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# Evaluation Of OsteopontinIn Diabetic Nephropathy In Patients With Type2 Diabetes Mellitus

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***Abstract***

***Background:****Diabetic nephropathy is the most common cause of end stage renal disease (ESRD) that is associated with high rates of morbidity and mortality. osteopontin (OPN) is a secreted phosphorylated glycoprotein that mediates diverse biological functions. The study aimed to determine the relation between serum OPN and the progression of diabetic nephropathy in patients with type 2 DM. That may open a door for early prediction of diabetic nephropathy.* ***Methods:****This case-control study was conducted in Internal Medicine, Endocrinology Unit and Biochemistry Departments in Zagazig University Hospital. This study was carried out on 96 patients devided into: Group 1: 20 age and sex matched healthy volunteers (control group). Group 2: 38 patients with type 2 DM without nephropathy (DWN group) . Group3:38 patients with type 2 DM with nephropathy(DN group).Serum osteopontin concentrations was measured for all cases .****Results:****HOMA-IR increased significantly in DN group(8.59 ± 1.34) compared with DWN group(4. 73 ± 0.59) and control group(0.90 ± 0.18). serum osteopontin level( ng/ml) increased significantly in the DN group(258.52 ± 46.93 ng/ml) compared with DWN group(159.12 ± 19.56 ng/ml) and control group (70.90 (± 20.47 ng/ml)(p value =0.000).there a high significant correlation between S. OPN and HOMA-IR .****Conclusion:*** *Type 2 diabetic patients with or with out nephropathy increased osteopontin levels than control group. Serum osteopontin may be considered as an early prognostic marker for the risk of nephropathy in patients with type 2 diabetes mellitus.*

***Key words:*** *osteopontin- diabetic nephropathy-prediction.*

## I. Introduction:

Diabetic nephropathy is the most common cause of end stage renal disease (ESRD) that is associated with high rates of morbidity and mortality.It is of utmost importance to emphasize the early identification and treatment of this chronic complication which would reduce the medical and economic burden associated with it **(1).**

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Although microalbuminuria remains the gold standard marker for early detection of DN, it is not a sufficiently accurate predictor of DN risk given some limitations. For example, not all diabetics with microalbuminuria will end up with ESKD and 30% of them may actually have normoalbuminuria, while several biomarkers of glomerular or tubular dysfunction can precede microalbuminuria, suggesting that microalbuminuria is present once significant renal injury has already occurred**(2).**

OPN is a secreted phosphorylated glycoprotein that mediates diverse biological functions. Originally isolated from bone, OPN was later shown to have a wider distribution . In adults, OPN expression is normally limited to the bone, kidney, and epithelial linings, and is secreted in body fluids including milk, blood and urine . In contrast to its restricted distribution in normal tissue, OPN is strikingly upregulated at sites of inflammation and tissue remodeling **(3).**

The study aimed todetermine the relation between serum OPN and the progression of diabetic nephropathy in patients with type 2 DM. That may open a door for early prediction of diabetic nephropathy.

## II. Patients and Methods

This case-control study was conducted in Internal Medicine, Endocrinology Unit and Biochemistry Department in Zagazig University Hospitals during the period from October 2018 to April 2019.

***Patients:***

This study was carried out on 96 patients devided into:

- **Group 1:**20 age and sex matched healthy volunteers (control group).
- **Group 2:** 38 patients with type 2 DMwithout nephropathy (DWN group) .
- **Group3:**38 patients with type 2 DM with nephropathy(DN group).

***Inclusion Criteria***

Age: 35- 70 years old

Diabetic patients with Normoalbuminuria when urinary albumin excretion (UAE) < 30 mg/24hr.

***Diabetic nephropathy patients with:***

- Microalbuminuria when the UAE in the range of 30–299 mg/24hr.
- Macroalbuminuria when the UAE ≥ 300 mg/24 hr .

***Exclusion criteria:***

- Conditions of obesity ,gravis disease and liver cell failure.
- Acute inflammatory illness (including a common cold, infections) as it can affect the serum OPN level.
- Autoimmune diseases as multiple sclerosis,rheumatoid arthritis and systemic lupus erythmatosis
- Malignancy as lung cancer and stomach cancer.
- Inflammatory bowel disease (IBD).
- Bone and muscle diseases as osteoarthritis and Duchenne muscular dystrophy.

