

**ECHOCARDIOGRAPHIC FEATURES OF CHILDREN PRESENTING WITH
RICKETS AT KENYATTA NATIONAL HOSPITAL**

A dissertation submitted in partial fulfillment for the degree of Master of Medicine
(Paediatrics) of the UNIVERSITY OF NAIROBI

BY

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DECLARATION

I hereby certify that this is my original work and that it has not to my knowledge been submitted in any other University for any degree.

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DEDICATION

This book is dedicated to my husband Duncan for his understanding and support and our daughters Lynette, Beryl, Barbara and Beracchah for their patience during the period of my postgraduate studies.

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ABBREVIATIONS

Ab	Antibody
ALP	Alkaline phosphatase
Ca ²⁺	Calcium
cCa ²⁺	Corrected Calcium
CHF	Congestive Heart Failure
DCM	Dilated Cardiomyopathy
Echo	Echocardiography
EDV	End Diastolic Volume
EF	Ejection Fraction
ESV	End Systolic Volume
Hb	Haemoglobin
HCM	Hypertrophic Cardiomyopathy
HIV	Human Immunodeficiency Virus
IQR	Interquartile range
JVP	Jugular venous pressure
KNH	Kenyatta National Hospital
LVDd	Left Ventricular dimension in diastole
LVDs	Left Ventricular dimension in systole
LVSD	Left Ventricular Systolic Dysfunction
OR	Odds Ratio
Po ₄ -	Phosphate
PTH	Parathyroid hormone
RCM	Restrictive Cardiomyopathy
1, 25(OH) ₂ D	1, 25 dihydroxycholecalciferol
25(OH) D	25 hydroxycholecalciferol

ABSTRACT

Background: The prevalence of rickets has remained high in our setup probably due to inadequate nutrition and insufficient exposure to sunlight. Rachitic children have significant morbidity and mortality, often due to severe pneumonia and heart failure.

Objective: To determine the prevalence, pattern and biochemical correlates of echocardiographic abnormalities in children with rickets at KNH.

Methodology: This was a Descriptive Cross sectional study conducted at KNH paediatrics wards. Children aged between 3 months and 36 months admitted with rickets were recruited to the study. All the study patients had serum calcium, phosphorus and alkaline phosphatase levels measured and Echocardiography done to determine the presence of cardiac abnormalities.

Results: A total of one hundred and seventy five patients were evaluated. There were 87 males (50%) and 88 females (50%). Their age range was 3 months to 18 months with a median of 7 months. Abnormal echocardiographic findings were present in 103 (59%) patients. Of these, left ventricular systolic dysfunction was found in 78 (45%), pulmonary hypertension in 35 (20%), diastolic dysfunction in 33 (19%) and pericardial effusion in 8 (4.5%) patients. Left ventricular systolic dysfunction was significantly correlated with low calcium levels, p-value <0.001, odds ratio 4.1 and 95% confidence interval (2.2 - 7.8). There was no difference between children with pneumonia and those without pneumonia with regards to echocardiographic abnormalities.

Conclusion: The study revealed a high prevalence of echocardiographic abnormalities in children with rickets (59%). Left ventricular systolic dysfunction was the most common finding (45%); it was significantly associated with severe rickets and hypocalcaemia.

Recommendations: Echocardiographic evaluation should be done on patients with rickets, particularly those with severe rickets and severe hypocalcaemia so that appropriate treatment can be instituted for cardiac dysfunction if present.

BACKGROUND AND LITERATURE REVIEW

Introduction

Rickets is a disease of growing bone that is unique to infancy and childhood. It is caused by failure of bone mineralization in a growing child. Failure of bone mineralization in an adult is called osteomalacia. Causes of rickets include vitamin D deficiency; mainly due to dietary deficiency and lack of exposure to sunlight. Intestinal malabsorption of fat, liver disease and renal tubular defects, as well as anticonvulsant drugs (e.g. phenobarbital, phenytoin) may also lead to insufficiency of vitamin D and rickets. Calcium and phosphorus deficiencies also cause rickets though less commonly. Calcium deficiency is mainly dietary whereas phosphorus deficiency can be dietary, X-linked or Autosomal dominant renal phosphate wasting, or fanconi's syndrome.

Rickets often is associated with chronic hypocalcaemia; chronic hypocalcaemia has been associated with myocardial dysfunction^{31, 32, 33, 34, 35}. A study done by Uysal et al in Turkey, demonstrated echocardiographic evidence of left ventricular dysfunction in children with vitamin D deficiency rickets.

The predominant cause of rickets in our set up is vitamin D deficiency due to insufficient exposure to sunlight and /or inadequate dietary intake^{1, 2, 3}. The other causes of rickets in order of frequency include familial hypophosphatemia, renal tubular defects and inherited disorders of vitamin D i.e. vitamin D dependent rickets¹.

Pathophysiology of rickets

The active form of vitamin D (i.e. 1, 25 (OH)₂ D) acts at three known sites to tightly regulate calcium metabolism. It promotes absorption of calcium and phosphorus from the intestine, increases reabsorption of phosphate in the kidney, and acts on bone to release calcium and phosphate. It may also directly facilitate calcification. These actions increase the concentrations of calcium and phosphorus in serum. The increase of calcium and phosphorus in serum, in turn, leads to the calcification of osteoid (bone matrix). Parathyroid hormone facilitates the 1-hydroxylation step in vitamin D metabolism.

In the vitamin D deficiency state, hypocalcaemia and hypophosphatemia develop early in the course of the disease. The fall in calcium leads to stimulation of PTH secretion, this in turn leads to restoration of a nearly normal serum calcium concentration because of increased bone mobilization and renal reabsorption of calcium. Meanwhile, it leads to a further fall in serum phosphate because of increased phosphaturia and reduced phosphate absorption in the kidneys. The loss of phosphorus further reduces deposition of calcium in the bone. Alkaline phosphatase levels are elevated due to increased osteoblastic activity. When the degree of vitamin D deficiency becomes severe, bone resorption decreases inspite of increased levels of PTH and chronic hypocalcaemia ensue⁴³.

Vitamin D deficiency rickets can therefore be divided into three biochemical stages as follows: (1) Stage 1- calcium level is decreased, phosphate is normal and 25-(OH) D is decreased, ALP is elevated, 1, 25-(OH)₂ D and PTH are normal. (2) Stage 2 - calcium is normal, phosphate is decreased, PTH is increased, 1, 25-(OH)₂ D is low normal or elevated. ALP is markedly elevated. (3) Stage 3 - calcium and phosphate are low, PTH is elevated, 25-(OH) D and 1, 25-(OH)₂ D are decreased. ALP is markedly elevated.

Epidemiology

Despite there being ample sunlight in developing countries, rickets especially due to vitamin D deficiency has remained an important and common problem^{1, 2, 3, 4, 5, 6, 7}. Evidence of vitamin D deficiency rickets among children in the developed countries also exists; especially among unsupplemented dark skinned infants and in breastfed infants who are not or whose mothers are not exposed to sunlight^{8, 9, 10}. In Kenya, studies carried out at Kenyatta National Hospital (KNH) show increasing prevalence of rickets from 3.4% in 1985 to 14.3% in 2000¹. Waris et al found the prevalence of vitamin D deficiency rickets to be 12.3% in healthy children attending paediatric surgical clinic of KNH², while Oyatsi et al reported a prevalence of 58.8% among preterm infants in the same institution⁴.

The incidence of rickets remains high in our set up. About 3-5 patients admitted daily into the paediatrics wards of KNH have clinical features of rickets⁵. The average daily admission is 30 patients.

