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Albuminuria Independently Determines Intact Parathyroid Hormone in Diabetic Nephropathy with Micro-albuminuria

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ABSTRACT
Background: Diabetes mellitus (DM) is a well-recognised cause of end-stage renal disease (ESRD) through diabetic nephropathy (DN). Elevated intact parathyroid hormone (intact PTH) in DN represents a serious metabolic threat.
Objective: We aimed to detect determinants of intact PTH changes during micro-albuminuria stage of DN.
Patients and Methods: An observational study included 294 patients with type 2 diabetes mellitus (T2D) divided into 2 groups. Case group; 220 patients of DN with micro-albuminuria versus control group; 74 patients without albuminuria. Participants were subjected to history taking, clinical examination and laboratory assessment of glycosylated haemoglobin (HBA1c), serum creatinine, blood urea nitrogen (BUN), urine albumin creatinine ratio (urine ACR), estimated glomerular filtration rate (eGFR), 25(OH) vitamin D, intact PTH, serum ionized calcium, phosphorus and magnesium.
Results: Among 220 patients of mean age 48.7 years and 57.7% male, albuminuria was significantly higher (P<0.001). We found non-significant difference between the study groups in estimated glomerular filtration rate (eGFR) and serum creatinine (P=0.375 and 0.294) respectively. Meanwhile, intact PTH was significantly elevated (P<0.001) in case group. Intact PTH was positively correlated with urine ACR, HBA1c, BUN, and serum creatinine and inversely correlated with eGFR. However, urine ACR was the only independent determinant of intact PTH in diabetic patients with micro-albuminuria (OR: 1.177; 95% CI: 1.074-1.290, P= 0.001).
Conclusion: Interestingly, urine albumin creatinine ratio was the only independent determinant of intact PTH in diabetic patients with micro-albuminuria.
Keywords: Diabetic nephropathy, Intact PTH, Micro-albuminuria.

INTRODUCTION

Diabetic nephropathy (DN) is a prevalent cause of end-stage renal disease (ESRD). Hyperglycaemia causes structural and functional disruption of the glomerular capillaries and renal tubules, resulting in a progressive illness [(1)].

Early detection of diabetic nephropathy (DN) represents a serious clinical challenge to reduce the burden of chronic kidney disease (CKD) [(2)]**.** Despite the contradictory data concerning sensitivity and specificity of the urine albumin creatinine ratio (urine ACR), it is often used to assess the severity of DN. Persistent micro-albuminuria (urine ACR =30-300 mg/g) is considered an important marker and predictor of DN and its progression to ESRD [(3,4)]. To date, the pathogenesis of DN is not fully understood. The increased urinary albumin excretion in DN may be attributed to defects in the glomerular membrane filtration and endothelial damage due to hyperglycaemia, oxidative stress, ischemia, and inflammation [(5,6)].

Mineral and metabolic bone disorders (MBD) associating chronic kidney disease (CKD) start early and worsen with the progression of CKD particularly among patients with DN.

In incipient CKD, the precise metabolic alterations that lead to secondary hyperparathyroidism is still unclear [(7)]. Management of metabolic bone disorder (MBD) is an integral component of CKD treatment. Serum intact parathyroid hormone (intact PTH) is the key target for the management of CKD-MBD [(8)]**.**

In the current study, we aimed to address the clinical and biochemical alterations in diabetic patients with micro-albuminuria. We tried to clarify the relationship between intact PTH and other metabolic parameters in this early stage of DN.

PATIENTS AND METHODS

An observational case-control study conducted at the outpatient department of our institutional hospital, during the period from July 2019 to December 2019.

Our study included 294 participants of two groups of patients with type 2 diabetes mellitus (T2D) of matched age and sex: Case group; 220 patients with micro-albuminuria (urine ACR=30-300mg/g) and control group; 74 patients without albuminuria. Egyptian patients aged (40-60) years with glycosylated haemoglobin (HBA1c) 7.0-9.5% and estimated glomerular filtration rate (eGFR) ≥ 50 mL/min/1.73 m² were enrolled in this study. After signing written informed consent, all participants were subjected to

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detailed medical history, clinical examination with anthropometric measures. Blood samples were obtained in the morning following a 12-hr overnight fasting. The sera were withdrawn for assessment of serum ionized calcium, phosphorus, magnesium, blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG). Morning spot-urine albumin/creatinine ratio (urine ACR) was repeated three times prior enrolment to confirm the existence of micro-albuminuria.

