ASSOCIATION OF OSTEOPONTIN (OPN) LEVEL IN DIABETIC NEPHROPATHY PATIENTS AS AN EARLY DIAGNOSTIC MARKER

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ABSTRACT

OBJECTIVES

The study aimed to find an association between OPN levels and diabetic nephropathy as a serum marker in DM patients for early detection.

METHODOLOGY

A cross-sectional study was conducted for 6 months on 277 diagnosed cases of DM with 10 positive and 10 negative controls collected through non-probability sampling. Fasting blood sugar, HbA1c, serum urea and creatinine levels of known diabetic patients were measured to confirm their disease status and OPN using an ELISA Kit and compared in DM patients and controls. Statistical analysis was performed using SPSS v22.0. DM patients and negative controls were compared using unpaired one-way ANOVA and least square methods. The p-value of ≤0.05 was deemed statistically significant.

RESULTS

Osteopontin has a significant relation with diabetes duration only. Serum biomarkers of the DM show significant osteopontin levels with 0.00 p values for HBA1C and all biomarkers of Diabetes. The least-square distribution of the different biomarkers with osteopontin level at 95 % confidence interval and standardized coefficients show lower and upper bound and significant levels. Osteopontin levels show 0.29 and 0.38 lower and upper bound at a 95% confidence interval, with a 0.79 considerable level and 0.25 t distribution, with 0.01 beta values for diabetes duration. Blood creatinine level is 0.02 and 0.05 lower and upper bound, with 0.36 significance level for Osteopontin and 0.02 standard error. Osteopontin levels 11ng/dl to 20 ng/dl have been found in 88 cases, while 09 in positive controls and 12 in negative controls. Meanwhile, only two positive controls were found>20 ng/dl osteopontin level.

CONCLUSION

Serum (OPN) was positively correlated in positive controls and low levels in negative control and regular diabetic patients with no nephropathy.

KEYWORDS: *Association, ANOVA, DM (Diabetes Mellitus), OPN (Osteopontin), Nephropathy*

INTRODUCTION

Diabetes Mellitus (DM) results from a prolonged elevation of blood glucose levels, either an absolute or relative insulin insufficiency.[1] Diabetes Mellitus (DM) is a widespread health condition worldwide, affecting about 8.5% of the population worldwide and 16% in Pakistan. This includes newly diagnosed cases and previously existing diabetic patients.[9] According to the World Health Organization, Diabetes Mellitus is the 4th most crucial non-communicable disease affecting the population worldwide.[2] Diabetes Mellitus affects almost all parts of the body, especially the urogenital system, cardiovascular system, and eyesight due to microangiopathies. Renal involvement of Diabetes mellitus may result in chronic renal failure requiring dialysis, which is a very costly procedure and treatment.[4] Current studies have shown the role of the RAS (Renin angiotensin) protein system in developing chronic kidney diseases, especially in diabetic patients.[5] Patients suffer proteinuria, perhaps due to modifications in the RAS (Renin Angiotensin) protein system responsible for kidney protein preservation. Once the Diabetes is established, it may result in kidney failure if left untreated.[6] Some studies suggest that people with both Type-I and Type-II diabetes may have fewer podocytes in their glomeruli. This decrease in podocytes may happen simultaneously with the start of protein in the urine.[7] Control of diabetic nephropathy mainly depends on early detection and prompt treatment. Early detection of diabetic nephropathy can be done using new biomarkers like Osteopontin.[8] Osteopontin (OPN) is a protein that sticks to calcium, the substance found in bone, the part that filters urine in

the kidneys and the cells that cover blood vessels.[9] The severity of Diabetic nephropathy largely depends on the serum osteopontin level. A study conducted by Yamaguchi et al. revealed a relationship between nephropathy and osteopontin levels in 301 diabetic nephropathy patients. They observed that plasma osteopontin concentration was raised compared to other biomarkers inside the urinary sample of diabetic patients compared to the control group.[10] The current study is done to mark the presence of osteopontin levels in diabetic nephropathic patients as an early detection marker as compared to other biomarkers.[11]