Vitamin D-deficiency rickets commonly presents between three months and three years of age, when growth rates (and calcium needs) are high and exposure to sunlight may be limited^{1, 11-14}. Notably among preterm infants rickets is commonest in babies assessed after 12 weeks of age³. The risk factors associated with development of rickets include: lack of exposure to sunlight which depends on the time spent outside, the amount of skin exposed, air pollution, cloud cover, time of day, latitude, and skin pigmentation^{3, 15}. Melanin found in the skin decreases the amount of vitamin D synthesized from sunlight. Therefore infants with dark skin need a longer time of exposure to sunlight to synthesize the same amounts of vitamin D₃ compared to infants with light skin¹⁶. Waris et al reported that duration of exposure to sunlight of less than 10 hours per week was associated with increased risk of developing vitamin D deficiency rickets². Overcrowding, multistorey buildings and heavy atmospheric pollution obstruct sunrays and therefore may lead to insufficient exposure to ultraviolet light³.

The normal infant diet which mainly is constituted of human milk or cow's milk contain small amounts of vitamin D. Human milk typically contains less than 25 IU of vitamin D per liter and even lower amounts are found in cow's milk¹⁸⁻²². The other infants' diets such as cereals, vegetables and fruits contain negligible amounts of vitamin D. The recommended adequate intake of vitamin D to prevent deficiency in normal infants and young children is 200 IU/day.

Dietary deficiency of calcium also contributes to rickets. Vegetables, fruits, cereal grains and legumes contain less calcium than do milk or dairy products. Cereal grains and legumes contain phytates whereas vegetables and fruits contain oxalates which bind calcium and further reduce its bioavailability^{23, 24}. High intake of fibres also decreases intestinal absorption of vitamin D and calcium. Studies done in South Africa and Nigeria suggest that a dietary deficiency of calcium may cause rickets and osteomalacia rather than just vitamin D deficiency^{25, 26, 27}.

Because of the high growth rates and calcium demand from 3 months of age, babies who are exclusively breastfed for up to 6 months and are not supplemented with vitamin D, or have insufficient exposure to sunlight, are at increased risk of developing rickets. The tradition of introducing vegetables, fruits and cereals as the main complementary diets also increase chances of these babies developing rickets.

Morbidity associated with rickets

Most children who have rickets present with pneumonia, bronchitis, enteritis and failure to thrive, leading to a further increase in the incidence of these already common conditions. Several studies have shown that there exists a relationship between rickets and lower respiratory tract infections; a study by Najada et al in Jordan found that 85% of children with rickets were admitted due to lower respiratory tract infections as compared to 30% among those without rickets²⁸. Muhe et al in Ethiopia found a 13-fold higher incidence of rickets among children with pneumonia than in controls²⁹. Locally, Nyakundi et al found that 34.5% of the children diagnosed to have rickets had been admitted with pneumonia¹. Rickets has also been associated with longer duration of hospital stay and an increased risk of dying from severe pneumonia^{28, 30}.

Rickets often is associated with chronic hypocalcaemia; chronic hypocalcaemia has been associated with myocardial dysfunction in numerous studies and case reports especially in adults^{31, 32, 33, 34, 35}. Although the adult studies were not on patients with osteomalacia, they demonstrated that hypocalcaemia affects cardiac contractility^{34, 35}. The only study reported in children was that done by Uysal et al in Turkey. This was a prospective study of 27 infants diagnosed with vitamin D deficiency rickets. They demonstrated echocardiographic evidence of left ventricular dysfunction in these children particularly in stage three of the disease. Of the 27 patients studied, 10 were classified in biochemical stage three of rickets out of which 8 had an increase in the ratio of interventricular septum thickness to left ventricular posterior wall thickness (I/L) which is a hypertrophic cardiomyopathic change. In this study, the other parameters which were found to be significantly different between the patients and controls included ejection time (ET), end-diastolic volume (EDV), stroke volume (SV) and ejection Fraction (EF). None of these patients however had signs of cardiac failure and the abnormality reversed to normal

following treatment of rickets. Electrocardiographic (ECG) changes like T-wave abnormalities, pronounced U wave and prolonged QT interval were also noted in some of the patients from all the groups which resolved with treatment.

The rest of the evidence of cardiac involvement in rickets are in form of case reports of patients with rickets presenting with dilated cardiomyopathy and congestive heart failure^{33, 36, 37, 38, 39}. In these case reports, the patients reported had signs of CHF and on echocardiography were found to have dilated cardiomyopathy. Wrist X-rays showed features of rickets, and hypocalcaemia was the most prominent biochemical derangement. In a local study by Nyakundi et al looking at the aetiology and clinical presentation of rickets, 24% of the children had features of congestive heart failure at presentation¹. In this study 10.3% of the patients died and they all had CHF and pneumonia, the author also noted that the CHF was refractory to the conventional antifailure treatment. In a study by Ochieng et al on the prevalence and aetiology of congestive heart failure, 14% of cases were found to be due to rickets and pneumonia⁴². These two studies further illustrate that cardiac abnormalities and congestive heart failure can occur in rickets.

Mechanism of cardiac dysfunction in rickets

Rickets is a constellation of hypocalcaemia, hypophosphatemia and reduced vitamin D levels. The most striking biochemical derangement is hypocalcaemia; most of the case reports have associated reduced myocardial contractility with hypocalcaemia. It is probable that the other biochemical derangements also contribute to the cardiac dysfunction other than just hypocalcaemia. Studies in adults with vitamin D deficiency and hypophosphatemia have demonstrated some effect on myocardial performance.

Calcium ions have a key role in the excitation as well as the contraction of cardiac muscle fibers hence a reduction in serum calcium level may affect ventricular contraction.

Several studies have shown that cardiac dysfunction occurs during acute reduction in serum calcium levels in children and adults as well as experimental animals. One study showed that at a constant stroke work, sustained hypocalcemia was associated with a marked depression of left ventricular function⁴¹. Chronic hypocalcaemia is a relatively uncommon and reversible cause of congestive heart failure. Several case reports of

hypocalcaemia induced cardiomyopathy have appeared in literature^{31, 32, 33, 34, 35}. The exact mechanism of cardiomyopathy is however not completely understood. Hypocalcaemia causes ECG changes which include prolonged Q-T interval and non specific ST and T-wave changes.

Hypophosphatemia can trigger a reduction in the contractility of the myocardium, arrhythmias and cardiomyopathies owing to fall in adenosine triphosphate available for cardiac muscle. Improvement in cardiac functions has been seen following administration of phosphates in surgical patients with hypophosphatemia where presence of total parenteral nutrition, use of diuretics and sepsis were the main risk factors.

Hypophosphatemia was found to be associated with depressed stroke work which improved with phosphate infusion. The stroke volume increased significantly in face of the same or higher afterload, therefore an increase in the ejection fraction⁴⁴.

The mechanism by which vitamin D deficiency affects the heart is not known, it could probably be through its role in calcium metabolism. A study in Germany designed to evaluate the association between vitamin D status and CHF in adults, found low vitamin D status to be associated with myocardial dysfunction in the congestive heart failure patients. All the patients in this study had low calcium and low vitamin D levels. They concluded that the low vitamin D status explained the alterations in calcium metabolism as well as myocardial dysfunction⁴⁰.

Hypocalcaemia leads to hypotonia, which results in weakness of intercostal muscles and the diaphragm, this coupled with the chest deformities in rickets results in reduced thoracic volume, poor coughing effort and inadequate clearing of the airway. These patients develop secondary bacterial infection which if not or are inadequately treated cause chronic pneumonitis that results in ventilation-perfusion mismatch leading to hypoxia. Chronic hypoxia may cause increased pulmonary resistance and cor-pulmonale.

Clinical presentation of rickets

The earliest symptoms of rickets are restlessness, apathy, flabby toneless muscles, excessive sweating on the head, distended abdomen and gastrointestinal upsets.

