# ANALYSIS OF SERUM CALCIUM AND PHOSPHORUS IN RICKETS AND NON RICKETS CHILDREN OF GONIN-GORA, KASO AND JANKASA COMMUNITIES IN KADUNA STATE

**BY**

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**DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY**

# AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

**MARCH, 2011**

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**(M. Sc/PHARM-SCI/00140/06-07)**

# A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE IN PHARMACEUTICAL AND MEDICINAL CHEMISTRY

**DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA**

# MARCH, 2011

# DECLARATION

I declare that the work in this thesis entitled ‘Analysis of serum calcium and phosphorus in rickets and non rickets children of Gonin-gora, Kaso and Jankasa communities of Kaduna state’ was performed by me in the Department of Pharmaceutical and Medicinal Chemistry under the supervision of Prof. (Mrs.) M. T. Bakare-Odunola and Prof. Magaji Garba of the Department of Pharmaceutical and Medicinal Chemistry, A.B.U., Zaria. No part of this work has been presented for another degree or diploma in any in any institution.

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# CERTIFICATION

This thesis titled “analysis of serum calcium and phosphorus in rickets and non rickets children of Gonin-gora, Kaso and Jankasa communities of Kaduna state ” meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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# DEDICATION

I dedicate this thesis to my dearest parents, my siblings and my lovely wife for relinquishing some of their comforts while I was undertaking this work. Without their help and support I could not have achieved this.

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# ABSTRACT

The beginning of the 20th century witnessed the epidemic of nutritional rickets among children in many countries of Asia, North America, Northern Europe and Africa. Nutritional rickets remain a problem in developing countries despite a decline in the prevalence of the condition in developed countries. Prevalence of rickets among infants and young children is high in Nigeria and in Gonin-Gora, Jankasa and Kaso in particular. It was therefore imperative to evaluate some biochemical parameters in rickets disease prevalence areas of Kaduna state namely: Gonin Gora, Jankasa and Kaso. This study aimed at determining the serum levels of calcium and phosphorus together with the levels of associated biochemical parameters for the affected family member in these communities; as an investigation into the scourge of rickets. Randox Diagnostic test kit was used to determine the serum levels of calcium and urea while creatinine and phosphorus serum levels were measured using Agappe Diagnostic kit, serum sodium and potassium levels were determined using flame photometric method. The results obtained showed that serum calcium levels were low with mean values of 2.29± 0.01 S.E.M., 2.34+

0.01 S.E.M and 2.24 ± 0.01 S.E.M in Gonin Gora, Jankasa and Kaso respectively compared with the 2.25-2.75 mmol/l normal limit. Phosphorous levels were toward the upper limit with mean values of 1.48 ± 0.02 S.E.M and 1.68 ± 0.02 S.E.M in Gonin gora and Jankasa respectively; compared with the normal limit of 0.8-1.9 mmol/l. However the mean serum calcium for rickets children from Kaso community (2.19 ± 0.03S.E.M) was below the normal range value of (2.25-2.75mmol/dL). None of the differences in measured levels was statistically significant. Rickets among rural children has been reported to be attributed to low serum calcium levels. The low serum levels of calcium and high serum phosphorus levels could be the major causes of the disease in these settlements especially during the period of the children growth. Also when the mean biochemical

parameters for Gonin-Gora, Jankasa and Kaso were compared, the results showed that calcium levels was much more significantly reduced in Kaso compared with the other two communities, and this could be the reason why more rickets children were found in Kaso compared to Gonin-Gora and Jankasa. The results of influence of sex among the rickets and non rickets males and females children showed that, sex had no significant influence in the parameters of rickets male and females children living in Gonin-Gora, Jankasa and Kaso communities.

In conclusion, the concentrations of serum calcium for rickets children were at the lower limit of normal range while the concentration of serum phosphorus were at the higher limit of the normal range which can be attributed to rickets disorder among children.

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# CHAPTER 1 INTRODUCTION

# RICKETS

Rickets causes bone deformities through the impaired mineralization of actively growing bone. Rickets is ranked among the 5 most prevalent diseases of young children in developing countries (Guesry et al., 1991), and is frequently found in Africa and Asia (Pettifor et al., 1978, Thacher et al., 1997; Thacher, 2003; Fischer et al., 1999 and Bhattacharyya et al., 1992). Up to 9% of children in central Nigeria have physical findings consistent with rickets (Pfitzner et al., 1998), including bowing of the legs, impaired mobility, pain, and pathologic fractures. Besides the long-term sequel associated with the bone deformities, rickets is also associated with an increase in acute morbidity. In Ethiopia, a case-control study described a 13-fold greater prevalence of rickets among children with pneumonia than in control children (Muhe et al., 1997).

Although nutritional rickets is often attributable to vitamin D deficiency (Holick et al., 1999). Recent reports suggest that an insufficient calcium intake is also an important cause of rickets (Oginni et al., 2003 , DeLucia et al., 2003). Children with calcium-deficiency rickets have higher serum concentrations of 1,25-dihydroxyvitamin D [1,25(OH)2D] and parathyroid hormone and lower serum concentrations of calcium and 25-hydroxyvitamin D [25(OH)D] than do children without rickets. Calcium supplementation, with or without vitamin D, heals rickets more rapidly in children than does vitamin D alone (Thacher et al., 1999). However, despite uniformly low calcium intakes, calcium intakes are not lower in Nigerian children with rickets than in those without rickets ( Thacher et al., 2000). Reduced calcium absorption or relative

resistance to 1,25(OH)2D could account for rickets in these children.(Thacher et al., 2000).

# TYPES OF RICKETS

1. **Vitamin D- deficiency rickets or nutritional rickets.**

The causes are vitamin D deficiency, phosphorus or calcium deficiency (rare), inadequate sunlight exposure, secondary to malabsorption syndrome (IBD), celic disease, cystic fibrosis (rarely). The clinical features include skeletal findings, abnormal gait, hypocalcemic tetany / seizures developmental delay and failure to strive.

