ASSESSMENT AND CORRELATION OF SERUM BIOCHEMICAL PARAMETERS AND PARATHYROID HORMONE IN SELECTED ADULT POPULATION SUFFERING FROM VARIOUS STAGES OF CHRONIC KIDNEY DISEASES (CKD)

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Abstract

To determine correlation with biochemical and hormonal parameter, which may exist in Chronic kidney diseases (CKD) patients who are at various stages of their diseases (including renal failure and end stage renal disease-ESRD), the present study detailed the assessment of calcium, inorganic phosphorus, alkaline phospahatse, uric acid, creatinine clearance (C.C), protein to creatinine ratio (P:C ratio) and parathyroid hormone (PTH) in 57 selected patients. They were grouped as, mild CKD (n = 6); moderate CKD (n = 27); severe CKD (n = 13) and ESRD (n = 11). Estimation of biochemical parameters, PTH, creatinine clearance and protein to creatinine ratio was performed according to established procedures and techniques. The results showed that C.C. and urinary P: C ratio and plasma concentration of mean PTH was altered with high significance ($P< 0.001$) in severe CKD and ESRD groups as compared to mild and moderate groups. Generally all patients of mild to ESRD stages have increased level of PTH, ALP, uric acid and decline levels of calcium and C.C. Similarly a pattern of mild to moderate levels of significance ($P< 0.05$ to $P< 0.01$) was noted when biochemical parameters, P:C ratio and C.C. were compared among mild to moderate, moderate to severe and mild to severe groups, respectively. It is concluded that the selected population of patients showed altered levels of several biochemical parameters, urinary creatinine clearance, and protein to creatinine ratio and notably PTH. This established that the altered levels are directly proportional to the extent of renal failure or dysfunctions and may caused hyper-parathyroidism.

Introduction

It is frequently reported in scientific journals that chronic kidney disease (CKD) and one of its very significant clinical associate, chronic renal failure (CRF) is attributed to the induction of many co-morbidities that affect patients at all stages of the disease (Patel *et al.*, 2007). The complications of CKD, although, are due to the disease itself, but may aggravate and cause a number of systemic disorders related to mineral and bone metabolism (Drueke, 2008; Patel *et al.,* 2007). Such systemic disorders are manifested by a single or a pattern of biochemical irregularity (deviant calcium, phosphorus, PTH, or vitamin D metabolism), bone abnormalities (anomalous bone turnover, mineralization, volume, linear growth, or strength) including vascular or other soft tissue calcification (Tentori *et al.,* 2008). Furthermore, chronic renal failure (CRF) also reported to induce an array of related metabolic abnormalities of calcium and phosphorus (Rahman *et al.,* 2005) resulting in secondary hyper-parathyroidism that develops at the early stages of chronic renal insufficiency (Arnaud, 1974; Malluche *et al.,* 1978; Rahman *et al.,* 2005). In general sense it was suggested due to the resulting low calcium levels (hypo-calcemia) in blood, hyper-compensated by (or vice versa) phosphate retention and deficiency in the production of 1,25 dihydroxy Vitamin D_3 (Salusky *et al.,* 1987). As a resultant mechanism (or probably compensatory dysfunction) the elevation of serum phosphorus concentration cause a decrease in production of 1,25 dihydroxy Vitamin D_3 which induces an increase in the release of parathyroid hormone (PTH) (Rahman *et al.,* 2005). It was argued that without treating it, the extent and acuteness of secondary hyperparathyroidism gets worse with intense decrease in minimal renal function, thus resulting in progressively severe and end stage of renal diseases (Rahman *et al*., 2005).

More recent studies and reports showed that chronic kidney disease (CKD) is also a major cause of malnutrition also, concerning approximately one-third of patients already distressing from advanced renal failure (RF) (Naunes *et al.*, 2008). Nephrologists and researchers have strongly advocated that CKD is a syndrome typify by extensive and rapid declining of the renal activity, resulting in ionic and biochemical imbalance that includes accumulation of nitrogen catabolism products, such as urea and creatinine. It is argued and suggested that the main component that can determine the disease and death in hemodialysis (HD) patients are biochemical parameters in blood including hormones, such as PTH, and to some extent the nutritional status of patients. Of all blood parameters, serum PTH levels seem to play significant role in maintenance of normal

functions. Certain observational studies showed that there is an increase in relative risk of mortality in CKD patients, in whom the levels of PTH showed highest and lowest values (Block *et al.,* 2004; Drueke, 2008; Moe and Drueke, 2003; Moe *et al.,* 2006). A recent study conducted in a selected population of elderly men noted a strong correlation between plasma iPTH, which was found within normal range and the cardiovascular mortality (Drueke, 2008; Hagstrom *et al.,* 2009).