The typical findings of advanced rickets include: enlargement of the costochondral junction which is visible as beading along the anterolateral aspects of the chest ("rachitic rosary"), enlargement of the wrist and bowing of the distal radius and ulna, parietal and frontal bossing, craniotabes (soft skull bones), delay in the closure of the fontanelles, progressive lateral bowing of the femur and tibia (bowlegs), the development of Harrison sulcus caused by the muscular pull of the diaphragmatic attachments to the lower ribs. A study in Kenya by Nyakundi, found rachitic rosary and widening of the wrists to be the commonest clinical presentation, present in 96.6%¹ of the patients, and bowlegs in 65.5% of the patients¹. A similar study by Tahir in Pakistan found widening of the wrists in 61%⁷ of patients, rachitic rosary in 36%⁷ of patients, bossing of the skull in 8.3%⁷ of patients and bowlegs in 8.1%⁷ of the patients.

Rickets can affect the musculoskeletal system with decreased muscle tone leading to delayed achievement of motor milestones. Delayed milestones were present in 93.1% and 33% of the patients^{1, 7} of the studies quoted respectively.

Hypocalcaemic seizures are a frequent presenting sign in the first year of life and were found in 5% of the patients⁷. Children with rickets are particularly prone to acquiring infectious diseases particularly lower respiratory tract infections (18%) and gastroenteritis (20%) of the patients⁷.

Diagnosis of rickets

The diagnosis of rickets is based on history of inadequate intake of vitamin D/calcium or inadequate exposure to sunlight and characteristic clinical signs. It is confirmed by biochemical and radiological examination.

The laboratory findings include increased alkaline phosphatase levels; which are usually markedly elevated over the age-specific reference range. Alkaline phosphatase is an excellent marker of activity of disease and a useful biochemical parameter in

confirmation of rickets as elevated levels is a constant finding in vitamin D deficiency rickets^{1,2,4,6}. Its activity increases due to osteoblastic proliferation, resulting in an increase in measurable serum ALP levels. Serum calcium and phosphorus levels vary with the stage of the disease as discussed under pathophysiology (page 2). Low phosphorus level is the predominant finding in hypophosphatemic rickets due to X-linked or Autosomal dominant renal phosphate wasting, or fanconi's syndrome.

Radiography is essential in confirmation and assessment of severity of rickets. The changes of rickets are best visualized at the growth plate of rapidly growing bones and include widening of the epiphyseal plate and loss of definition of the zone of provisional calcification at the epiphyseal/metaphyseal interface which appear early in the disease and therefore, define early (mild) rickets. In advanced (severe) disease there is cupping, splaying, formation of cortical spurs, and stippling and osteopenia of shaft of long bones with thinning of the cortices. In healing rickets, there are metaphyseal bands visible at the epiphyseal /metaphyseal interface. A study by Tahir in Pakistan found 85% of the patients to have radiological changes of rickets⁷. Another study done in Saudi Arabia found active(severe) rickets in 53% of patients, minimal change(mild) rickets in 32% and healed rickets in 15% of the patients⁶.

Diagnosis of Cardiac dysfunction

Clinical signs of cardiac disease are non specific and when present are often ascribed to pulmonary disease. Some of the clinical features that may be suggestive include excessive sweating, poor feeding and fatigability in an older child. Left ventricular systolic dysfunction may be asymptomatic or present with features of congestive heart failure like tachycardia, cardiomegally, hepatomegally, tachypnoea, edema, raised JVP. See appendix IV for criteria for diagnosis of heart failure⁵⁵.

Echocardiography is used as gold standard for diagnosis of structural and functional abnormalities of the heart. It provides an assessment of systolic and diastolic function as well as an estimation of left and right heart filling pressures and pulmonary pressures^{46,47,48,49,50,51,52}.

STUDY JUSTIFICATION

There is evidence of increasing prevalence of rickets in Kenya as seen from local studies at KNH and current trends in the wards^{1, 2}. Rickets is an important cause of morbidity leading to frequent and prolonged hospital admissions with its complications often leading to mortality. There is some evidence of cardiac dysfunction including dilated cardiomyopathy and congestive heart failure mainly due to hypocalcaemia of rickets. The dysfunction is usually reversible with supplementation of vitamin D and calcium. However, if left untreated it may lead to intractable congestive heart failure requiring life support³⁹.

Rickets associated mortality is more often related to severe pneumonia and heart failure³⁰. It is possible that features of cardiac dysfunction when present are ascribed to pulmonary disease due to their non-specific nature, and hence early diagnosis missed until they develop overt features of congestive heart failure.

This study will determine the magnitude and pattern of cardiac dysfunction in children with rickets.

STUDY UTILITY

The data obtained from this study will help determine basis for routine screening of patients with rickets for cardiovascular dysfunction and early treatment to avoid decompensation of the patient's cardiovascular status and thereby reducing the morbidity and mortality related to rickets.

PRIMARY OBJECTIVE

To determine the prevalence and pattern of Echocardiographic abnormalities in children presenting with rickets at Kenyatta National Hospital.

SECONDARY OBJECTIVE

To define the biochemical correlates of Echocardiographic abnormalities in children with rickets at Kenyatta National Hospital.

METHODOLOGY

Study design

Descriptive Cross sectional Study.

Study Area

KNH paediatrics wards. KNH is a teaching hospital for University of Nairobi (UON) and Tertiary referral for Nairobi and the country-Kenya. It caters for low to middle socio-economic population of Nairobi and nearby districts. There are four general paediatrics wards admitting serially with approximately 30 admissions per day.

Study Population

Children aged 3 years and below admitted to the general paediatrics wards of KNH with rickets.

Definitions

Definition of Rickets

In this study, rickets was defined by the presence of 2 or more of the following clinical features - bossing of the skull, widened wrists, rachitic rosary, Harrison's sulcus, craniotables and wide anterior fontanelle³.

AND confirmed by the presence of 2 or more of the following radiological changes on Wrist X-ray – widening of the epiphyseal plate, fraying, cupping, splaying, cortical spurs, and /or osteopenia³.

Radiological grading of rickets^{7, 43}

In this study, the radiological features of rickets were graded as follows;

- (a) Mild rickets was defined by widening of the epiphyseal plate and loss of definition of the zone of provisional calcification at the epiphyseal/metaphyseal interface.
- (b) Severe rickets was defined by marked cupping, splaying, formation of cortical spurs, and stippling and/or osteopenia of shaft of long bones with thinning of the cortices.
- (c) Healing rickets was defined by presence of metaphyseal bands at the epiphyseal /metaphyseal interface.

Definition of Echocardiographic cardiac abnormalities

- (a) Dilated cardiomyopathy was defined by presence of dilatation of heart chambers with poor contractility of ventricles, ejection fraction (EF) <55% and reduced stroke volume⁴⁸
- (b) Left ventricular systolic dysfunction was defined by presence of reduced stroke volume, increased ESV and EDV; EF<55%^{48, 52}.
- (c) Diastolic dysfunction was defined by presence of abnormal filling pattern of left ventricle on Pulse wave Doppler. This was either restrictive, pseudonormalized or reversed⁵⁰.
- (d) Hypertrophic cardiomyopathy was defined by presence of left ventricular hypertrophy especially the interventricular septum. A ratio between the interventricular septal thickness to left posterior wall thickness (IVST/LPWT) of >1.3 was considered to be hypertrophic cardiomyopathy⁵¹.
- (e) Pulmonary Hypertension was defined by mean pulmonary pressures >30mmHg, dilated pulmonary trunk, dilated right ventricle, pulmonary and tricuspid valve regurgitation⁴⁹.
- (f) Pericardial effusion was the presence of fluid in the pericardial space demonstrable on 2D real time -echocardiography. It was either, anterior, posterior or circumferential.

