Is There A Place for Plasma Osteopontin as Key Mediator in Patients With Diabetic Nephropathy?
Wafaa Mohi El-Deen Abd El-Fatah* and Mona Abd El-Raof Abd El-Kader**
*Department Of Medical Biochemistry, **Department Of Internal Medicine Faculty of Medicine for Girls Al-Azhar University

ABSTRACT
Background: micro- and macro-vasculopathies, such as nephropathy and coronary artery disease (CAD), respectively, are common in diabetes and constitute the major causes of death for in these patients. Pro-inflammatory cytokines play a critical role in the pathogenesis of diabetic complications through various biochemical and cellular pathways. Osteopontin (OPN) has been identified as a key regulator of many metabolic and inflammatory diseases including obesity, diabetes and diabetic nephropathy. The aim of this study was to evaluate plasma level of osteopontin in different stages of diabetic nephropathy in type II DM, and to correlate it with the stage of nephropathy and with other measured parameters. Patients and methods: the study was conducted on 58 patients with diabetic nephropathy as well as 15 apparently healthy subjects as a control group. Patients were classified into 2 main groups according to the level of glycated hemoglobin (HbA1c) Group I: controlled type II DM (HbA1c 5.55%-7.6%). Group II: uncontrolled type II DM (HbA1c > 7.6 %). Each group was subdivided into two subgroups (A and B) according to the presence of microalbuminuria or macroalbuminuria (degree of nephropathy). In addition to, Group III: DM type II with end stage renal disease (serum creatinine ≥ 5mg/dl) and just starting hemodialysis (1-3 sessions Only) plasma osteopontin was measured by ELISA. Results of the study revealed significant increase of serum osteopontin in all studied groups.

Results: compared to normal control subjects (P<0.001). There was a statistically positive correlation between serum osteopontin versus all variables in group I and II; except HbA1C in group I, and FBS in group II. But, no statistical correlation change between serum osteopontin versus all variables in group III (P>0.05). Cut ROC curve of osteopontin levels of all cases of diabetic nephropathy indicates high validity of OPN to detect positive cases of diabetic nephropathy with accuracy of 100%, and OPN is considered a high validity test in prediction of end-stage renal disease (ESRD) more than prediction of microalbuminuria. Conclusion: plasma level of osteopontin increases with the progression of diabetic nephropathy and osteopontin may be useful as a biomarker to trace disease progression as well as a potential diagnostic biomarker for the prediction of diabetic ESRD.

Keywords: Osteopontin, Microalbuminuria, Macroalbuminuria, Diabetic Nephropathy, End-Stage Renal Disease.

INTRODUCTION
Diabetic nephropathy (DN) occurs in approximately 30% of diabetic patients and leads to renal failure in most countries. It is the leading cause of chronic renal disease in patients starting renal replacement therapy. (1) DN has been classically defined as increased protein excretion in urine. Early stage is characterized by a small increase in urinary albumin excretion (UAE), also called microalbuminuria or incipient DN, (2) but doesn't cover all patients with renal impairment. (3) More advanced disease is defined by the presence of macroalbuminuria or proteinuria. Although microalbuminuria is considered a risk factor for the development of macroalbuminuria, not all patients progress to this stage, and some may regress to normoalbuminuria. (4) DN screening must be performed when DM is diagnosed in patients with type II DM. (5) High glucose concentration can induce production of many cytokines and initiate all kinds of pathophysiologic process. (6) Thus, the elevated glucose concentrations observed in patients with diabetes may directly affect the calcification process. (7)

Pathologically, there are two patterns of vascular calcification. Typical atherogenesis and medial calcification (also called Mo¨nckeberg’s calcification or medial calcinosis). Medial calcification occurs initially in the
medial layer and is not associated with lipid laden macrophages or intimal hyperplasia. As Mo"nckeberg’s calcification progresses, it forms a dense circumferential sheet of calcium crystals in the centre of the media, bounded on both sides by vascular smooth muscle cells (VSMCs) and can contain bone trabeculae and osteocytes. It is most commonly described in distal vessels of patients with diabetes, advanced aging and renal failure.\(^7\)