The present study describes the assessment and correlation between biochemical and related components including parathyroid hormone in patients suffering from various stages of chronic kidney diseases (CKD). The results will be helpful in reviewing the status and extent of alteration that may occur in blood concentration of calcium, inorganic phosphorus, creatinine clearance, protein to creatinine ratio, uric acid and alkaline phosphate in renal dysfunctions including failure and end stage renal disease (ESRD).

Materials and Methods

Patients: A total of 57 consecutive patients suffering from various stages of CKD in our tertiary care hospital were studied. The groups are classified as mild CKD (n = 6); moderate CKD (n = 27); severe CKD (n = 13) and ESRD (n = 11) as described earlier (Rahman *et al.,* 2005). The mean age of the patients with mild CKD was 54.12 ± 9.0 years, in the moderate group was 53.10 ± 10.6 years, in the severe group it was 51.20 ± 16.11 and in the ESRD group 57.05 ± 14.15 years. Total 40, aged and gender-matched subjects (n = 17 female; n = 23 males) were included as controls. Known cases of coronary artery disease, pulmonary disease and smokers were excluded from the study. The selected patients underwent routine clinical examination and the relevant biochemical investigations as and when required.

Procedures: Protocols of Rehman *et al.,* (2005) was followed all procedural steps. From each CKD patients and control, 6 mL of blood samples were collected in Li-heparin tubes for estimation of calcium (Ca), inorganic phosphorus (Pi), alkaline phosphatase (ALP) and uric acid (UA). Collection of sample in the morning was chosen for PTH because of its ability to be altered at night time (Ohe *et al.,* 2003). Therefore for PTH, 5 mL blood samples were collected in EDTA tubes and transferred on ice packs to laboratory. Serum was separated and stored at -20^{o}C until further use.

Biochemical and Hormonal analysis: Estimation of biochemical parameters was done on Hitachi 912 Chemistry analyzer (Roche Diagnostics, Basil) where as PTH assay was done on Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Basil) with Electrochemi-luminescence technology according to manufacturer's instructions. Creatinine clearance was calculated using earlier described Schwartz formula (Rahman *et al.,* 2005). Statistical analysis for all determinants and significance of difference between groups were calculated by Student's *t*-test., whereas correlation coefficients were calculated using Pearson correlation. Normal reference ranges of serum and urinary biochemical parameters are creatinine clearance = M = 91-125 ml/min/1.73 m^2, F = 91-119 ml/min/1.73 m^2; urinary protein to creatinine ratio = less than 1 (no decline in GFR), greater than 1 (decline in GFR and suggestive of ESRD); PTH = 12-50 pg/ml; calcium = 5-20 yrs = 9.2-11.0 ng/dL, adults = 8.6-10.2 ng/dL; inorganic phosphorus = 2.5-4.5 mg/dL; ALP = M = < 129 U/L F = < 104 U/L; uric acid = M = < 7.0 mg/dL, F = < 5.7 mg/dL.

Results

Results are summarized in Table I and Fig 1. CKD patients were classified into four groups according to the stage of their diseases (mild, n = 6; 11%; moderate, n = 27; 47%; severe, n = 13; 23% and ESRD, n = 11; 19%). The values of creatinine clearance (C.C) and urinary protein to creatinine ratio (P:C ratio) were taken as criteria for classifications. Thus CKD patients exhibiting C.C of 80.49 ± 15.10 ml/min/1.73 m^2 and P:C ratio of 0.59 (Control group 110.20 ± 19.25 ml/min/1.73 m^2; P:C 0.20) was considered in mild group (n = 6) where as those showing C.C of 18.23 ± 9.15 ml/min/1.73 m^2 and P:C ratio of 1.20 was grouped as severe CKD (n = 13). Furthermore ESRD group consist of patients (n = 11) whose C.C. were at the lowest, 5.10 ± 1.30 ml/min/1.73 m^2 and P:C ratio at a higher value of 4.10, confirming extreme severity of the disease and the end state of renal function. PTH, Ca and Pi levels corresponds to the different stages of groups of CKD viz highest level of PTH, 359.60 ± 51.20 pg/ml (control group 18.15 ± 4.10 pg/ml), lowest Ca levels of 5.12 ± 2.15 mg/dl (control group 9.10 ± 2.35 mg/dl) and Pi levels of, 7.81 ± 3.10 mg/dl (control 3.10 ± 0.65 mg/dl) was noted in ESRD groups. Levels of ALP and uric acid also showed elevation trends as the disease stage progressed to severity towards ESRD. Comparison of biochemical parameters, C.C and P:C ratio among various groups of CKD showed that most parameters in ESRD groups showed highly significant ($P< 0.001$) elevated levels or declined values when compared with mild and moderate stages of CKD patients. However moderate level of significance ($P< 0.01$) was noted for Ca, Pi, ALP and uric acid in ESRD group when compared with severe CKD patients with the exception of C.C, urinary P: C ratio and PTH levels which showed strong significance ($P< 0.001$). A pattern of

