Pregnancy-associated plasma protein-A in patients on Maintenance Hemodialysis

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ABSTRACT

A high level of serum pregnancy associated plasma protein-A (PAPP-A) has been observed in patients suffering from renal impairment. The aim of the present study is to evaluate the level of PAPP-A and to elucidate its relationship with renal osteodystrophy and renal functions in patients maintained on hemodialysis (HD). Intact parathyroid hormone (i-PTH), calcium (Ca) and phosphorus (P) levels and alkaline phosphatase activity (ALP) were measured in the serum as markers of renal osteodystrophy while the level of blood urea and serum creatinine were evaluated as markers of renal functions. The results obtained showed that for patients maintained on HD, the levels of PAPP-A, i-PTH, P, urea and creatinine, were significantly higher than controls. Significant positive correlations were obtained between PAPP-A and each of i-PTH, ALP and creatinine in the same group. After dialysis session, the level of PAPP-A increased significantly, compared to its pre-dialysis level. According to the results obtained in the current study, it could be concluded that the increase in PAPP-A level in the serum of patients maintained on hemodialysis is probably the result of chronic inflammation and impairment of kidney functions rather than renal osteodystrophy.

Key Words: PAPP-A/ Renal Osteodystrophy/ Patients Maintained on Hemodialysis.

INTRODUCTION

Pregnancy-associated plasma protein A (PAPP-A) was described by Lin et al. in 1974 as a high molecular weight component of serum obtained from pregnant women in later pregnancy. These molecules detected with anti-third trimester pregnancy were not reactive with normal human serum and were alphabetically named (PAPP-A-B, PAPP-C and PAPP-D) according to their precipitation order in the immunodiffusion method used. Later it was revealed that the molecule detectable in pregnancy plasma that has been named PAPP-A was actually a covalent complex of PAPP-A, and the proform of eosinophil major basic protein (pro MBP) in equimolar amounts. More specifically, the molecule is a heterodimer consisting of two PAPP-A subunits and two pro MBP subunits connected by disulfide bridges (PAPP-A / Pro MBP complex). PAPP-A is a zinc-binding proteinase.

PAPP-A is produced in high concentrations by trophoblasts during pregnancy, however, low serum levels of PAPP-A were detected in first and second trimester of certain genetic fetal developmental disorders, such as Down's syndrome and Cornelia de Lange syndrome, respectively. Although PAPP-A expression has been reported in the endometrium, testis, kidney, bone, colon and other adult and fetal tissues. Although great progress has been made in renal replacement therapy, renal osteodystrophy is still a common problem in chronic renal failure, end-stage renal failure and dialysis patients. Bone growth and turnover are influenced by the metabolism of calcium, phosphate, and a number of enzymes including alkaline phosphatase (ALP) and hormones including parathormone (PTH).

The aim of this study was to evaluate PAPP-A levels in dialysis patients (before and after the
dialysis session) in comparison with levels in healthy subjects. Possible relationships between osteodystrophy parameters, renal function and PAPP-A were also investigated.

SUBJECTS AND METHOD

The present study included 30 chronic hemodialysis patients (15 men and 15 women). Their ages ranged from 43 to 89 with a mean age of 68±12 years. They were selected from those attending the hemodialysis unit of Kasr-Eleni. All were dialyzed three times per week during 4 hours using synthetic membranes (Fresenius polysulfone UF 4.0; Fresenius Medical care, Hamburg, Germany). All patients fulfilled the criteria of adequate dialysis (Kt/V > 1.2 according to the Daugirdas II formula). Blood samples were withdrawn from the arterio-venous fistula of hemodialyzed patients before starting the dialysis session and after the dialysis session (240 min). The control group consisted of 30 healthy subjects (15 men and 15 women). Their ages ranged between 53-77 years with mean age 61±8 years. Samples from controls were obtained via venipuncture of the cubital vein. All sera obtained were stored at -40 until analysis.