Definition of pneumonia

Pneumonia was defined by presence of cough and tachypnoea (**see appendix IV for reference ranges**) WITH OR WITHOUT any of the following: difficulty in breathing, cyanosis, grunting, head nodding, lower chest wall indrawing and inability to breastfeed or drink⁵⁴.

Case selection

Inclusion criteria

- (1) Children with rickets who were 3 years and below.
- (2) Informed written consent by a parent or guardian.

Exclusion criteria

- (1) Children known to have structural heart disease (congenital or acquired)
- (2) Children with conditions that compromise cardiac function:
 - (a) Severe Malnutrition (defined as weight for age <60%, visible severe wasting, +/- nutritional oedema)
 - (b) Severe anaemia (Hb<7 g/dl)
 - (c) HIV antibody positive for those >18months
 - (d) HIV abs positive and without DNA PCR or positive DNA PCR if <18 months.
- (3) Children discharged before echocardiography was done.

These conditions were ruled out through history, physical examination and laboratory tests obtained from the case notes (HIVab test, DNA PCR, Hb).

Sample Size

The sample size was estimated using the following formula:

$$n = \frac{Z_c^2 \times P(1-P)}{D^2}$$

Where n = required sample size

P = Prevalence of cardiac abnormalities among patients with rickets (24%)¹

D = Precision of the study set at 0.065

Z_{crit} is the cut off points along the x-axis of the standard normal probability distribution that represents probabilities matching the 95% confidence interval (1.96).

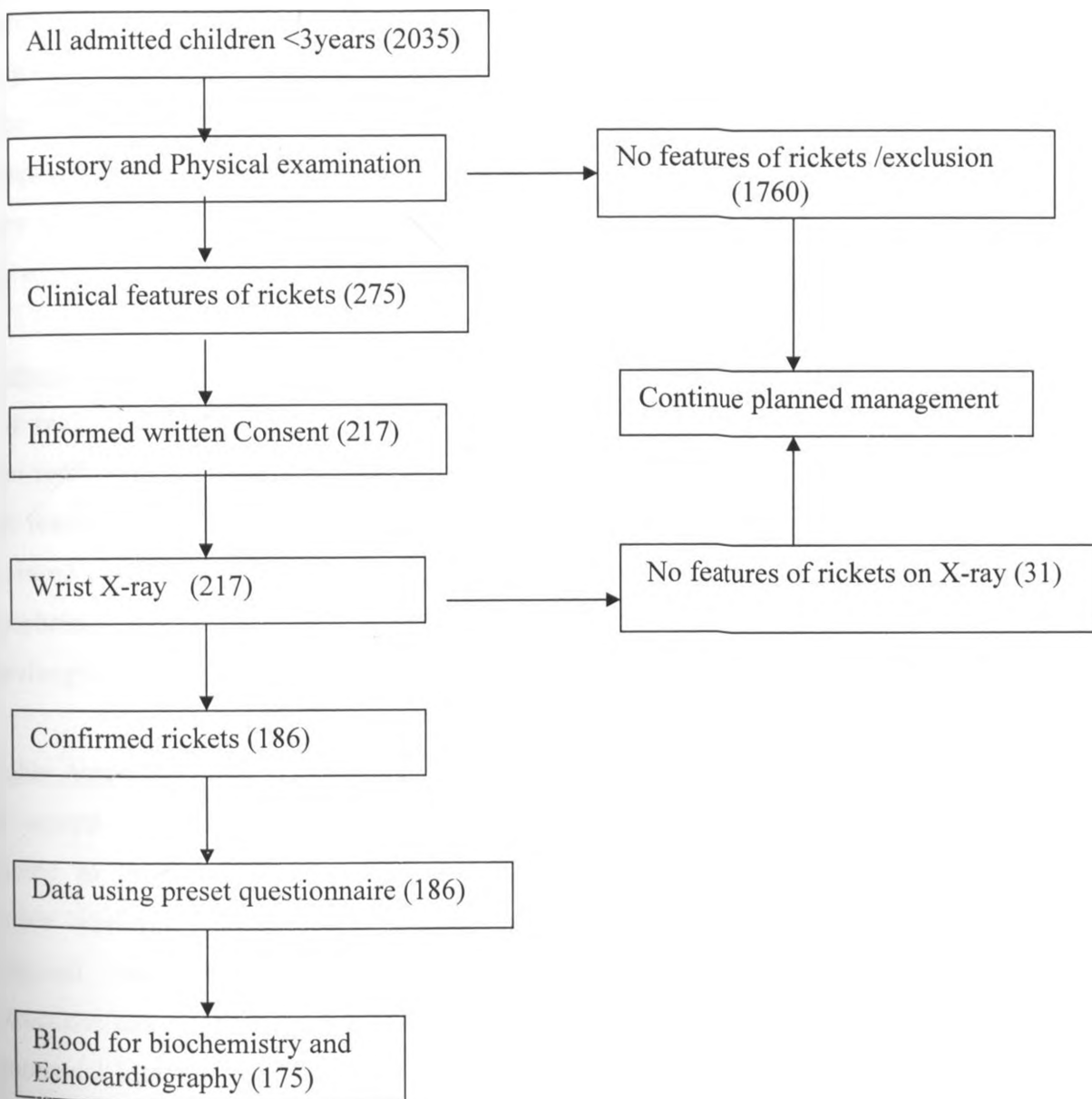
Substituting the above in the formulae we got;

$$n \approx 165$$

Recruitment

The Principal Investigator visited the post admissions' ward of KNH every day between 8 a.m and 5 p.m. All admitted patients were screened as per the flow chart (**figure 1**); those who met the inclusion criteria were selected until the required sample size was attained using consecutive sampling.

Figure 1: Flow chart for patient selection



Study procedures

Radiographic procedure

Plain antero-posterior view X-ray of both wrists was done. The films were examined by the investigator and reported with the help of 2 independent qualified radiologists.

Clinical procedures

Blood collection

After obtaining a written consent, a venous sample of 3ml was collected in a plain tube using sterile procedures. It was allowed 30 minutes to clot; serum was then extracted and placed into plastic serum vials for estimation of calcium, phosphorus, alkaline phosphatase and albumin. The samples were stored at -20 °C and tests run in batches on every alternate day. Samples to be run on the same day were stored at 2-8°C until the time of analysis.

Laboratory analysis

A Humalyzer 2000 spectrophotometer and reagents from Human Diagnostics Laboratory were used. Calcium was measured by spectrophotometry of coloured complex formed after reaction with o-cresolphthalein-complexone at wavelength 570nm, phosphorus was measured photometrically following its reaction with molybdate at 340nm and alkaline phosphatase was measured by release of p-nitrophenol at PH 9.8, temp 37°C and wavelength 405nm. **See appendix V** for reference values and biochemical procedure.

Quality Assurance

All aspects of quality control were adhered to. Standard operating procedures were adhered to through collection [aseptic technique, avoid haemolysis], identification [proper labeling], separation and storage and analysis. All precautions for handling biohazard materials as recommended were observed. The manufacturers instructions/specifications on analysis and calibration of equipments were adhered to and commercial internal quality control were included in each batch. Results were only accepted if controls were within the acceptable limits.

Cardiac Evaluation

Echocardiography was used as gold standard for demonstrating structural and functional cardiac abnormalities in this study. Transthoracic echocardiography was done by the cardiologist assisted by the investigator in the wards. A general ultrasound Vivid I cardiovascular system model from General Electric Healthcare Technologies was used by applying the subcostal, parasternal, apical and suprasternal views. The modalities of Echocardiography used were M-Mode, 2-Dimensional real-time, Pulsewave, Continuous and Special Doppler color flow. The findings were recorded on a data sheet as per **appendix III.**

M-mode echocardiography was used to study chamber sizes, interventricular septum and posterior wall thickness and to calculate the indices of contractility. Left ventricular systolic function was quantified as the left ventricular ejection fraction. An operational definition of systolic dysfunction was an ejection fraction of $<55\%$ ^{48, 52}.

