DETERMINATION OF PARATHYROID HORMONE, TOTAL CALCIUM AND PHOSPHORUS IN PATIENTS WITH CHRONIC RENAL FAILURE

By
Mohammed Ahmed Eltigani Osman
B.Sc Basic Medical Sciences-Biochemistry
Faculty of Medicine and Health Sciences
Shendi University, 2002

Supervisor
Dr. Barakat El Hussein Mohammed
Head Department of Biochemistry
Faculty of Veterinary Medicine
University of Khartoum

Co. Supervisor
Dr. Ishraga Ahmed Faragalla Osman
B.Sc (Medicine) University of Khartoum
Ph.D. (pathology) Jordan University

A thesis submitted in partial fulfillment for the requirement of the degree of master in Biochemistry.

Department of Biochemistry
Faculty of Veterinary Medicine
University of Khartoum
September 2005
بسم الله الرحمن الرحيم

خلقني، وَيَهْدِينِي الَّذِي ِهِ، وَيُسِقِينِي

قَدُّرتُ ِذِي ِهِ، وَإِذَا ِشَفَيْنِي

يَحْيِينِي ثَمَّ يَمِيتْنِي وَالَّذِي

يُطَعْمُني، وَالَّذِي

يُنَّيرُني، وَالَّذِي

يُعْفِفُ عِنْي، وَالَّذِي

يُؤْمِنُونِي، وَالَّذِي

يَقْدِرُونِي، وَالَّذِي

يَحْبُسُونِي، وَالَّذِي

يَعْطِئُونِي، وَالَّذِي

يَقْلِدُونِي، وَالَّذِي

يَجْعَلُونِي، وَالَّذِي

يَوْمُ الْخَطِيَّةِ لِيَغْفِرُ 

(82-78) َهَدْيَةَ ُنَبِيَّ
Dedication

To who taught me what is the meaning of life,
mother's soul.

To who trained me how I can change better to the best,
dear father.

To the deepest feeling who support me always,
dear brother "Abd Elkarim".

To who shares me all moments of happiness and sadness, my
sisters "Arafa & Mona".

To who lead me to the way of success,
my teachers.

To my friends and colleagues.
All thanks to "Allah" from the start to the end.

I would like to express my deepest appreciation and gratitude to my supervisor Dr. Barakat El Hussein Mohamed for help, advice and encouragement.

I would be grateful to the co-supervisor Dr. Ishraga Ahmed Fragalla Osman for her smooth valuable guidance.

Thank also due to all the staff of the Department of Haemodialysis Unit in Ahmed Gasim Hospital.

Thank due to all staff of the Department of Radio-Immunio-Assay in the Sudanese atomic energy corporation.

Thank to all my friends and all who helped me in my work.

My last, but not least thank to all my family for their patience, support and enthusiasm throughout the course of this study.
ABSTRACT

This study was conducted in Department of Biochemistry, Faculty of Veterinary Medicine. Samples were collected from Ahmed Gasim Hospital, Cardiac Surgery and Renal Transplantation Center, Haemodialysis Unit, in the period from April to May 2005, to determine the parathyroid hormone levels, total calcium levels and phosphorus levels in patients with chronic renal failure.

Forty patients with chronic renal failure were selected for this study. Their ages range from (18-78 years), including 31 males, 9 females, the duration of dialysis ranged from (6-48 months).

The study also included 20 healthy personnel subjects as control group.

Sera were analyzed for parathyroid hormone concentration, total calcium concentration and phosphorus concentration.

The results showed that the parathyroid hormone level was significantly ($P < 0.05$) increased in the patients group compared to the control.
Total calcium concentration was significantly (P<0.05) decreased in the patients group compared to the control, 57.5% with hypocalcemia, 20% within the normal range and 22.5% with hypercalcemia. Phosphorus concentration was significantly (P<0.05) increased in the patients group compared to the control, 52.5% were within the normal range, 47.5% with increased level (hyperphosphatemia), and no one observed with hypophosphatemia.
الإجراء ملخص

دراسة هذه الأجراء البيطري الكليية الكيمياء في مقالة 2005 أجرىiguam نحى

численه: العينات

الكلي بالإنسان، المركزية للقلب الجراحية، الكلي، غسل وحدة إلى أبريل مرن، سنتين 2005 مريض لديها دوق الغريب

الدراسة، وشملت شهور 2010 مراقبة كمجمعة شخص.

النتائج تبين في ذلك:

• القيمة المتوسطة للبروتين الجريب من الكلي (1145 ± 455 p mol/L).

• القيمة المتوسطة للكالسيوم (6.4 ± 2.3 mg/dl) تبين أنه أساسي المستويات.

