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Original Article

Biochemistry Section

Study of Red Cell Fragility in Different Stages of Chronic Kidney Disease in Relation to Parathyroid Hormone

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ABSTRACT

Introduction: Anaemia is one of the common complications associated with Chronic Kidney Disease (CKD) responsible for the increase in the morbidity and mortality in such patients. Several factors have been attributed to cause renal anaemia, amongst which hyperparathyroidism is one of the less recognised reasons. Most studies have been conducted in this regard in CKD patients undergoing haemodialysis. The level of PTH in early stages of chronic kidney disease has not been much studied. The excess amount of Parathyroid Hormone (PTH) secondary to CKD has been suggested to be a causative factor for anaemia.

Aim: To evaluate the serum PTH level in CKD patients before haemodialysis and to study the association of the haemoglobin status with the parathyroid hormone.

Materials and Methods: Forty CKD patients above 18 years of age before haemodialysis and 25 age and sex matched healthy controls were included in the study. Routine biochemical and haematological parameters such as Routine Blood Sugar (RBS), urea, creatinine, Na^+, K^+, Ca^{2+}, PTH and Hb% were perfomed. Red cell osmotic fragility was measured by serial dilutions of whole blood with varying concentrations of sodium chloride ranging from 0.1% to 0.9%.

Results: The study revealed a significant fall in Hb%, along with a rise in Median Osmotic Fragility (MOF) and PTH in the CKD patients when compared to the control group. Linear regression of PTH with Hb% revealed significant negative association between both the parameters with a R^2 value of 0.677. Multilinear regression analysis of MOF and other independent variables such as Hb%, Na^+, K^+, Ca^{2+}, urea, PTH and creatinine highlighted the variance of MOF by 72%, maximal variance contributed by PTH. Receiver Operating Curve (ROC) analysis revealed an area under the curve of 0.980 with a sensitivity of 100% and specificity of 87% in detecting osmotic fragility at a cut off value of PTH ≥100 pg/ml.

Conclusion: The underlying cause of anaemia should be identified early in the CKD patients before haemodialysis. Secondary hyperparathyroidism should be ruled out as a causative factor of anaemia to slow down the progression of the disease process.

Keywords: Anaemia, Hyperparathyroidism, Osmotic fragility

INTRODUCTION

Hyperparathyroidism is commonly associated with declining kidney function, due to alteration of the bone and mineral metabolism [1]. Altered levels of vitamin D and phosphate are responsible for this increase in PTH, which develops quite early in the course of the disease process and remains unrecognised and untreated. Anaemia is a common complication in CKD patients, who have a mortality rate 20-100 times higher than the normal population [2].

Several studies have revealed the association of hyperparathyroidism with CKD [3-5]. Parathyroid hormone (PTH) is a major uremic toxin which may be responsible for long term consequences in CKD such as renal osteodystrophy, vascular calcification, altered cardiovascular function, immune dysfunction and anaemia [6,7]. Hyperparathyroidism induces anaemia in patients with normal kidney function. Therefore, raised PTH secondary to chronic kidney disease may result in unfavourable influence on the haemoglobin level of uremic patients [8]. Patients undergoing haemodialysis have low haemoglobin which may be attributed to their PTH level as cited by various authors [9-11].

Secondary hyperparathyroidism is often an unrecognized incident and develops early in the course of the disease before dialysis initiation [12]. Correction of anaemia in the CKD patients is essential for the prevention of associated cardiac complications and other debilitating features such as fatigue, weakness, anaemia and sleep disturbance. Low haemoglobin levels also increases the length of the stay in the hospital leading to higher consumption of health care resources and increased risk of mortality [13,14]. Anaemia induced by PTH may be due to bone marrow fibrosis as suggested by Rao DS et al., [15] or suppression of peripheral burst forming units or by causing an enhanced osmotic fragility.

Though various pathophysiological mechanisms have been outlined by in vitro and in vivo studies, regarding the worsening effect of raised parathyroid levels on the haemoglobin levels, no definitive mechanism has been identified. The objective of the present study was to evaluate the serum PTH levels in various CKD patients before haemodialysis and assess the association of haemoglobin level with the hormone. The possible underlying mechanism is also studied by examining the fragility of the red blood cell at different concentrations of PTH.

MATERIALS AND METHODS

The present study was undertaken in the Department of Biochemistry, in association with the Department of Nephrology of SCB Medical College, Cuttack, Odisha, India, during the period of August 2015 to August 2016. A total of 40 CKD patients attending the OPD or admitted to the indoors, above 18 years and 25 healthy age and sex matched controls who were healthy volunteers, were included in the study group.