Laboratory investigations:

The level of PAPP-A was determined by Enzyme Linked Immunosorbent Assay (ELISA) using DRG PAPP-A (EIT-3397) RUO kit(7). Intact PTH (i-PTH) levels were determined by Intact PTH assay by chemiluminescent microparticle immunoassay on the ARCHITECT I system provided by ABBOTT Diagnostics Division, Biokit S.A., 08186 Barcelona, Spain(8). Serum calcium, phosphorous, alkaline phosphatase, creatinine and blood urea were determined by standard clinical chemistry methods using the semi automated chemistry analyzer BY 224. Assessment of both calcium and alkaline phosphatase was performed using the CPC method and DEA reagent respectively, provided by Biolabo SA, 02160, Maize, France(9). The level of phosphorous was determined by the colorimetric Molybdenum, blue method provided by Biotecnicia Instruments-vis Licenza, 18-00156 ROMA(10). Assessment of creatinine by colorimetric method Jaffe without deproteinization by Biotecnicia instruments via Licenza, 18-00156 ROMA(11). Blood urea was determined according to the enzymatic kinetic method of Randox(12).

For controls and HD patients (before starting the dialysis session) PAPP-A level, Intact PTH assay, Serum calcium, phosphorous, alkaline phosphatase, creatinine and blood urea were determined. After the dialysis session urea and PAPP-A were determined.

Statistical analysis:

For comparison between controls and HD patients before the session, student t-test was used. Differences between obtained values (mean ±SD) as regard urea and PAPP-A in the studied groups, were carried out by one way analysis of variance (ANOVA) followed by the Tukey-kramer multiple comparison test. A P-value of 0.05 or less was taken as a criterion of a statistically significant difference. Correlation coefficient was used to calculate the significance of the difference between the mean effects of the studied group compared with the control group. Statistically significant values were defined as P < 0.05(13).

RESULTS

Table (1) shows comparative study of the laboratory investigations between dialysis patients and controls regarding: blood urea, serum creatinine, calcium, phosphorous, alkaline phosphatase, Intact parathyroid hormone (i-PTH) and pregnancy associated plasma protein-A (PAPP-A). The results indicated that the mean values of urea, creatinine, phosphorous, iPTH and PAPP-A were
highly significantly elevated in dialysis patients compared to healthy controls (P < 0.001). However, serum calcium levels in dialysis patients were insignificantly lower than controls (P > 0.05) on the other hand, alkaline phosphatase levels were insignificantly elevated in dialysis patients compared to controls (P > 0.05). No differences were obtained between male and female patients (P > 0.05).

Table (1): Biochemical alterations in the serum of controls subjects and patients before dialysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (n=30)</th>
<th>Patients before dialysis (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>29.9±6.4</td>
<td>146.67±52.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9±0.2</td>
<td>8.3±3.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.7±0.5</td>
<td>8.5±1.0</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Phosphorous (mg/dl)</td>
<td>3.6±0.6</td>
<td>5.9±1.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/dl)</td>
<td>110.5±9.0</td>
<td>114.5±74</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>i-PTH (Pg/ml)</td>
<td>69±8.7</td>
<td>288.5±214</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PAPP-A (µg/ml)</td>
<td>0.48±0.05</td>
<td>1.41±0.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD (n=30).
P>0.05: not significant, P<0.01: highly significant, P<0.001: very highly significant.

The results regarding the effect of hemodialysis on PAPP-A showed that the increase in the level PAPP-A was significantly higher in post-dialysis patients, compared to its level before dialysis (Figure 1). On the other hand, the level of urea in post dialysis patients was significantly lower than pre-dialysis although still significantly higher that the control level (Figure 2).

