Effect of type II diabetes mellitus on intact parathyroid hormone level in end stage renal disease patients on maintenance hemodialysis

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Abstract: *Introduction:* Osteodystrophy is more common among hemodialysis patients than normal population. Earlier the higher incidence of osteodystrophy among maintenance hemodialysis (MHD) patients was attributed to high Intact Parathyroid Hormone (iPTH) level (150-300 pg/ml). Osteodystrophy due to high iPTH level is called High Turnover Bone Disease (HTBD). It was later found that another type of osteodystrophy, which can be attributed to low iPTH level and called Low Turnover Bone Disease (LTBD), also afflicts a subset of hemodialysis population, the diabetic End Stage Renal Disease (ESRD) patients. In our study, we propose to ascertain if diabetic ESRD patients on MHD have lower iPTH level than their non-diabetic counterparts. *Methods:* Total 193 patients were enrolled into the study. Of them, 98 had diabetic nephropathy as primary cause of ESRD, 69 had Chronic Glomerulonephritis, 13 had Hypertensive Nephropathy, 8 had Polycystic Kidney Disease, 3 had Urolithiasis and 2 had Drug Induced Nephrotoxicity as primary cause of ESRD. All of them had been on MHD for more than 6 months. We measured the iPTH level of all the patients enrolled in the study. *Result.* Serum iPTH level was significantly lower in diabetic group than in non-diabetic group ($P < 0.001$). *Conclusion:* Type 2 Diabetes Mellitus contributes towards relatively low iPTH level in diabetic ESRD patients on MHD.

Keywords: Type 2 Diabetes Mellitus, Maintenance Hemodialysis, Intact Parathyroid Hormone

Introduction

Globally, the numbers of ESRD patients on Maintenance Hemodialysis (MHD) have gone up in virtually all countries in last two decades. Although the incidence rate of Chronic Kidney Disease (CKD) in developing countries (150 per million population (pmp)) is lower than developed countries, the developing countries also did not emerge unscathed, with India being no exception [1]. At the end of 2004, 1,783,000 patients worldwide were receiving treatment for ESRD, of which 77% were on dialysis and 23% had a functioning renal transplant (RT) [2].

In India, the approximate prevalence of CKD is 800 per million population (pmp), and the incidence of End Stage Renal Disease (ESRD) is 150–200 pmp [3]. In India, with a population base of one billion and an estimated incidence of ESRD of 100 pmp, approximately 100,000 patients develop ESRD each year [4]. Etiologically, diabetes (41%), hypertension (22%), chronic glomerulonephritis (16%), chronic interstitial disease (5.4%), ischaemic nephropathy (5.4%), obstructive uropathy (2.7%), miscellaneous (2.7%) and unknown cause (5.4%) constituted the spectrum of primary disease among CKD patients in India [5]. This etiological distribution pattern may show regional variation.

ESRD patients on MHD more often than not have altered iPTH level. Different subsets of ESRD patients on MHD are afflicted with alteration of iPTH level in opposite direction. Nevertheless, both higher and lower than normal iPTH level (10-65 pg/ml) give rise to osteodystrophy, which has been given the general name of renal osteodystrophy.

Consequently, renal osteodystrophy can be of two types [6-7] - High Turnover Bone Disease (HTBD) due to high iPTH level and Low Turnover Bone Disease (LTBD) due to comparatively low iPTH level. It has been revealed in some studies that usually diabetic ESRD patients on MHD have lower iPTH level than non-diabetic ESRD patients on MHD [8-10]. Initially, low iPTH level in diadetic ESRD patients on MHD was thought to confer a protective effect from the secondary skeletal manifestation of hyperparathyroidism [11]. But it was later found that LTBD may be associated with low Bone Mineral Density (BMD), vascular calcification, cardiovascular morbidity and higher overall mortality [12-14].

The studies showing diabetic ESRD patients on MHD have lower iPTH level than non-diabetic ESRD patients on MHD have been conducted abroad. No such studies have been conducted in India. The objective of this study to verify if the statistically significant difference in iPTH level between diabetic and non-diabetic ESRD patients on MHD found by other similar studies [10,15] conducted elsewhere holds true for our patient population i.e. ESRD patients on MHD in south India.

Material and Methods

The study samples were collected from the adult hemodialysis unit at the Department of Nephrology in Sri Ramachandra Medical Centre. The study subjects included both male and female diabetic ESRD patients in the age group of 30-75. The mean age of subjects was 57.26 years. The study period spanned from May 2010 to November 2011.