Informed consent was obtained from cases and controls followed by medical history, clinical examination and routine biochemical investigations after Institutional Ethical Clearance. Those patients with stage 5 CKD requiring dialysis treatment and other associated kidney disease, therapy with iron, erythropoetin stimulating agents, associated bleeding disorders or need for blood transfusion which interfere with the haematocrit value, anaemia due to other causes such as Vitamin B12, folate, iron deficiency, haemoglobinopathies were excluded from the study.

Blood samples were collected and the parameters such as RBS, urea, creatinine, Na^+, K^+, Ca^{2+} were carried out by TBA 120 FR Autoanalyser. The eGFR was calculated using MDRD equation taking serum creatinine, age and sex of the patient. Haematocrit was measured by bioelectrical impedance method in percentage. Serum intact Parathyroid Hormone (iPTH) was measured using chemiluminiscence immuno assay by Beckmann Coulter. Red cell osmotic fragility was evaluated by incubating 50 µl of the RBC of the patients sample with gradually increasing concentrations of NaCl ranging from 0.1% to 0.9% [16]. After one hour, the incubated solutions were centrifuged and the absorbance of the supernatant was taken at 540 nm. A curve was plotted based on the concentration of NaCl and absorbance reading. The concentration of NaCl causing 50% haemolysis is taken as the Median Osmotic Fragility (MOF).

STATISTICAL ANALYSIS

Quantitative variables were expressed as Mean±SD. Linear regression and multi linear regression analysis were used to test the possible association between PTH, red cell osmotic fragility and eGFR. ROC curve analysis was done to determine the sensitivity and specificity of PTH in determining median osmotic fragility. Statistical analysis was done using SPSS version 21.0. Student's t-test was applied to calculate the p-value and p-value<0.05 was considered to be statistically significant.

RESULTS

The study revealed that 20 out of 40 CKD patients had Hb% level ≤ 10 (50%) in comparison seven out of 25 patients among the control group (28%). The rest of the biochemical parameters such as serum urea, creatinine, serum PTH also registered a higher value in the case group. Median osmotic fragility of RBC also revealed a significant rise in the CKD patients in comparison to the controls, i.e., 50% of RBC were lysed at 0.5±0.12 in comparison to 0.4±0.06 in the healthy controls [Table/Fig-1].

[Table/Fig-2] depicted a significant fall in Hb% along with a rise in the serum PTH and MOF when the patients were stratified based on the estimated GFR values (eGFR).

In [Table/Fig-3], multilinear regression analysis of MOF and other independent variables such as Hb%, Na^+, Ca^{2+}, K^+, urea, PTH and creatinine highlighted the variance of MOF by 72% i.e., 72% of change in the MOF is contributed by combined effect of all these predictors where as [Table/Fig-4] revealed significant variation in MOF (74.7%) is contributed by unit change in PTH.

Linear regression of PTH with Hb% in [Table/Fig-5] reveals significant negative association between both the parameters with a R^2 value of 0.677 i.e., 67.7% of the alteration in MOF is caused due to unit change in serum PTH.

[Table/Fig-6] depicts the linear regression analysis of MOF with the PTH. MOF is the dependent variable and PTH is the independent variable and 70.6% of the variation of MOF is contributed by unit change in PTH.

ROC analysis in [Table/Fig-7] revealed an area under the curve of 0.980 with a sensitivity of 100% and specificity of 87% in detecting osmotic fragility at a cut-off value of PTH ≥100 pg/ml.

Parameters	Controls (n=25) Mean±SD	Cases (n=40) Mean±SD
RBS (mg/dl)	96.4±12.2	105.6±34.97
Urea (mg/dl)	46.0± 4.4	77.35±25.5*
Creatinine (mg/dl)	1.4 ± 0.14	2.8±1.1*
Na^+ (mmol/L)	140.1 ± 4.2	138.9±7.8
K^+ (mmol/L)	3.9± 0.15	4.5±0.6*
Ca^{2+} (mmol/L)	1.08± 0.11	0.97±0.15*
PTH (pg/ml)	47.0 ±10.6	179.8±80.5*
Hb (gm/dl)<10	7 (28%)	20 (50%)
MOF (%)	0.4 ±0.06	0.55±0.12*

[Table/Fig-1]: Baseline biochemical parameters of the cases and controls.
* p<0.05

