

Screening Study for Determination Serum Calcium and Phosphorus Levels in Renal failure Patients in Tikrit City

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Abstract

The present study was designed to determine the calcium and phosphorus levels in patients (N=30) suffering from renal failure in (Al-Qadissia hospital- Tikrit city) and comparing with healthy volunteers. This method is based on using specific kits for these measurements to calculate the concentration of these two elements in (mg/dl) in patients and healthy volunteers with ($p < 0.05$) and show good correlation between higher level of calcium and lower level of phosphorus in serum .

دراسة مسحية لتقدير مستوى الكالسيوم والفسفور في مصل الدم عند مرضى
الفشل الكلوي في تكريت

حسن احمد حسن

المستخلص

صممت هذه الدراسة لتقدير مستوى الكالسيوم والفسفور لدى المرضى (ن=30) الذين يعانون من الفشل الكلوي في (مستشفى القادسية - مدينة تكريت) ومقارنتها مع المتطوعين الأصحاء. هذه الطريقة تعتمد على استخدام العدة المعنية لهذه القياسات لتقدير تركيز الكالسيوم والفسفور لدى مرضى الفشل الكلوي والمتطوعين بنسبة إحصائية $p = 0.05$ وتظهر علاقة جيدة بين ارتفاع نسبة تركيز الكالسيوم وانخفاض تركيز الفسفور.

Introduction

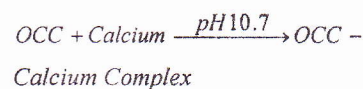
Calcium exists in the blood in three forms: ionized (13%), complexed (47%) and bound to protein, mainly albumin (40%). When calcium determinations are performed, the total calcium concentration is determined regardless of the amount of calcium present in each form [1-6]. A depressed concentration of total calcium can be due to hypoproteinemia, but the concentration of physiologically active (ionized) calcium in such case may be normal [7]. For this reason, a protein determination should accompany each calcium analysis so that the calcium value can be interpreted properly [8].

Depressed serum calcium levels usually accompany hypoparathyroidism, some bone diseases, certain kidney diseases [9] and low protein levels [10]. Elevated serum calcium levels occur in hyperparathyroidism, vitamin-D poisoning and sarcoidosis [11]. Human body contains approximately 600 gram of phosphates expressed as phosphorus of which about 85% is bound to calcium in bones and the rest principally in other tissue cells such as phospholipids, nucleic acids, and high energy compounds [12]. An elevation of phosphorus in serum is often associated with bone diseases, renal failures, hypoparathyroidism, hypervitaminosis D. Decreased serum phosphorus concentration in case of osteomalacia, vitamin D deficiency, primary hyperparathyroidism [13]. The measurement of serum calcium is fraught with possible errors; several means of contamination might lead to false elevations of serum calcium concentration. False low levels are less common, so if several measurements are obtained, the lowest is usually the most accurate [14].

Venous occlusion of the arm during venipuncture may increase the total concentration of serum [15]. This results from an increase in plasma protein concentration caused by hemodynamic change [16].

Experimental work

This research was conducted in Tikrit Governorate, Al -Qadissia hospital. The samples included (30) patients suffering from renal failure aged between (20-60) years. The determination of calcium in the serum of patients is based on the specific binding of o-cresolphalein complexone (OCC), a metallochromic indicator, and calcium at alkaline pH (10.7) with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of total calcium in the sample as in the following equation:



The calcium in serum was determined using spectrophotometric method at 570 nm, calculated as following :

$$\frac{A_{sample}}{A_{standard}} * C_{standard} = mg / dl \quad \text{the total}$$

calcium in the sample. Where A is the absorbance and C is the concentration of standard usually used 10mg/dl for calcium and 5mg/dl for phosphorus. Phosphate ions form phosphomolybdic complex with ammonium molybdate in acid medium .The absorbance measured at 340nm is proportional to the concentration of phosphate ions in the sample .The determination of phosphorus in the patients suffering from renal failure is based on the same principle that used

for calcium analysis .Figures (1) and (2) interpreted the serum calcium and phosphorus in renal failure and healthy volunteers respectively.

Materials and Apparatus

- Amino -2-methyl-2-propanol (1.70 mol/l)
- Hydrochloric acid (0.21mol/l).
- O-cresolphthalein complexone (7.8×10^{-5} mol/l).
- Hydroxy-8-quinoline(3.36×10^{-3} mol/L)
- Ethylenediamine-Tetra acetic acid (EDTA) (0.01mol/l).
- Standard calcium (2.5×10^{-3} mol/l)
- Ammonium molybdate (0.63×10^{-3} mol Sulfuric acid (0.21 mol/l).
- Phosphorus standard 5 mg/dl (1.61×10^{-3} mol/l).
- Distilled water .
- Spectrophotometer (CE-1) with automatically calibration curve.
- Quartz cell (1 cm) .
- Micropipette (25 μ l&1ml).

with healthy volunteers ,and Table (2) showed mean serum phosphorus levels were significantly ($P < 0.001$) higher in healthy volunteers compared with patients. And from Table (3) the mean \pm SD for serum calcium levels was significantly ($P < 0.05$) higher than the mean \pm SD for serum phosphorus level in patients.

