), Kt/V [a dimensionless index that measures the fractional urea clearance during dialysis: K = blood urea clearance (liters per hour), t = dialysis length (hour), V = distribution volume of urea (liters)]; PD, peritoneal dialysis; RRF, residual renal function.

Results

Both study groups were comparable in age, gender, presence of diabetes, BMI, PD modality, PD vintage, residual renal function (RRF), peritoneal membrane characteristics, Kt/V and serum albumin levels (Table 1). All PD patients had ADI < 1.0 and “pseudo” hypoalbuminemia.

Table 1. The characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>Group A: Patients with ADI &lt; 0.5</th>
<th>Group B: Patients with ADI ≥ 0.5</th>
<th>p$^{ab}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.1±11.2</td>
<td>54.7±4.0</td>
<td>56.9±2.3</td>
<td>0.317*</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>28.5±5.3</td>
<td>27.2±1.3</td>
<td>29.7±1.7</td>
<td>0.128*</td>
</tr>
<tr>
<td>Males/Females (n)%/(n)%</td>
<td>(17) 70.8/(7) 29.2</td>
<td>(9) 75/(3) 25</td>
<td>(8) 66.7/(4) 33.3</td>
<td>0.215†</td>
</tr>
<tr>
<td>Diabetes mellitus (n)%</td>
<td>10 (41.7)</td>
<td>(5) 41.7</td>
<td>(5) 41.7</td>
<td>1.0†</td>
</tr>
<tr>
<td>CAPD/APD (n)%/(n)%</td>
<td>(14) 58.3/(10) 41.7</td>
<td>(7) 58.3/(5) 41.7</td>
<td>(7) 58.3/(5) 41.7</td>
<td>1.0†</td>
</tr>
<tr>
<td>PET: HA/LA (n)%/(n)%</td>
<td>(12) 50/(12) 50</td>
<td>(7) 58.3/(5) 41.7</td>
<td>(5) 41.7/(7) 58.3</td>
<td>1.0†</td>
</tr>
<tr>
<td>Weekly Kt/V</td>
<td>2.0±0.13</td>
<td>2.02±0.13</td>
<td>2.05±0.14</td>
<td>0.311*</td>
</tr>
<tr>
<td>RRF (ml/min/1.73 m$^2$)</td>
<td>5.28±1.01</td>
<td>5.39±0.25</td>
<td>5.18±0.32</td>
<td>0.308*</td>
</tr>
<tr>
<td>PD vintage</td>
<td>24.8±14.4</td>
<td>23.9±16.6</td>
<td>25.8±12.6</td>
<td>0.309*</td>
</tr>
</tbody>
</table>

*, Wilcoxon rank-sum test; †, Fisher’s exact test; * group A: patients with ADI < 0.5; ‡, group B: patients with ADI ≥ 0.5; p$^{ab}$, statistical significance between groups A and B; ADI, albumin detection index; BMI, body mass index; CAPD, continuous ambulatory peritoneal dialysis; APD, automated peritoneal dialysis; PET, peritoneal equilibration test; HA, high average; LA, low average; Kt/V [a dimensionless index that measures the fractional urea clearance during dialysis: K = blood urea clearance (liters per hour), t = dialysis length (hour), V = distribution volume of urea (liters)]; PD, peritoneal dialysis; RRF, residual renal function.
Mean ADI was 0.53±0.12 in all patients, 0.43±0.01 in patients with ADI < 0.50 and 0.62±0.03 in patients with ADI ≥ 0.50 (P < 0.001). Extracellular-Intracellular water ratio (E/I ratio) was lower in patients with ADI < 0.5 than in patients with ADI ≥ 0.5 (P = 0.002) (Table 2). Oncotic pressure and hsCRP were higher in patients with ADI < 0.5 than in patients with ADI ≥ 0.5 (P = 0.024, P = 0.032, respectively (Table 2), nPNA, RRF and LTI were similar in both groups.