• أوزوز 520% ظاهرة في الكلى. 7.5% ليو الخ

• العينات استفحلت لـ 50% من الـ KDPZ 22.5%
التقييم الفاصل 

(5.4 ± 2.1 mg/dl) 

تعلم أن الأسبة 

المؤهلات 

فترة 

簿 

17.5% 

簿 

52.7% 

簿 

KA 

簿 

簿 

簿 

簿
LIST OF TABLES

Table 1: The level of parathyroid hormone, total calcium and phosphorus in patients with chronic renal failure 29

Table 2: The level of parathyroid hormone, total calcium and phosphorus in chronic renal failure patients compared to control group 29

Table 3: The distribution of age in the study population 48
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Factors involved in plasma calcium homeostasis</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Correlation between parathyroid hormone level and age in</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Patients with chronic renal failure</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Correlation between parathyroid hormone level and duration of dialysis</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Correlation between parathyroid hormone level and total calcium</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>in patients with chronic renal failure</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Correlation between parathyroid hormone level and phosphorus</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>in patients with chronic renal failure</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Distribution of sex in the study population</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>Distribution of duration of dialysis in the</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>study population</td>
<td></td>
</tr>
</tbody>
</table>
# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>I</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td>II</td>
</tr>
<tr>
<td>Abstract</td>
<td>III</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>V</td>
</tr>
<tr>
<td>List of tables</td>
<td>VI</td>
</tr>
<tr>
<td>List of figures</td>
<td>VII</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
</tbody>
</table>

## CHAPTER ONE

LITERATURE REVIEW .......................................................... 3

1.1. Parathyroid hormone ........................................... 3  
   1.1.1. Anatomy and synthesis .................................. 3  
   1.1.2. Control of parathyroid hormone secretion .......... 4  
   1.1.3. Parathyroid hormone action ......................... 5  

1.2. Calcium in the body ............................................ 6  

1.3. Effect of parathyroid hormone on calcium and phosphate concentration in the extra-cellular fluid ............ 7  

1.4. Vitamin D ...................................................... 8  
   1.4.1. Vitamin D action .................................... 10  

1.5. Chronic renal failure ....................................... 10  

1.6. Hyper-parathyroidism ........................................ 11  
   1.6.1. Secondary hyperparathyroidism ..................... 11  
      1.6.1.1. Control of parathyroid hormone secretion in secondary hyperparathyroidism .................. 12  

1.7. Hypocalcemia in chronic renal failure ...................................................... 16
1.8. Hyperphosphatemia in chronic renal failure .......................................... 17

CHAPTER TWO
MATERIALS & METHODS ............................................................................. 19
2.1. The study area and population ............................................................... 19
2.2. Samples collection .................................................................................. 19
2.3. Biochemical analysis ............................................................................. 20
   2.3.1. Parathyroid hormone determination .................................................. 20
   2.3.2. Total calcium determination ............................................................... 24
   2.3.3. Serum phosphorus determination ....................................................... 25
2.4. Data analysis ......................................................................................... 27
2.5. Statistical analysis ................................................................................. 27

CHAPTER THREE
RESULTS ........................................................................................................... 28
3.1. Serum parathyroid hormone ................................................................. 28
3.2. Serum total calcium ............................................................................... 31
3.3. Serum phosphorus ................................................................................ 31

CHAPTER FOUR
DISCUSSION
DISCUSSION .................................................................................................... 33
CONCLUSION .................................................................................................. 37
REFERENCES ................................................................................................ 38
APPENDIX ....................................................................................................... 47
INTRODUCTION

Chronic renal failure (CRF) produces a number of abnormalities of calcium and phosphorus metabolism. Secondary hyper-parathyroidism develops early in the course of chronic renal insufficiency (Arnaud, 1974), even at the glomerular filtration rate (GFR) of 50-80ml/1.73m² (Malluche, et al. 1976).

Secondary hyperparathyroidism is generally thought to result from hypocalcemia as a result of phosphate retention and deficient 1,25 (OH)₂D₃ synthesis (Salusky, et al.1987).

In response to an increase in serum phosphorus concentration, production of 1,25 (OH)₂D₃ is decreased and secretion of parathyroid hormone (PTH) is increased, which in turn increases urinary excretion of serum phosphorus to maintain normal serum calcium and phosphorus level. Therefore, PTH is an important factor in the regulation of calcium and phosphorus metabolism and the key target organ of parathyroid hormone action are the kidney and skeleton (Hruska and Khan, 2000). Without treatment the severity of
secondary hyperparathyroidism generally worsens with progressive
decline in renal function (Rahman, et al. 2005).

In Sudan, data regarding parathyroid hormone in relation to
different biochemical parameters in patients with chronic renal
failure are not available.

Therefore, this study was conducted to estimated
parathyroid hormone levels and to correlate these levels with other
biochemical parameters, such as total calcium and phosphorus, and
also to correlate the levels of the PTH with other factors such as age
and duration of dialysis.
LITERATURE REVIEW

1.1. Parathyroid hormone:

1.1.1. Anatomy and Synthesis:

Normally there are four parathyroid glands in the human being, they are located immediately behind the thyroid gland, one behind each of the upper and each of lower poles of the thyroid (Gyton and Hall, 1996).