![PAPP-A](#)

**Figure (1):** PAPP-A in control subjects, hemodialysis patients before and after dialysis.
In patients before the dialysis session a significant positive correlation ($r^2 = 0.88$) was detected between PAPP-A and i-PTH (Figure 3), between PAPP-A and alkaline phosphatase ($r^2 = 0.189$) (Figure 4) and between PAPP-A and creatinine ($r^2 = 0.78$) (Figure 5). Yet correlations between PAPP-A and each of urea, calcium and phosphorous were not statistically significant ($P>0.05$).
DISCUSSION

The insulin like growth factor (IGF) axis is a multi-component ubiquitously involved in the regulation of growth, proliferation, and differentiation of a variety of cell types. This axis is integrated by growth hormone (GH), IGF-1 and IGF-2, six IGF-binding proteins (IGFBP) and IGF-binding proteins proteases (IGFBP-protease). Several abnormalities in IGF axis have been reported in patients with chronic renal failure \(^{(14)}\) and dialysis patients \(^{(6)}\).

IGFBP-4 protease, identified as PAPP-A \(^{(15)}\), is the major IGFBP protease produced by human osteoblasts. It has been shown to be abundantly expressed in the kidney and to play an important role in renal development and physiology. High serum levels of PAPP-A have recently been observed in patients with renal impairment and cardiovascular events such as plaque instability and acute coronary
syndrome. Furthermore, there is increasing evidence of a relationship between inflammation and PAPP-A\(^{(16)}\).

Previous studies demonstrated that the level of PAPP-A transiently increased during hemodialysis then return to the baseline after the session\(^{(17&18)}\) contrary to the present study. In the current study, the level of PAPP-A was elevated in patients maintained on hemodialysis, which could represent a new acute phase reactant. The results corroborate the findings of\(^{(19)}\) who speculated that artificial membranes may induce PAPP-A production or release during dialysis. Other study explained that the continuous interaction of blood with artificial membranes activates the circulating neutrophils and monocytes and increase the production of reactive oxygen species and cytokines, which possibly contribute to chronic inflammation\(^{(20)}\). In the same way\(^{(21)}\) explained that the behavior of such a high molecular weight protein might be associated with a complex biological response to the extracorporeal procedure, which starts immediately after the contact of blood with the artificial surfaces and includes leukocytes and complement activation, the release of enzymes stored intracellular. However\(^{(22)}\) suggested that heparin, which is administrated intravenously during dialysis is the cause of the increase in serum PAPP-A concentrations.

Patients on chronic hemodialysis (HD) treatment usually suffer from various complications which are both uremic and dialysis associated. Inflammation seem to be at least partly responsible for accelerated atherosclerosis and, thus, increased cardiovascular morbidity and mortality\(^{(2)}\). In the current study the level of PAPP-A in patients maintained on HD was significantly increased after the dialysis session (4hrs), which is in accordance with the findings of\(^{(19,22)}\) while in contradiction with\(^{(18)}\), who documented that inflammatory marker PAPP-A increased after the start of dialysis and returned to pre-treatment value. Previous study,\(^{(17)}\) attributed the transient increase in PAPP-A to limited renal clearance of the protein in chronic renal failure.

In the current study, the increase in PAPP-A in post-dialysis patients might be due to an increased production due to HD treatment or systemic vascular damage. Systemic vasculopathy is a well-established complication in chronic renal failure. Patients on long term hemodialysis show marked changes in bone metabolism variously attributed to secondary hyperparathyroidism, osteomalacia, and a dynamic bone disease\(^{(23)}\). In the present study i-PTH, and phosphorous levels in HD patients were significantly higher than controls. In the current study, positive correlations between PAPP-A and both i-PTH and ALP levels were recorded in patients maintained on hemodialysis group. While the correlations between serum PAPP-A and calcium and phosphorous were not statistically significant (P > 0.05). The results obtained showed also a statistically significant +ve correlation between PAPP-A and creatinine, which corroborate the findings of\(^{(21)}\).

**CONCLUSION**

According to the results obtained in the present study, it could be concluded that the significant increase in the level of PAPP-A recorded in hemodialysis patients probably result from alterations in kidney functions and chronic inflammation rather than dysfunctions in bone metabolism since consistent correlations with all markers of bone metabolism were not found.

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