The Study of Hemodialysis on the Change Rate of Serum Osteopontin, Sialic Acid, and Nitric Oxide Levels

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Abstract

Objective : Chronic renal failure (CRF) is one of the most prevalent diseases of human societies, especially in Iraq. Despite all advances that have been made so far, hemodialysis with all its complications is being applied as the novel treatment strategy for such individuals.Osteopontin (OPN) is a multi-functional secreted glycoprotein that plays a crucial role in and inflammatory process.

Our aim was to ascertain whether circulating osteopontin,sialic acid ,and NO levels are altered in patients with CRF before and after dialysis.

Design and Methods: A total of 126 patients with HD were enrolled in this study. Serum OPN levels were measured using a commercial enzymelinked immunosorbent assay kit.

Results: Osteopontin and Sialic acid levels were significantly higher in haemodialysed CKD patients than predialysed CKD patients and normal healthy controls. The levels of NO was significantly lower in CRF patients pre HD when compared with healthy controls and significantly increased in post HD as compared to pre HD.

Conclusion: In our study, OPN **SA, NO** were found to be a valuable marker of severity of CKD.

Keywords: *Hemodialysis; Osteopontin; Chronic renal failure*

Introduction

The kidneys are involved in the secretion of several, toxins, and regulate the volume and composition of the extracellular fluid to maintain homeostasis by constantly processing the plasma by filtration, reabsorption, and secretion of substances, thereby help in preserving the internal environment of the body[(1-3)].

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Chronic renal failure (CRF) is an irreversible kidney condition that leads to end-stage renal disease (ESRD).It is the progressive and irreversible loss of normal functioning of kidneys that leads to end-stage renal disease (ESRD)[(4)].Itis a major global public health problem, Characterized by the sustained presence of either kidney damage (albuminuria) or reduced kidney function (estimated glomerular filtration rate (eGFR] <60 ml/min/1.73 m^2) leading to loss of normal kidneys capability to eliminate toxic molecules from the body, which detected by renal injury markers, including urinary and hematological alterations. ESRD patients require replacement interventions, such as kidney transplant or hemodialysis. ESRD leads to death[(5-7)].

HD is one of the replacement therapy. In this technique, body waste product like urea, Cr and free water are removed from the blood when the kidneys are impaired [8].

Osteopontin (Eta-1) also known as secreted phosphoprotein 1 or bone sialoprotein 1,is a 34 kDa phosphorylated acidic glycoprotein,found in bone, acute and chronic inflammatory cells, smooth muscle, epithelial, and endothelial cells, neurons, and fetal renal tissue and is expressed in the thick ascending limb of the loop of Henle,it is plays an essential role in bone remodeling largely due to its function in osteoclast adhesion and boneresorption. Osteopontin is rich in aspartic acid, glutamic acid, and a polyaspartic acid motif, and it can be highly phosphorylated at serine and threonines, all of which allows it to bind to both ionic calcium and to hydroxyapatite, making it a potent inhibitor of calcification[9-10].OPN induces the accumulation of extracellular matrix by binding to type I collagen, fibronectin, and osteocalcin, thereby contributing to tissue fibrosis[11].OPNbesides proinflammatory functions, physiologically, is a potent inhibitor of mineralization, prevents ectopic calcium deposits, and is a potent inducible inhibitor of vascular calcification[12-13].

Our aim was to ascertain whether circulating Osteopontin,sialic acid ,and NO levels are altered in patients with CRF before and after dialysis as compared with healthy control group.

Subjects and Methods

The trial was designed as a prospective observational study and was conducted between July 2019 to March 2020in the dialysis unit in Tikrit Teaching Hospital in Tikrit city. We consecutively recruited 100 patients of whom 54 patients were females and 46 males; mean age of (45 ± 10.8years) and one hundred apparently normal volunteers of whom 41were females & 59 male age & sex with matched.The average duration of HD therapy for the CRF patients in the present study was2.19 ± 0.76.

All patients were receiving hemodialysis dialysis process at least once every month since 6 month at list. Excluded patients, are those with Diabetes, the Chronic smokers, Patients with active inflammatory disease, and Seropositive hepatitis.

Venous blood samples were collected from the antecubital veins ,following at least 12 h of fasting. Samples were separated by centrifugation for 15 min at 2000 g after clotting for 30 min at room temperature. The serum samples were subsequently stored in aliquots at K80 8C prior to the analysis.Serum OPN was performed using an enzyme-linked immunosorbent assay (ELISA) ,nitric oxide by the method of Smarason*et al*[14].The study was conducted with the approval of the local ethics committee on clinical investigations.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics, version 22.Mean ±SD used to expression of results . (ANOVA) used to find a correlation between the two groups.*P* values <0.05 were considered significant.

