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Serum asporin levels in maintenance hemodialysis patients without osteoarthritis

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ABSTRACT

Aims: Several human and experimental studies have shown that small leucine-rich proteoglycans might play a significant role in inflammation and fibrosis in various renal diseases. However, as far as we know, no study has reported asporin levels in patients with advanced renal disease. The primary aim of this study was to determine serum asporin levels in hemodialysis (HD) patients without symptomatic osteoarthritis.

Methods: This single-center, cross-sectional study prospectively enrolled maintenance HD patients and healthy control subjects. Subjects with clinically clear osteoarthritis were excluded. Serum asporin level was measured via Human ASPN (Asporin) ELISA Kit (Elabscience Biotechnology Inc. Houston, Texas, USA) in fasting blood samples.

Results: The study included 25 (mean age: 43.3±13.5 years, 60% were females) patients and 29 control subjects (mean age: 38.0±8.8 years, 37.9% were females). Patients and controls were similar in age and sex. Serum asporin levels were significantly higher in HD patients compared with the controls 2.4 (0.9-4.8) ng/mL vs. 0.3 (0.2-0.6) ng/mL, respectively, $p<0.001$). Asporin levels were not correlated with age ($r=0.344$, $p=0.092$) and the duration of HD ($r=0.385$, $p=0.077$). Among HD patients, asporin level was not significantly correlated with C-reactive protein, parathyroid hormone, calcium, or phosphorus levels.

Conclusions: This study showed that serum asporin levels were significantly elevated in patients undergoing HD. Further studies must elucidate the possible origins of increased asporin in these patients.

Introduction

Small leucine-rich proteoglycans (SLRPs) are non-collagen constituents of extracellular matrix (ECM) that play important roles in cell proliferation and function (1,2). Asporin is a member of the SLRP family. Since its discovery in 2001, it has been increasingly shown to be involved in the pathogenesis of various disease processes, including osteoarthritis (OA), cardiovascular disease, and cancer (3-6). The pathophysiologic roles of SLRPs stem from the fact that they can interact with several extracellular receptors, such as transforming growth factor-β1 (TGF-β1), fibronectin, and bone morphogenic protein-4 (7,8). Despite several studies suggesting the association of aspartic acid repeat polymorphism of the asporin gene with OA risk, some meta-analyses have not reported similar findings (9,10). However, D-repeat polymorphism was associated with an increased risk of OA only in male patients (11).

The plausible explanations supporting the role of asporin in the pathogenesis of OA and the heterogeneity of data in the meta-analysis suggest that larger and more detailed studies are needed to clarify this issue. Asporin can inhibit the binding of TGF-β1 to its receptor and consequently impairs TGF-β1 driven chondrogenesis (7). Due to the chondrogenesis and osteogenesis-blocking effects of TGF-β1, asporin can induce OA when expressed in increased amounts (3). Moreover, asporin can bind collagen and help its mineralization (12).

Musculoskeletal disorders are prevalent in the hemodialysis (HD) population. In a cross-sectional study of HD patients, Hage et al. (13) reported that 54% of the patients had OA changes at least in one skeletal area. The most frequently affected area was the spine. The relatively advanced age of end-stage kidney disease patients might in part account for this increased prevalence of OA. However, renal osteodystrophy is a heterogeneous syndrome involving several distinct bone pathologies, namely, osteoporosis, osteomalacia, osteitis fibrosa, and adynamic bone disease (14). Some studies reported changes in serum asporin levels in some disease states other than OA (15). Asporin has been mentioned in the literature as a promising extracellular tissue-specific protein for pharmacogenomic approaches in bone and joint diseases, but there is little published data, and its level is increased in common bone and joint diseases (5,16).