mild to moderate levels of significance (P< 0.05 to P< 0.01) was noted when biochemical parameters, P:C ratio and C.C. were compared among mild to moderate, moderate to severe and mild to severe groups, respectively.

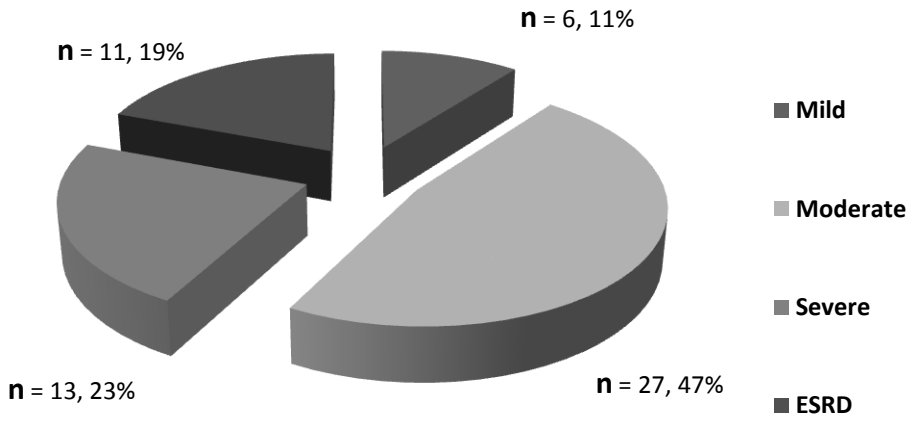

Fig. 1. The total number and percentage incidence of various stages of CKD in selected adult population of patients (n = 57).

Table I. Mean ± SD values of biochemical parameters, Protein to creatinine ratio (P:C), parathyroid hormone (iPTH) and creatinine clearance (C.C) in various chronic renal disease groups

Groups	CC ml/min/1.73 m^2	Urinary P:C	PTH pg/ml	Calcium mg/dl	Phosphorus mg/dl	ALP U/L	Uric acid mg/dl
Control N = 40	110.20 ± 19.25	0.20	18.15 ±4.10	9.10 ±2.35	3.10 ± 0.65	112.25±12.38	4.86±1.25
Mild N = 6	80.49 ± 15.10^{a,b}	0.59^{a}	59.40 ±12.12^{a}	8.00 ±4.10^{b}	4.29 ± 2.10^{b}	155.20±21.50^{a}	5.15±2.35^{a}
Moderate N = 27	51.20 ± 11.32^{a,b,c}	0.91^{a}	101.30 ±16.10^{a}	7.05 ±5.20^{b}	6.00 ± 2.10^{c}	245.38±19.25^{a}	5.90±1.41^{c}
Severe N = 13	18.23 ± 9.15^{a,c}	1.20^{a}	120.25 ±20.56^{a}	6.00 ±2.10	6.90 ± 4.15	312.45±61.14^{c}	6.56±3.34
ESRD N = 11	5.10 ± 1.30^{a}	4.10^{a}	359.60 ±51.20^{a}	5.12 ±2.15^{b}	7.81 ± 3.10^{b,c}	459.11±60.25^{a,c}	7.11±1.66^{a,c}

Normal reference ranges: creatinine clearance (C.C) = M = 91-125 ml/min/1.73 m^2, F = 91-119 ml/min/1.73 m^2; Urinary Protein to creatinine ratio (P:C) = less than 1 (no decline in GFR), greater than 1 (decline in GFR and suggestive of ESRD); parathyroid hormone (PTH) = 12-50 pg/ml; calcium (Ca) = 5-20 yrs = 9.2-11.0 ng/dl, adults = 8.6-10.2 ng/dL; inorganic phosphorus (iP) = 2.5-4.5 mg/dL; alkaline phosphatase (ALP) = M = < 129 U/L F = < 104 U/L; Uric acid (UA) = M = < 7.0 mg/dL, F = < 5.7 mg/dL. ^{a}Significance at <0.001; ^{b}significance at <0.01, ^{c}significance at <0.05.