# Vitamin D –dependent rickets.

Type 1 is also known as pseudovitamin D deficiency rickets and it is caused by deficiency of renal 25 (OH) D3-1-alpha-hydroxylase. Inheritance pattern is autosomal recessive and the clinical features for younger than two years are hypocalcemic tetany, severe bony changes and seizures.

# Type 11 or hereditary 1-alpha,25-dihydroxyvitamin D-resistant rickets.

It is caused by defective interaction between calcitriol and receptor. Inheritance pattern is autosomal recessive and clinical features for younger than one year, severe bony changes and alopecia.

# Vitamin D-resistant rickets.

There are two types of vitamin D resistant rickets which are;

di) Familial hypophosphatemic rickets or X-linked hypophosphatemic rickets caused by impaired proximal renal tubular reabsorption of phosphorus and inappropriate normal calcitriol levels. Inheritance pattern is X-linked dorminant and clinical features include short status, leg bowing and dental abnormalities.

dii) Hereditary hypophosphatemic rickets with hypercalciurea.

It is caused by impaired proximal renal tubular reabsorption of phosphorus and increased calcitriol. Inheritance pattern are autosomal recessive and autosomal dorminant. The clinical features are bone pain and muscular weakness.

# Miscellaneous

These include renal rickets or renal osteodystrophy which is caused by loss of functional renal parenchyma caused by chronic renal failure leads to mineral derangements and decreased calcitriol production. The clinical features include bone pain, arthralgias, fractures, muscle weakness and failure to thrive.

# Rickets of prematurity

The cause is multifactorial with osteopenia and fractures as clinical features.

1. Tumor-induced or oncogenic rickets caused by tumor-induced inhibition of renal 25 (OH)D3 -1-alpha-hydroxylase. The clinical features include fractures, bone pain and muscle weakness.(American family physician,2006).

# VITAMIN D DEFICIENCY

The peak age at which rickets is most prevalent is 3-18 months (Salimpour, 1975). Factors that have been shown to be important in the pathogenesis of rickets at this age include exclusive breast-feeding, maternal vitamin D deficiency, living in temperate climates, lack of sunlight exposure, and darkly pigmented skin. In the Middle East and other more-tropical climates, social and religious customs that prevent sunlight exposure appear to be important (Molla et al., 2000 Bassir et al., 2001 Atiq et al., 1981).

It is well recognized that breast milk normally contains insufficient concentrations of vitamin D or its metabolites (estimated as 20–60 IU/L) (Specker et al., 1985; Hillman et al., 1986). To ensure the normal vitamin D status of the nursing infant relatively high-dose maternal vitamin D supplements (2000 IU/d) are needed to increase maternal breast milk concentrations to levels that maintain the vitamin D status of the breast-fed infant (Ala-Houhala et al., 1986).

Breast-fed infants are generally protected from vitamin D deficiency rickets during the first few months of life, because vitamin D metabolites, especially 25- hydroxyvitamin D [25(OH)D], do cross the placenta, such that neonatal 25(OH)D concentrations are approximately two-thirds of maternal values (Hillman et al., 1995). It is estimated that the half-life of serum 25(OH)D is about 3 weeks; therefore, even if neonates do not receive an exogenous supply of vitamin D during the first weeks of life, 25(OH)D concentrations should decrease to values associated with vitamin D deficiency only towards the second month, provided that the maternal vitamin D status is adequate during pregnancy.

Several studies from the Middle East, North America, and northern Europe have highlighted the prevalence of low circulating concentrations of 25(OH)D during pregnancy (Henriksen et al., 1995; Daaboul et al., 1997; Datta et al., 2002). Factors found to be important include increased skin pigmentation, immigration from non- European countries to countries of high latitude, limited skin exposure as a result of religious and social customs, and vegetarian diets. Congenital rickets has been observed in such situations, although its occurrence is rare (Mohapatra et al., 2003; Anatoliotaki et al., 2003; Zeghoud et al., 1997), and neonatal hypocalcemia is more frequent among neonates born to mothers with low 25(OH)D concentrations than among those born to mothers with normal vitamin D status (Zeghoud et al., 1997).

The development of clinical vitamin D deficiency rickets is dependent not only on the severity of the vitamin D deficiency [circulating concentrations of 25(OH)D] but also on the duration of the deficiency, on the rate of the child's growth (which influences calcium demands), and on the dietary calcium content. Studies from northern Europe and North and South America have highlighted the marked seasonal fluctuations in serum 25(OH)D concentrations, with values being lowest in late winter and highest in late summer or early autumn (McLaughlin et al., 1974, Olivieri et al., 1993; Harris et al., 1998). Several studies have documented spontaneous healing of radiologically evident rickets during the summer months (Gupta et al., 1974) and seasonal fluctuations in serum parathyroid hormone (PTH) ( Guillemant et al., 1995) and 1,25-dihydroxyvitamin D [1,25(OH)2D] (Woitge et al., 2000) concentrations in association with changes in serum 25(OH)D concentrations. Clinical vitamin D deficiency rickets is also well recognized to have seasonal fluctuations in prevalence, with the highest prevalence being in spring and early summer (Salimpour.,1975).

The seasonal changes in 25(OH)D concentrations, the lag period between the decrease in 25(OH)D concentrations and the development of biochemical, radiologic, or clinical rickets, and the influence of diet on the development of rickets have made it difficult to define a clear division between vitamin D deficiency and sufficiency on the basis of serum 25(OH)D concentrations. Nevertheless, there is widespread agreement in the pediatric literature that vitamin D deficiency should be defined as 25(OH)D concentrations of <10–12 ng/mL (Greer et al., 2003 and Shaw et al., 2002). The value varies, however, depending on the assay method used to determine 25(OH)D concentrations (Lip et al., 1999). In the past decade, considerable discussion has taken place regarding the definition of vitamin D sufficiency and what should be considered the normal range for serum 25(OH)D concentrations (Viet, 1999). In population studies, the term vitamin D insufficiency has been used to indicate serum 25(OH)D concentrations between those associated with vitamin D deficiency and those considered to be optimal. Vitamin D insufficiency is associated with mildly elevated PTH concentrations, although values remain within the normal reference range (Jesudason, 2002). Few studies have been conducted among infants and children to determine whether the concept of vitamin D insufficiency is valid. Among young infants, it appears that PTH concentrations increase only when 25(OH)D concentrations are in the vitamin D3-deficient range (Zeghoud et al, 1997). Studies with adolescents reported increased PTH concentrations when 25(OH)D concentrations decreased below 12–16 ng/mL (Guillemant et al., 2001 and Outila et al, 2001), whereas Docio et al (1998) suggested that perturbations in calcium homeostasis occur among prepubertal children when 25(OH)D concentrations are between 12 and 20 ng/mL. Therefore, it appears that, if the concept of vitamin D insufficiency is valid for children, values are

very close to the upper limit of what is defined as vitamin D deficiency, a pattern that is very different from that reported for adults.