- Patients with end-stage renal disease or on dialysis.

***Methods:***

All subjects were submitted to the following:

- **Patient consent was obtained from all subjects.**
- **Full history taking:** including age, sex,
- **Anthropometric measurements:** Weight in kilograms, height in meters) and Body Mass Index (BMI). BMI = weight (kg) / height (m2)
- **Laboratory investigations:**

***Measurement of insulin resistance***

By homeostasis model assessment (HOMA IR) index was calculated by the following formulaHOMA-IR calculated by this equation

**HOMA-IR =** $\frac{\mathbf{FBG\ (mg/dL\ )XFINS\ (\ mU/L)}}{\mathbf{405}}$ **according to ADA**

HOMA-B calculated by this equation **HOMA-B =** $\frac{\mathbf{360XFINS\ (mU/L)}}{\mathbf{[FBG\ (mg/dL)\ -63\ ]}}$ **according to ADA .**

***Measurment of Serum OPN by ELISA technique***

***principle***

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Osteopontin(OPN)in samples.

***Statistical Analysis***

A collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage , quantitative continues group represent by mean ± SD , the following tests were used to test differences for significance;. difference and association of qualitative variable by Chi square test ($X^2$) . Differences between quantitative independent groups by t test, correlation by Pearson's correlation or Spearman's . P value was set at <0.05 for significant results &<0.001 for high significant results. Data were collected and submitted to statistical all the following statistical tests and parameters were used .ONE Way ANOVA followed by post hoc analysis using LSD test. Receiver operating characteristic curve (ROC) was used to assess the best cut off point for predictors of severity with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC).The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:P-value> 0.05: Non significant (NS)P-value < 0.05: Significant (S)

## III. Results:

This table shows that, no significant difference between the studied groups regarding age and sex, p value >0.05. On other hands shows highy significant difference regarding body mass index(BMI), systolic blood pressure, Diastolic blood pressure and duration of diabetes mellitus**(Table 1)**.

| | | **Control group** | **DWN group** | **DN group** | **Test value** | **P-value** | **Sig.** | **Post Hoc analysis by LSD** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **No. = 20** | **No. = 38** | **No. = 38** | | | | **P1** | **P2** | **P3** |
| **Age (years)** | Mean± SD | 47.85 ± 7.88 | 45.53 ± 5.93 | 48.97 ± 5.14 | 3.114• | 0.052 | NS | — | — | — |
| | Range | 37 – 64 | 37 – 65 | 40 – 62 | | | | | | |
| **Sex** | Females | 10 (50.0%) | 19 (50%) | 19 (50%) | 0.603* | 0.740 | NS | — | — | — |
| | Males | 10 (50.0%) | 19(50%) | 19(50%) | | | | | | |
| **BMI (kg/m$^2$)** | Mean± SD | 22.89 ± 1.57 | 30.29 ± 3.04 | 29.27 ± 3.00 | 49.700 • | 0.001 | HS | 0.001 | 0.001 | 0.115 |
| | Range | 20.3 – 25.6 | 24.8 – 34.8 | 23.7 – 37.6 | | | | | | |
| **Systolic BP (mmHg)** | Mean± SD | 119.00 ± 3.08 | 134.03 ± 5.83 | 140.53 ± 8.14 | 72.845 • | 0.001 | HS | 0.001 | 0.001 | 0.001 |
| | Range | 110 – 120 | 128 – 150 | 130 – 160 | | | | | | |
| **Diastolic BP (mmHg)** | Mean± SD | 76.00 ± 5.03 | 86.74 ± 4.71 | 87.82 ± 4.71 | 44.622 • | 0.001 | HS | 0.001 | 0.001 | 0.327 |
| | Range | 70 – 80 | 78 – 90 | 78 – 95 | | | | | | |
| **Duration of DM (years)** | Mean± SD | – | 2.69 ± 1.82 | 15.58 ± 3.96 | -18.220 •• | 0.001 | HS | — | — | — |
| | Range | – | 0.58 – 11 | 10 – 23 | | | | | | |

**Table (1):**Comparison of the mean values ± SD of demographic parameters among the different groups by ANOVA test and LSD.