Glycosylated haemoglobin (HBA1c) estimation, radioimmunoassay of 25(OH) vitamin D, intact PTH level measured by enzyme-amplified sensitive immunoassay, and eGFR calculated using the Modification of Diet in Renal Disease (MDRD) equation [9] were applied.

Abdominal and neck ultrasonography were done for all participants meanwhile, neck computed tomography (CT) scan with contrast was done for suspected cases of parathyroid adenoma. All participants were settled on their regular medications for more than 6 months prior enrolment.

Exclusion criteria

We excluded patients with macro-albuminuria; urine ACR > 300 mg/g, patients with eGFR<50 mL/min/1.73m^2, other causes of CKD like lupus nephritis, obstructive nephropathy, infection induced nephropathy, drug-induced kidney injury, pre-renal, ischemic or congenital renal disorders, history of diabetic ketoacidosis, co-administration of medications that may alter renal function or electrolyte state such as (NSAIDS, steroid, immunosuppressant, diuretics, hormonal therapy, etc.), patients with parathyroid adenoma and patients with severe comorbidities such as malignancy, heart, respiratory or hepatic failure.

Ethical approval and consent to participate:

The study was approved by the Institutional Review Board for Clinical Research Committee (IRB) of Mansoura University with approval number (No.R.19.07.562).

All procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was approved by the IRB and signed by all participants.

Statistical analysis

Data were fed to the computer and analysed using IBM SPSS Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using number and percentage. Quantitative data were described using mean ± standard deviation for parametric data after Kolmogrov-Smirnov test of normality.

Linear correlations were performed by Pearson's correlation coefficient test. Forward stepwise logistic regression analysis of all variables related to high PTH with odds ratio and 95% confidence interval (95% CI) was applied. Significance was judged at the (0.05) level.

RESULTS

Among 220 patients with micro-albuminuria of a mean age 48.7 years, and 57.7% male, the duration of DM, body-weight (BW), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly elevated in case group (P<0.001).

Serum albumin values were significantly reduced in case group than control group (P= 0.0264). We found non-significant difference regarding HBA1c (P = 0.339), eGFR (P= 0.375) and serum creatinine (P=0.294) between the study groups meanwhile, urine ACR and BUN were significantly elevated in case group (P <0.001).

Interestingly, 25(OH) vitamin D was significantly elevated in case group (P<0.001). Despite the non-significant difference between the study groups regarding serum ionized calcium (P= 0.310), intact PTH and serum phosphorus were significantly elevated in case group (P<0.001) (Table 1).

Table (1): Demographic characteristics of the study groups.

Variable	Control group		Case group		P-value
	Mean	(±)SD	Mean	(±)SD	
Age (year)	47.71	7.12	48.71	8.62	0.464
Sex Male No (%)	41	53.94%	127	57.72%	0.188
Duration (year)	5.56	3.56	7.85	3.46	<0.001*
Weight (kg)	87.55	7.26	96.55	11.77	<0.001*
Height (cm)	169.38	7.016	166.70	7.779	0.3733
BMI (kg/m²)	30.74	3.45	34.68	3.59	0.1812
SBP (mmHg)	137.03	10.52	148.02	9.79	<0.001*
DBP (mmHg)	86.51	8.40	93.86	6.81	<0.001*
ALT (IU/L)	38.86	5.59	27.30	5.54	<0.001*
AST (IU/L)	27.73	5.76	21.12	4.50	<0.001*
Albumin (g/dL)	4.512	0.58	4.189	0.441	0.0264
Serum creatinine (mg/dL)	1.013	0.193	1.001	0.186	0.294
Estimated GFR(ml/ min/1.73m²)	76.23	5.015	78.32	2.959	0.375
BUN(mg/dL)	15.025	3.85	24.900	4.77	<0.001*
Urine ACR(mg/g)	16.22	4.94	135.71	6.62	<0.001*
HBA1c (%)	8.52	1.32	8.72	1.22	0.3339
25(OH)Vitamin D(ng/mL)	31.78	5.62	34.40	5.9	<0.001*
Intact PTH (ng/L)	27.99	5.87	97.26	8.85	<0.001*
Ionized calcium (mg/dL)	1.203	0.074	1.179	0.091	0.3104
Phosphorus (mg/dL)	3.18	0.33	3.75	0.50	<0.001*
Magnesium (mg/dL)	2.01	0.25	1.94	0.25	0.0215*