# CALCIUM DEFICIENCY

The role of low dietary calcium intakes in exacerbating the development of vitamin D deficiency rickets has been known for many years. More than 80 years ago, Mellanby (1919) showed the deleterious effects of low dietary calcium intakes on the development of rickets among vitamin D-deficient animals. Sly et al (1984) demonstrated a similar effect with the addition of unrefined maize to a vitamin D- deficient diet for baboons. However, the mechanisms were not known.

Among humans, one of the most well-studied communities with a high prevalence of rickets has been the Asian community in the United Kingdom. Since the early 1960s, numerous studies have highlighted the predisposition of this community to rickets and osteomalacia (Dunnigam et al., 1962; Ford et al., 1976; Hodgkin et al., 1973). Several pathogenetic mechanisms have been proposed, including lack of sunlight exposure, increased skin pigmentation, lack of dietary vitamin D intake, genetic predisposition, low-calcium diets, and high phytate contents in the diet. Clement et al (1987) using a rat model, demonstrated that the elevation of 1,25(OH)2D concentrations through feeding of the rats with low-calcium or high-phytate diets resulted in increased catabolism of 25(OH)D to inactive metabolites and increased excretion of these products in the stool, with resultant reduction of 25(OH)D concentrations. Similarly, infusion of 1,25(OH)2D led to a reduction in the serum 25(OH)D half-life and a 7-fold increase in 24,25-dihydroxyvitamin D production by the kidney (Halloran et al., 1986). In human studies, the half-life of 25(OH)D was reduced by nearly 40% among patients with partial gene, secondary hyperparathyroidism, and

elevated 1,25(OH)2D concentrations (Davies et al., 1997), and similar findings were noted among patients with intestinal malabsorption (Batchelor et al.,1982) and subjects consuming high-fiber diets (Batchelor et al., 1983). The administration of 1,25(OH)2D to normal subjects was shown to reduce the circulating 25(OH)D half-life and to induce vitamin D deficiency among those with relatively low 25(OH)D concentrations (Clement et al., 1992).

Therefore, it was proposed by Clement (1989) that the pathogenesis of rickets in the Asian community in the United Kingdom is attributable to the high-cereal, low- calcium diet, which induces mild hyperparathyroidism and elevation of 1,25(OH)2D concentrations, with a resultant reduction in vitamin D status. In situations in which the vitamin D status is marginal, because of reduced sun exposure, increased skin pigmentation, and/or limited dietary vitamin D intake, the reduction in 25(OH)D half- life is sufficient to produce vitamin D deficiency and rickets. It follows that rickets in the Asian community can be treated either by increasing the vitamin D intake or by reducing the phytate content of the diet. Both of these treatments have been found to be effective (Ford et al., 1972; Pietrek et al., 1976).

The role of low dietary calcium intakes in the pathogenesis of vitamin D deficiency is probably greater than originally recognized. This has been proposed as a mechanism for rickets among young children in India (Balasubramanian et al., 2003) and among toddlers in the United States (DeLucia et al., 2003) and probably accounts for the lower 25(OH)D concentrations among rachitic subjects, compared with control subjects, in Nigeria (Thacher et al., 1999).

# STATEMENT OF RESEARCH PROBLEM

Visitors to Gonin-Gora, Jankasa and Kaso communities with a population of about four thousand people can not fail to notice the prevalence of rickets disorder. Most of the children in these communities appear to be suffering from a disease condition called Rickets. Medical experts attribute the causes of rickets to nutritional disorder characterized by softening and weakening of bones in children resulting in skeletal deformities. Giving birth in these villages is normal, but the feelings that run through parents can better be imagined. However, to a discerning mind or visitor to the area the reason is not far fetched as children born here end up with deformities. The prevalence rate of the disease in these villages is high as there is hardly a family without a child afflicted with the disease. Children in these villages with the disease were not born with these deformities; it began when they started walking. Driven by some kind of superstition, men here see their wives and mothers of the children as being possessed by some evil spirit. This belief as an explanation for the medical condition of the children is strongly rooted amongst the Gbaygi people, such that when new babies are born, parents are seldom happy. What hits them is the thought of what will become of their children. According to Kitz (1997), when the case was first reported in 1997 only about twelve (12) cases were known. Today, over two hundred and fifty (250) cases are known and the fear is that in a few years time it may be doubled.

# AIM AND OBJECTIVES OF THE STUDY

This present work aims at investigating the probable causes of rickets in Gonin- Gora, Jankasa and Kaso communities by estimating the serum levels of calcium, phosphorus and associated biochemical parameters.

The above aim will be achieved by the following objectives;

* + - Collecting blood samples from the rickets and non rickets children from families with a rickets child or children in Gonin-Gora, Jankasa and Kaso communities of Kaduna state with their informed consent.
		- Estimating the sodium, potassium, calcium, phosphorus, urea and creatinine levels in the sera samples collected from rickets and non rickets children. Randox diagnostic kit, Agappe diagnostic kit and flame photometric method will be for these analyses.
		- Comparing the serum levels of the estimated parameters for the rickets children with those of the non rickets children.

# CHAPTER 2 LITERATURE REVIEW

# THE COMMUNITIES

Jankasa and Kaso are rural land locked villages located in the remote interior of Chukun Local Government Area of Kaduna State Nigeria. They are twenty five kilometers (25kms) South of Kaduna town on the Kaduna-Kachia road. While, Gonin- gora, unlike Jankasa and Kaso, is just a few kilometers South of Kaduna town on the Kaduna-Abuja road. The natives of these villages are Gbaygi, Kadara, Fulani and Hausa. Though there are very few minority tribes scattered all over the region.