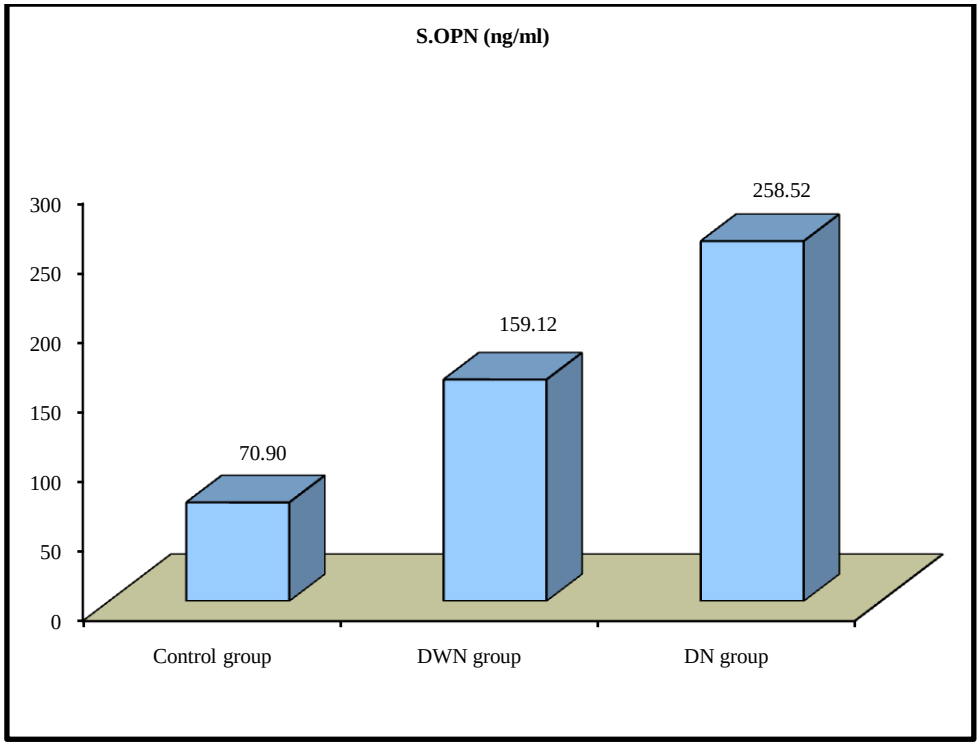


**Figure (1): Mean of serum OPN among all studied groups.**

**Table (2) and figure (1)** show highly significant difference regarding OPN(ng/ml) among all studied groups which was more in DN group than the other groups and less in control group.

**Table (2):** Comparison among all studied groups as regarding Serum OPN by ANOVA test and LSD.

| | | **Control group** | **DWN group** | **DN group** | **Test value** | **P-value** | **Sig.** | **Post Hoc analysis by LSD** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **No. = 20** | **No. = 38** | **No. = 38** | | | | **P1** | **P2** | **P3** |
| **S.OPN (ng/ml)** | Mean±SD | 70.90 ± 20.47 | 159.12 ± 19.56 | 258.52 ± 46.93 | 219.401 | 0.001 | HS | 0.001 | 0.001 | 0.001 |
| | Range | 42.9 – 103.4 | 125.7 – 204.8 | 204.8 – 389.7 | | | | | | |

**Table(3)**cut off point, sensitivity and specificity of serum OPN for detection of diabetic nephropathy between DN group and Control group **.**

| Parameter | AUC | Cut off Point | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| S.OPN(ng/ml) | 0.895 | >137.3 | 82.05 | 85.00 | 97.0 | 73.1 |

**Table (3)** showed that the best cut off point of serum OPN level was >137.3 between DN group and Control group with sensitivity to detect diabetic patients with nephropathy (82.05%) and specificity to exclude diabetic patients without nephropathy(85%).