Parameters	Stage 2 (n=2)	Stage 3A (n=18)	Stage 3B (n=15)	Stage 4 (n=5)
eGFR(ml/min)	65.8±1.56	42.3±8.5	19.8±4.7	11.2±2.44
Hb%(gm%)	13.75±0.21	10.12±1.43	9.24±1.29	7.98±1.36
PTH(pg/ml)	69.5±9.19	129.2±44.4	219.9±53.8	577.12±43
MOF(%)	0.35±0.12	0.48±0.11	0.62±0.07	0.65±0.66

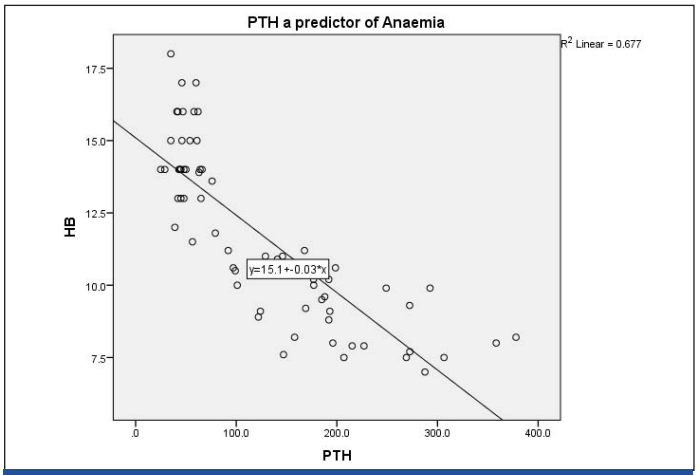
[Table/Fig-2]: Different parameters stratified according to stages of CKD.
*p<0.05

Model	R	R^2	Adjusted R^2	Standard Error of the Estimate
1	0.851a	0.724	0.685	0.07277

[Table/Fig-3]: Multilinear regression analysis of MOF with combined effect of different predictors.
Predictors: (Constant), HB%, Na^+, Ca^{2+}, K^+, urea, PTH, creatinine
Dependent Variable: MOF

Parameters	Standarized Coefficient B	Standard Error	t	p-value
PTH (pg/ml)	0.747	0.000	4.909	<0.001*
Urea (mg/dl)	0.133	0.001	0.899	0.372
Creatinine (mg/dl)	-0.089	0.015	-0.548	0.586
Na^+ (mmol/L)	0.087	0.001	-1.170	0.247
K^+ (mmol/L)	-0.006	0.008	-0.071	0.944
Ca^{2+} (mmol/L)	0.057	0.082	0.716	0.477
Hb (gm/dl)	-0.127	0.006	-0.978	0.332

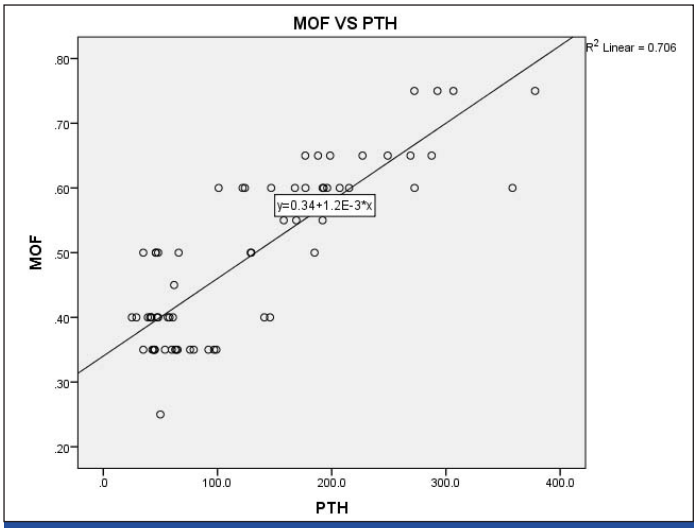
[Table/Fig-4]: Multilinear regression analysis of MOF with individual effect of different predictors.