In terms of origin, the Hausa and Fulani people claim to descend from the Fulani (Bororo) origin who were scattered all over and originated from North Africa. Some of the Bororo lost their cattle and settled in these areas for farming. According to them, cattle were the up-keep and as they dwindled, another means of livelihood had to be found. These communities believe they would have been bigger than they are except for the effects of tribal feuds, harsh climates and tribal wars combined to keep the population down. Their population began to grow with the arrival of the British and Missionaries who brought peace, medical help and educational services. As seen, the people live in a non- mountainous, dry and plain land. Evidently, the cause of this was the need for more land for farming and better homes. Each of these inhabitants has their own language. The Gbaygi, speaks Gbaygi language, Kadara speaks Kadara language, the Fulanis speak Fulatanchi whereas the Hausa speaks Hausa which is the most commonly used language. Communication in Hausa is done through out these areas. As the case is in traditional African society where everybody is a worker, Gbaygi and Kadara are all farmers, hunters and herdsmen. There is no other way of earning a living

from these communities. Fulani still keep their cattle as a means of livelihood but not in large number. They milk cows and process the milk for commercial purpose. While the Hausa’s, are both farmers and traders. Some of them buy farm produce and transport it to a distance for sale to earn interest.

# RELIGION AND CULTURE

The religion of these people is a combined belief in Allah (a Supreme God) with belief in gods, the spirit of ancestors and fetishes. About ten percent (10%) of the population practice animism while about sixty percent (60%) are Christian and the remainder practice Islam.

At the base of their traditional religion is the concept of the “ultimate Reality”. The conception of God or the Supreme Being came into being to explain the origin of man and the world. At the base of Gbagyi and Kadara religious philosophy, “God is the abstract idea, the cause”. He created the Earth and Man. He is an all knowing and all seeing God. He is “transcendent,” living in heaven from where he rules the universe. The foundation of this religion and others rests on the mystery of the origin of man and the universe. These people religion relates the beliefs in deities to daily life (which has led to the creation of still lesser gods or spirits which control moral and social life), and enterprises such as agriculture, hunting, marriages etc. They also believe in the spirits of dead ancestors. The dead ancestors living in the spirit world serve as “solicitors” and “advocators” on behalf of their living relatives. Despite the infiltration of Christianity and Islamic ideas into the Area, the religious concepts are still deeply entrenched in the minds of the inhabitants of these areas including those who have accepted Christianity or Islam. These Christians or Muslim can not be regarded as consummate converts.

They accepted Christianity or Islam because it affords them an additional channel of reaching God.

As part of their religious believes and culture these people especially the Gbaygi and Kadara care for each other (provided he/she is willing to work). People work for one another. The work is essentially distributive. They practice aid (gai-ya), a term for aid where people take turns helping each other working in the fields. It also means the host either is just married or is building a new house and needs assistance from the community. This type of occasion requires entertainment after a full day of working hours.

# REVIEW ON ANALYSIS OF CALCIUM, SODIUM, POTASSIUM AND PHOSPHORUS

Aersenazo III dye assay method which uses aqueous composition at pH of about 7.5 and 10 to form complex calcium with hydroxyquinoline has been reported for the analysis of calcium. (United states Patent 5215922 (2004-2010). http.[www.freepatentsonlin.com/5215922.html).](http://www.freepatentsonlin.com/5215922.html%29)

An improved reagent system for determining calcium by the orthocresolphthalian complexone colour reaction has also been described. The reagent utilizes an alkaline buffer containing an amino lower alkanol at a pH of about 10.2 –

10.5. (United State Patent 3938954. http.[www.freepatientsonlin.com/3938954.html).](http://www.freepatientsonlin.com/3938954.html%29)

Daisy et al., (2010) reported the determination of serum phosphate by molyebdivanadate method, using colorimetric assay. It is based upon the reduction of molybdicaciid to molybdenum blue and subsequent estimation of the intensify of the blue colour formed.

An improved photometric method for the determination of inorganic phosphate in fluid samples was reported. The method made used of two reagents, an acid reagent and ammonium molybdate reagent. The concentration of inorganic phosphate in the said sample was determined by comparing the difference in light absorbance for a standard having a known concentration of inorganic phosphate. (United states patent 4599316 http/ww.freepatentsonline.com 4599316.html).

Serum calcium and phosphorus can also be analyze by using Atomic Emission spectrometry. (Polyakova and Shuvaeva, 2005).

A direct method for spectrometric determination of inorganic phosphate in blood serum was proposed by Daly and Gerhard (1974). The process requires only a single reagent addition and comprises reacting the phosphate with an ammonium molybdate solution and measuring the absorbance within a specific time interval.

An enzymatic colorimetric method for the determination of inorganic phosphorus in serum and urine was reported by Berti et al (1988). Phosphate ions react with inosine in the presence of purine nucleoside phosphorulase to form hypoxanthine; this is oxidized by xanthine oxidase to uric acid with the production of hydrogen peroxide. The later is determined with the aid of chromogen system peroxidase/4 – amnopheniazone/N- ethyl – N (3-methylphenyl)–N1 – acetylethylene diamine, the coloured.

Busch et al (1974) has described a vidicon flame spectrometer which permits simultaneous multielement (sodium, potassium and calcium) analysis by flame emission. Serum samples were introduced into a flame source by injection of micro volume samples without dilution or pretreatments using a hypodermic syringe. The

transient signals produced by flame emission are dispersed using a greating monochromotor and detected using a silicon intensified target vidicon tube.

A manual spectrophotometric method for the measurement of serum sodium and potassium by enzyme activation was reported by Mazzachi (1994).The assays requires only minimal modification of reagents already available for BM/Hitachi analyzers and are performed in an end point mode, allowing up to 20 assays per run.