**Discussion**

The main finding in this study is the detection of “pseudo” hypoalbuminemia in all PD patients participated in the study. The hypoalbuminemia was “pseudo” owing to the presence of undetectable OSA fraction by the BCG assay. Inaccurate diagnosis of “true” rather than “pseudo” hypoalbuminemia may mislead medical staff decisions. Although OSA may contribute to better oncotic pressure and hydration status of these patients, its pathogenic ability to accelerate atherosclerosis should be kept in mind.

Two study groups with ADI < 1, suggesting the presence of OSA, were evaluated in the present study: Group A that included 12 hypoalbuminemic PD patients with ADI < 0.50 (indicating the presence of higher levels of OSA in their blood samples) and group B that included another 12 hypoalbuminemic PD patients with ADI ≥ 0.50 (indicating the presence of lower levels of OSA in their blood samples). Both groups were similar in their characteristics including age, BMI, gender, the presence of diabetes, PD modality, peritoneal membrane characteristics, Kt/v, RRF, PD vintage and serum albumin levels measured by the BCG assay.

The mean LTI of participates in both groups was similar and within the reference range suggesting that all patients had normal nutritional status (Table 2). The mean nPNA of participates in both groups was similar and only slightly lower than the recommended target of 1.2 g/kg/day for peritoneal dialysis patients suggesting an acceptable nutritional status (Table 2). The mean weekly KT/V of participates was similar in both groups and within the recommended targets suggesting well-dialysis adequacy (Table 2). Thus, these results suggest that inadequate protein nutrition and dialysis inadequacy were not the main causes of the development of hypoalbuminemia in the study population. Although feeling of fullness, decreased appetite, slow gastric emptying, anorexia and protein loss into the peritoneal cavity and urine are considered common causes that may contribute to the development of hypoalbuminemia in PD patients, the aggravated inflammation and oxidative stress seem to play an important role on the generation of OSA and its role in the recognition of “pseudo” hypoalbuminemia [3-12].

**Table 2.** Serum albumin measured by bromocresol green, albumin-detection index, nPNA, hsCRP, oncotic pressure, E/I ratio and LTI in hypoalbuminemic peritoneal dialysis patients with ADI < 0.5 and those with ADI ≥ 0.5

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>Group A: Patients with ADI &lt; 0.50</th>
<th>Group B: Patients with ADI ≥ 0.50</th>
<th>pab</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>2.59±0.36</td>
<td>2.52±0.09</td>
<td>2.67±0.11</td>
<td>0.158</td>
</tr>
<tr>
<td>Albumin-detection index</td>
<td>0.53±0.12</td>
<td>0.43±0.01</td>
<td>0.62±0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>nPNA (g/kg/day)</td>
<td>1.075±0.08</td>
<td>1.07±0.09</td>
<td>1.08±0.08</td>
<td>0.414</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>13.4±5.1</td>
<td>15.50±1.91</td>
<td>11.64±0.84</td>
<td>0.032</td>
</tr>
<tr>
<td>Oncotic pressure (mmHg)</td>
<td>22.16±3.04</td>
<td>23.48±0.76</td>
<td>21.05±0.86</td>
<td>0.024</td>
</tr>
<tr>
<td>E/I ratio</td>
<td>0.94±0.14</td>
<td>0.81±0.04</td>
<td>1.00±0.04</td>
<td>0.002</td>
</tr>
<tr>
<td>LTI (kg/m²)</td>
<td>13.6±1.9</td>
<td>14.1±0.32</td>
<td>13.2±0.14</td>
<td>0.139</td>
</tr>
</tbody>
</table>