Two-dimension real time echocardiography was used to visualize the cardiac chamber sizes and wall thickness, visual contractility as well as any other abnormalities including valvular abnormalities, wall motion abnormalities and pericardial effusion.

Continuous doppler echocardiography was used for measuring the pressures across valves. The right ventricular systolic pressure which is equivalent to the pulmonary artery systolic pressure in the absence of obstruction of the right ventricular outflow tract was estimated by measurement of the systolic regurgitant tricuspid flow velocity. Pulmonary pressures were then derived using the Bernoulli's equation, which states:

$$P = 4v^2, \text{ where}$$

P = pulmonary pressure

V = maximum velocity of tricuspid regurgitation

A value of 5mmHg (an estimate of right atrial pressure) was added to the tricuspid regurgitation gradient to estimate pulmonary pressures.

Pulmonary hypertension was present if mean pulmonary artery pressure $>25\text{mmHg}$ at rest or 30mmHg with agitation.

Data Collection & Management

The data was collected using a structured questionnaire and entered into a database in MS Excel. The data was then analysed using Statistical Package for Social Sciences software (SPSS – Version 12.0). Chi-square was used to establish the significant associations between the discrete variables. The data was presented in tables and figures where applicable. Level of significance was set at 0.065.

Ethical Considerations

The permission to carry out this study was sought and obtained from KNH Research and Ethics Committee.

The parents /guardians gave informed consent for participation in the study.

The study was voluntary and any parent /guardian who wished not to participate or to withdraw from the study at any time did so without being denied appropriate investigations and treatment thereafter.

The results were availed to the primary clinician and caregiver as soon as they were obtained, for treatment to be instituted.

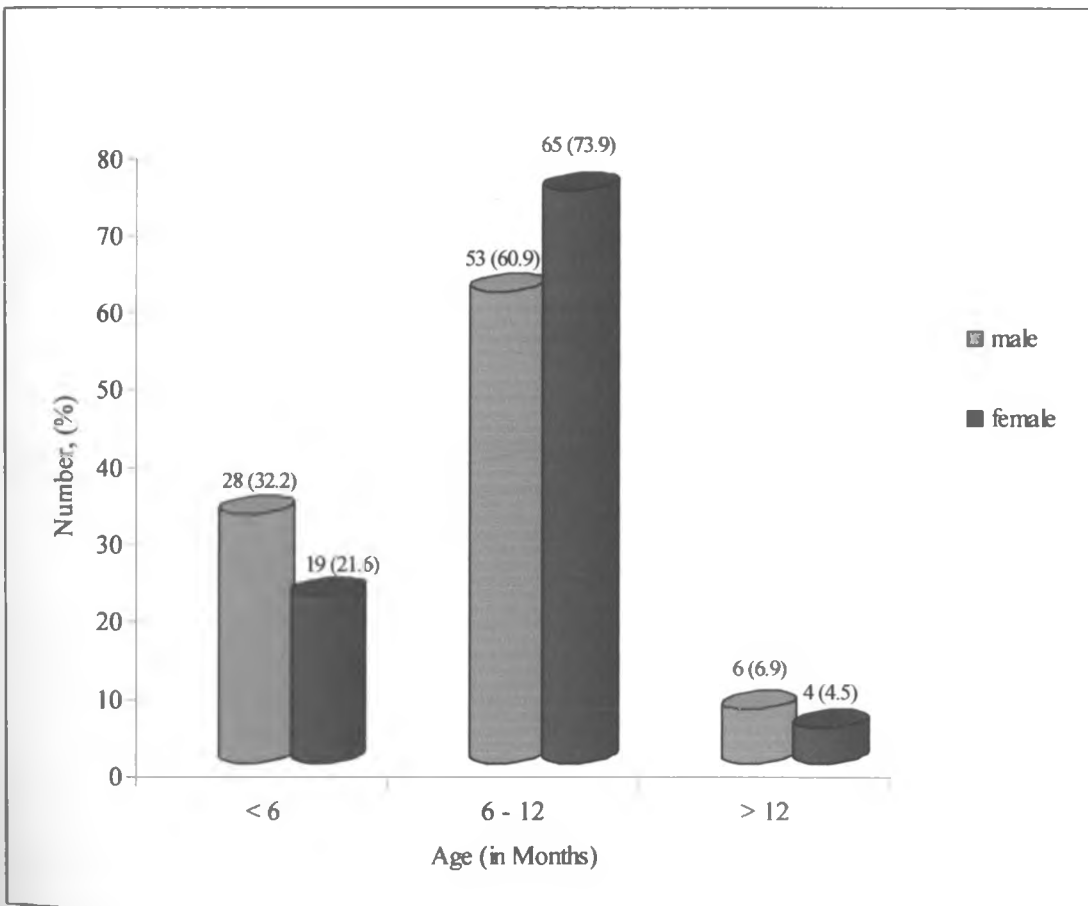
The patient did not incur any extra costs.

Confidentiality was maintained at all times.

RESULTS

During the study period of February 2008 to May 2008, one hundred and eighty six (186) consecutive patients who met the inclusion criteria were recruited into the study. Eleven patients were discharged before echocardiography and therefore excluded from the study leaving 175 patients. Of the 175 patients, 103 had echocardiographic abnormalities giving a prevalence of 59%. There were 87 males (50%) and 88 females (50%) giving a sex ratio of 1:1. More than half of the children in the study were in the age group 6-12 months. This is depicted in Figure 2 below.

Figure 2: The distribution of Age and Sex of the Patients



The socio-demographic characteristics of the study population were as depicted in Table 1 below.

Table 1: Socio-Demographic characteristics of the study population

Characteristics	Median / Number (n=175)	IQR / Percentage
Age (months)	7	9 -15
Sex male	87	50
Nutritional status		
normal	97	55
underweight	78	45
Exposure to sunlight		
yes	143	82
no	32	18
duration of exposure (minutes)	30	
Birth history		
term	158	90
preterm	17	10
Exclusive breast feeding		
yes 0-3 mo	119	68
3-6mo	47	27
Housing		
flats	86	49
single units	89	51

The ages of the children in the study were between 3 months and 18 months. Weight range was between 4.1 kg-10 kg, median weight was 6.5 kg. Majority lived in single rooms occupied by 4-7 family members. The median duration of exposure to sunlight

Echocardiography findings

There were 103 (59%) patients with abnormal echocardiographic findings while 72 (41%) were normal. Table 2 below shows the profile of the echocardiographic findings.

Table 2: The profile of echocardiographic findings of the patients

Echocardiographic feature	Number(n=175)	percentage
Normal	72	41%
Left ventricular systolic dysfunction	78	45%
Diastolic dysfunction	33	19%
Dilated Cardiomyopathy	57	32%
Pulmonary hypertension	35	20%
Hypertrophic Cardiomyopathy	31	18%

The commonest echocardiographic finding was left ventricular systolic dysfunction which was found in 78 (45%) patients. Most patients had an overlap of more than one echocardiographic abnormality. Figures 4-7 below are copies of some of the echocardiographs.

Figure 4: apical four chamber view showing dilated left ventricle with poor systolic function, EF 23%.



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Figure 4: apical four chamber view showing dilated left ventricle with poor systolic function, EF 23%.

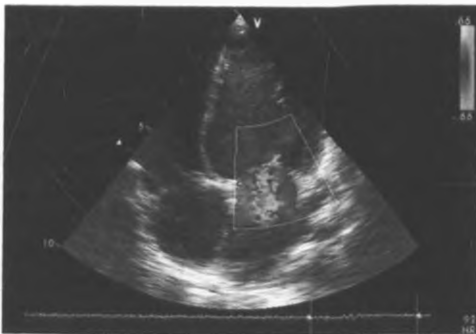


Figure 5A: apical four chamber view showing involvement of right and left ventricles.