The discrete flame atomic emission method described by Chuang et al (1973) has been applied for the determination of calcium, sodium and potassium in several blood samples. The method involves injection of blood sample (in some cases diluted with appropriate chemical) directly into a suitable carrier solution, which transport the serum plug into a H2/N20 flame. The resulting integrated emission signal is corrected for a blank emission signal and the amount of potassium, sodium and calcium in the blood serum is readily on a suitable analytical curve.

# REVIEW ON ANALYSIS OF CREATININE AND UREA

A flow-through enzyme reactor system for the determination of urea using conductimetric measurement has been described. Samples were introduced as pulses in a continuous flow of buffer which is passed through a dialyser. The dialysis solution on the other side of the dialysis membrane collects urea molecules from the samples which diffuse through the membrane and is pumped through an enzyme reactor containing urease immobilized to porous glass. Conductivity electrodes are used to measure the increase in conductivity of the dialysis solution resulting from the hydrolysis of urea into charged products. The system is used for the determination of urea in standard solutions as well as in human blood serum samples.(Thavarungkul et al.,1999).

Another method is, the common direct method for the determination of urea which employs the Fearon reaction where urea reacts with diacetyl to form a coloured chromagen, which is then quantified photometrically.

A colorimetric process for the quantitative determination of creatinine in blood serum or urine wherein an alkaline pH in the approximate range of 11.0 to 13.5 is maintained during colorimetry evaluation and in which urea and a detergent are caused to react simultaneously and synergistically with the serum or urine protein to prevent extraneous chromogen formation in the conventional alkaline-picrate reaction for creatininem (Serwin 1974 United States Patent 3894843).

# COLORIMETRIC METHODS OF ANALYSIS

Colorimetry is a branch of spectrometry which is concerned with the measurement of absorbance of coloured substances. A drug with little or no useful absorption in the visible region can be more sensitively determined by modifying it to a more highly absorptive chromophore; or a drug, its impurity or a metabolite can be selectively transformed so that its spectrum is shifted to the visible region and away from interference caused by anoyher drug, formulation components or biological substances. These can be accompanied by the use of colour reagents (selective) or colour reaction. Several factors can affect the success of the determination:

* + 1. The colour reagent should be selective for the drug molecule itself, discriminating against degradation products, impurities and formulation excipients likely to be present.
		2. The effect and control of the drug parameters likely to affect the colour reaction should be established, viz solvent, pH (use of a buffer often considered),

temperature (optimum), time (standing time for full-colour development after addition of the reagent), reagent concentration (optimum concentration to be established), order of mixing reagents (a specified sequence is frequently important), stability (some reagents may need to be stabilized and ,for unstable absorbing complexes formed, absorbance measurement should be taken as soon as possible ).

* + 1. The time required to established a stable absorbance plateau and the stability of the chromophore generated should be carefully assessed and validated.
		2. In general, analytical performance should be established in terms of recovery, precision, sensitivity, linear range and robust behaviour.

In visual colorimetry, natural or artificial white light is generally used as light source. When the eye is replaced by a photoelectric cell, the instrument is termed a photoelectric colorimeter or absorptiometer. This is usually employed with light contained within a comparatively filters. The filters (which are manufactured by Kodak,llford and Coming) are used to isolate any desired spectral region. The best filter to use in a particular determination is that which gives the maximum transmission for a given concentration of the absorbing substance.

Examples of commercial colorimeters/absorptiometers include the Eel, Gallenkamp and Bausch Colorimeter, the Lomb Spectronic 20 Colorimeter, the Hilger Biochemical Absortiometer and the Unicam SP-6-550 Absorptiometer. (Olaniyi, 2000)

# CHAPTER 3

**MATERIALS AND METHOD**

# MATERIALS

# Chemicals

The reagents used include the following: Trichloroacetic acid

Randox diagnostic kit, Randox laboratories Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.

Agappe diagnostic kit, Agappe Hill: Dist: Ernakulam, kerala, India\_683- 562

Deionised distilled water Ammonium molybdate Methylated spirit

# Equipment and Glassware

Retort stand

Beakers 50ml, 100ml Volumetric flasks 25ml, 50ml Glass vials

Flame photometer (model; Corming 410, UK)

Colorimeter (Model;Beckman Cuolter, Germany) Polyethene bags

Conical flask Glass Petri-dishes Glass spreader

Measuring cylinder:50ml Scape vein needles Samples bottles

Cotton wool

# METHOD

# Study Protocol

Children with deformities characteristics of rickets were identified, clinical history obtained and examination points documented. 5ml of blood sample were obtained from anticubital vein into capped, clean plastic vials from each of the 30, 40 and 57 rickets( R ) and 30,40 and 57 non rickets (N R ) living in Gonin Gora, Jankasa and Kaso communities of Kaduna state respectively. The non rachitic healthy children from the same communities serves as controls. The sera were obtained and analysed for the content of sodium , potassium, calcium, phosphorus ,cretinine and urea.

# Analysis of serum Calcium, Phosphorus, Urea, Creatinine, Sodium and Potassium

# Analysis of calcium

Randox diagnostic kit was used for the determination of serum calcium urea. The test was carried out in three different test tubes; blank, standard and sample. The blank contains 1000ul of working reagent, the standard contains 1000ul of working reagent and 10ul of standard reagent and the sample contains 1000ul of working reagent and 10ul of serum. All these were mixed and incubated at room temperature for 5 minutes. The absorbance of standard and sample were read against the blank at 570nm (Ray et al.,1967 Barnell et al.,1973).

Concentration(mmol/l) = absorbance of sample x 2.50

Absorbance of standard

# Analysis of Phosphorus

Agappe diagnostic kit was used of the determination of inorganic phosphorus. The test was carried out in three different test tubes; blank, standard and sample. The blank contains 1000ul of working reagent, the standard contains 1000ul of working reagent and 20ul of standard reagent and the sample contains 1000ul of working reagent and 20ul of serum. All these were mixed and incubated at room temperature for 5 minutes. The absorbance of standard and sample were read against the blank at 570nm (Tietz,1983).