¶Data expression: mean ± SD & No (%), * significant value (P<0.05)

Table (2) shows the significant correlations between intact PTH and various studied parameters in patients with DN. Intact PTH was positively correlated with urine ACR, HBA1c, BUN, serum creatinine and inversely correlated with eGFR.

Table (2): Correlation between intact PTH with clinical and biochemical parameters in patients with DN.

Parameters	Intact PTH	
	r	P-value
Age (years)	.049	0.472
Body weight (kg)	.013	0.846
Height (cm)	0.039	0.568
BMI (kg/m²)	-.050	0.462
HBA1c (%)	0.286	<0.001*
Serum creatinine (mg/dL)	0.195	0.004*
BUN (mg/dL)	0.287	<0.001*
eGFR (ml/ min/1.73m²)	-0.215	0.001*
Urine ACR (mg/g)	0.756	<0.001*
25(OH) vitamin D (ng/mL)	-0.078	0.251
Ionized Calcium (mg/dL)	-0.013	0.847
Phosphorus (mg/dL)	0.039	0.570
Magnesium (mg/dL)	0.129	0.057
Serum albumin (g/dL)	0.021	0.762
ALT (IU/L)	-0.019	0.779
AST (IU/L)	0.034	0.611

¶r= correlation coefficient, * significant value (P<0.05)

With multiple logistic regression analysis of the significant determinants of high intact PTH, urine ACR was the only independent determinant of intact PTH level in patients with micro-albuminuria (OR 1.177, 95% CI 1.074-1.290, P = 0.001) as shown in table (3).

Table (3): Multiple logistic regression analysis of high intact PTH determinants.

Variable	Odds ratio	95% Confidence interval	P-value
Urine ACR (mg/g)	1.177	1.074-1.290	0.001*

* Significant value (P<0.05)

DISCUSSION

In the current study, intact PTH was significantly elevated in patients with micro-albuminuria. Intact PTH was positively correlated with urine ACR and inversely correlated with eGFR. Urine ACR was the only independent determinant of intact PTH level in patients with micro-albuminuria. Our results agreed with **Wang *et al.*** [7] who carried out a community cross-sectional study and reported the positive correlation between albuminuria and log intact PTH that was inversely correlated with eGFR even with subset analysis and adjustment of 25(OH) vitamin D, calcium and phosphorus. In accordance with **Muntner *et al.*** [10] who reported higher prevalence of hyperparathyroidism among patients with moderate CKD with a stepwise increase of intact PTH with progressive decline of eGFR. Similarly, **Okamoto *et al.*** [11] reported a positive linear correlation between intact PTH and CKD progression, along with a significant negative correlation between intact PTH and eGFR in moderate CKD.

In our study, though serum ionized calcium values were within normal range and showed non-significant difference between the study groups, intact PTH and serum phosphorus were significantly elevated in patients with micro-albuminuria. Surprisingly, 25(OH) vitamin D showed significant higher values in case group.