nPNA, normalized protein equivalent of total nitrogen appearance; hsCRP, high sensitivity C-reactive protein; E/I ratio, extracellular/intracellular water ratio; LTI, lean tissue index; ADI, albumin-detection index; pab, statistical significance between groups A and B.
pared to those with ADI ≥ 0.5, (Table 2). The oncotic pressure correlates with total serum albumin, even when modified or oxidized [14]. Thus, the oncotic pressure was higher in patients with ADI < 0.50 than those with ADI ≥ 0.50 leading to a better hydration status as it is reflected by the lower E/I ratio (Table 2). In contrast, patients with ADI ≥ 0.50 had lower levels of plasma hsCRP and smaller OSA fraction resulting in lower oncotic pressure and higher fluid overload as it is indicated by the higher E/I ratio (Table 2). The OSA, which seems to be capable of improving the oncotic pressure and hydration status, may concurrently initiate or accelerate atherosclerotic processes. On the other hand, true hypoalbuminemia in PD patients may impede the ability to maintain volume status, leading to the development of fluid overload that may be accompanied by elevated blood pressure and cardiovascular complications [18-20].

Various studies have utilized the serum albumin levels for epidemiological and clinical surveys without assessing the OSA fraction. It is impossible to relate all the findings in such studies to true hypoalbuminemia without excluding the possibility of “pseudo” hypoalbuminemia, especially in dialysis patients where oxidative stress and inflammation are aggravated. Hypoalbuminemia associated with low ADI indicates the presence of pathogenic modified serum albumin molecules. By triggering and injuring various cells such as endothelium, these molecules may contribute to vascular complications via various molecular mechanisms such as over production of apolipoprotein B, affecting neutrophils and endothelial cells, and through decreasing the antioxidant capacity [12, 21, 22]. It should be recalled that these mechanisms suggest that OSA is a pathogenic mediator with the ability to initiate and accelerate the development of atherosclerosis.

There are many causes that may provoke the inflammatory processes in PD patients. Declining renal function may inversely affect the plasma levels of inflammatory molecules such as hsCRP, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) [23-25]. Vascular congestion due to fluid overload in PD patients may result in altered permeability of the gastrointestinal tract, thereby leading to accumulation of endotoxins that may in turn stimulate monocytes and the increased release of pro-inflammatory cytokines [26-28]. Moreover, molecules that are not cytokines such as advanced glycosylated end-products (AGEs), which result from carbonyl stress, may also accumulate and provoke an inflammatory response [29]. Oxidative stress, which occurs when there is an excessive free-radical production or low antioxidant levels, could be an important condition for the development of endothelial dysfunction, inflammation, and atherogenesis [30-32]. Some dialysis patients with chronic inflammation develop a negative protein balance, despite an intact appetite [33, 34]. In this setting, there may be a shift in protein synthesis from muscle to acute-phase proteins as renal function declines [33-35]. Albumin synthesis is suppressed when plasma hsCRP is elevated [36, 37]. In chronic kidney disease patients, serum albumin decreases and pro-inflammatory cytokines accumulate as renal function deteriorates [38-40]. Among well-dialized patients, activation of the acute-phase response also correlates with lower serum albumin levels due to decreased albumin synthesis [40].

In PD patients where there are varying degrees of inflammation and oxidative stress, the commonly used BCG assay cannot identify the OSA fraction leading to the possibility of the inaccurate diagnosis of “true” rather than “pseudo” hypoalbuminemia that may mislead medical staff decisions. OSA may play an essential role in hypoalbuminemic peritoneal dialysis patients and may be considered an important factor not only for its beneficial oncotic effect but also for its pathogenic ability to initiate and/or accelerate atherosclerosis in this population.

Limitations

The present study was performed in one medical center and included a relatively small number of patients. However, the findings may provide useful information for further multi-center studies to determine the opposed role of OSA in maintaining the hydration status and accelerating the atherosclerosis in PD patients.

Conclusions

“Pseudo” hypoalbuminemia, results from the presence of undetectable OSA fraction by the BCG assay, seems to be a very common finding
and may mislead medical staff decisions in PD patients. Although OSA may contribute to better oncotic pressure and hydration status of these patients, its pathogenic ability to accelerate atherosclerosis should be kept in mind.

Disclosure of conflict of interest

None.

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