Concentration(mmol/l) = absorbance of sample x 5

Absorbance of standard

# Cretinine Test

Cretinine was also determined using Agappe diagnostic kit. The test was carried out in three different test tubes; blank, standard and sample. The blank contains 1000ul of working reagent, the standard contains 1000ul of working reagent and 100ul of standard reagent and the sample contains 1000ul of working reagent and 100ul of serum. All these were mixed and incubated at room temperature for 5 minutes. The absorbance (T1) of standard and sample were read against the blank at 520nm a second reading was taken immediately after 60s (T2) (Allen et al.,1982; Tanganelli et al., 1982).

Concentration (µmol/l) = T2-T1 of sample x 2

T2-T1 of standard

# Analysis of Urea

Urea was determined using Randox diagnostic kit. The test was carried out in three different test tubes; blank, standard and sample. The blank contains 100ul of working reagent, the standard contains 100ul of working reagent and 10ul of standard reagent and the sample contains 100ul of working reagent and 10ul of serum. All these were mixed and incubated at 37oC for 15 minutes. The absorbance of standard and sample were read against the blank at 550nm (Scott et al., 1960).

Concentration (mmol/l) = Absorbance of sample x 10

Absorbance of standard

# Analysis of Sodium and Potassium

Serum sodium and potassium were determined using flame photometric method. In this method, 0.1ml of serum was added to 9.9ml of deionised distilled water

in a universal bottle. The contents were mixed and the reading was taken with flame photometry; for sodium, the galvanometer was set with the working standard and the absorbance was measured at 590nm. And for potassium, the galvanometer was set with potassium Working standard and the absorbance was measured at 770nm. (Chuang et al., 1973).

Sodium concentration (mmol/l) = Absorbance of sample x 0.2

Absorbance of standard

Potassium concentration (mmol/l) = Absorbance of sample x 0.1

Absorbance of standard

# CHAPTER 4 RESULTS

* 1. **DETERMINATION OF MEAN SERUM BIOCHEMICAL INDICES IN RICKETS AND NON RACHITIC CHILDREN IN GONIN-GORA COMMUNITY**

The mean + S.E.M biochemical parameters of rickets and non rachitic children from Gonin-Gora communities are shown in figure 4.1. The values for rickets and non rachitic were not significantly (p> 0.05) different. However higher values were estimated for non rachitic children compared with rickets children in all parameters expect in creatinine and phosphorus.

Concentration (Mmol/L)

# Figure 4.1 Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Goni-gora community of Kaduna State.

160

140

120

100

80

60

Rickets

Non Rickets

40

20

0

Na

K

Urea

Creatinine

Ca

P

* 1. **MEAN SERUM BIOCHEMICAL INDICES IN RICKETS AND NON RACHITIC CHILDREN OF KASO COMMUNITY.**

The results of sodium (Na), potassium (K),urea (H2NCOONH2), creatinine (Cr), calcium(Ca) and phosphorus (P) levels estimated for rickets and non rachitic children of Jankasa community are presented in Figure 4.2, potassium and calcium levels were significantly lowered in rickets children compared to the non rachitic children.

The values of calcium in rickets and non rickets were 2.19 mmol/L S.E.M +0.03 and 2.30 mmol/L S.E.M +0.02 and that of potassium in rickets and non rickets were

3.69 mmol/L S.E.M +0.09 and 7.76 mmol/L S.E.M +4.10. Normal calcium and potassium levels are 2.25-2.75 mmol/L and 3.6-5.2 mmol/L (A.B.U. Teaching Hospitals Chemical Pathology Department).



# Figure 4.2 Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Kaso community of Kaduna State.

* 1. **MEAN SERUM BIOCHEMICAL INDICES FOR RICKETS AND NON RICKETS CHILDREN OF JANKASA COMMUNITY.**

The mean (+) S.E.M biochemical parameters of rickets and non rachitic children from Jankasa community are shown in Figure 4.3. The serum values for rickets and non rachitic are significantly different (p<0.05 ) for sodium and calcium.

The mean biochemical serum values for sodium in rickets and non rachitic children were 138.44 mmol/L S.E.M +0.53 and 137.77 mmol/L S.E.M +0.06 and that of calcium for rickets and non rickets were 2.35 mmol/L S.E.M+0.02 and 2.32mmol/L S.E.M+ 0.07 respectively. Normal calcium and sodium levels are 2.25-2.75mmol/L and 136-145mmol/L ( A.B.U. Teaching Hospitals Chemical Pathology Department).

Concentration (mmol/L)

# Figure 4.3 Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Jankasa community of Kaduna State.

160

140

120

100

80

Rickets

Non Rickets

60

40

20

0

Na

K

Urea

Creatinine

Ca

P

-20

* 1. **COMPARISON OF MEAN SERUM BIOCHEMICAL PARAMETERS FOR RICKETS AND NON RACHITIC CHILDREN OF GONIN-GORA, JANKASA AND KASO COMMUNITIES.**

The results of group analysis of values determined for the rickets and non rachitic children in the three communities are shown in Figure 4.4. Calcium and potassium were significantly reduced for rickets children.

Contration (mmol/L)

# Figure 4.4 Mean (+) S.E.M Serum Biochemical parameters for Rickets and Non Rachitic Children in Gonin-Gora, Jankasa and Kaso communities.

160

140

120

100

80

Rickets

Non Rickets

60

40

20

0

Na

K

Urea

Creatinine

Ca

P

* 1. **COMPARISON OF THE EFFECT OF MEAN SERUM BIOCHEMICAL PARAMETERS IN RICKETS AND NON RACHITIC MALE AND FEMALE CHILDREN IN GONIN-GORA, JANKASA AND KASO COMMUNITIES.**

The results of influence of sex on the biochemical parameters of rickets and non rachitic children in Gonin-Gora, Jankasa and Kaso communities are shown in Figures 4.5, 4.6 and 4.7 respectively. There was no significant difference in the values estimated for male and female children in the communities.