Wahl *et al.* [12] reported an earlier onset of disrupted mineral bone metabolism in patients with DN rather than other causes of CKD. They found lowered levels of 25(OH) vitamin D in early stages of DN; 23.9$\pm$13.3 ng/mL, in contrary to our study, 25(OH) vitamin D was slightly elevated in patients with DN; 34.4$\pm$5.9 ng/mL than control counterparts. This inconsistency might be attributed to the more adherence of patients with DN than others. **Wang *et al.*** [7] reported a progressive rise of intact PTH even before 25 (OH) vitamin D-calcium-phosphate axis changes as well as **Vassalotti *et al.*** [13] who demonstrated elevated intact PTH even with normal calcium and phosphorus levels in early DN. Therefore, intact PTH may represent an early predictor of impaired renal function as well as a serious potential marker of CKD progression [14]. Moreover, **Bhuriya *et al.*** [15] considered elevated intact PTH as an early predictor of cardiovascular disease in patients with mild to moderate CKD rather than serum calcium or phosphorus alterations. This finding was attributed to the pro-calcific and pro-fibrotic effects of intact PTH through promoting expression of micro-RNA, advanced glycation end products and interleukin-6. [16].

Drüeke *et al.* [17] **and Koizumi *et al.*** [18] attributed disrupted homeostasis of intact PTH, in early stages of CKD to the disruption of fibroblast growth factor 23 (FGF23), 1, 25 dihydroxy vitamin D, soluble α-Klotho and Wnt-b-catenin pathway inhibitors that may potentiate CKD-MBD. Moreover, the down regulation of vitamin D receptor and calcium-sensing receptor in the parathyroid glands as well as enhanced parathyroid hyperplasia and proliferation without balanced apoptosis may impair control of PTH synthesis and secretion.

In our study, intact PTH was positively correlated with HBA1c. **Ismail-Beigi *et al.*** [19] considered hyperglycaemia as an independent risk factor of DN with an inverse correlation between glycaemic control and progression of DN. On the other hand, **Ali *et al.*** [20] **and Haque *et al.*** [21] reported lowered intact PTH level with poor glycaemic control during moderate and advanced stages of DN. This inconsistency was attributed to the divergent pathogenesis of CKD-MBD with elevated PTH during high bone turn-over stage and lowered PTH accompanying a dynamic bone disease stage.

In the current study, we aimed to address intact PTH correlations and determinants during relatively early stage of DN. We insisted to select patients with micro-albuminuria and excluding patients with macro-albuminuria. Micro-albuminuria was confirmed three times before enrolment.

We assessed intact PTH not the whole PTH to get more accurate insight. Sample size of the case group was reasonable for a single-centre study. We strived to assess all available renal and metabolic bone parameters in our institution. However, we had many limitations such as lack of fibroblast growth factor 32 assessment due to unavailable tools, we considered urine ACR rather than staging of CKD, single ethnicity, and single centre protocol. Multi-centre trials with larger scale of multiple ethnicities and invasive tools are needed in the future studies.

CONCLUSION

In micro-albuminuria stage of diabetic nephropathy (DN), intact PTH was significantly elevated, positively correlated with glycosylated haemoglobin (HBA1c), urine albumin creatinine ratio (urine ACR), blood urea nitrogen (BUN), and serum creatinine and inversely correlated with estimated glomerular filtration rate (eGFR). However, urine ACR was the only independent determinant of intact PTH changes.

ABBREVIATIONS

Diabetes mellitus (DM), end-stage renal disease (ESRD), diabetic nephropathy (DN), intact parathyroid hormone (intact PTH), type 2 diabetes mellitus (T2D), glycosylated haemoglobin (HBA1c), blood urea nitrogen (BUN), urine albumin creatinine ratio (urine ACR), estimated glomerular filtration rate (eGFR), chronic kidney disease (CKD), metabolic bone disorders (MBD), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG), the Modification of Diet in Renal Disease (MDRD), the Institutional Review Board (IRB).