Chronic inflammation and fibrosis are common features of chronic kidney disease (CKD). ECM deposition is central to the evolution of kidney disease as it can lead to impaired matrix composition and ultimately scar formation. It also acts as a network for different molecular mediators, such as enzymes, growth factors, and cytokines (17). Among the various ECM components, SLRPs seem to play a crucial role in renal inflammation and fibrogenesis and can lead to loss of organ function (18). It has become increasingly known that SLRPs can induce anti-inflammatory responses as well as act as “classic damage-associated molecular patterns” in renal inflammation and fibrosis (19). Several human and experimental studies have shown that SLRP might play a significant role in inflammation and fibrosis in various renal diseases (20). These studies also unveiled the dual role of SLRPs in renal disease: pro- or anti-inflammatory, depending on the context (19). Several studies reported increased serum SLRP levels; however, data regarding asporin levels and SLRP levels in human disease are not sufficient.

As far as we know, no study has reported asporin levels in patients with advanced kidney disease. Understanding changes in the serum levels of asporin in advanced renal disease is considered important because several disease markers show altered levels in patients with kidney disease independent of their disease associations. Moreover, several markers are either metabolized in or excreted from the kidney. Thus, changes in renal function might affect the serum levels of the markers being studied. In the asporin example, prevalent bone and cartilage changes in the setting of CKD might be another reason for altered serum levels. Hence, for the first time, we evaluated serum asporin levels in maintenance HD patients in a cross-sectional case-control study.

Methods

This was a cross-sectional study that compared serum asporin levels in HD patients and gender- and age-matched control subjects. The association of the asporin level with the variables of calcium-phosphorus metabolism was also studied in HD patients.

The study was conducted at Medicana International Ankara Hospital, Türkiye. Outpatients who were in the chronic HD program due to end-stage renal disease, who were followed up/evaluated for transplantation in the nephrology and renal transplantation outpatient clinic, and who volunteered to participate in the study were consecutively included. Control subjects were the volunteers from the check-up clinics. Exclusion criteria were as follows: known symptomatic OA, patients who are not on a regular HD program, peritoneal dialysis patients, and patients ≤18 years of age. All study participants gave written informed consent. The Medicana International Ankara Hospital’s Ethics Committee approved the study protocol (BŞH-2022/11). All study procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Age, sex, body weight, and body height were recorded. Midweek predialysis blood samples were used to measure serum asporin and calcium and phosphorus metabolism parameters, including calcium, phosphorus, 25(OH) vitamin D, and parathormone (PTH). Control subjects also provided blood samples.

Asporin was measured via Human ASPN (Asporin) sandwich ELISA Kit (Elabscience Biotechnology Inc. Houston, Texas, USA). The micro ELISA plate in the kit was pre-coated with a human ASPN, and then Avidin-Horseradish Peroxidase (HRP) conjugate was added to each microplate well and incubated. After washing away the free components, the substrate solution was added to each well. Only wells that included ASPN, detection antibody, and Avidin-HRP conjugate appeared blue. stop solution was added to end the enzyme-substrate reaction. The optical density was determined by spectrophotometry at 450 nm±2 nm. ASPN level in samples was calculated by comparing their optical density against the standard curve.

Statistical Analysis

IBM Statistical Package for the Social Sciences Statistics for Windows, Version 25.0. (IBM Corp., Armonk, NY: USA) was used for statistical calculations and to prepare boxplot graphics. Continuous variables were expressed as mean±standard deviation or median (minimum-maximum), whereas categorical variables were reported as numbers and percentages. The chi-square test was used to compare categorical variables between HD patients and controls. To detect whether the distribution of continuous variables is normal or not, we used the Shapiro-Wilk test, histogram, and Q-Q plots. To compare the groups in terms of age, body mass index, and PTH level, the independent t-test was performed. Serum phosphorus, albumin, corrected calcium, and asporin levels were compared between the two groups by the Mann-Whitney U test. The statistical significance level was set as $p<0.05$.

Results

The study included 25 maintenance HD patients and 29 control subjects. The mean age of patients vs. controls was similar (43.3±13.5 years vs. 38.0±8.8 years, p=0.102). The median duration of HD was 30 months (9-75 months). In the patient group, 10 had hypertension, two had hypertension + hypothyroidism, one had diabetes mellitus, one had hypertension + hyperlipidemia, one had Alport syndrome, one had coronary artery disease + hypertension, and one had hypertriglyceridemia. Eight HD patients did not have additional comorbidities. The two groups were also comparable in terms of sex. Mean serum phosphorus and PTH levels were significantly higher in HD patients compared with controls (Table 1).