Parathyroid hormone has been isolated in a pure form. It is first synthesized on the ribosomes in the form of a prepro hormone, a polypeptide chain of 110 amino acids residues. This is cleaved first to a prohormone with 90 amino acids, then to the hormone itself with 84 amino acids residues by the endoplasmic reticulum and golgi apparatus, and finally packaged in secretory granules in the cytoplasm of the cells. The final hormone has a molecular weight of about 9500. Smaller compounds with as few as 34 amino acids residues adjacent to the N terminus of the molecule have also been isolated from the parathyroid glands that exhibit full parathyroid hormone activity. In fact, because the kidneys rapidly remove the
whole 84-amino acids hormone within minutes but fail to remove many of the fragments for hours, a large share of the hormonal activity is caused by the fragments (Gyton and Hall, 1996).

1.1.2. Control of parathyroid hormone secretion:

The parathyroid glands are not under anterior pituitary control nor under nervous control. The main mechanism known to regulate PTH release is a negative feedback with plasma ionized calcium (Ca$^{+2}$). A decrease in plasma Ca$^{+2}$ level stimulates the parathyroids to release PTH. Conversely an increased plasma ionized calcium level decreases hormonal release (Sukkar, et al. 2000) (see Fig.1).

Many other factors are known to influence PTH secretion, including β-adrenergic agonists, vitamin D metabolites, growth hormone and somatostatin, vitamin A, prostaglandin, prolactin, aluminum, and divalent cations such as magnesium and strontium (Weatherall, et al. 1996).
1.1.3. Parathyroid hormone action:

Parathyroid hormone increases ionized plasma calcium and lowers plasma phosphate concentration. It acts on the bone and kidney and indirectly on the gastrointestinal tract (see Fig.1). Its actions on the bone and kidney appear to be mediated by cAMP. PTH increases the rate of bone reabsorption by stimulating the activity of the osteocytes and osteoclasts. The presence of vitamin D is needed for these actions. These effects are important in long-term regulation of plasma calcium while its actions on the kidney appear to be more important in compensating for short-term changes (Bray, et al. 1994).

In the kidney, PTH increases the tubular reabsorption of calcium and decreases that of phosphate. The resulting fall in plasma phosphate stimulates calcium release from bone and prevents the deposition of calcitriol in the kidney. Thus, the absorption of calcium in the gastrointestinal tract is increased as a consequence of an increase in calcitriol. In the long-term, increased secretion of PTH may result in a net loss of calcium from the body through the kidney. Under these conditions, increased ionized plasma calcium increases
the filtered load by an amount that exceeds the additional calcium reabsorbed in the tubules (Bray, et al. 1994).

1.2. Calcium in the body:

Calcium is an important intra-and extra-cellular ion, which comprises a major component of the skeleton and is needed for blood cloting and for the activity of many enzyme systems. Movement of calcium across cell membranes occurs all the time and change in flux is an important event, with physiological consequences. The importance of changes in calcium within the cell, acting as a messenger for hormones. Release of calcium within the cell can be an important intra-cellular signal, leading, for example, to activation of secretion processes or to muscular contraction. Even small alterations in extra-cellular calcium concentration affect the excitability of cells, and a low serum calcium concentration, hypocalcaemia, can lead to epilepsy or tetany (Brook and Marshall, 1996).
1.3. Effect of parathyroid hormone on calcium and phosphate Concentrations in the extra-cellular fluid:

The rise in calcium concentration is caused principally by two effects:

1- An effect of parathyroid hormone in causing calcium and phosphate absorption from the bone.

2- A rapid effect of parathyroid hormone in decreasing the excretion of calcium by the kidneys. The decline in phosphate concentration, on the other hand, is caused by a strong effect of parathyroid hormone on the kidneys in causing excessive renal phosphate excretion, an effect that is usually great enough to over ride increased phosphate absorption from the bone (Gyton and Hall, 1996).
1.4. Vitamin D:

Vitamin D is a steroid pro-hormone. It is represented by steroids that occurs in animals, plants and yeast. By various metabolic changes in the body, they give rise to a hormone known as
calcitriol, which plays a central role in calcium and phosphate metabolism (Murray, et al 1996).

Ergocalciferol (vitamin D) may be made commercially from plants in this way, whereas, in animals, cholecalciferol (vitamin D3) is formed from 7-dehydrocholesterol in exposed skin (Murray, et al. 1996).

Vitamin D3 formed from 7-dehydrocholesterol by the action of sunlight, and dietary vitamin D3 (or D2), after absorption from cells in the intestine followed by transport in the lymphatics, circulates in the blood bound to a specific globulin, vitamin D-binding protein. Vitamin D3 is taken up by the liver, where it is hydroxylated on the 25 positions by vitamin D3-25 hydroxylase. 25-hydroxy vitamin D3 is the major form of vitamin D in the circulation and the major storage form in the liver (Adams, 1982).