Shanahan et al.\(^8\) found that in distal peripheral arteries with calcification from patients with diabetes, VSMCs expressed a number of osteocytic/ chondrocytic markers. The exposure of VSMC to high glucose activates several signal transduction networks responsible for mediating the proliferative and growth-promoting response \(^9\). High-glucose concentrations increased the expression of the osteoblast differentiation factor, (Cfα1) and osteocalcin and alkaline phosphatase activity in BVSMCs. High glucose also significantly enhanced calcification of BVSMCs \(^10\). Moreover, excess glucose attaches nonspecifically proteins in a process called glycosylation. Glycosylated proteins trigger inflammatory reactions which injure the lining of blood vessels.\(^6\)

Osteopontin (OPN) is a multifunctional phosphoprotein which secreted by many cell types such as osteoclasts, lymphocytes, macrophages, epithelial cells, and vascular smooth muscle cells (SMC)\(^11\), and acts by facilitating cell adhesion and migration.\(^12\)

Osteopontin OPN is a secreted matricellular protein that was first identified in 1985 by Heingard et al. as sialoprotein derived from bovine bone matrix.\(^13\) The most commonly used name osteopontin is derived from “osteon”, the Greek word for bone, and “pons”, the Latin word for bridge illustrating its function as a linking protein and crucial factor in bone homeostasis.\(^14\)

OPN is a negatively charged aspartic acid-rich, N-linked glycosylated phosphoprotein composed of 314 amino acid residues.\(^15\) The human gene for OPN has been localized on the long arm of chromosome 4q13 directly related to four similar genes encoding for bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extra cellular phosphoglycoprotein (MEPE).\(^16\) Due to common functional motifs and domains these are categorized as the so-called SIBLING proteins (small integrin-binding ligand N-linked glycoproteins)\(^17\).

It is encoded by the SPP1 gene, which is transcribed into three mRNA isoforms,\(^11\) as a result of alternative splicing, alternative translation and different posttranslational modifications (PTMs), which allow for a molecular weight ranging from 41 to 75 kDa.\(^18\)

Its availability to interact with integrin receptors through an Arg-Gly-Asp(RGD) sequence and isoforms of CD44 receptor, also known as extracellular matrix receptor type III, has established OPN as an important attachment and signaling molecule.\(^19\) Extracellular OPN functions through its interactions with multiple cell surface receptors including various integrins\(^20,21\).

In addition, OPN is highly secreted by macrophages at sites of inflammation where it mediates monocyte adhesion, migration, differentiation, and phagocytosis. It is now well recognized that OPN induces chemotaxis of monocytes and promotes cellular motility via direct interaction with its receptors.\(^22\)

OPN expression is affected by a number of substances including hormones, cytokines, several inflammatory mediators and growth factors such as interleukin-1 (IL-1), tumor necrosis factor alpha, and platelet-derived growth factor are known to stimulate OPN transcription via activation of protein kinase C (PKC).\(^23\) Increase in PKC activity has been demonstrated in several tissues from diabetic animals.\(^24\) It was also noted that high glucose concentrations stimulated osteopontin OPN expression via a protein kinase C(PKC)-dependent pathway and the hexosamine pathway in cultured rat aortic SMC.\(^25\)

Pro-inflammatory cytokines stimulate OPN gene transcription and expression. For example, activation of macrophages with lipopolysaccharide (LPS) and nitric oxide (NO) induces OPN gene expression and protein secretion.\(^23\) Other mediators that can induce OPN up regulation include angiotensin II, transforming growth factor β (TGF β), hyperglycemia and hypoxia.\(^26\) In addition, several cis and trans-regulatory elements within
OPN have been investigated to establish the mechanisms by which OPN gene transcription occurs. (27)