Asporin level was significantly higher in HD patients [2.4 (0.9-4.8) ng/mL] relative to the control group [0.3 (0.2-0.6) ng/mL] (Table 1, Figure 1). In HD patients, the median asporin level was 2.5 (0.9-4.3) ng/mL in females, and 2.4 (1.0-4.8) ng/mL in male patients (p=0.523). Among HD patients, serum asporin levels showed no significant correlation with C-reactive protein (CRP), PTH, calcium, or phosphorus levels (Table 2). No correlation was detected between age and asporin level. However, there was a strong positive correlation between serum asporin level and dialysis duration (Table 2).

Discussion

In this study, we demonstrated that HD patients had significantly higher serum asporin levels compared with control subjects. Serum asporin level significantly increased in parallel with the duration of dialysis. To the best of our knowledge, this is the first study reporting serum asporin levels in patients with CKD.

Dialysis patients have many comorbid diseases, including musculoskeletal diseases (21). Renal osteodystrophy, now known as the mineral and bone disorder of CKD, affects almost all HD patients (22). The strong correlation between the dialysis vintage and the serum asporin level in the current study may point to a more severe underlying renal osteodystrophy in patients with a longer dialysis duration. Although we excluded patients with clinically manifest OA from the study, asymptomatic or unreported OA might have been missed in some patients. Thus, increased serum asporin levels might reflect the underlying asymptomatic OA changes, particularly in the spine. However, many disease markers are altered in HD patients due to changes in their metabolism of renal excretion, which might have also contributed to elevated serum asporin levels in our patients (23).

Experimental studies have revealed the anti-inflammatory and anti-fibrotic roles of several SLRPs. Particularly biglycan, an SLRP, interacts with interleukin-1-beta and nicotinamide adenine dinucleotide phosphate oxidase (NOX2) (19). Another SLRP, decorin, neutralizes the fibrotic effects of TGF-β1 and connective tissue growth factor (24). However, no such data exist for asporin regarding its effects on inflammation and renal fibrosis. CKD is considered a condition with increased inflammation (25). Increased asporin levels in HD patients thus might be among the reasons for inflammation or represent a compensatory response. However, there was no significant correlation between asporin and CRP levels in our patients.

Although not studied for serum asporin levels, there are data on the association of elevated serum levels of some SLRPs, i.e., decorin and biglycan and with albuminuria in lupus nephritis patients. In a murine study, serum biglycan level showed a correlation with the emergence of albuminuria (26). Another experimental study showed that in the renal ischemia

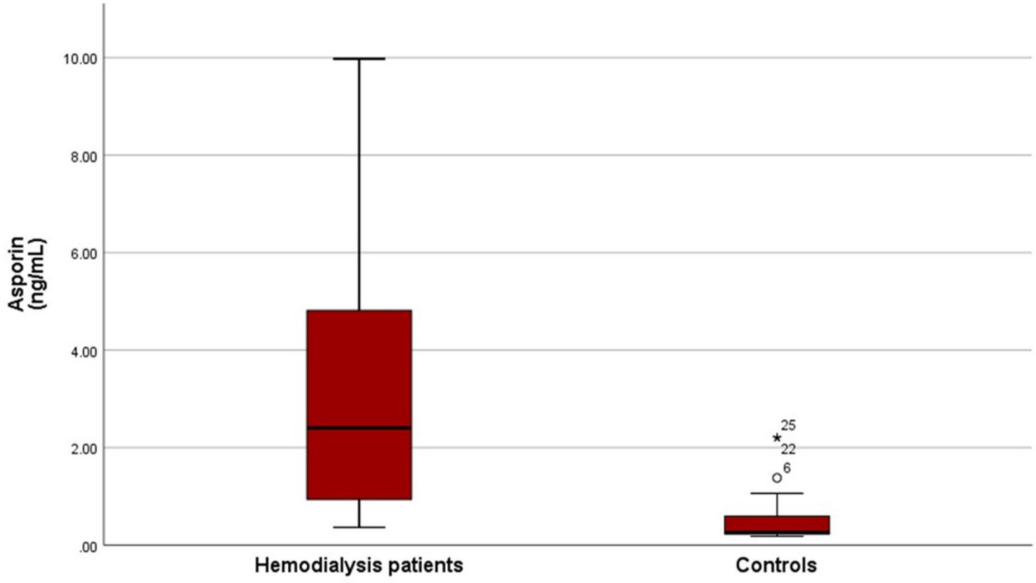

Figure 1. Boxplot diagram of serum asporin levels in hemodialysis patients and control subjects