In the renal tubules, bone and placenta, the 25-hydroxy vitamin D3 is further hydroxylated in position 1 by 25-hydroxy vitamin D3-1-hydroxylase, a mitochondrial enzyme. The product is $1\alpha$, 25-dihydroxy vitamin D3 (calcitriol) (Murray, et al .1996).
1.4.1. Vitamin D action:

In addition to increasing calcium absorption from the intestine, 1,25-dihydroxycholecalciferol facilitates calcium reabsorption by the kidneys. It also acts on bone, where it mobilizes calcium and phosphate, by increasing the number of mature osteoclasts. It also stimulates osteoblasts, but the net effects are still calcium mobilization (see Fig. 1) (Ganong, 1997).

1.5. Chronic renal failure:

Renal failure is a common problem worldwide, it is now more frequent in Sudan. One of the most important functions of the kidney is the endocrine and metabolic function. This is done in association with the parathyroid gland. Chronic renal failure (CRF) is defined as a persisting and progressive deterioration of renal function leading to retention of waste products of metabolism (Brenner, et al. 1998).

Chronic renal failure is complicated by hypertension, bleeding tendency due to defect on platelets function, severe anaemia due to bone marrow suppression and/or erythropoietin deficiency,
infection, hyperuricemia, hyperphosphatemia and hyperkalemia (Brenner, et al. 1998).

The management of CRF is mainly directed towards the underlying cause, so as to delay the progression of renal damage, and the complications (Brenner, et al. 1998).

1.6. Hyper-parathyroidism:

The term hyperparathyroidism is applied to those clinical disorders characterized by an increase in circulating concentration of PTH. These can be arbitrarily classified into primary, secondary, tertiary and ectopic (Pseudo-) hyperparathyroidism (Weatheral, et al. 1996).

1.6.1. Secondary hyper-parathyroidism:

It is due to hypo-calcaemia, such as seen in vitamin D deficiency or chronic renal failure, which results in the over secretion of PTH and lead to hyperplasia of the parathyroid glands (Weatheral, et al. 1996).
1.6.1.1. Control of parathyroid hormone secretion in secondary hyperparathyroidism:

In secondary hyper-parathyroidism, hyper function of the parathyroid glands is the result not of an abnormality of the glands themselves but of continued stimulation by low concentration of calcium ion in the circulatory system. This could result from excessive losses of calcium in the urine, feces, or sweat, insufficient dietary intake of calcium or inadequate calcium absorption from the intestine, or decreased translocation of calcium from the solid calcium (Prient, et al. 1976).

The store of the PTH in the parathyroid glands is small in relation to the amount secreted. For example, in human beings normal parathyroid glands contain less than 500 units/g of fresh tissue (Kenny, et al.1964). Normal parathyroid glands in aggregate usually weight less than 150 mg (Roth, 1962).

The storage capacity of the parathyroid glands for PTH is low and the turnover of hormone is high, the parathyroid glands must increase the rate of hormone synthesis and release per cell as well as
increase the number of cells involved to meet the demands of the chronic stimulation of hypocalcemia (Prien, et al. 1976).

Factors to be considered in control of the rate of synthesis of PTH per cell are rate of entry of amino-acid precursors into the cell, rate of incorporation of amino acids into polypeptide chains, conversion of possible prohormone to active hormone, transport of active hormone within the cell packaging, and secretion. If increased synthesis is not adequate to meet the demands of chronic stimulation, more cells would have to be recruited (Prien, et al. 1976).

In view of the small amount of hormone stored in the parathyroid glands, persistent hypocalcemia might be expected to result in increased mass or volume of the parathyroid gland (Stoerk and Carnes, 1945), although such an increase in itself would not be sufficient to conclude that the glands are hyperfunctioning. There is evidence, however, that secondary hyperfunction in human beings and secondary hyperfunction induced experimentally in animals is characterized by an increase in relative and absolute numbers of chief cells, an increase in the ratio of cells to interstitum, increased ratios of cytoplasm to nucleus (Capen, et al. 1968). An absence of or marked
decrease in fat is a characteristic of secondary hyperplasia of the parathyroid glands in human beings (Roth, 1962).

The biosynthetic activity of the parathyroid cells can be estimated by evaluating the secondary state of the changes in structure which are presumably related to function (Prien, et al. 1976).

The question may now be asked whether in secondary hyper-parathyroidism the increase in total mass and number of cells is accompanied by augmented synthesis and release of PTH. There is evidence of several different types that parathyroid tissue in secondary hyper parathyroidism responds qualitatively as expected to alterations in ionic environment (Prien, et al. 1976).

Parathyroid tissue from patients with the secondary hyper-parathyroidism of chronic renal failure, when cultured in vitro, decreases the release of PTH in response to increasing the calcium and/or magnesium concentration in the ambient medium (Sherwood, et al.1969).