Statistical analysis

Data were analyzed with Chi-square test and utilizing SPSS program .All reported values are given as mean values \pm standard deviation (SD) and $P < 0.05$ was considered as significant.

Results

Thirty patient with renal failure and thirty healthy volunteers served as control were used in this study , the results obtained were listed in the Tables (1) and (2) in the form of mean values .Table(1) showed mean serum calcium levels were significantly ($P < 0.001$) higher in patients compared

Table (1):-Calcium and Phosphorus Levels in Serum (Renal Failure)

Element	Patients No. (N)	Mean (mg/dl)	Std. Deviation (SD)
Calcium	30	8.6	0.6
Phosphorus	30	3.8	0.8

**Table (2):-Calcium and Phosphorus Levels in Serum Healthy volunteers
(Control)**

Element	Healthy Volunteers NO. (N)	Mean (mg/dl)	Std. Deviation (SD)
Calcium	30	6.6	0.8
Phosphorus	30	6.9	1.1

Table (3):-Calcium and Phosphorus Levels in Serum (Renal Failure and Healthy volunteers)

Element	Samples NO. (N)	Renal Failure Mean \pm SD (mg/dl)	Healthy volunteers Mean \pm SD (mg/dl)	t-test*
Calcium	30	8.6 \pm 0.6	6.6 \pm 0.8	significant
Phosphorus	30	3.8 \pm 0.8	6.9 \pm 1.1	significant

* P < 0.05

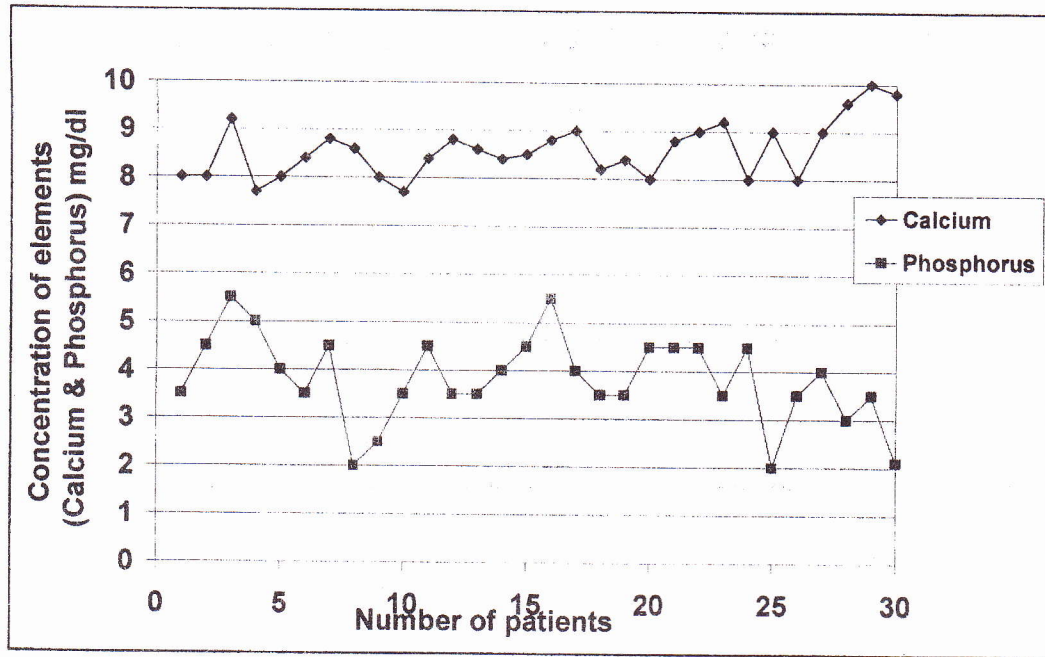
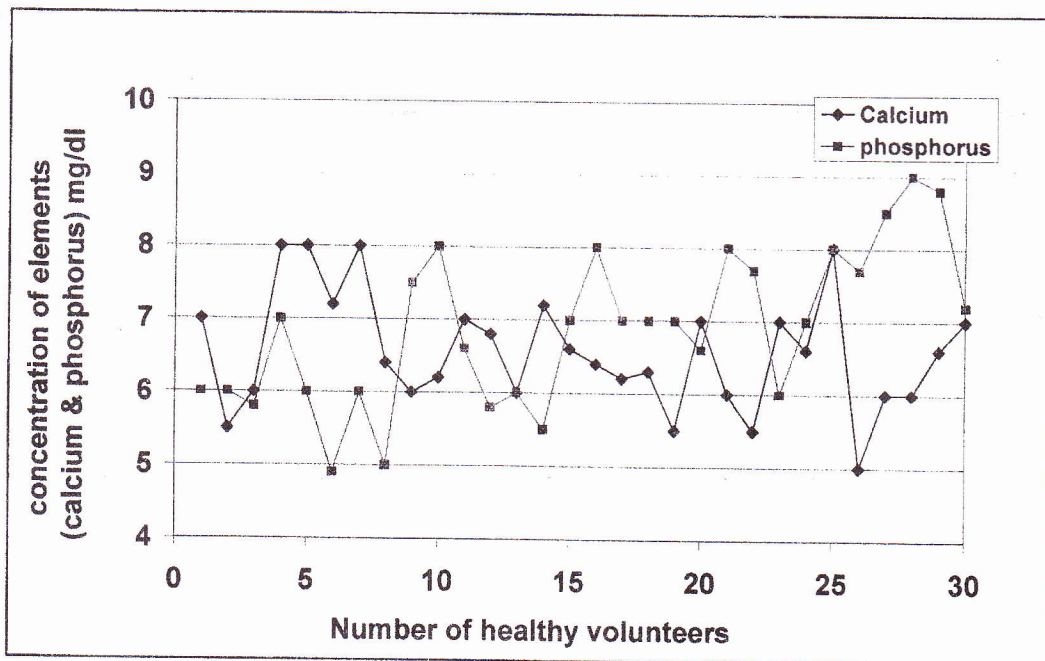