Results

A total of 100 consecutive CKD patients were included in the study. Females (n=54) outnumbered males (n=46). The mean age of all patients was 45 ± 10.8years (range 25-70 years). The average duration of HD therapy for the CRF patients in the current study was 2.19 ± 0.76 (Figures 1-3).

Table 1: Demographic characteristics of study groups.

	CRF patients	Healthy controls
Age (years)	45 ± 10.8	43.6 ± 13
Sex (males /female)	46/54	59 /41
Duration of hemodialysis (years) =2.19 ± 0.76		

Table 2. Biochemical parameters of study and control groups:

Parameters	Control	Pre-HD	HD
Osteopontin (ng/mL)	17.9 ±4.9	34.7 ±10.01	43±14.5
Sialic acid (mg/dl)	60.74 ± 2.13	89.7 ± 3.56	100±5.10
NO (μ mol / L)	38± 13.36	24.04 ± 7.23	90.35± 20.62
Urea (mmol/L)	5.024 ± 2.1	33±4.1	11.1±4.3
Creatinine (mmol/L)	100± 18.3	557 ± 19	297±15.7

$P < 0.05$

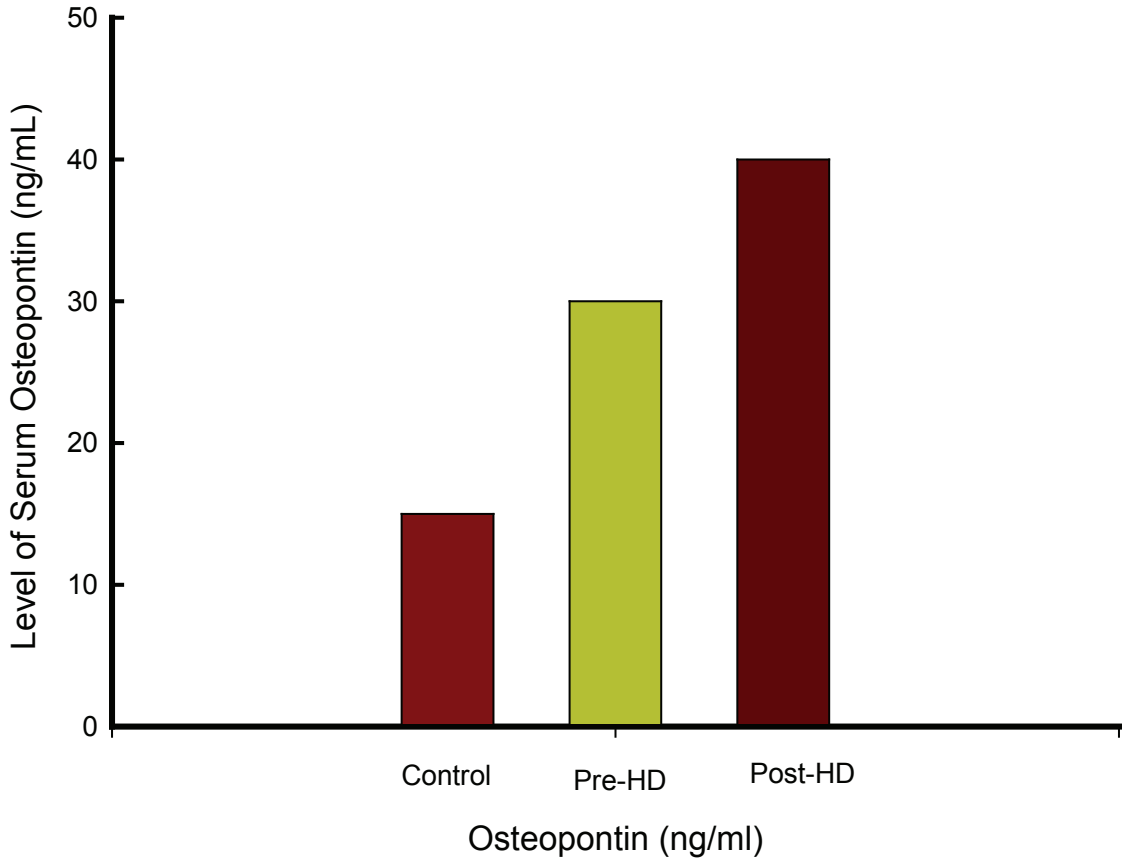

Figure (1):-The level of Serum Osteopontin (ng/mL) in study groups.