Osteopontin (OPN), has emerged as an active player in biominalization, tissue remodeling and inflammation. (28) Thus, OPN is thought to play multiple roles in the progression of atherosclerotic plaque including diabetic vascular complications. (29)

At first, OPN has been reported to be highly expressed in the tubular epithelium of the renal cortex and in glomeruli in rat and mouse models of diabetic nephropathy. (30)

This was associated with extensive macrophage accumulation in the kidney interstitium indicating that OPN upregulation and macrophage recruitment may play a role in the tubulo-interstitial injury in diabetic nephropathy. (31)

The aim of our study was to assess the relation between plasma level of osteopontin, and the severity and progression of diabetic nephropathy, as well as the validity of OPN to be used as a marker of diabetic nephropathy.

SUBJECTS AND METHODS

This study was conducted on 58 patients suffering from type II diabetes mellitus and nephropathy from Alzahraa University Hospital, as well as 15 healthy volunteers as a control group matched by age and sex, with an age range between 44 and 72 years. The healthy subjects did not have any systemic diseases as diabetes, hypertension, cardiovascular disease, or renal insufficiency.

Patients were chosen and classified into TWO main groups according to the level of glycosylated hemoglobin (HbA1c):

Group I: twenty five patients with controlled type II DM (HbA1c 5.5%-7.6%) and

Group II: twenty three patients with uncontrolled type II DM (HbA1c ≥ 7.6%). Each group was subdivided into two subgroups according to the presence of microalbuminuria or macroalbuminuria (degree of nephropathy).

Subgroup IA: fourteen patients having controlled DM with microalbuminuria (Albumine excretion rate (AER) 30-300mg/L).

Subgroup IIB; Eleven patients having controlled DM with macroalbuminuria (AER ≥ 300mg/L).

Subgroup IIA: Twelve patients having uncontrolled DM with microalbuminuria (Albumine excretion rate (AER) 30-300mg/L).

Subgroup IIB: Eleven patients having uncontrolled DM with macroalbuminuria (AER ≥ 300mg/L).

Group III: ten patients having type II DM with end stage renal disease and just starting hemodialysis (1-3 sessions Only). For subjects of all groups oral consent and history was taken.

All patients and controls were subjected to thorough history taking with a special emphasis on age, symptoms of diabetes mellitus, full clinical examination (Special attention was paid to the body mass index), and routine laboratory investigations including: fasting and postprandial blood glucose according to Spin react kit (32), lipid profile (Total cholesterol Spin react kit (33) H.D.L to Spin react kit (33), L.D.L according to the Friedewald Formula, triglycerides according to Diamond kit (34), blood urea and serum creatinine by Roche / Hitachi 912 (Roche Dignostic , Indianapolis, IN USA). (35) Blood glycated (HbA1c) using fresh blood Colorimetric method using StanbioGlycohemoglobin kits, Texas (Procedure No. 0350) according to. (36)

- Microalbuminuria was measured using micral strips (negative cases were excluded)
- 24 -hours urinary protein excretion was measured by Pyrogallol red direct colorimetric method according to Watanebe et al. (37)
- Plasma OSTEOPONTIN (OPN) was done by ELISA kit provided by R&D System, Inc. USA.

Blood samples were collected after an overnight fast. Plasma samples were separated and stored at −80°C until assay. Urine samples were collected from all subjects in the morning and were stored at −80°C within 2 h of collection until analysis. Analysis of data was done by IBM computer using SPSS (statistical program for social science version 16). (38)

P value >0.05 insignificant
P<0.05 significant
P<0.001 highly significant
RESULTS

The results of the study are summarized and illustrated on table 1 to 5 and figure 1 to 5, the study revealed the following:

1- The serum level of osteopontin was significantly elevated in group IA, IB (groups of controlled DM with microalbuminuria and macroalbuminuria) group II A, II B (groups of uncontrolled DM with microalbuminuria and macroalbuminuria) and group III(type II DM with end stage renal disease and just starting hemodialysis) respectively when compared to normal control subjects (P<0.001). (Table 1)

2- There were significant changes in the level of osteopontin among all studied groups compared to each other (P <0.001) (Table 1, 3& Figure 1)

3- The serum levels of fasting blood sugar, urinary protein excretion, blood urea, serum creatinine and HbA1C% were significantly elevated in group IA, IB, II A, II B and group III respectively when compared to normal control subjects (P<0.001) (Table 1&2)

4- There were significant changes in the levels of all variants among all studied groups compared to each other (P <0.001), Except F.B.S between subgroup IA and subgroup II B (P>0.05) (Table 1&2).

5- Statistically positive correlation between serum osteopontin versus all variables in group I and II; except HBA1C in group I, and FBS in group II by using Spearman correlation coefficient test (P <0.001) (Table 4).

6- No statistical correlation change between serum osteopontin versus all variables in group III. (P>0.05) (Table 4)

7- Osteopontin levels shows best cut off 65ng/ml, 100% sensitivity and 89% specificity in group III. (Table 5& Figure 4). While, it shows 67%, 90% sensitivity and 30%, 47% specificity in patients with micro- and macroalbuminuria respectively(Table 5& Figure 2, 3), which proof that OPN is considered a high validity test in prediction of ESRD more than prediction of microalbuminuria.

8- Osteopontin levels shows best cut off 60ng/ml in control group and Roc curve of all cases of diabetic nephropathy shows 100% sensitivity, which indicate high validity of OPN to detect positive cases of diabetic nephropathy with accuracy of 100% (Table 5& Figure 5).

DISCUSSION

Diabetic nephropathy is a leading cause of chronic renal failure in western world. (39) In the past, several mechanisms have been suggested to involve in the initiation and deterioration of diabetic nephropathy, including hemodynamic and genetic factors, intracellular metabolic anomalies, and advanced glycation end products. (40) Emerging evidence suggests that inflammation is crucially contributed in the pathophysiology of diabetic nephropathy. (41) The appearance of microalbuminuria is a detectable early marker of DN, but doesn't cover all patients with renal impairment. (3) The detailed molecular mechanisms underlying the correlation between albuminuria and DN remain elusive. (29)

A number of cytokine systems including transforming growth factor-β (TGF-β), angiotensin II, and osteopontin (OPN) have been implicated in the pathophysiology of diabetic nephropathy. (42)

OPN has been reported to be directly or indirectly related to the pathogenesis of DM target organ damage including nephropathy. High glucose induces OPN expression in renal fibroblasts, vascular smooth muscle cells (43), monocytes (44) and mesangial cells. (45) OPN has been reported to be highly expressed in the tubular epithelium of the renal cortex and in glomeruli in rat and mouse models of diabetic nephropathy. This was associated with extensive macrophage accumulation in the kidney interstitium indicating that OPN upregulation and macrophage recruitment may play a role in the tubulo-interstitial injury in diabetic nephropathy. (28)

The results of the current study showed that plasma level of osteopontin was significantly increased in all studied groups when compared to healthy control subjects (P<0.001), also all other laboratory results including FBS, 24 hr. urinary protein excretion, blood urea, serum creatinine and HbA1C were significantly increased(P<0.001) in all groups when compared to control.

Moreover, there was a significant increase in plasma level of osteopontin and all laboratory results among all studied groups when compared to each other(P<0.001), except
for HbA1c between controlled patients with microalbuminuria and uncontrolled patients with macroalbuminuria (P >0.001).

Cointicing to the currant study, Yan et al. conclude that patients with DM have higher concentrations of plasma OPN than non-DM controls .(46)

Takeda et al. who examined OPN expression in human samples, and imply that OPN plays a role in the development of diabetic vascular complications .(47).