Figure (1):- Serum Calcium & Phosphorus Levels in Renal Failure



Figure(2):- Serum Calcium & Phosphorus Levels in Healthy Volunteers

Discussion and conclusion

The serum level of calcium is greatly affected by the serum level of inorganic phosphate, there is an inverse relationship between calcium and inorganic phosphate [16], the determination of inorganic phosphate in serum was done in present work in the same serum. The mean level \pm SD of calcium and phosphorus in serum of healthy volunteers and patients with renal failure disease are listed in Table (3), which ensure the inverse relationship between serum levels of calcium and phosphorus. The manifestations and hence the clinical significance of hypercalcemia consist of five effects: soft tissue calcification, tubulo interstitial renal disease, anorexia, nausea and an acute brain syndrome [17]. Three sites of soft tissue calcification occur with hypercalcemia even in the absence of serum phosphate elevations. These are corneal and / or conjunctival calcification, chondrocalcinosis and renal calcification [18]. While corneal calcifications are usually asymptomatic, conjunctival calcification often are quite irritating. It is obvious from the results of present study, that there were significant ($P < 0.05$) differences between measured serum calcium and phosphorus levels in patients and that levels for healthy volunteers, which they are increase in calcium levels and decrease phosphorus levels in patients compared with that healthy levels as explained in tables and figures. Therefore estimation of calcium and phosphorus levels in human serum are very important for diagnosis of disease such as hypothyroidism and hyperthyroidism and because they have an important role in physiological functions e.g. muscle contraction, blood coagulation, and kidney function. The highest values of calcium are found in

blood may be due to an increase of calcium concentration caused by such hormones like parathyroid hormone (PTH) [19], in contrast, the level of phosphorus concentration will be reduced. The biochemistry and metabolism of calcium and phosphorus is complex and differ in their excretion in gut, kidney, and bone.

References

1. Tietz NW. Text book of Clinical chemistry. 3rd Ed. C.A. Burtis, E.R, Ashwood, WB Sanders.1999;1406- 1457
2. Jays IT. Classification of otitis media and surgical principle. Clinics of north America. J.1995,61;114
3. Mortin LG . Direct method of Inorganic phosphate. Am .J.Clin. Path.1974,45;290
4. Bishop ML. Determiation of Essential element in serum. 2005 ;202-206
5. Technical sheet. Determiation of Phosphate Biolabo Reagent.2005
6. Nickless M. Determiation of calcium in serum . Talanta.J. 1975 ,22;201
7. Marton JD. Assay of Calcium in serum . J.chem. Br. 1990,78;141
8. Young DS. Effect of Drug on clinical Laboratory Test.4th ed.1995 ;456- 462
9. Macmaland HB. Determiation of calcium in serum .Biochem.J.1988,285;108.
10. Marshal ZD .Determiation of calcium in serum .Med.J.1977,1041;297.
11. Handson HM. Determiation of phosphorus in serum. Clin.Lab.Sci.J.1979,271;11.
12. Jakson B.Z., "Determiation of Calcium in serum.Anal. Chemistry.J.1980,90;1.

13. Mik MM .Determination of phosphorus in serum. *Clinical endocrinal .J.*1975,454;4.
14. Flant AG. Determination of calcium and phosphorus in serum. *J.Med.*1978.201;287.
15. Waston DV. Analysis of Element. *pharma. J.*2000,115;240.
16. Butan KL. Determination of calcium. *Environ. Contam.Toxical.*2003, 44; 417.
17. Skoog KN.Analysis of serum. *Clinical .chem.*1973, 19; 476.
18. Martin MM. Detection of Phosphate. *Clinical chem.*1971,17;1038.
19. Bertram G. Katzung. *Basic & Clinical pharmacology.* 2007; 38-39.