METHODOLOGY

A cross-sectional study with positive and negative control was conducted at various tertiary care Hospitals, including Pakistan Kidney Center Abbottabad, King Abdullah Teaching Hospital Mansehra and Ayub Medical College Abbottabad for 6 months. Sample Size was collected for 277 diagnosed cases of DM with 10 positive controls (established cases of diabetic nephropathy patients) and 10 negative controls (normal individuals) having no history of Diabetes collected through non-probability consecutive sampling. Patients with diabetic nephropathy were taken as the cases, while the positive control were the patients with no diabetic nephropathy despite a positive history of Diabetes. Negative controls were the normal individuals with no diabetes and diabetic nephropathy. Patients with post-menopausal women, Osteomalacia and patients having inflammatory conditions. Duration of Diabetes mellitus in the cases was recorded on pre-structured proforma along with other demographic data. Fasting blood sugar, HbA1c, serum urea and creatinine levels of known diabetic patients were measured to confirm their disease status. According to the manufacturer's instructions, Osteopontin was measured using an ELISA Kit (BioAssay USA). Osteopontin levels of the DM patients and controls were compared. Statistical analysis was performed using (SPSS) v22.0. DM patients and negative controls were compared by using unpaired one-way ANOVA. The p-value of ≤0.05 was deemed statistically significant. Investigated data was displayed on charts and a tabletop.

RESULTS

Table 1 Demonstrates ANOVA grades of the different demographic variables based on serum osteopontin levels. Diabetes duration is significantly related to osteopontin level with p values 0.00 and no significant relation with other demographic variables. Table 2 shows that the different serum biomarkers of Diabetes mellitus changed due to fluctuating blood sugar levels in the body variable based on serum osteopontin levels. It shows that osteopontin level shows 0.00 p values for HBA1C, with osteopontin level, which means Osteopontin has only a significant relation with all biomarkers of Diabetes. Table 3 shows the serum osteopontin level in three different study groups, including positive control groups, negative control groups and diabetic patients in the study groups. Table 4 shows the serum osteopontin level in three different study groups, including positive control groups, negative control groups and diabetic patients in the study groups in which significant levels were shown in diabetic nephropathic patients.

Table 1: The Different Variables Based on Serum Osteopontin Levels

		Total of Squares	df	Total Mean Squares	F	Sig.
Gender	Cases	15.81	72	0.22	0.88	0.73
	Controls	50.90	204	0.25		
	Total	66.71	276			
Diabetes Duration	Cases	53.62	72	0.74	1.70	0.00
	Controls	89.12	204	0.43		
	Total	142.75	276			
Age in Years	Cases	8345.41	72	115.90	0.90	0.68
	Controls	26093.00	204	127.90		
	Total	34438.419	276			

Table 2: ANOVA Results of Different Variables Like Serum

		Total Squares	Df	Mean of Squares	F	Sig.
HBA1c	Cases	20626.14	72	286.47	59.18	0.00
	Controls	844.67	204	4.14		
	Total	21470.82	276			
Micro-albumin level mg/dl	Cases	1210522.13	72	16812.80	11.10	0.00
	Controls	308797.81	204	1513.71		
	Total	1519319.95	276			
Blood Creatinine level mg/dl	Cases	7787.92	72	108.16	7.83	0.00
	Controls	2815.22	204	13.800		
	Total	10603.14	276			
Blood Urea (mg/dl)	Cases	498599.94	72	6924.99	5.97	0.00
	Controls	236505.72	204	1159.34		
	Total	735105.66	276			
Fasting Blood Sugar(mg/dl)	Cases	492982.47	72	6846.97	2.05	0.00
	Controls	680352.43	204	3335.06		
	Total	1173334.90	276			