Another study was performed by Miri et al. showed that the expression of OPN is up-regulated in rat aortic VSMCs by diabetes .(48).

Previous studies have shown that advanced glycation end-products and angiotensin II can stimulate OPN synthesis by a variety of cells, and initiate local effects of cell spreading, adhesion, and proliferation .(49, 12)

Yuji et al. reported that high glucose levels increase osteopontin production in rat dental pulp tissues .50 Moreover, Hsieh et al. conclude that, osteopontin gene expression is upregulated in diabetic rat proximal tubular cells .(51)

In rat models of streptozotocin- or high-fat diet-induced diabetic nephropathy, OPN expression is significantly upregulated in the renal cortex and aorta .(46)

OPN expression has been shown to be upregulated in the vascular wall of diabetic patients and diabetic animal models, which might be induced by high glucose and advanced glycation end product .(52, 53).

Furthermore, Moe et al. reported that in patients with ESRD positive immunostaining for OPN in the artery is stronger in diabetes than in non-diabetes .(54).

OPN knockout mice were protected from diabetes-induced albuminuria and mesangial expansion .(55).

The results of our study agreed with Yamaguchi et al. who showed that plasma OPN level increased significantly with progression of diabetic nephropathy (DN) especially at the stage of renal failure .(51).

Lorenzen et al. stated that OPN contributes to the development of nephropathy and atherosclerosis in diabetes and inhibition of OPN may become a novel therapeutic strategy for these patients .(55).

Once more there was high significant positive correlation between osteopontin versus all variables Except HBA1C in group I, and FBS in group II.A high significant positive correlation with 24hr. urinary protein excretion level in patients with diabetic nephropathy(P<0.001) indicating that that plasma OPN level increased significantly with progression of diabetic nephropathy(DN).

Recently, it was reported that quantification of OPN immunostaining revealed a marked increase in the percentage of OPN-positive proximal tubular cells in human DM kidneys, which correlated strongly with the degree of cortical scarring .(42).

The results of our study agreed with Hsieh and his colleagues who conclude that a strong correlation between higher OPN levels and more severe diabetic albuminuria and glomerulosclerosis .(51). Several gene array studies have suggested that osteopontin (Opn) expression strongly correlates with albuminuria and glomerular disease .(55).

While, this study concluded that there was no significant correlation between osteopontin versus all variables in group III.

Al-Malki observed that urinary osteopontin were not correlated with other laboratory markers such as plasma glucose, creatinine, and HA1C .(54). Furthermore, Yamaguchi et al. stated that plasma OPN level increased significantly with aging and the progression of diabetic nephropathy, especially at the stage of renal failure (p<0.05). However, the level was not related to the progression of retinopathy or neuropathy, or to laboratory findings, such as HbA1c or serum lipids .(56).

While, other study stated that in patients with diabetic nephropathy, OPN expression is closely related to the degree of cortical interstitial scarring .(57).

Yan et al. found that plasma OPN concentrations were proportional to the severity of renal dysfunction and were an independent risk factor for the presence and severity of renal insufficiency. Conversely, the presence of renal insufficiency could lead to elevated plasma OPN concentrations, forming a vicious cycle that exaggerates diabetic nephropathy and atherosclerosis .(46).
The possible explanation of all the previous results is that previous studies suggest that OPN may promote the development of atherosclerosis. Isoda et al. 2002 showed that OPN overexpression in mice results in increased medial thickening with age and a larger neointima formation after mechanical injury. The involvement of OPN in vascular remodeling may be related to the association between OPN and collagen. Collagen plays a pivotal role in vascular remodeling and has been shown to interact with OPN. In fact, it has recently been shown that OPN null mice demonstrate vessels with a more loosely organized collagen matrix.