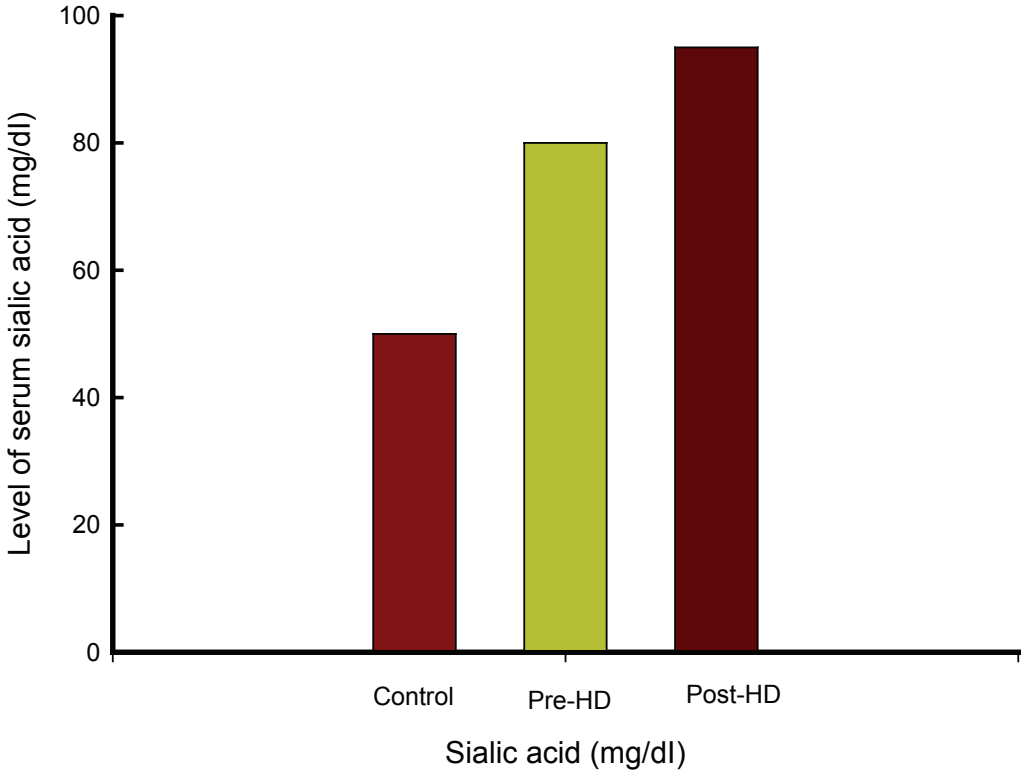

Figure (2):-The level of serum sialic acid (mg/dI) in study groups.

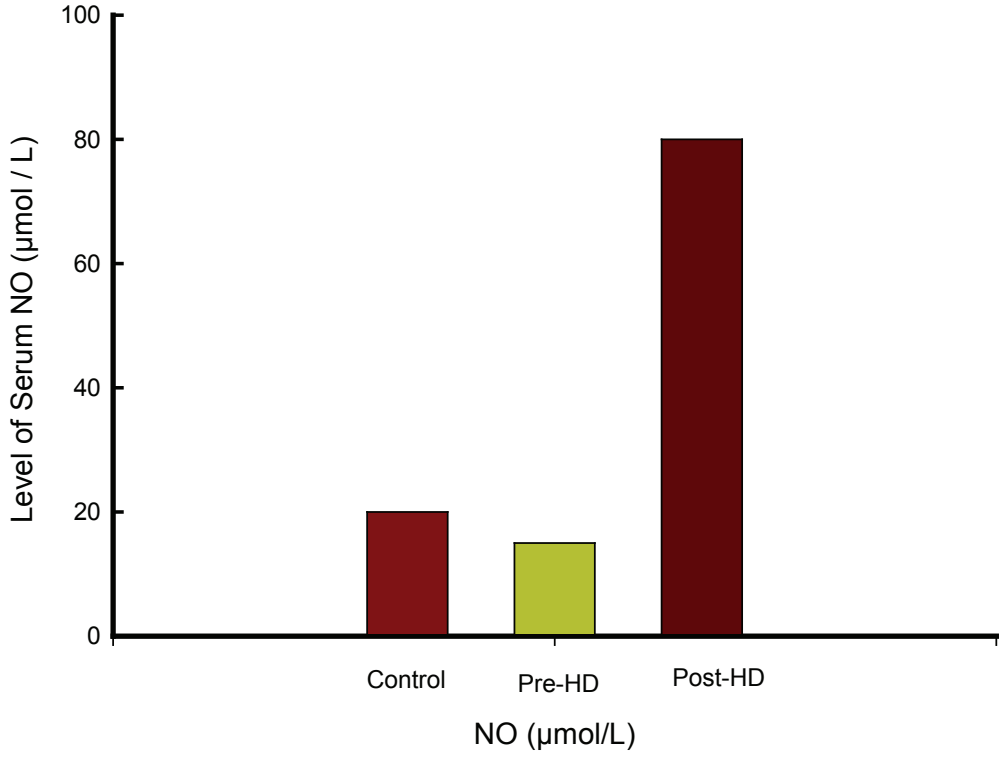

Figure (3):-The level of Serum NO (µmol / L) in study groups.