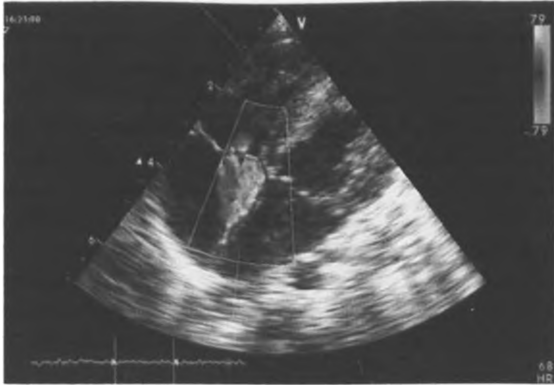
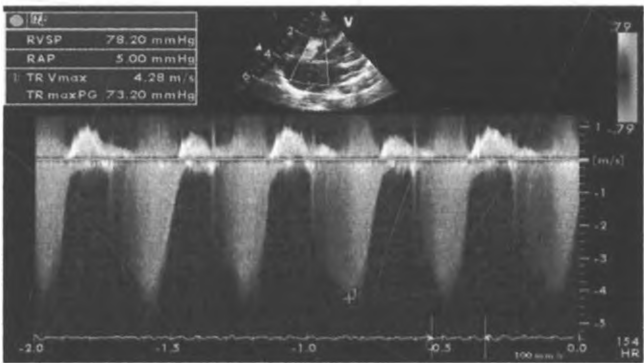


Figure 5B: Continuous wave Doppler showing tricuspid regurgitant jet of 4.28m/sec with estimated pulmonary pressures 78mmhg.

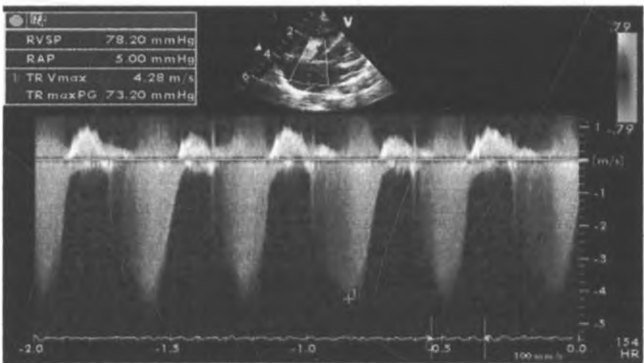


The above echocardiographic features are for a patient who had left and right heart involvement. This patient had severe biochemical derangements with serum ca^{2+} 1.65mmol/L, Po_4 0.71mmol/L, ALP 1480 and severe radiological rickets on wrist X-ray.

Figure 5A: apical four chamber view showing involvement of right and left ventricles.



Figure 5B: Continuous wave Doppler showing tricuspid regurgitant jet of 4.28m/sec with estimated pulmonary pressures 78mmhg.



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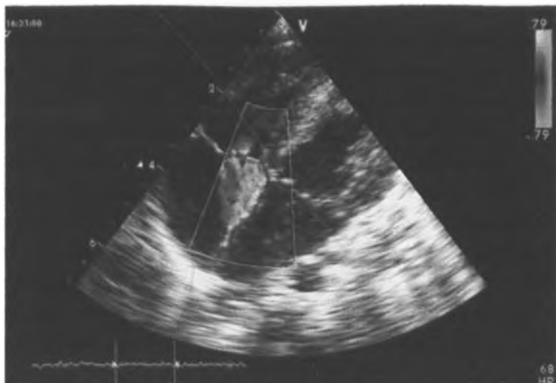
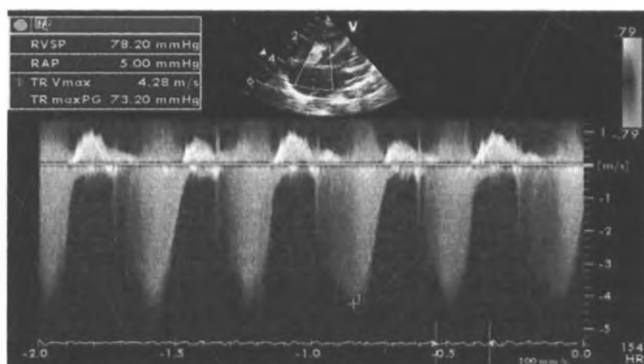


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The above echocardiographic features are for a patient who had left and right heart involvement. This patient had severe biochemical derangements with serum ca^{2+} 1.65mmol/L, po_4 0.71mmol/L, ALP 1480 and severe radiological rickets on wrist X-ray.

Figure 6A: Transmitral pulsed wave Doppler showing reversed diastolic dysfunction.

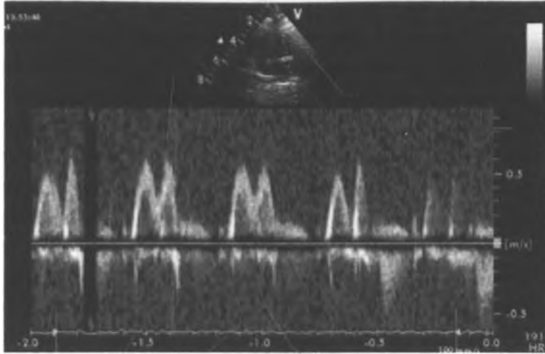


Figure 6B: Transmitral pulsed wave Doppler showing restrictive diastolic dysfunction

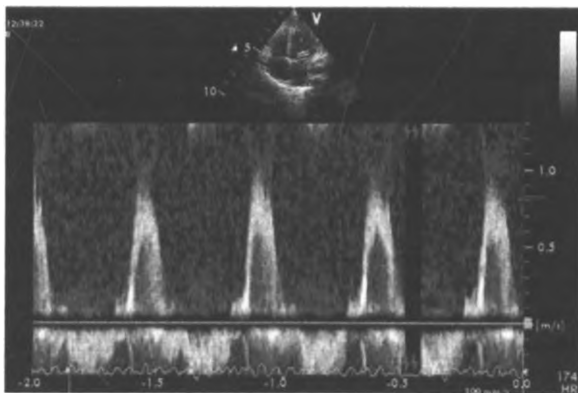


Figure 7: Parasternal long axis view showing hypertrophied left ventricle.



The echocardiographic abnormalities were distributed across all the age groups and sex with no age group or sex more likely to have echocardiographic abnormalities than the other, ratio 1:1. This is depicted in table 3 below.

Table 3: Distribution of echocardiographic abnormalities according to age and sex

	Age (months)		Sex	
	<6, n (%)	>6, n (%)	Male, n(%)	Female, n(%)
Ejection fraction Poor	31(44)	47(45)	39(45)	39(44)
Good	40(56)	57(54)	48(55)	49(55)
Diastolic function Normal	15(21)	18(17)	17(20)	16(18)
Abnormal	56(79)	86(83)	70(80)	72(82)
Pulmonary HTN Present	14(20)	21(20)	18(20)	17(19)
Absent	57(80)	83(80)	69(80)	71(81)
Hypertrophic CM Present	13(18)	18(17)	15(17)	16(18)
Absent	58(82)	86(83)	72(83)	72(82)

Gender and age difference were not statistically significant in relation to cardiac abnormalities (p-values > 0.065).