Table 3: ANOVA Test Comparisons among All Studied Groups

		Positive Control Group	Negative Control Group	Diabetic Group	Test Value	P-value
		10	10	277		
S.OPN(ng/ml)	Mean ± SD	71.80 ± 20.37	169.12 ±20.56	278.42 ± 48.83	229.301	0.001
	Range	41.8 – 101.4	135.7 – 214.7	214.8 – 379.8		

Table 4: Comparison of Adverse Effects in Both Groups

		Osteopontin level ng/dl			Total
		<1 to 10	11 to 20	>20	
Study Population	Cases	183	88	00	271
	Positive Controls	02	09	02	13
	Negative Controls	01	12	00	13
Total		186	109	02	297

DISCUSSION

Osteopontin (OPN) is a multifunctional protein associated with Type II Diabetes and disorders affecting patients' vascular architecture. However, the specific role of Osteopontin in individuals with Type-1 diabetes remains unclear.[14,15] Our study aims to investigate the role of Osteopontin in the progression of Diabetes mellitus, utilizing it as a biomarker for the study. Several studies have highlighted the inflammatory role of Osteopontin in diabetic nephropathy, with elevated levels of Osteopontin being consistently observed.[16] Numerous studies have explored the link between osteopontin levels and the impact of Diabetes mellitus in affected individuals.[17] Various investigations have indicated the inflammatory role of Osteopontin in diabetic nephropathy, with higher concentrations of Osteopontin being observed.[17] Our study found that patients with diabetic nephropathies with a duration exceeding five years exhibited significantly higher osteopontin levels.[18] The investigation's results illustrate the serum levels of various diabetic indicators, with fasting blood sugar averaging 267 mg/dl. In line with our research, Yamaguchi and colleagues explored osteopontin levels, observing diabetic complications in microvascular scenarios among 229 patients with Type II Diabetes. This manifested as advanced retinopathy, noticeable neuropathy, and more evident nephropathy in both plasma and urine. Notably, osteopontin levels showed a substantial and marked increase as nephropathy advanced. However, discernible changes in osteopontin development were not noted in retinopathy or neuropathy.[18] It has been determined that osteopontin levels are a significant predictor exclusively in end-stage disorders of renal origin, with no notable impact. In a study within a multiethnic cohort by Zhang, it was observed that Osteopontin levels were significantly higher in subjects with advanced established diabetic nephropathy (64.7 ng/mL) compared to diabetic patients without diabetic nephropathy (51.7 ng/mL; $p<0.001$). These elevated Osteopontin levels have been linked to the onset and severity of diabetic nephropathy, establishing Osteopontin as a potential biomarker for diabetic nephropathy. These findings align with the research conducted by El Dayem, which examined the correlation between high Osteopontin levels and diabetic nephropathy in eighty patients with Type I Diabetes.[19] Elevated OPN is intricately involved in severe and extensive vascular calcification and plays a role in mineral metabolism. Osteopontin is a crucial element in the development of calcification and dysfunction in vascular epithelial structures, resulting in nephropathy.[20] Individuals with diabetic nephropathy exhibited a prolonged DM duration compared to those with Type II Diabetes without nephropathy. Additionally, a noteworthy increase in BMI was noted in both cases and controls. This aligns with existing research, indicating a predisposition to insulin resistance and diabetic nephropathy in individuals with higher BMI.[21] Consistent with earlier research, all diabetic patients in our study exhibited elevated osteopontin levels, particularly those with diabetic nephropathy, marked by positive microalbuminuria.[22]

LIMITATIONS

The limitation of this research stems from its single-centre focus. Moreover, assessing vascular dysfunction as a cause of microvascular issues relies solely on vascular markers, neglecting alternative factors. It's crucial to acknowledge the potential influence of medications on Osteopontin, necessitating confirmation across diverse populations.

CONCLUSIONS

Serum (OPN) levels marked in diabetic nephropathy patients and positive controls were high as compared to low levels in negative control.

CONFLICT OF INTEREST: None

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