All patients had been on regular bicarbonate hemodialysis for more than 6 months using polysulfone membrane dialyzer; 4 hours per episode and 3 times/week, with a dialysis fluid of 3.0 mEq/L of calcium concentration, and there was no difference in dialysis frequency and efficacy among patients (Urea Reduction Ratio URR>65%). 1-α-(OH) D3 and calcium carbonate were used routinely for all patients in a low dose of 0.25 μg –0.75 μg daily.

Total 193 patients were enrolled into the study. Of them, 98 had diabetic nephropathy as primary cause of ESRD, 69 had Chronic Glomerulonephritis, 13 had Hypertensive Nephropathy, 8 had Polycystic Kidney Disease, 3 had Urolithiasis and 2 had Drug Induced Nephrotoxicity as primary cause of ESRD. Patients with history of fasting plasma glucose level > 126 mg/dl on multiple occasions and/or on anti-diabetic medication were defined as diabetic. The applied selection criteria were as follows:

Inclusion Criteria: Only patients meeting the following criteria were included in the study: ESRD patients on MHD

Exclusion Criteria: Patients with any one or more of the following criteria were not made part of the study:

1. Liver Disease
2. Cardiac Disease
3. Active Infection
4. Acute Illness
5. Parathyroidectomy
6. Pre-menopausal Female Patients
7. Patients who were on the following drugs:
    a. Hormone Replacement Therapy
    b. Bisphosphonates
    c. Aluminium Hydroxide
    d. Steroids

Study Protocol: Sample Collection, Analysis: Before collection of data or blood sample, each patient was explained the details of the study including rationale, expected benefits, risk profile, confidentiality safeguards and study protocol. For some patients, help of appropriate interpreter(s) was taken. Only those patients who were willing to follow the study protocol and gave their written consent were included in the study. There was neither any financial cost nor any financial incentive for the patient for being part of the study.

1. Appropriate blood samples were collected from ESRD patients on MHD. For estimation of serum Intact Parathyroid (iPTH) level, serum Albumin (Alb) level, serum Calcium (Ca) level, serum Phosphorus (P) level, fasting blood sample were drawn into serum (without anticoagulant) gel containing yellow colour capped BD Vacutainer tubes before starting hemodialysis. All samples were
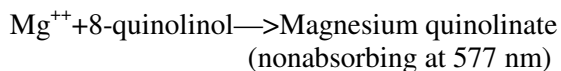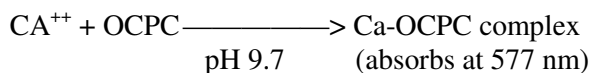
immediately centrifuged and stored at 2-8°C until analysis for the relevant biochemical parameters. All analysis were performed within 3 hours of sample collection.

2. Serum iPTH was measured by ADVIA CENTAUR instrument using Chemiluminescence principle.The ADVIA Centaur iPTH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti-human PTH antibodies in the Lite Reagent. The first antibody is a polyclonal goat anti-human PTH (N-terminal 1–34) antibody labeled with acridinium ester. The second antibody is a biotinylated polyclonal goat anti-human PTH (39–84 region) antibody. Streptavidin in the Solid Phase is covalently coupled to paramagnetic latex particles. A direct relationship exists between the amount of PTH present in the patient sample and the amount of relative light units (RLUs) detected by the system.
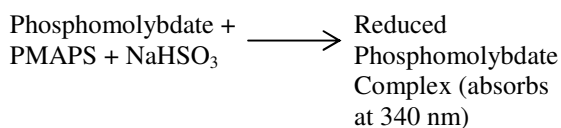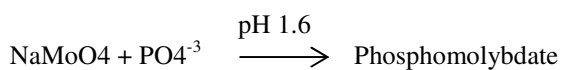
3. Serum Alb was measured by Dimension RXL Max instrument using Bromo Cresol Green Dye Binding principle. In the presence of a solubilizing agent, BCP binds to albumin at pH 4.9. The amount of albumin-BCP complex is directly proportional to the albumin concentration. The complex absorbs at 600 nm and is measured using a polychromatic (600, 540, 700 nm) endpoint technique.

pH 4.9

Albumin + BCP dye-----> Albumin-BCP complex (nonabsorbing at 600 nm)   (absorbs at 600 nm)
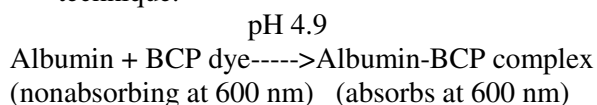
4. Serum Ca was measured by Dimension RXL Max instrument using Arsenazo III principle. Calcium reacts with OCPC to form a purple complex. The amount of complex thus formed is proportional to the calcium concentration and is measured using a bichromatic (577, 540 nm) endpoint technique. Magnesium ions, which also form a colored complex with OCPC, are removed from the reaction by complexation with 8-quinolinol.