Osteopontin and Sialic acid levels were significantly higher in haemodialysed CKD patients(43±14.5), (100±5.10:0.001) respectively than pre-dialysed CKD patients (34.7±10.01: p= 0.001),and(89.7 ± 3.56: 0.001) respectively and normal healthy controls (17.9±4.9: p= 0.00),(60.74 ± 2.13: 0.001). Significant difference in OPN levels has been shown between pre-dialysed CKD patients and normal healthy controls (p= 0.00)

The levels of NO was significantly lower in CRF patients pre HD (24.04 ± 7.23: <0.01)when compared with healthy controls(38± 13.36: <0.01), and significantly increased in post HD(90.35± 20.62) as compared to pre HD.

Serum Urea and Cr were significantly higher in CRF patients pre HD process (33±4.1), (557 ± 19)when compared with healthy controls(100± 18.3),(5.024 ± 2.1) and their levels were significantly decreased after HD(11.1±4.3) and (297±15.7) as compared to pre HD.

Discussion

Osteopontin (OPN) is a phosphorylated glycoprotein secreted by activated macrophages, leukocytes, and activated T lymphocytes, and is present in extra cellular fluids, at sites of inflammation, and in the extra cellular matrix of mineralized tissues[15]. However, Jono*et al*[16].recently demonstrated that the phosphorylation of osteopontin was critical for this activity: non-phosphorylated osteopontin (converted by the addition of alkaline phosphatase) did not inhibit mineralization, whereas phosphorylated osteopontin did inhibit mineralization[16].The exact excretion pathway of OPN from the body is not known. Although the exact mechanism is not clear, this may be due to the kidneys' role in OPN metabolism and clearance.The serum OPN levels in the present study were increased in patients with CKD in comparison to healthy volunteers. may be due to its upregulation in chronic inflammatory states and/or due to bone mineral density (BMD)[13].which is similar to the study done by Lorenzen *et al*,[17]..Significantly higher values for serum OPN were noted in HD groups than preHD, and control groups which may be related to uremic stimulation and the dialysis procedure itself[17] consistent with similar study done by Chaitanya,[12], and Lorenzen *et al*,[17].

Nitric oxide is extremely reactive signaling molecule and it is remarkable regulator for cellular functions including vasodilatation, inhibition of platelet aggregation, neutrophil adhesion, scavenging superoxide (O^{-2}) radical and inhibition of xanthine oxidase activity[19].The mean serum NO concentration has found to be decreased in CKD patients but they was increased in post HD when compared with the controls.

This may result from arginine deficiency caused by a loss of functional renal mass, increased endogenous NO synthase (NOS) inhibitors that accumulate in renal failure, and/or other causes, such as increased reactive oxygen species. In addition to being caused by CRD, low NO production may contribute to and/or exacerbate the progression of CRD by both hemodynamic and renal growth-promoting actions[20,21].

In the process of haemodialysis triggers a series of events that increase the levels of inflammatory cytokines as TNF α, IL-I and interferon γ (INF γ) by neutrophils and monocytes, the increase of these inflammatory cytokines appear to be additional reasons for enhanced formation of NO in uraemia, as IL-1 and TNF α are potent inducers of iNOS where in NO is released in micromolar amounts in cellular systems[22].These results are compatible with the studies ofMeenakshi et al [23].,but disagree with SaharEladawy[24].

The increased levels of serum sialic acid in CRF may be the result of increased synthesis and catabolism of glycoproteins and glycolipids and ,and releasing of sialic acid from the cell into the bloodstream due to cell damage in chronic renal failure patients[25,26].The elevation in mean levels of AS are observed in the post haemodialysis treatment due to increased cytokine production.Our results agreed with the findings of[27,28].

Blood tests for Blood urea nitrogen (BUN) and creatinine are the simplest way to monitor kidney function. but the test for creatinine is more sensitive than urea[29].However, neither creatinine nor urea are directly toxic and are just substances used to measure kidney function .Urea is an organic compound produced by the liver and excreted by the kidneys. and plays a vital role in the metabolism of nitrogen-containing compounds[29,30].Our observations we found urea, and creatinine concentration, plasma creatinine and urea concentrations were observed to be significantly higher

in CRF subject were in accordance with the study.

Creatinine is a waste product, formed from creatine and creatine phosphate in muscle diffuses from various tissues into blood. it is constantly excreted via kidneys without reabsorption. The kidneys' ability to get rid of creatinine in the blood decreased compared to the high creatinine in the blood which leads to mechanical damage to the renal cells[31,34].Urea accumulation in serum patients arises from the degradation of food and tissues such as muscle. The high level of urea in blood leads the body very sick unless remove it from the blood streams by kidneys[35].

Conclusion

The concentration of osteopontin is also influenced by renal dysfunction. The extent of renal failure must be considered when these markers are used in routine practice.

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