In secondary hyper-parathyroidism the increase in number of active cells may be accompanied by alterations in parathyroid
function as assessed quantitatively (Prien, et al. 1976). It has been noted that the number of secretory granules in active parathyroid cells decrease rather than increase when parathyroid tissue in culture is switched abruptly from high calcium to a low calcium environment (Roth and Raisz, 1966).

Since the amount of stored hormone is small, then a cell already releasing hormone at high rate may not be able to release much more when the ambient calcium concentration is lowered. Since the total number of cells is also increased the net effect on hormone release by increasing the hypocalcemia is not readily predictable (Prien, et al. 1976).

Evidence suggests that the chronic stimulus of hypocalcemia is responsible for the increase in parathyroid cell mass as well as hyperfunction of each cell in secondary hyper-parathyroidism (Melson, 1966).

In secondary hyper-parathyroidism in human beings the increase in parathyroid mass is guide variable and ranges from slight increases to weights over 100 times normal (over 10 g) (Prien, et al, 1976).
1.7. Hypocalcemia in chronic renal failure:

The biochemical mechanisms involved in the pathogenesis of the disturbances of calcium regulation and their effects in causing metabolic bone disease in patients with chronic renal failure are multi-factorial and still require considerable further elucidation (Wills, 1971).

Chronic renal failure leads to the retention of metabolic end-products, and on the main line this leads to the development of acquired vitamin D resistance with consequent hypocalcaemia. The latter is responsible for both osteomalacia and parathyroid gland stimulation. The response of the parathyroid glands with increased hormone secretion causes osteitis fibrosa. However, in some patients the efficacy of parathyroid hormone action is blocked either as a result of the acquired vitamin D resistance or because of the marked degree of hypocalcaemia. It would seem probable that the resistance to parathyroid hormone action results in excessive parathyroid hormone secretion, (parathyroid hormone overdosage), and osteosclerosis. As associated features of the retention of metabolites
in chronic renal failure there is both metabolic acidosis with bone dissolution and phosphate retention (Wills, 1971).

The absence of tetanic episodes in patients with chronic renal failure and hypocalcemia is classically explained by the concomitant acidosis, the protective role of acidosis has been attributed either to an increase in the concentration of the ionized calcium due to a decrease in the protein-bound fraction or to a specific effect of hydrogen ion on neuromuscular irritability (Walser, 1962).

In addition to both osteomalacia and osteitis fibrosa, patients with chronic renal failure also show osteosclerosis, which was probably the most common bone lesion seen in chronic renal failure, and may be present simultaneously with either osteomalacia or osteitis fibrosa (Kaye and Silverman, 1965).

The bone lesions in these patients are seen histologically as an excess of bone, or hyperostosis, rather than the production of abnormally dense bone (Garner and Ball, 1966).

1.8. **Hyperphosphatemia in chronic renal failure:**

In chronic renal failure the plasma phosphate concentration rises. Plasma inorganic phosphate is derived from the diet and may
be liberated from organic compounds such as phospholipids and nucleoproteins in catabolic states. The increase in the plasma concentration could be considered to be due simply to a reduction of functional renal mass. Although phosphate clearance decreases with advancing chronic renal disease the rate of clearance falls proportionately less than of glomerular filtration. Thus the average rate of phosphate excretion per residual nephron increases as the nephron population is reduced. There is evidence that in this mechanism parathyroid hormone is the important factor rather than the increase in glomerular filtration per residual nephron (Wills, 1971). The high phosphate concentration in itself is not known to produce biochemical disturbances but, in the classical concept of bone disease in chronic renal failure, phosphate is considered to have a secondary effect in lowering the plasma calcium concentration. It is well accepted that oral or intravenous administration of phosphate salts lowers the plasma calcium concentration in both normal and hypercalcaemic subjects (Wills, 1971).
2.1. The study area and population:

This study was conducted in Khartoum State in Ahmed Gasim Hospital, in the period from April to May 2005. Forty patients with chronic renal failure were selected randomly from Haemodialysis Unit. All patients underwent dialysis two times weekly, four hours per session, and treated with vitamin D sterol and calcium supplementation. The study also included 20 normal subjects as control group.

2.2. Sample collection:

Blood samples were obtained after an overnight fast. All samples were drawn immediately before the dialysis session. Five ml of venous blood were taken from antecubital vein by plastic disposable syringes. The blood was then transferred into a plane glass tubes. After one hour at room temperature (after clot retraction) centrifugation of the blood was done at a relative centrifugal force of 1000 g for 5 minutes. Afterward, sera were removed by disposable
pasture pipettes and transferred into glass containers. Sera were stored at (-20°C) to be analyzed in patches.

2.3. Biochemical analysis:

2.3.1. Parathyroid hormone determination:

Parathyroid hormone is determined by using radioimmunoassay (RIA) kit (M-44-68).