CA^{++} + OCPC ————————> Ca-OCPC complex
                    pH 9.7   (absorbs at 577 nm)

Mg^{++}+8-quinolinol—>Magnesium quinolinate
(nonabsorbing at 577 nm)

5. Serum P was measured by Dimension RXL Max instrument using UV End Point principle. Inorganic phosphate combines with molybdate (MoO_4) in an acid solution to form a complex which is reduced by p-methylaminophenol sulfate (PMAPS) and bisulfite. The absorbance of the reduced phosphomolybdate solution is proportional to the inorganic phosphorus concentration and is measured using a bichromatic (340+700) endpoint technique.

pH 1.6

$NaMoO4$ + $PO4^{-3}$ ——————> Phosphomolybdate

Phosphomolybdate + PMAPS + $NaHSO_3$ ——————> Reduced Phosphomolybdate Complex (absorbs at 340 nm)

6. Patients' recent-most blood/plasma/serum values of the afore-mentioned biochemical parameters were noted if already available, provided they were done on the same day. The serum levels of iPTH, Ca, P and Alb of the two groups were compared for presence or absence of statistically significant differences.

Statistical Analysis: The statistical software R version 2.11.1 was used to analyze the data. As serum Intact Parathyroid (iPTH) was not normally distributed, it was expressed as median and range (Figures 1, 2). Other values were expressed as mean $\pm$ one standard deviation unless otherwise indicated, and differences in mean values between two groups were analysed using Student's t-test (Figure 3, Table 1 & 