Also, the activation of protein kinase C by high concentrations of glucose has been proposed as a mechanism for the development of diabetic vascular complications. High concentrations of glucose increases the expression of OPN at the transcriptional level, possibly through the activation of protein kinase C as well as the hexosamine pathway. High glucose stimulates de novo synthesis of diacylglycerol from glycolytic intermediates, which is involved in a variety of cellular functions, thus leading to cellular responses and gene expressions.

Another possible explanation is that vascular calcification is more common in patients with diabetes and is associated with increased mortality, stroke and amputations. In patients with kidney disease, diabetes is a prominent risk factor for vascular calcification.

This study examined the validity of OPN level to be used as an effective marker at different stages of nephropathy by using ROC curve to find out the best cut off value, and validity. Osteopontin levels shows 100% sensitivity and 89% specificity in group III, with less sensitivity and specificity in patients microalbuminuria and macroalbuminuria which proof that OPN is considered a high validity test in prediction of ESRD more than prediction of microalbuminuria.

Moreover, ROC curve of all cases of nephropathy (microalbuminuria, macroalbuminuria and ESRD) collectively, shows area under the curve is 100% as cut off value of healthy control subjects is 60 ng/ml. That results mean that any diabetic patients with OPN level equal or more than 60 ng/ml must have a degree of diabetic nephropathy with 100% accuracy.

All these observations suggested a possible role of OPN in accelerated the development of renal disease in diabetes mellitus.

In conclusion, plasma level of osteopontin increases with the progression of diabetic nephropathy and osteopontin may be useful as a biomarker to trace disease progression as well as a potential diagnostic biomarker for the prediction of diabetic ES.

REFERENCES


32. Young DS (2001): Effects of diseases on clinical lab.tests;4thed AACC


Table (1) Comparison between the studied groups as regard different laboratory parameters.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group IA N=14</th>
<th>Group IB N=11</th>
<th>Group IIA N=12</th>
<th>Group IIB N=11</th>
<th>Group III N=10</th>
<th>Controls N=15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood sugar FBS (mg/dl)</td>
<td>150±16*</td>
<td>184±16.5*</td>
<td>212±20*</td>
<td>159±19*</td>
<td>197±32*</td>
<td>105±12</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>Urinary protein excretion (gm/24hr)</td>
<td>0.08±0.03*</td>
<td>0.8±0.4*</td>
<td>0.18±0.01*</td>
<td>1.1±0.5*</td>
<td>2.7±1*</td>
<td>0.03±0.01</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>36.8±4*</td>
<td>66.7±10*</td>
<td>47.2±3*</td>
<td>67.5±8.58*</td>
<td>196±15*</td>
<td>20.5±2</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.97±0.07*</td>
<td>2.3±0.5*</td>
<td>1.06±0.2*</td>
<td>2.6±0.6*</td>
<td>8.1±0.3*</td>
<td>0.57±0.09</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>HbA1C%</td>
<td>6.5±1*</td>
<td>6.8±0.9*</td>
<td>9±0.8*</td>
<td>9.9±0.8*</td>
<td>7.5±0.6*</td>
<td>4.8±0.7</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>osteopontin (ng/ml)</td>
<td>45.5±3.6*</td>
<td>53.3±3.1*</td>
<td>58±3.4*</td>
<td>66.2±0.5*</td>
<td>80.9±9*</td>
<td>21.6±3.4</td>
<td>0.0001 HS</td>
</tr>
</tbody>
</table>

This table shows statistically significant difference between the studied groups as regard different variables by using one way ANOVA test.