2.3.1.1. Principle:

The RIA method incorporated a competitive-binding reaction in which a fixed amount of radiolabeled antigen and antigen in the samples compete, for limited number of specific antibody-binding sites. Both unlabeled and radiolabeled antigens are bound by the antibody forming precipitable complexes. Bound and free radiolabeled antigens must then be separated before the radioactivity of the bound radioisotope can be measured (Yalow, 1993).

2.3.1.2. Reagents:

RIA China kit consisting of 6 vials of parathyroid hormone standards: standards are prepared by adding 2.0 ml incubation solution and mixed by gentle inversion, ready to be used with known
concentration of parathyroid hormone (63.5, 176.3, 352.6, 705.2, 1763 and 3526 p mol/L) respectively.

- One vial of $^{125}$I-PTH solution.
- One vial incubation solution.
- One vial antibody solution.
- One vial precipitate solution.

2.3.1.3. Equipments:

1- Micropipettes with disposable tips (10 - 100 µL).

2- Test tubes and Vortex mixer.

3- Test tube racks.

4- Incubater.

5- Centrifuge.

6- Gamma counter suitable for measuring iodine-125.

2.3.1.4. Procedure:

1- The tubes were labeled in duplicates.

2- 100 µL of each standard (STD) and samples were pipetted, 100µL of incubation solution were added to zero STD tube and 200 µL of incubation solution were added to non specific binding (NSB) tube.
Then 100 µL of $^{125}$I-labeled PTH were added to each tube and 100 µL of PTH antibody were added to zero STD and STDs and samples, as seen in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>NSB</th>
<th>OSTD</th>
<th>STD 1-6</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation solution</td>
<td>-</td>
<td>200</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard solution</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Samples</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>$^{125}$I-labeled PTH</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PTH antibody</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

3- The tubes were mixed and incubated at 4°C for 16 hours.

4- 500 µL of cold precipitate were added to each tube and mixed.

5- The tubes were centrifuged for 15 minutes at 3000 g.

6- The bound fractions were separated from the free fractions by decantation.

7- The precipitates were counted in gamma counter.

2.3.1.5. Calculations:

Results were calculated by using automated computerized method.
The method: using logit-log plotting:

i. Express the counts $B$ for each of the standards and unknown as a percentage of the mean counts of zero standards ($B_0$).

$$\text{i. } \frac{B}{B_0} \times 100\% = \frac{B_{\text{standard or unknown}}}{B_0} \times 100\%$$

ii. Plot the percentage values obtained for the PTH concentration on logit-log graph paper and construction as standard curve.

iii. Read the PTH concentration directly from curve of unknown samples.

![Diagram of logit-log graph showing $B/B_0$ against concentration of antibody]
2.3.2. Total serum calcium determination:

Total calcium was determined by the method described by (Connerty, et al. 1966).

2.3.1. Principle:

The method is based on the specific binding of cresolftalein complexone (OCC), a metallochromic indicator, and calcium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the cromophore formed is proportional to the concentration of total calcium in the sample.

\[
\text{OCC + Calcium} \quad \text{pH 10.7} \quad \text{OCC-calcium complex}
\]

2.3.2.2. Working reagent:

**R1:** occ indicator.

**R2:** occ buffer.

One volume of R1 were added to one volume of R2 and mixed.

2.3.2.3. Procedure:

1- Reagents and samples were brought at room temperature.

2- They were pipetted into labeled test tubes as it is shown in the following table:
3- The tubes were then mixed and incubated at room temperature for 2 minutes.

4- The absorbance (A) of the sample and STD were read against blank reagent at 570 nm using a colorimeter.

### 2.3.2.4. Calculations:

\[
\text{Total serum calcium (mg/dl)} = \frac{A \text{ of sample}}{A \text{ of STD}} \times \text{STD concentration}
\]

### 2.3.3. Serum phosphorus determination:

Serum phosphorus was determined by the method described by (Drewes, 1972).

#### 2.3.3.1. Principle:

Inorganic phosphate reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour with intensity proportional to the amount of phosphorus present in the sample.
P₃⁻ O₄ + H⁺ + (NH₄)₆ MO₇O₂₄ → Pi Phosphomolybdic complex
Phosphomolybdic complex pH >10 → Molyodenum blue reductant

2.3.3.2. Working reagent:

R₁: Molybdate Reagent.

R₂: Reducing solution.

R₃: Color developer.

One volume of R₁ were added to one volume of R₂ and mixed.

2.3.3.3. Procedure:

1- Reagents and samples were brought at room temperature.

2- They were pipetted into labeled test tubes as it is shown in the following table:

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Blank</th>
<th>Samples</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Samples</td>
<td>_</td>
<td>50 μL</td>
<td>-</td>
</tr>
<tr>
<td>STD</td>
<td>-</td>
<td>-</td>
<td>50 μL</td>
</tr>
</tbody>
</table>

3- The tubes were mixed and incubated at room temperature for 1 minute.
4- 0.5 ml of developer (R3) were added to each tube.