2). Descriptive information regarding categorical variable were presented as frequency. Mann-Whitney Test was used to compare the iPTH values. Fischer's exact probability test was used for comparison of categorical data. All tests were two tailed and considered statistically significant if p-value < level of significance, 0.05.
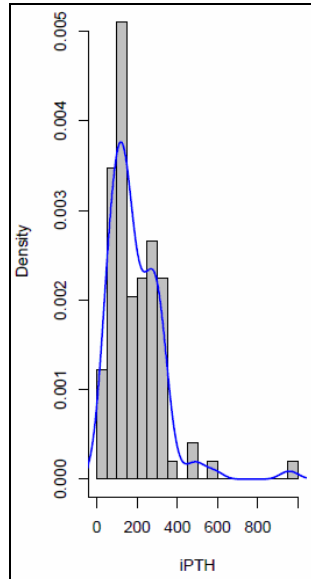
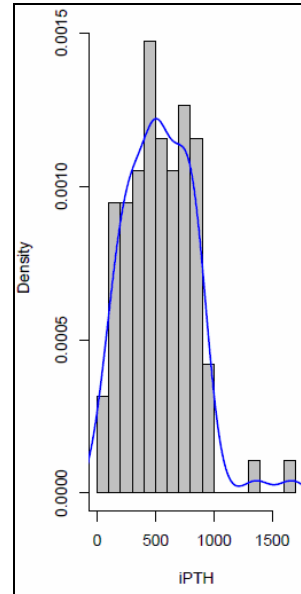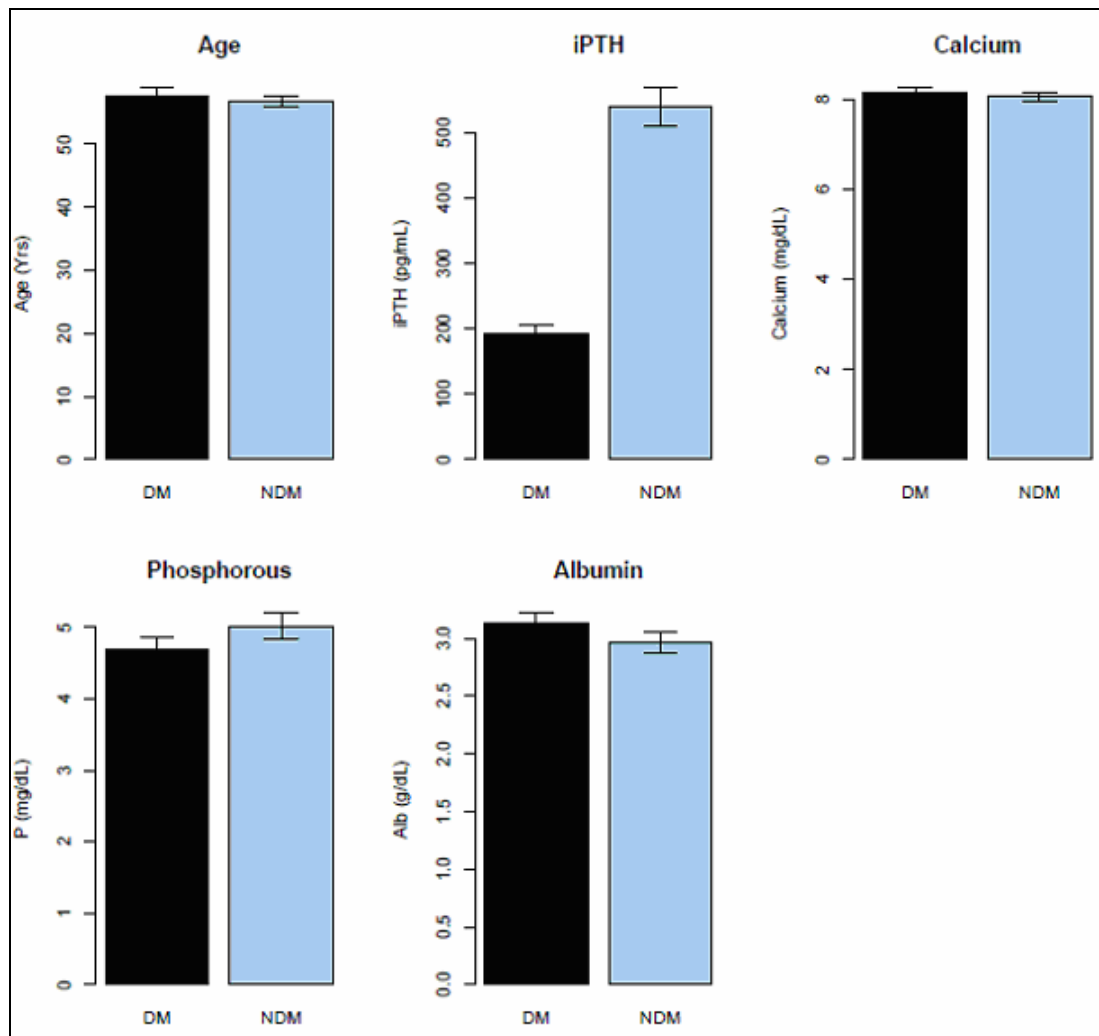**Fig-1: Distribution of iPTH in diabetic (DM) group**   **Fig-2: Distribution of iPTH in non-diabetic group**

Fig-3: Comparison of Age, iPTH, Calcium, Phosphorus and Albumin levels of diabetic (DM) and non-diabetic (NDM) groups

Table-1: Comparison Between Male and Female							
Var. Name	Male			Female			P-Value
	OBS.	Mean	S.D.	OBS.	Mean	S.D.	
iPTH	112	352.1	261.67	81	377.45	308.83	0.54
Ca	112	8.12	1.02	81	8.07	1.07	0.75
P	112	4.83	1.69	81	4.87	1.73	0.86
Alb	112	3.15	0.89	81	2.9	0.87	0.06
HbA1c	57	6.92	1.67	41	7.03	1.66	0.76

Table-2: Comparison Between Male and Female Within DM and Non DM Patients						
	Var. Name	DM		NDM		P-Value
		Mean	SD	Mean	SD	
Female	iPTH	218.0854	173.5567	540.8	332.6793	<0.001
	Ca	8.031707	1.061706	8.115	1.090648	0.3
	P	4.843902	1.659525	4.905	1.819263	0.88
	Alb	3.04878	0.876961	2.7575	0.854217	0.47
Male	iPTH	174.1193	91.84857	536.5491	252.8116	<0.001
	Ca	8.22807	0.953893	8.012727	1.076526	0.88
	P	4.584211	1.617183	5.089091	1.736927	0.08
	Alb	3.187719	0.828309	3.110909	0.963534	0.28