Table (2) Comparison between the studied groups as regard other laboratory data:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>167±17*</td>
<td>185±20*</td>
<td>197±32*</td>
<td>105±12</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>Urinary protein excretion (gm/24hr)</td>
<td>0.44±0.02*</td>
<td>0.64±0.04*</td>
<td>2.7±1*</td>
<td>0.03±0.01</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>51.7±6*</td>
<td>57.3±5*</td>
<td>196±15*</td>
<td>20.5±2</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>Serum cr.(mg/dl)</td>
<td>1.6±0.2*</td>
<td>1.8±0.5*</td>
<td>8.1±0.3*</td>
<td>0.57±0.09</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>HbA1C</td>
<td>6.6±1.4*</td>
<td>6.8±0.9*</td>
<td>7.5±0.6*</td>
<td>4.8±0.7</td>
<td>0.0001 HS</td>
</tr>
<tr>
<td>OPN(ng/ml)</td>
<td>49±3.6*</td>
<td>62±4*</td>
<td>80.9±9*</td>
<td>21.6±3.4</td>
<td>0.0001 HS</td>
</tr>
</tbody>
</table>

This table shows statistically significant difference between the studied groups as regard different variables by using one way ANOVA test.
Fig. 1: Box plot for comparison of different groups as regard osteopontin

Table (3) Comparison between the studied groups as regard osteopontin

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I A</th>
<th>Group I B</th>
<th>Group II A</th>
<th>Group II B</th>
<th>Group III</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I A</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td></td>
</tr>
<tr>
<td>Group I B</td>
<td>0.001 HS</td>
<td>0.01 S</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td></td>
</tr>
<tr>
<td>Group II A</td>
<td>0.01 S</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td></td>
</tr>
<tr>
<td>Group II B</td>
<td></td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td>0.001 HS</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001 HS</td>
</tr>
</tbody>
</table>

LSD (least significant difference) post hoc test that shows that controls and group I A had the lower level compared to other groups with significant difference by ANOVA test.
### Table (4) Correlation between OPN versus different variables among different groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I r/P</th>
<th>Group II r/P</th>
<th>Group III r/P</th>
<th>Controls r/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>0.67/0.0001* HS</td>
<td>0.17/0.33</td>
<td>0.22/0.11</td>
<td>0.51/0.05</td>
</tr>
<tr>
<td>24 hr. urinary protein excretion (mg/24hr)</td>
<td>0.77/0.0001* HS</td>
<td>0.60/0.0001* HS</td>
<td>0.09/0.67</td>
<td>0.20/0.33</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>0.80/0.0001* HS</td>
<td>0.55/0.0001* HS</td>
<td>0.13/0.40</td>
<td>0.17/0.30</td>
</tr>
<tr>
<td>Serum cr.(mg/dl)</td>
<td>0.76/0.0001* HS</td>
<td>0.63/0.0001* HS</td>
<td>0.20/0.40</td>
<td>0.09/0.60</td>
</tr>
<tr>
<td>HBA1C</td>
<td>0.07/0.14</td>
<td>0.62/0.0001* HS</td>
<td>0.30/0.12</td>
<td>0.13/0.66</td>
</tr>
</tbody>
</table>

*r-value shown in the table and P represented as * for significant*

This table shows statistically positive correlation between serum osteopontin versus all variables except HBA1C in group I, and FBS in group II by using Spearman correlation coefficient test.

### Table (5) Validity of OPN in different stages of nephropathy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Microalbuminuria&lt;300</th>
<th>Macroalbuminuria&gt;300</th>
<th>ESRD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best cut off</td>
<td>44</td>
<td>50</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>0.25</td>
<td>0.49</td>
<td>0.98</td>
<td>0.100</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>67%</td>
<td>90%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>30%</td>
<td>47%</td>
<td>89%</td>
<td>70%</td>
</tr>
<tr>
<td>PPV</td>
<td>40%</td>
<td>50%</td>
<td>94%</td>
<td>78%</td>
</tr>
<tr>
<td>NPV</td>
<td>70%</td>
<td>92%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

This table shows that OPN is highly valid in overall prediction of nephropathy and ESRD more than prediction of microalbuminuria.
Figure 2: Prediction of Microalbuminurea
Figure 3: Prediction of Macroalbuminurea
Figure 4: Prediction of ESRD
Figure 5: Overall Prediction of Nephropathy