5- The absorbance of the sample and the STD were read against blank reagent at 740 nm using a colorimeter.

2.3.3.4. Calculations:

\[
\text{Phosphorus (mg/dl)} = \frac{A \text{ of sample}}{A \text{ of STD}} \times \text{STD concentration}
\]

2.4. Data analysis:

Clinical data were collected through a questionnaire sheet. Parathyroid hormone, total serum calcium and phosphorus were obtained from the laboratory.

The percentage of ages, sex and duration of dialysis were analyzed as seen in the appendix (Table 3, Figures 6 and 7).

Relation between parathyroid level and total calcium level, phosphorus level, ages and duration of dialysis were studied.

2.5. Statistical analysis:

All the data were presented as the mean ±SD. Student t-test was used to evaluate the statistical significance of various parameters and (P. value) of ≤ 0.05 was considered significant (Babiker, 2001).
RESULTS

In this study a total of 40 patients with chronic renal failure in the haemodialysis unit at Ahmed Gasim Hospital, Cardiac and Renal Transplantation Center were examined for the serum level of PTH, total calcium and phosphorus. The study also included 20 healthy personnel subjects as control group.

3.1. Serum parathyroid hormone:

In this study, it was found that the parathyroid hormone level was high in all chronic renal failure patients (Table 1). As shown in Table 2, the level of parathyroid hormone is significantly (P<0.05) higher in the patients group compared to the control group. No significant correlation between PTH level and age (Fig. 1), and PTH level and duration of dialysis was found (Fig. 2).
Table (1): The level of PTH, total calcium and phosphorus in patients with chronic renal failure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High Serum level</th>
<th>Normal</th>
<th>Low serum level</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>PTH (n = 40)</td>
<td>40</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total calcium (n = 40)</td>
<td>9</td>
<td>22.5</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Phosphorus (n = 40)</td>
<td>19</td>
<td>47.5</td>
<td>21</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Table (2): The level of parathyroid hormone total calcium and phosphorus in chronic renal failure patients compared to control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CRF patients (mean ± SD)</th>
<th>Control group (mean ± SD)</th>
<th>National reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (P mol/l)</td>
<td>1145 ± 455&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83 ± 30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70 – 100</td>
</tr>
<tr>
<td>Total calcium (mg/dl)</td>
<td>6.4 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.4 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.4 – 10.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.4 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 – 4.5</td>
</tr>
</tbody>
</table>

Means within the same raw having different superscript letters are significantly different (P <0.05).
Fig. 2. Correlation between parathyroid hormone level and age in patients with chronic renal failure

Fig. 3. Correlation between parathyroid hormone level and duration of dialysis
3.2. Serum total calcium:

In the chronic renal failure patients hypocalcaemia was found in 57.5% (Table 1). As shown in (Table 2) the level total calcium concentration was significantly (P<0.05) lower in the patients group compared to the control group. Also no significant correlation between total calcium level and parathyroid hormone level (Fig. 3).

3.3. Serum phosphorus:

In the chronic renal failure patients hyperphosphatemia was found in 47.5% (Table 1). As shown in (Table 2) the level of serum phosphorus concentration was significantly (P<0.05) higher in the patients group compared to the control group. Also no significant correlation between phosphorus level and parathyroid hormone level (Fig. 4).
Fig. 4. Correlation between parathyroid hormone level and total Ca in patients with chronic renal failure.

Fig. 5. Correlation between parathyroid hormone level and total phosphorus (P) in patients with chronic renal failure.

DISCUSSION
The present study was undertaken to assess PTH in patients with chronic renal failure and to correlate the levels with other biochemical parameters such as total calcium and phosphorus.

In this study; it was observed that plasma PTH was significantly increased in patients with chronic renal failure as compared to the control group. Sherrard, (1993) reported secondary hyperparathyroidism is a common finding in dialysis patients. In this study, significant levels of hypocalcemia and hyperphosphataemia were present in patients with chronic renal failure. This in line with Hruska et al., (1975) who reported about hypocalcemia resulting from phosphate retention and calcium malabsorption which has been shown to cause over production of parathyroid hormone (PTH) in advanced renal disease.

In early renal failure, alterations in vitamin D metabolism play a key role in the development of secondary hyperparathyroidism. Low levels of calcitriol and decreased expression of the vitamin D responsive element may allow greater synthesis and secretion of PTH. Phosphorus independent of serum calcium and calcitriol
increases PTH synthesis and secretion by a post-transcriptional mechanism (Slatopolsky, et al. 1999).