Results

The present study confirms the finding of earlier studies that serum iPTH level in diabetic ESRD patients on MHD is lower than their non-diabetic counterparts. There were no significant differences in age and serum levels of calcium, phosphorus, albumin between the two groups. The rate of the administration of calcium-based phosphate binders was not different either. The mean serum calcium levels in both diabetic and non-diabetic groups were slightly lower than the normal reference interval used in our laboratory. It may be attributed to chronic nutritional deficiency. Serum inorganic phosphorus levels in both the groups were within the normal reference interval (2.5-4.9 mg/dl) of serum inorganic phosphorus used in our laboratory. Although many CKD patients have hyperphosphatemia, most of the participants in our study had normal serum inorganic phosphorus level. This may be attributed to use of phosphate binders. In our study, serum iPTH level in diabetic group was below the target range (150-300 pg/ml) for ESRD patients on MHD as per KDOQI guideline, while serum iPTH level in non-diabetic group was above the target range.

Discussion

Chronic Kidney Disease – Mineral Bone Disease (CKD-MBD) is probably one of the commonest non-renal manifestations of ESRD. It presents with enormous diversity both in terms of the components of bone mineral system involved and the magnitude of derangement of individual components. Diabetes mellitus being one of commonest risk factors in development of ESRD, its role in development of CKD-MBD has always been in focus. Various hypotheses have been put forward to explain the relative hypoparathyroidism found in diabetic ESRD patients on MHD vis-à-vis their non-diabetic counterpart.

The present study showed serum iPTH level in diabetic ESRD patients on MHD to be lower than their non-diabetic counterparts. Studies were done previously to find if diabetes mellitus has role in decreasing PTH level in ESRD patients on MHD and whether all diabetic patients were equally susceptible to suppression of PTH secretion, as has been proved in a study conducted in Japan [16].

The PTH level of patients (HbA1c > 7.0) in 'Poor Glycemic Control' group was found to be significantly lower than that in 'Good Glycemic Control' group (HbA1c < 7.0). So, glycemic control, rather than diabetes mellitus per se, turned out to be the real determinant of PTH level in ESRD patients on MHD. Another study found similar result among diabetic ESRD patients on MHD in Egypt [17].

Glycemic status is one of the determinants of iPTH level in T2DM ESRD patients on MHD. Two mechanisms have been postulated to be behind relative hypoparathyroidism caused by T2DM. They are Advanced Glycation End Products (AGEs) mediated suppression of parathyroid hormone secretion and hyperinsulinemia / hyperglycemia mediated suppression of parathyroid hormone secretion. In a study published in 1991, it was reported that AGEs level in diabetic ESRD patients was much higher than non-diabetic ESRD patients and higher than diabetic non-ESRD subjects. So, high AGEs level is caused by both T2DM as well as ESRD [18]. Hyperglycemia and hyperinsulinemia are both features of Type 2 Diabetes Mellitus. Both these states have been found to suppress PTH secretion. In an in-vitro study involving bovine parathyroid cells, exposure to 50mM concentration of glucose suppressed PTH secretion which was found to be reversible 19]. In a euglycemic hyperinsulinemic and hypoglycemic hyperinsulinemic clamp mediated double blind crossover study involving 16 men, it was found that induction of hyperinsulinemia resulted in a reduction in iPTH (27% +/- 5; $P < 0.01$) [20]. The inhibitory effect of poor metabolic control on low calcium-mediated iPTH secretion has also been documented [21].

Conclusion

Tight metabolic control of the diabetic process is very important to avoid hypoparathyroidism and low bone turnover in these patients. Alongside, individual titration of Ca and 1-α-Vit D supplementations in ESRD patients on MHD may be fruitful in keeping iPTH level within target range. Further studies should be done to investigate whether targeting iPTH and other determinants involved in renal bone disease in dialysis patients may prevent or delay the development of vascular calcifications. Cellular studies may be undertaken to ascertain the role of AGE, hyperinsulinemia, hyperglycemia in development of relative hypoparathyroidism in diabetic ESRD patients on MHD. Theses research endeavours may lead to favourable ramifications in the form of discovery of candidate drug molecules that may be able to address the issues of LTBD and vascular calcification.

Acknowledgement

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