Table 1. Age, sex distribution, calcium-phosphorus metabolism parameters and serum asporin levels in patients and controls

	Patients (n=25)	Controls (n=29)	p value
Age (years), mean±SD	43.3±13.5	38.0±8.8	0.102
Sex			
Female, n (%)	10 (40.0)	18 (62.1)	0.179
Male, n (%)	15 (60.0)	11 (37.9)	
Comorbidities			
Diabetes mellitus, n (%)	1 (4)	2 (6.9)	
Hypertension, n (%)	2 (8)	2 (6.9)	1.000
Ischemic heart disease, n (%)	1 (4)	1 (3.5)	
Duration of hemodialysis (months), median (min-max)	36 (6-300)	-	-
Body mass index (kg/m^2), mean±SD	25.2±4.2	25.8±4.0	0.653
Phosphorus (mg/dL), median (min-max)	4.4 (3.3-5.8)	3.4 (3.1-3.7)	**0.014**
Albumin (g/dL), median (min-max)	4.4 (4.2-4.7)	4.4 (4.3-4.5)	0.480
Corrected calcium (mg/dL), median (min-max)	8.7 (8.4-9.5)	9.1 (9.0-9.4)	0.090
Parathyroid hormone (pg/mL), mean±SD	499.1±432.6	43.9±14.9	**<0.001**
Asporin (ng/mL), median (range)	2.4 (0.9-4.8)	0.3 (0.2-0.6)	**<0.001**
C-reactive protein (mg/dL), median (min-max)	3.1 (2-23.1)	-	-
25(OH) vitamin D (ng/mL), median (min-max)	11.0 (6.5-17.5)	21 (11.3-37.1)	**0.002**

*Median (min-max).
NA: Not applicable, min-max: Minimum-maximum, SD: Standard deviation

Table 2. Pearson correlation analysis between asporin and age, dialysis duration and calcium-phosphorus metabolism parameters

		Age	BMI	Phosphorus	Calcium	PTH	25(OH) vitamin D	Duration of HD
Asporin level	r	0.344	-0.408	0.118	0.273	-0.077	0.133	0.610
	p value	0.092	0.074	0.575	0.187	0.713	0.546	**0.003**

BMI: Body mass index, HD: Hemodialysis, PTH: Parathormone

perfusion injury model, serum biglycan level correlated with kidney injury (27). However, despite some experimental data, human studies are lacking. Furthermore, we still do not know the exact effect of glomerular filtration rate on serum SLRP levels, particularly on asporin.

Study Limitations

Some limitations of this study should be acknowledged. First, we did not perform a comprehensive evaluation of bone and joint lesions of renal osteodystrophy in the study population. Second, although we excluded clinically clear OA, asymptomatic patients with OA might have been included, resulting in elevated serum asporin levels. However, despite these limitations, this is the first study to report significantly increased serum asporin levels in maintenance HD patients.

Conclusion

In conclusion, this study showed significantly elevated serum asporin levels relative to healthy control subjects in patients receiving maintenance HD. To evaluate the association of asporin with renal osteodystrophy lesions, further studies involving groups of HD patients with and without OA are needed.

Ethics

Ethics Committee Approval: The Medicana International Ankara Hospital's Ethics Committee approved the study protocol (BŞH-2022/11).

Informed Consent: All study participants gave written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.D.D., C.D., Design: A.D.D, C.D., Data Collection or Processing: A.D.D, C.D., Analysis or Interpretation: A.D.D, C.D., Literature Search: C.D., Writing: A.D.D., C.D.

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