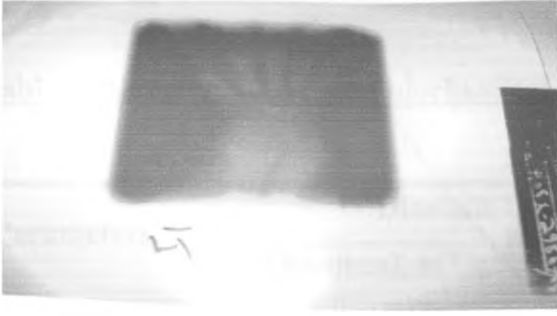
Radiological and biochemical findings

Wrist X-rays were done in all the patients at entry into the study to confirm the diagnosis of rickets. Three categories of patients were identified from radiography: mild rickets, severe rickets and healing rickets. The radiological grading was according to the report by the radiologists and was not based on the clinical features at the time of recruitment into the study. Biochemical profiles were also done on all patients. When the patients were grouped into the 3 stages of rickets, we found 3 patients (2%) in stage 1, 89 patients (51%) in stage 2 and 70 patients (40%) in stage 3. Thirteen (7%) did not fall in any group as they had both low calcium and low phosphate levels. Table 4 below shows the distribution of the radiological and biochemical characteristics of the study population.

Table 4: Radiological and Biochemical markers of the study population

Marker	Number (n=175)	percentage
Radiological grading		
Mild	122	70
Severe	32	18
Healing	21	12
Biochemical		
Calcium (2.02-2.6) Low	73	42
Normal	102	58
Phosphorus (1.30-2.26) Low	159	91
Normal	16	9
ALP (upto 320) High	143	82

Figure 8: Wrist X-ray showing severe rickets.



To determine the association between the biochemical parameters and the echocardiographic findings, an analysis was carried out using chi-square tests. Table 5 shows the association between biochemical parameters and EF Percent (LVSD).

Table 5: Association between biochemical parameters and EF Percent

Parameters	EF Percent		OR 95% CI	P-value
	<55%, n (78)	>55%, n (97)		
Calcium				
• <2.02mmol/L	47 (60.3)	26 (26.8)	4.1 (2.2 – 7.8)	<0.001
• >2.02mmol/L	31 (39.7)	71 (73.2)		
Phosphate				
• <1.30mmol/L	73 (93.6)	86 (88.7)	1.9 (0.6 – 5.6)	0.261
• >1.30mmol/L	5 (6.4)	11 (11.3)		

Hypocalcaemia was statistically significant in relation to left ventricular systolic dysfunction (odds ratio 4.1, 95%CI 2.2-7.8; p-value <0.001). On further correlation, patients with calcium levels <1.5mmol/L had a 20 fold increased odds of having LVSD (OR 20.6, 95%CI 4.9-137; p-value <0.001) as compared to patients with calcium levels between 1.5 -2.02mmol/ L (OR 2.77, 95%CI 1.32-5.83; p-value 0.003).

Tables 6 and 7 below show the association between biochemical parameters and diastolic function, and pulmonary hypertension respectively.

Table 6: Association between biochemical Parameters and Diastolic function

Parameters	Diastolic function		OR 95% CI	P-value
	Abnormal, n(33)	Normal, n(142)		
Calcium				
<2.02mmol/L	10 (30.3)	63 (44.4)	0.5 (0.2 – 1.2)	0.140
>2.02mmol/L	23 (69.7)	79 (55.6)		
Phosphate				
<1.30mmol/L	30 (90.9)	129 (90.8)	1.0 (0.3 – 3.8)	0.991
>1.30mmol/L	3 (9.1)	13 (9.2)		

Table 7: Association between biochemical Parameters and Pulmonary HTN

Parameters	Pulmonary HTN		OR 95% CI	P-value
	Abnormal, n(35)	Normal, n (140)		
Calcium				
• <2.02mmol/L	18 (51.4)	55 (39.3)	1.6 (0.8 – 3.4)	0.193
• >2.02mmol/L	17 (48.6)	85 (60.7)		
Phosphate				
• <1.30mmol/L	32 (91.4)	127 (90.7)	1.1 (0.3 – 4.1)	0.896
• >1.30mmol/L	3 (8.6)	13 (9.3)		

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	Abnormal, n(33)	Normal, n(142)		
Calcium				
<2.02mmol/L	10 (30.3)	63 (44.4)	0.5 (0.2 – 1.2)	0.140
>2.02mmol/L	23 (69.7)	79 (55.6)		
Phosphate				
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Parameters	Pulmonary HTN		OR 95% CI	P-value
	Abnormal, n(35)	Normal, n (140)		
Calcium				
• <2.02mmol/L	18 (51.4)	55 (39.3)	1.6 (0.8 – 3.4)	0.193
• >2.02mmol/L	17 (48.6)	85 (60.7)		
Phosphate				
• <1.30mmol/L	32 (91.4)	127 (90.7)	1.1 (0.3 – 4.1)	0.896
• >1.30mmol/L	3 (8.6)	13 (9.3)		

Table 8 below shows the association between biochemical parameters and hypertrophic cardiomyopathy.

Table 8: Association between biochemical Parameters and Hypertrophic cardiomyopathy

Parameters	Hypertrophic cardiomyopathy		OR 95% CI	P-value
	present, n (31)	absent, n (144)		
Calcium				
• <2.02mmol/L	11 (35.4)	62 (43.0)	0.7 (0.3 – 1.7)	0.439
• >2.02mmol/L	20 (64.6)	82 (57.0)		
Phosphate				
• <1.30mmol/L	24 (77.4)	135 (93.7)	0.23 (0.07-0.76)	0.004
• >1.30mmol/L	7 (22.6)	9 (6.3)		

On further analysis using the biochemical staging of rickets, patients in stage 3 of rickets were more likely to have left ventricular systolic dysfunction than those in stage 2 (OR 3.92 95%CI 1.92-8.06; p-value <0.001). Stage 1 was not used for comparison because of the small numbers. These numbers can be explained by the fact that patients in stage 1 are less likely to have overt clinical features of rickets. The entry point to our study was patients with clinical features of rickets hence the small numbers in this group. There was no statistically significant association between the biochemical staging and the other echocardiographic abnormalities.

An analysis was also carried out to determine the association between the severity of rickets according to radiography and the echocardiographic abnormalities. Table 9 shows the association between the echocardiographic abnormalities and severity of rickets.

Table 9: Association between echocardiographic abnormalities and severity of rickets according to radiography.

	Severe rickets (n=22)	Mild rickets (n=132)	OR 95%CI	p-value
Poor EF(LVSD)	22 (69%)	46 (38%)	3.6 (1.5-9.1)	0.002
Pulmonary hypertension	11 (34%)	16 (13%)	3.5 (1.3-9.4)	0.004
Diastolic dysfunction	9 (28%)	18 (15%)	2.3 (0.8-6.2)	0.076
Hypertrophic Cardiomyopathy	7 (22%)	19 (16%)	1.5(0.5-4.4)	0.398

Patients with severe rickets had 3 fold increased odds of having poor ejection fraction (OR 3.6, 95%CI 1.5-9.1; p-value 0.002) and 3 fold increased odds of having pulmonary hypertension (OR 3.5 95%CI 1.3-9.4; p-value 0.004) as compared to those with early rickets.

To evaluate the additional effect of pneumonia on cardiac dysfunction, we compared children with pneumonia and those without pneumonia with regards to cardiac dysfunction.

Table 10: Association between Pneumonia and cardiac dysfunction

	Pneumonia (n=101)	No pneumonia (n=74)	OR95%CI	P-value
Poor Ejection fraction	45 (44.5%)	33 (44.5%)	1.0 (0.5-1.8)	0.996
Hypertrophic cardiomyopathy	21 (20.7%)	10 (13.5%)	1.7 (0.7-4.2)	0.214
Pulmonary hypertension	24 (23.8%)	11 (14.9%)	1.7 (0.8-3.9)	0.146
Diastolic dysfunction	20 (19.8%)	13 (17.6%)	1.1 (0.5-2.5)	0.709

There was no statistically significant difference between the two groups (p-values >0.065).