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REFERENCES

1. **International Diabetes Federation (IDF) (2015):** Diabetes Atlas 7th edition. idf.org. http://www.diabetesatlas.org
2. **Fawzy M, Abu AlSel B (2018):** Assessment of vitamin d-binding protein and early prediction of nephropathy in type 2 Saudi diabetic patients. J Diabetes Res., 2018: 8517929.
3. **Radcliffe N, Seah J, Clarke M *et al.* (2017):** Clinical predictive factors in diabetic kidney disease progression. Journal of Diabetes Investigation, 8(1):6–18.
4. **Campion C, Sanchez-Ferras O, Batchu S (2017):** Potential role of serum and urinary biomarkers in diagnosis and prognosis of diabetic nephropathy. Canadian Journal of Kidney Health and Disease, 4:2054358117705371.
5. **Behl T, Kaur I, Goel H *et al.* (2014):** Diabetic nephropathy and diabetic retinopathy as major health burdens in modern era. WJPPS., 3(7):370–87.
6. **Moresco R, Sangoi M, De Carvalho J *et al.* (2013):** Diabetic nephropathy: traditional to proteomic markers. Clin Chim Acta, 421:17–30.
7. **Wang W, Chen L, Lee C *et al.* (2017):** Association between parathyroid hormone, 25 (OH) vitamin D, and chronic kidney disease: A population-based study. Biomed Res Int., 2017: 7435657.
8. **Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group (2013):** KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Inter., 3:1–150.
9. **Levey A, Bosch J, Lewis J *et al.* (1999):** A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med., 130:461–470.
10. **Muntner P, Jones T, Hyre A *et al.* (2009):** Association of serum intact parathyroid hormone with lower estimated glomerular filtration rate. Clinical Journal of the American Society of Nephrology, 4(1):186–194.
11. **Okamoto K, Fujii H, Goto S *et al.* (2020):** Changes in the whole/intact parathyroid hormone ratio and their clinical implications in patients with chronic kidney disease. J Nephrol., 33(4):795-802.
12. **Wahl P, Xie H, Scialla J *et al.* (2012):** earlier onset and greater severity of disordered mineral metabolism in diabetic patients with chronic kidney disease. Diabe., 35(5):994-1001.
13. **Vassalotti J, Uribarri J, Chen S *et al.* (2008):** Trends in Mineral Metabolism: Kidney Early Evaluation Program (KEEP) and the National Health and Nutrition Examination Survey (NHANES) 1999-2004. Am J Kidney Dis., 51(4 Suppl 2): 56-68.
14. **Chowdary R, Nellutla R, Reddy P (2015):** Relationship between parathyroid hormone and serum creatinine levels in chronic kidney disease patients. J Med Sci Res., 3(1):17-21.
15. **Bhuriya R, Li S, Chen S *et al.* (2009):** Plasma parathyroid hormone level and prevalent cardiovascular disease in CKD stages 3 and 4: An Analysis from the Kidney Early Evaluation Program (KEEP) Am J Kidney Dis., 53(4 Suppl 4): 3-10.
16. **Evenepoel P, Bover J, Ureña Torres P (2016):** Parathyroid hormone metabolism and signalling in health and chronic kidney disease. Kidney International, 90(6):1184–1190.
17. **Drüeke T, Feingold K, Anawalt B *et al.* (2018):** Hyperparathyroidism in Chronic Kidney Disease. In: Endotext (Internet). South Dartmouth (MA): MDText.com, Inc. https://pubmed.ncbi.nlm.nih.gov/25905209/
18. **Koizumi M, Komaba H, Fukagawa M (2013):** Parathyroid function in chronic kidney disease: role of FGF23-Klotho axis. Contrib Nephrol., 180:110-23.
19. **Ismail-Beigi F, Craven T, Banerji M *et al.* (2010):** ACCORD Trial Group. Effect of intensive treatment of hyper glycaemia on micro vascular outcomes in type 2 diabetes: An analysis of the ACCORD randomised trial. Lancet, 376:419–430.
20. **Ali A, Emam A, Assal H *et al.* (2009):** Intact parathyroid hormone in Egyptian type 2 diabetics with chronic haemodialysis: impact of glycaemic control. Kidney, Kidney. DOI: 10.1007/s00596-009-0116-z.
21. **Haque W, Rahim M, Mitra P *et al.* (2017):** Serum intact parathyroid hormone level is inversely correlated with glycated haemoglobin in diabetic chronic kidney disease stages 3-5 predialysis patients. BIRDEM Med J., 7(2): 110-113.