Concentration (mmol/L)

# Figure 4.5 Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Gonin-gora community.

160

140

120

100

80

Rickets

Non Rickets

60

40

20

0

Na

K

Urea

Creatinine

Ca

P

Concentration (mmol/L)

**Figure 4.6 Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Jankasa community.**

160

140

120

100

80

Rickets

Non Rickets

60

40

20

0

Na

K

Urea

Creatinine

Ca

P

Concentration (mmol/L)

# Figure 4.7 Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Kaso community.

160

140

120

100

80

Rickets

Non Rickets

60

40

20

0

Na

K

Urea

Creatinine

Ca

P

**CHAPTER 5 DISCUSSION**

# MEAN SERUM BIOCHEMICAL INDICES FOR RICKETS AND NON RICKETS CHILDREN OF GONIN-GORA COMMUNITY.

The mean serum biochemical indices (sodium, potassium, urea, creatinine, calcium and phosphorus) were within the normal range for each parameter.

The serum calcium levels for both rickets and non-rachitic children were at lower limits of the normal range while the serum phosphorus values were at the higher limit of the normal range.

However, the serum values determined for calcium, sodium, potassium and urea were lowered while the serum values for phosphorus and creatinine were higher for rickets children compared to non-rachitic children.

Although, nutritional rickets is often attributed to vitamin D deficiency; low calcium intake with high phosphorus is also an important cause of rickets in children (Koof et al.,1972; Legiuse et al., 1989; Pettifor, 2004).

# MEAN SERUM BIOCHEMICAL INDICES FOR RICKETS AND NON RICKETS CHILDREN IN KASO COMMUNITY.

The mean serum biochemical values for sodium, potassium, urea, creatinine, calcium and potassium showed that only potassium and calcium serum levels were significantly lowered in rickets children as compared to the non rachitic children in Kaso community. The calcium level for the rickets children in this community is below the lower limit of the normal range. The serum phosphorus level is higher than that of

the non-rachitic children. These findings also implied nutritional rickets. The fact that the calcium level is below the normal value may explain why this community is mostly affected with the rickets scourge compared with Jankasa and Gonin-Gora. Access to medical facilities may also be low since it is far from Kaduna city compared with the other communities.

# MEAN SERUM BIOCHEMICAL INDICES FOR RICKETS AND NON RICKETS CHILDREN OF JANKASA.

All the parameters determined for the children in Jankasa are within the normal range (A.B.U. Teaching Hospitals Chemical Pathology Department.)

However, the results still followed similar pattern. Calcium mean serum value was at the lower limit of the normal range while phosphorus was at the higher limit of the normal range.

Higher serum levels were determined for the parameters of non-rachitic children compared with the rickets children except for phosphorus. Thus implying inadequate intake of dietary calcium.

# COMPARISON OF MEAN SERUM BIOCHEMICAL VALUES FOR RICKETS AND NON RICKETS CHILDREN FOR THE GROUP ANALYSIS IN GONIN-GORA, JANKASA AND KASO COMMUNITIES.

The values obtained for the group analysis in rickets and non rickets children in Gonin-Gora, Jankasa and Kaso communities showed that serum calcium and sodium were significantly lowered in rickets children in all the areas.

Calcium and phosphorus serum levels were significantly (p<0.05) lowered for rickets children compared with non-rachitic children when the level were compared for these communities. The reduction was found to be between Kaso compared with the Gonin-Gora community. This again explained the reason for more rickets children identified in Kaso community. The mean serum calcium determined for the three communities were just at the lower level of normal range (A.B.U. Teaching Hospitals Chemical Pathology Department). This further implies the need for more calcium rich food intake in the communities.

# THE INFLUENCE OF SEX ON THE SERUM BIOCHEMICAL PARAMETERS IN RICKETS AND NON RICKETS CHILDREN OF GONIN-GORA, JANKASA AND KASO.

There is no significant difference in the serum levels of the parameters determined for the male children compared with female children. However, since the sex of the children were not uniformly distributed among rickets and non-rachitic children; comparison of sex could not be considered based on all the children sampled.

# CHAPTER 6

**SUMMARY, CONCLUSION AND RECOMMENDATIONS**

# SUMMARY

Agappe diagnostic test kits and official methods were used to determine the levels of calcium and phosphorus in blood samples in Gonin Gora, Jankasa and Kaso rickets prevalent areas in Kaduna State.

The biochemical parameters in the three study settlements (Gonin Gora, Kaso and Jankasa) are low in serum calcium but high in serum phosphorus. The highest mean concentration of serum calcium was found to be 2.35 mmol/L ± 0.02 (S.E.M) and it was observed in Jankasa.

The low serum calcium with high serum phosphorus levels among infants and children has been shown to be attributed to the development of Rickets (Koof et al., 1972; Legius et al., 1989; Pettifor, 2004). Further studies are recommended to check the genetic implication of the disorder in these communities.

# CONCLUSION

The results of the determination of serum sodium, potassium, calcium, phosphorus, urea and creatinine in rickets and non-rachitic children in Gonin-Gora, Kaso and Jankasa communities showed that nutritional rickets which might have resulted from low or inadequate intake of dietary calcium coupled with high phosphorus intake is one of the causes of the rickets scourge in the communities. It particularly showed very low dietary calcium intake in Kaso.

# RECOMMENDATIONS

A mass enlightenment campaign in the communities is recommended because most men believe their wives are responsible for this problem.

* + - Government should as a matter of urgency provide vitamin D-fortified formular per day for the children of these communities.
		- Surgical intervention is also necessary to repair severe bone abnormalities in children with rickets. This should be done after the biochemical derangements have resolved so that optimal healing occurs at the surgical site.
		- Studies should be carried out to investigate the genetic implication of the rickets scourge, due to the multiple affected children born to one wife of polygamous men.