Discussions

The present study was undertaken for the assessment and correlation of biochemical parameters including creatinine clearance, urinary P:C ratio and PTH with various stages of CKD and ESRD. In the present study it was observed that C.C. and urinary P: C ratio and plasma concentration of mean PTH was altered with high significance level in severe CKD and ESRD groups as compared to mild and moderate groups and extremely with age and gender matching controls with normal renal activities. Generally all adult patients, that has been assessed for biochemical, hormonal and related parameters and belongs to mild to ESRD stages exhibited increased level of PTH, ALP, uric acid and lower than normal levels of Ca and C.C. As far as P:C ratio is concerned, it was only found increased beyond unit 1 in severe and ESRD groups. It has been postulated many times that such renal impairment state may occur due to hyper-parathyroidism, consistent with high PTH levels. Concomitantly, the concentration of PTH was higher during progressive stages of renal failure, thus

corroborating the relationship of severity of hyper-parathyroidism to the extent of renal impairment (Rahman *et al.,* 2005).

It has been reported that the incidence of ESRD patients in Pakistan is estimated to be 100 patients/million population (Abbas *et al.,* 2009; Naqvi *et al.,* 2000). These patients suffer high mortality rate, low quality life and increased hospitalization. Mortality rate of ESRD patients is currently 20% annually in USA. The cause of death for these ESRD patients, who were on hemo-dialysis is reported to be diverse ranging from cardio-vascular disease, protein energy mal-nutrition and chronic inflammation (Abbas *et al.,* 2009; Owen *et al.,* 1993; US Panel data system, 1999; Yeun *et al*., 2000).

Several incongruities exists in the nutritional, macro and micro components related to physiological functions in patients suffering from renal diseases or conditions. Regarding the sequence of metabolic anomalies in CRF and ESRD patients, it was argued that the retention of phosphate and the compensatory decline in plasma calcium levels was the key reason of hyperparathyroidism (Drueke, 2008; Llach *et al.,* 1977; Slatopolsky *et al.,* 1971). A study conducted in a large US population sample (NHANES III) with CKD stage 3, identified slight increases of plasma phosphate with a low creatinine clearance in comparison with healthy population without renal disease (Hsu *et al.,* 2002). Relating to this study, there is a possibility of milder adjustments in blood circulating and curbed factors involved in the control of phosphate metabolic balance, resulting in effects on PTH and the actual concentrations of plasma phosphorus in CKD patients (Rahman *et al.,* 2005). It is generally noted that in later stages of CRF, hyperphosphatemia is the most common feature in the stimulation of increased PTH secretion (Glorieux *et al.,* 1998; Patel *et al.,* 1995). For low levels of calcium which was generally observed in CKD patients and in present study, as well, it was suggested by several researchers and scientists that hypocalcemia set off hyperparathyroidism in early renal failure (Hsu *et al.,* 2002). In an another study, it was noted that the elevated levels of serum PTH was a major factor in early renal failure stages, and was recommended to be due to decreased calcium level and elevation in the concentration of inorganic phosphate during initial to later stages of renal failure. Some scientific studies, though, have reported a normal total calcium concentration in milder renal failure forms (Rahman *et al.,* 2005; Wilson *et al*., 1985), which sometimes occurs probably due to compensatory physiological mechanisms. On the contrary it was reported that significant levels of hypocalcemia and hyperphosphatemia exists in later stages of renal failure cases, such as grave CRF and ESRD (Rahman *et al*., 2005) thus inducing grimness in the condition. It was also determined that substantial high levels of phosphate (hyperphosphatemia) occurs usually, however, only in later stages of renal failure, when GFR levels decrease upto 30 mL/min/l.73 m2 (Portale *et al.,* 1982; Rao, 1983). Similarly, several abnormal uremic state, that occurs in CRF/CKD patients causing accumulation of toxic uremic substances, or calcification, even in new borns, have been shown to halt or interfere with renal functions, vitamin D metabolism, thus normal functions of PTH (Drueke and Massy, 2011; Glorieux *et al.,* 1998; Jorgetti and Drueke, 2010; Patel *et al.,* 1995; Portale *et al*., 1982).

Conclusions

In conclusion, our study showed altered levels of several biochemical parameters, urinary creatinine clearance, urinary protein to creatinine ratio and parathyroid hormone in selected adult patients groups suffering from various stages of CKD ranging from mild to ESRD. Notable amongst it was high PTH level, even at an early renal failure stage, establishing that the elevated levels are directly proportional to related to the extent and magnitude of renal failure. Similarly, progressing low calcium levels and high phosphate concentrations at all levels of CKD also depicts that they are actually the instigating factors for the progression of CKD associated hyperparathyroidism.

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