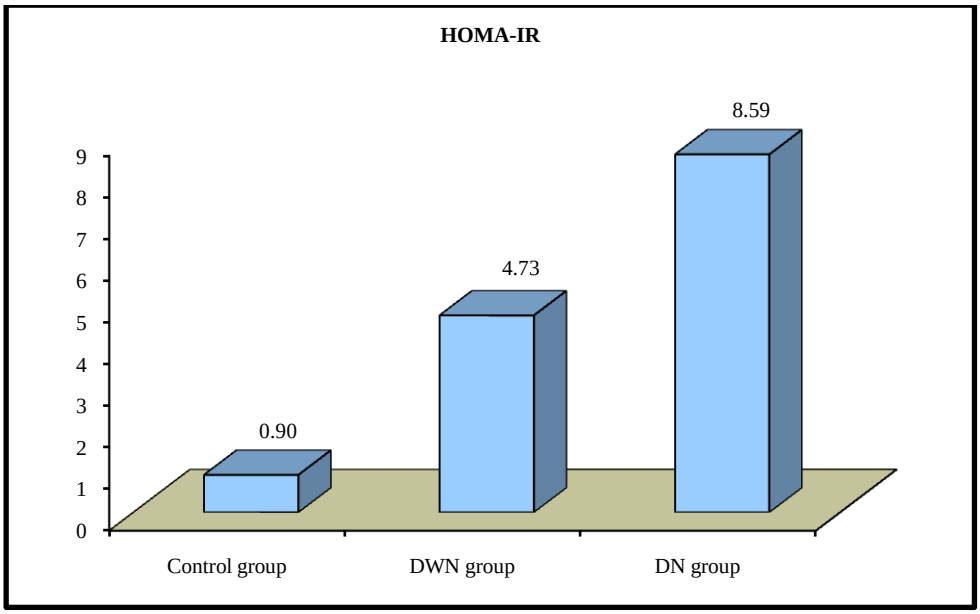


**Figure (2): Mean value of HOMA-IR among all studied groups**

**Figure (2)** shows higher (HOMA-IR) in DN group more than other groups and high HOMA-IR in DWN group more than control group.

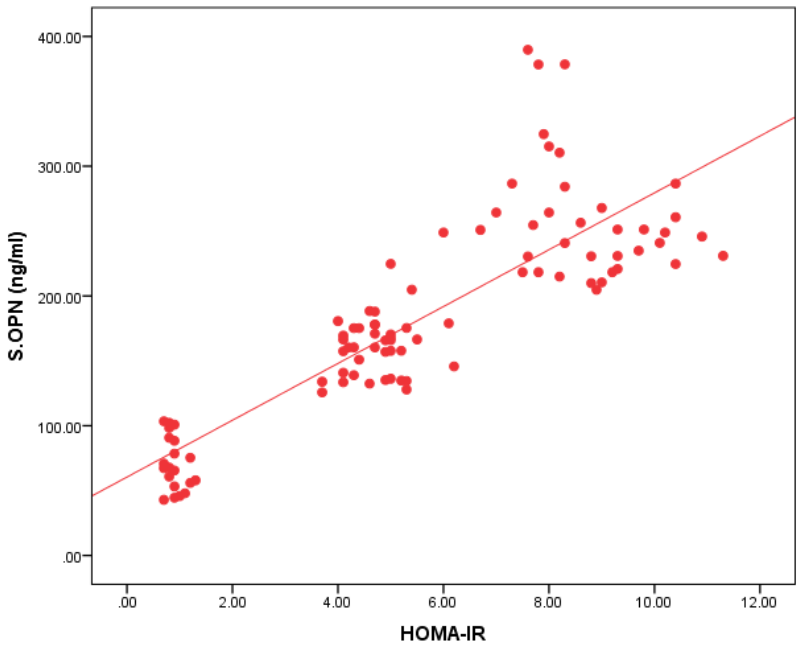


**Figure (3)** Correlation between serum OPN and HOMA-IR.

**Figure (3)**shows a positive correlation between serum OPN and HOMA-IR(r=0.855.p=0.001).

## IV. Discussion

In our study, we found a significant difference in the duration of (DM) between groups as the patients with diabetic nephropathy showed longer duration of DM (15.58 ± 3.96 ) years than patients with type 2 diabetes without nephropathy (2.69 ± 1.82)years.

In our study, BMI significantly increased among DWN group ( 30.29 ± 3.04 kg/$m^2$) compared to DN group ( 29.27 ± 3.00 kg/$m^2$) and Control group ( 22.89 ± 1.57 kg/$m^2$) (p value is <0.001).The present result support previous findings which show that patients with high BMI are more risk for insulin resistanceand diabetic nephropathy than those patients with low BMI. This was consistent with the results of ***Dennis, et al.*****(4)**Obesity (BMI ≥30 kg/$m^2$) is significant risk for insulin resistance thus obese patients more risky for insulin resistance than those patients with low BMI.