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APPENDIX

# Appendix 1

**Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Goni-gora community of Kaduna State.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter Mmol/L** | **Rickets** | **Non-Rickets** | **P-value** | **Normal Value** |
| Sodium (Na) | 137.71+ 1.77 | 139.47+ 0.95 | P< 0.83 | 136-145 |
| Potassium (K) | 4.86 + 0.25 | 4.90 + 0.14 | P< 0.24 | 3.6-5.2 |
| Urea (H2NCOONH2) | 3.29 + 0.21 | 3.41 + 0.19 | P< 0.74 | 2.5-6.5 |
| Creatinine**C H N 0 4 7 3**(Umol/L) | 54.43 + 4.18 | 51.93 + 2.17 | P< 0.39 | 9-26 |
| Calcium (Ca) | 2.24 + 0.02 | 2.31 + 0.01 | P< 0.65 | 2.25-2.75 |
| Phosphorus (P) | 1.58 + 0.06 | 1.29 + 0.05 | P< 0.09 | 0.8 -1.9 |

# p<0.05 considered significant

**Appendix 2**

# Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Kaso community of Kaduna State.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parmeters****Mmol**/L | **Rickets** | **Non-Rickets** | **p-value** | **Normal Value** |
| Na | 136.50+0.82 | 136.72+0. 79 | P< 0.42 | 136-145 |
| K | 3.69 + 0.09 | 7.76 + 4.10 | P< 0.03 | 3.6-5.2 |
| Urea | 3.04 + 0.17 | 3.03 + 0.19 | P< 0.58 | 2.5-6.5 |
| Creatinine Umol/L | 49.14 + 1.65 | 50.25 + 1.47 | P< 0.31 | 9-26 |
| Ca | 2.19 + 0.03 | 2.30 + .020 | P< 0.02 | 2.25-2.75 |
| P | 1.48 + 0.06 | 1.31 + .040 | P< 0.69 | 0.8 -1.9 |

P<0.05 considered significant

# Appendix 3

**Mean (+) S.E.M Serum Biochemical indices for Rickets and Non Rachitic Children in Jankasa community of Kaduna State.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parametersmmol/L | **Rickets** | **Non-Rickets** | **p-value** | **Normal Value** |
| Na | 138.44 + .53 | 137.77 +0. 06 | P< 0.10 | 136-145 |
| K | 3.82 +0 .08 | 3.78 + 0.07 | P< 0.63 | 3.6-5.2 |
| Urea | 2.73 + 0.13 | 2.90 + 0.16 | P< 0.47 | 2.5-6.5 |
| Creatinine Umol/L | 52.48 + 1.56 | 53.46 + 2.17 | P< 0.95 | 9-26 |
| Ca | 2.35 + 0.02 | 2.32 +0 .07 | P< 0.02 | 2.25-2.75 |
| P | 1.67 +0 .06 | 1.71 + .008 | P< 0.93 | 0.8 -1.9 |

P<0.05 considered significant

# Appendix 4

**Mean (+) S.E.M Serum Biochemical parameters for Rickets and Non Rachitic Children in Gonin-Gora, Jankasa and Kaso communities.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters****mmol/L** | **Rickets** | **Non-Rickets** | **p-value** | **Normal Value** |
| Na | 137.27 +0 .53 | 137.63+ 0.52 | P< 0.48 | 136-145 |
| K | 3.84 + 0.07 | 6.18 + 2.17 | P< 0.05 | 3.6-5.2 |
| Urea | 2.94 + 0.11 | 3.09 + 0.12 | P< 0.75 | 2.5-6.5 |
| Creatinine Umol/L | 50.77 + 1.15 | 51.37 + 1.06 | P< 0.25 | 9-26 |
| Ca | 2.25 + 0.02 | 2.31 + 0.18 | P< 0.04 | 2.25-2.75 |
| P | 1.55 + 0.03 | 1.39 + 0.04 | P< 0.44 | 0.8 -1.9 |

P<0.05 considered significant

# Appendix 5

**Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Gonin-gora community.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters**mmol/L | **Mean + S.E.M (Male)** | **Mean + S.E.M (female)** | **P-value** | **Normal Value** |
| ***Na*** | 139.28 + 1.13 | 138.25 + 1.32 | P<0.86 | 136-145 |
| K | 4.81 + 0.17 | 5.01 + 0.14 | P< 0.22 | 3.6-5.2 |
| Urea | 3.41 + 0.20 | 3.30 + 0.20 | P< 0.55 | 2.5-6.5 |
| Creatinine Umol/L | 52.28 + 2.27 | 53.50 + 3.82 | P< 0.62 | 9-26 |
| Ca | 2.30 + 0.02 | 2.28 + 0.02 | P< 0.14 | 2.25-2.75 |
| P | 1.36 + 0.06 | 1.44 + 0.07 | P< 0.41 | 0.8 -1.9 |

P<0.05 considered significant

# Appendix 6

**Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Jankasa community.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters Mmol/L** | **Mean + S.E.M (Male)** | **Mean + S.E.M (Female)** | **P-value** | **Normal Value** |
| ***Na*** | 138.25 + 0.54 | 138.17 + 0.63 | P<0.64 | 136-145 |
| K | 3.82 + 0.05 | 3.80 + 0.11 | P< 0.09 | 3.6-5.2 |
| Urea | 2.89 + 0.15 | 2.68 + 0.14 | P< 0.29 | 2.5-6.5 |
| Creatinine Umol/L | 53.65 + 1.83 | 51.89 + 0.71 | P< 0.50 | 9-26 |
| Ca | 2.38 + 0.04 | 2.30 + 0.03 | P< 0.74 | 2.25-2.75 |
| P | 1.73 + 0.06 | 1.63 + 0.08 | P< 0.09 | 0.8 -1.9 |

P<0.05 considered significant

# Appendix 7

**Mean S.E.M (+) Serum Biochemical levels of Rickets and non rachitic male and female children in Kaso community.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters****mmol/L** | **Mean + S.E.M (Male)** | **Mean + S.E.M (female)** | **P-value** | **Normal Value** |
| Na | 136.77 + 0.85 | 136.40 + 0.77 | P<0.36 | 136-145 |
| K | 7.08 + 3.34 | 3.63 + 0.10 | P< 0.07 | 3.6-5.2 |
| Urea | 3.06 + 0.17 | 3.00 + 0.18 | P< 0.85 | 2.5-6.5 |
| Creatinine Umol/L | 48.85 + 1.60 | 50.48 + 1.60 | P< 0.71 | 9-26 |
| Ca | 2.22 + 0.30 | 2.25 + 0.32 | P< 0.80 | 2.25-2.75 |
| P | 1.44 + 0.21 | 1.36 + 0.04 | P< 0.09 | 0.8 -1.9 |

P<0.05 considered significant