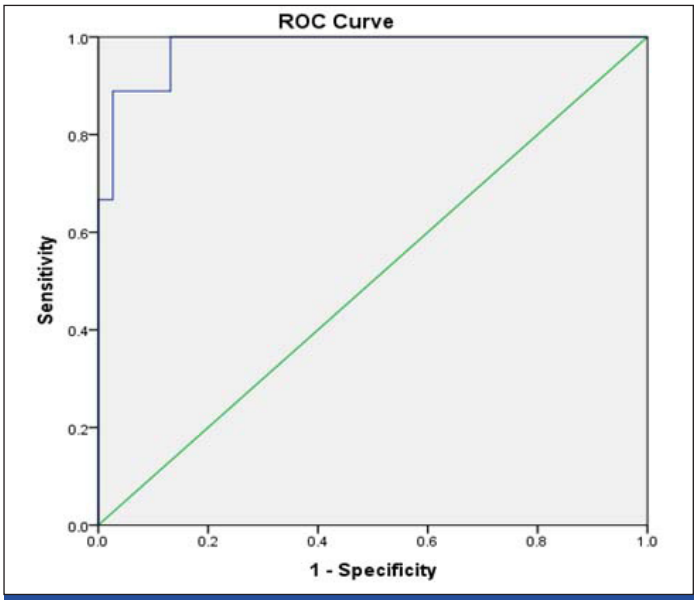
[Table/Fig-5]: Linear regression analysis of PTH with Hb%.
X-Axis-PTH in pg/ml, Y -Axis-Hb in gm/dl

DISCUSSION

Anaemia is one of the common complications in CKD patients associated with decline in the number of functional nephrons. Primary cause of anaemia is due to decrease in the erythropoetin production due to loss of renal functional mass [17]. Also, the



[Table/Fig-6]: Linear regression analysis of MOF with PTH.
X- Axis PTH in pg/ml, Y- Axis- Median Osmotic Fragility in %



[Table/Fig-7]: ROC of PTH as diagnostic of osmotic fragility.

impaired renal excretory function- leads to accumulation of toxin substances. Imbalance in the calcium phosphate, acid base and electrolytes resulting from impaired renal function affects the red cell shape and survival [18]. All these factors are responsible for low haemoglobin with declining kidney function. In our study, there was a significant fall in the haemoglobin level with a fall in the GFR [Table/Fig-2].

Parathyroid hormone is considered as uremic toxin [19]. Hyperparathyroidism secondary to CKD is caused due to underlying hyperphosphatemia, low calcium and calcitriol level. Anaemia has been recognized as a possible complication of hyperparathyroidism [7]. PTH is known to play a significant inhibitory role in RBC production and survival. Significant negative association was observed in between PTH and Hb% (R^2=0.677, p<0.001). This is in line with other authors [20,21] who have identified that secondary hyperparathyroidism results in anaemia by various mechanisms such as, inhibiting erythropoesis, inducing marrow fibrosis and increase in the blood loss by reducing platelet aggregation.

In the present study, three patients with stage 1 attended the OPD for treatment, but they were unwilling for serum PTH assay and red cell fragility test. Hence, they were excluded from the study.

Median osmotic fragility revealed a significant rise with a fall in eGFR, [Table/Fig-2] highlighting the shortened survival of RBC with the progression of renal dysfunction. Erythrocyte survival is reduced in patients with advanced renal failure [22,23]. RBC from uraemic patients has normal survival when infused into normal subjects, whereas a shortened survival of RBC was observed when infused in uremic patients [22]. Several extra corpuscular factors may result in this reduced survival. Multilinear regression analysis revealed PTH to be the major factor responsible for the increased osmotic fragility of the RBC. The action of PTH on the osmotic fragility of the RBC is related to enhanced calcium entry into the cells [24]. The increased calcium influx inside the RBC leads to the stimulation of Ca^{2+} activated ATPase, which in turn results in ATP depletion and erythrocyte fragmentation. Increased PTH resulted in the increase in the Ca^{2+} concentration in the RBC leading to the cross-linking of membrane protiens. This affects the structure and stability of the red blood cells resulting in its lysis [25,26]. ROC curve analysis revealed an area under the curve of 0.980 in predicting osmotic fragility of RBC at a cut off value of PTH≥100 pg/ml which is highly significant. However, the extent to which PTH contributes to anaemia, remains controversial.

LIMITATION

The present study has several limitations which includes a smaller sample size, other factors such as serum Vitamin D, iron, ferritin were not estimated to find out the causal factors of anaemia and previous records of the haemoglobin status was not examined.

CONCLUSION

Secondary hyperparathyroidism, an unrecognised and untreated condition develops early in chronic kidney disease. Several studies have revealed the reverse association of anaemia with hyperparathyroidism. Mechanisms of anaemia due to Secondary Hyperthyroidism (SHPT) are unclear, severe bone marrow fibrosis with a concomitant reduction of space for erythrogenesis is an important cause of anaemia among these patients. This study reveals that renal secondary hyperparathyroidism has considerable effects on erythrocyte survival, contributing to increased fragility and anaemia. Emphasis should be laid on the early detection of serum PTH and the concomitant fall in haemoglobin levels should be treated, which in turn will help in slowing the progression of CKD and other associated comorbidites.

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