A prolonged decrease in serum Ca\(^{2+}\) and 1,25 (OH)\(_2\)D\(_3\), or increase in serum phosphorus, such as in patients with chronic renal failure, leads to the appropriate secondary increase in serum PTH. This secondary hyperparathyroidism involves increases in PTH gene expression, synthesis, and secretion, and if chronic, to proliferation of the parathyroid gland (PT) cells. Low serum Ca\(^{2+}\) leads to an increase in PTH secretion, PTH mRNA stability, and PT cell proliferation. Phosphate also regulates the PT in a similar manner. The effect of Ca\(^{2+}\) on the PT is mediated by a membrane Ca\(^{2+}\) receptor. 1,25(OH)\(_2\)D\(_3\) decreases PTH gene transcription. Ca\(^{2+}\) and phosphate regulate the PTH gene posttranscriptionally by regulating the binding of PT cytosolic proteins (Silver, et al. 2002).

In secondary hyperparathyroidism there is down regulation of the calcium receptor (CaR) protein, and for any increase in serum Ca\(^{2+}\) there is a less efficient inhibition of PTH secretion. As a result, for a particular serum Ca\(^{2+}\) concentration there is an enhanced secretion of PTH, which is the essence of the so-called "shift" in the

The PT has a limited amount of preformed secretory granules containing mature PTH. The PTH in these granules is itself under regulatory control. In the face of persistent hypercalcemia, there is a rapid degradation of the mature PTH in the PT cell. With the stimulus of hypocalcemia there is a rapid secretion of PTH that is rapidly renewed by the synthesis of new hormone. It has been shown that the mechanism of this regulation in vivo to be mainly posttranscriptional by an increase in PTH mRNA stability (Kilav ,et al.1995, Moallem, et al. 1998). This is in contrast to the effect of 1,25(OH)\textsubscript{2}D\textsubscript{3} to markedly decrease PTH gene expression, which is a transcriptional effect (Silver, et al.1986). It was shown in vitro that 1,25(OH)\textsubscript{2}D\textsubscript{3} decreases PTH gene expression, and in the following year shown in vivo in rats that 1,25(OH)\textsubscript{2}D\textsubscript{3} dramatically decreased PTH gene transcription (Silver, et al.1986, Silver, et al.1985). Naveh-Many, et al. (1995) studied rats with experimental uremia and showed that there was an increase in PT cells
proliferation compared with control rats. Similar results were found by (Yi , et al. 1995), who showed that rats with experimental uremia had an increase in serum PTH, PTH mRNA, and PT cell proliferation, all of which were prevented by mild dietary phosphorus restriction. 1,25(OH)₂D₃ may have a role in regulating PT cell proliferation in chronic renal failure in addition to its role in decreasing PTH gene transcription. Szabo et al., (1989) showed that the thymidine incorporation into isolated PT glands from uremic rats was decreased by prior treatment with 1,25(OH)₂D₃. Cozzolino, et al. (2001) showed that the PT cell hyperplasia in rats with experimental chronic renal failure was decreased by a high dietary Ca²⁺ or treatment with 1,25(OH)₂D₃. In this study, hypercalcemia was observed in 22.5% of patients with chronic renal failure. This hypercalcaemia may be due to the treatments used for the correction of hypocalcaemia, these medicines contain calcium supplementation, phosphate binders and active vitamin D sterols in various combination this agree with Quarles et al., (2003) who mentioned that usage of vitamin D and calcium for the treatment of hyperparathyroidism lead to the elevation of calcium and phosphorus levels.
CONCLUSION

It is concluded from this study that chronic renal failure results in hyper-parathyroidism. Also hypocalcaemia and hyper-phosphataemia were presented.

Age and duration of dialysis were found to have no effects on the PTH concentration.
REFERENCES


QUESTIONNAIRE
The PHT in haemodialysis Patients

(1) Personal data:
Name: .................................................. Age:.........................
Sex .............. Residence: ......................... Occupation:.............

(2) Duration of CRF:
(1) 1-6 months □ (2) 1 year □ (3) 2 year □
(4) more than 3 years □

(3) Type of diet:
(1) Proteins □ (2) Carbohydrates □ (3) Vitamins □
(4) Others □ ...........................................................

(4) Number of dialysis per week:
(1) One □ (2) Two □ (3) three □

(5) BP.
(1) Increase □ (2) Decreased □ (3) Normal □

(6) Past medical history:
(1) Glomerulonephritis □ (2) DM □
(3) Hypertension □ (4) Chronic pyelonephritis □
(5) Unknown □

(7) General symptoms:
(1) Bone pain □ (2) Muscle pain □
(3) Fracture □ (4) Others: .....................

(8) Treatment: ..............................................................

(9) Investigation: (1) PTH ................. (.2) Total calcium...........
(3) Phosphorus.............

(10) Other notes:....................................................................
Table (3): The distribution of age in the study population (n = 40)

<table>
<thead>
<tr>
<th>Years</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 20</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>21 – 40</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td>41 – 60</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>61 – 80</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>
Fig. 7. Distribution of duration of dialysis in the study population