Also, **Kim *and* Park**[(5)]shows thatbody mass index (BMI) has been widely used as a significant predictor for diabetes mellitus, hypertension, and dyslipidemia. In the United States, the National Health and Nutrition Examination Survey (NHANES) conducted from 2003 to 2006 showed that the BMI was positively associated with the prevalence of metabolic syndrome based on the National Cholesterol Education Program's Adult Treatment Panel III (NCEP/ATP III) criteria**.**

***Lotta et al.*****[(6)]**showed that the decreased capacity of adipocytes to store and retain triglyceride in obesity, causing ectopic fat accumulation and 'lipotoxicity' in the liver and muscle and the disruption of beneficial factors secreted from adipocytes is postulated to trigger insulin secretion, and thereby cause hyperinsulinemiaand hypertriglyceridemia that support obesity as a strong riskfactor and a potential cause of the primary insulin resistance**.**

In our study there is no significant difference between all groups regarding genderthat disagree with ***Qiu et al*** **[(7)]**who found that women before menopause, have a lower incidence of insulin resistance than men of a similar age. However, this protective effect disappears after menopause, with the incidence of insulin resistance becoming similar in men and women , which suggests that estrogen could have a protective effect. That may indicate that 17β-oestradiol protects pro-opiomelanocortin (POMC) neurons from developing insulin resistance.

In the current study, HOMA-IR increased significantly in DN group(8.59 ± 1.34) compared with DWN group(4.73 ± 0.59) and control group(0.90 ± 0.18), these findings cope with ***Silva et al***who reported that HOMA-IR is increased in patients with diabetic nephropathy and also it was reported that increased HOMA-IR index predicted insulin resistance ***(8)***.

In the present study, there is a highly statistically significant difference between study groups regarding HOMA-IR as DN group had a higher level than other groups. The mean value of HOMA-IR in DN group is 8.59 (± 1.34) and in DWN group is 4.73 (± 0.59) also the mean value of HOMA-IR in control group is 0.90 ± 0.18 (p<0.001) .

This is in agreement with ***Purohit and*****Tiwari**[(9)]who revealed that statistical significant increase of the median value of HOMA IR score is 2.91 in diabetic nephropathy group with mean value of ( 4.15 ± 3.56) and the median value of group withoutdiabetic nephropathy is1.97with mean ( 2.03 ± 0.64) (p<0.0001).

In our study, serum osteopontin level( ng/ml) increased significantly in the DN group(258.52 ± 46.93 ng/ml) compared with DWN group(159.12 ± 19.56 ng/ml) and control group (70.90 (± 20.47 ng/ml)(p value =0.000).

This was in agreement with **Yan et al [(10)]**who showed that Plasma levels of OPN was significantly higher in patients with T2DM compared with controls and there was a significant correlation between OPN and the severity of nephropathy**.**

**Al-Rubeaan et al [(11)]**also investigated the diagnostic profile of pro-inflammatory cytokines among them serum osteopontin,Which proved that the most important cytokine that were found to have significant diagnostic value was serum osteopontin which had shown an excellent diagnostic value for patients with macroalbuminuria and good diagnostic value for patients with microalbuminuria**.**

These findings were consistent with what had been earlier reported among both type 1 and type 2 diabetic patients for osteopontin and in the study among Japanese type 2 diabetic patients for IL-18(**12)**.

Previous studies have shown that advanced glycation end-products and angiotensin II can stimulate OPN synthesis by a variety of cells, including mesangial cells and podocytes, and initiate local effects of cell spreading, adhesion, and proliferation**(13).**

In our study there a high significant correlation between S. OPN and HOMA-IR .

This is in agreement with **Al-Rubeaan et al.[(11)]**who found a postive correlation between S.OPN and HOMA-IR (r= 0.174;p= 0.002)**.**

## V. Conclusion:

Type 2 diabetic patients with or with out nephropathy increased osteopontin levels than control group. Serum osteopontin may be considered as an early prognostic marker for the risk of nephropathy in patients with type 2 diabetes mellitus.

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