Logistic regression Model

The biochemical, radiological and clinical characteristics of the study patients were entered into a logistic regression model. The results of that analysis are as presented in table 11 below.

Table 11: Logistic regression model to determine correlates of echocardiographic abnormalities

Factor	Par estimate (SE)	ODD	95%CI	P-value
Calcium	-1.58 (0.49)	1.22	1.08-1.72	0.001
Phosphate	-0.79 (0.48)	1.57	1.19-3.20	0.1
Age	0.096 (0.06)	3.01	2.66-3.46	0.116
Sex female	Reference			
male	0.049 (0.34)	2.86	1.71-7.85	0.888
Mild rickets	Reference			
Healing rickets	0.87 (0.53)	10.9	2.34-82.0	0.098
Severe rickets	1.25 (0.47)	32.4	3.99-98.7	0.008
Pneumonia	0.08 (0.37)	2.95	1.69-9.33	0.832

After controlling for age and sex, low calcium levels and severe radiological rickets were independently associated with development of left ventricular systolic dysfunction.

Discussion

This study found the prevalence of echocardiographic abnormalities to be 59%. Though on the higher side, this prevalence was similar to that reported by Uysal et al³¹. Uysal's study was prospective, and like ours the entry point was children with rickets diagnosed according to clinical, radiological and laboratory findings. However, the numbers were smaller with only 27 patients being evaluated as compared to our study which had 175 patients. In Uysal's study both echocardiography and electrocardiography were used for evaluation of the cardiac functions. Electrocardiographic changes were reported in 48% of their study patients; echocardiographic studies revealed left ventricular systolic dysfunction in the pretreatment stage. They also reported hypertrophic cardiomyopathy as the most striking echocardiographic change; found in 8 out of the 10 patients in biochemical stage three of rickets.

The commonest echocardiographic feature in our study was left ventricular systolic dysfunction, which was found in 78(45%) patients. The severity of left ventricular systolic dysfunction was variable. There were 6(3%) patients with severe LVSD (EF<30), 32(18%) patients with moderate LVSD (EF 30-44) and 40(23%) patients with mild LVSD (EF 45-54)^{46, 48}. In Uysal's study, left ventricular systolic dysfunction was presented as mean of the indices of left ventricular function whereby they reported a significant difference in the mean of the ejection fraction, ejection time, stroke volume and end-diastolic volume between the patients and the controls³¹. Diastolic dysfunction was present in 33(19%) of our patients, some patients had both systolic and diastolic dysfunction and thus the two components of left ventricular function were not mutually exclusive. The patterns of diastolic dysfunction that were found included: restrictive filling pattern in 19(11%) patients, pseudonormalised filling pattern in 4(2%) patients and reversed filling pattern in 10(6%) patients. Uysal et al did not assess the diastolic function of their study subjects.

Hypertrophic cardiomyopathy was found in 31(18%) patients. This was lower than that reported by Uysal et al at 30%³¹. All the patients with hypertrophic cardiomyopathy in

Uysal's study were classified in biochemical stage three of rickets. From our study, 10 out of the 70 patients in stage 3 had hypertrophic cardiomyopathy. These findings can be explained by the large number of patients in our study.

Dilated cardiomyopathy was found in 57(32%) of our study patients. Uysal et al did not report dilated cardiomyopathy. Several case reports of dilated cardiomyopathy and congestive cardiac failure in children with rickets have appeared in literature. In all the case reports, the children had low calcium levels^{32, 33, 36, 37, 38, 39}. Forty five out of the 57 patients with dilated cardiomyopathy in our study had low calcium levels; a finding which was consistent with the findings in the case reports quoted above. In one of the case reports, Olgun et al reported dilated cardiomyopathy in a 9 month old girl who presented with clinical features of rickets and cardiomegally on chest X-ray; wrists X-rays revealed severe rickets. She was found to have severe hypocalcaemia and echocardiography revealed a dilated left ventricle with reduced ejection fraction. The echocardiographic findings improved with treatment which included vitamin D and calcium supplementation and antifailure medication³². In all the case reports the cardiac dysfunction improved with supplementation of vitamin D and calcium showing that rickets was responsible for the dilated cardiomyopathy. All our study patients were started on vitamin D and calcium supplementation following the evaluation, those found to have severe left ventricular systolic dysfunction were in addition, started on antifailure medication. However, our study being cross-sectional we did not follow up the study patients after treatment. In a recent review, Maiya et al evaluated data of children who presented with dilated cardiomyopathy associated with vitamin D deficiency in South East England, between 2000 and 2006. They found that all the 16 patients they reviewed were profoundly hypocalcaemic and 10 patients had radiological rickets. Unlike our study, this was a retrospective review with the entry point being those children who were admitted in high dependency units and intensive care units with clinical heart failure with most requiring ventilatory and inotropic support⁵³.

We further found pulmonary hypertension and pericardial effusion in 35 (20%) and 8(4.5%) of the patients respectively. These findings had not been reported in Uysal's

study and case reports. Two (1%) patients had severe pulmonary hypertension (>50mmhg) with the rest having mild to moderate pulmonary hypertension (30-49mmhg). Mitral regurgitation was found in patients with dilated cardiomyopathy; this can be explained by stretching from valve incompetence. No isolated valvular abnormalities were demonstrated. Pericardial effusion was probably as a result of infection.

The main diagnoses at admission were pneumonia, gastroenteritis and convulsion. Other diagnoses included fever, bronchiolitis, asthma and hypocalcaemia. However, they were not mutually exclusive, some patients had more than one diagnosis. Pneumonia is known to be associated with development of pulmonary hypertension and acute congestive cardiac failure, and could have been a confounder in the assessment. However from our study the presence of pneumonia was not associated with an increased likelihood of developing cardiac dysfunction [OR1.0, 95%CI 0.5-1.8; p-value 0.996] suggesting that the poor contractility of the left ventricle found in most of our patients was probably due to low calcium levels. There was also no statistically significant association between pneumonia and pulmonary hypertension [OR1.7, 95%CI 0.8-3.9; p-value 0.146].

When an analysis was done to evaluate the significance of the biochemical parameters, low calcium levels was significantly associated with ejection fraction. Children with low calcium levels (<2.02mmol/L) were 4 times more likely to develop left ventricular systolic dysfunction. The odds increased with severity of hypocalcaemia, the patients with calcium levels below 1.5mmol/L had 20 times more likelihood of developing left ventricular systolic dysfunction.

On further analysis using stage 3 and stage 2 for comparison to evaluate the significance of the biochemical stages with regards to echocardiographic abnormalities, poor ejection fraction (LVSD) was found to be significantly associated with stage 3 of the disease. There was no association between the biochemical stage and the other echocardiographic abnormalities. Thirteen patients had normal calcium and normal phosphorus and could not fit into any of the above stages. This can be explained by the fact that some patients

were reported to have healing rickets on radiography and this could have contributed to the normalization of the biochemical parameters. However, it is important to note that none of the study patients had been started on vitamin D and /or calcium supplementation prior to recruitment to the study. The features of healing rickets noted on radiography can be explained by exposure to sunlight in a child who previously had developed rickets. The small number of patients in stage 1 can be explained by the fact that the patients we recruited into the study had clinical features of rickets. Stage 1 implies to the early stages of rickets before onset of clinical signs.

Severe rickets as defined by radiology, was found to be significantly associated with left ventricular systolic dysfunction and pulmonary hypertension. This indicates that radiology may be a useful screening tool for children with rickets who are suspected to have cardiac dysfunction.

Twenty seven (15%) of patients were diagnosed to have symptomatic heart failure at the time of admission. Most of the others did not fulfill the Framingham's criteria for diagnosis of heart failure. This can partly be explained by the fact that over 40 (50%) patients of those with LVSD had mild left ventricular systolic dysfunction. It is also possible that the presence of CHF may have been misinterpreted as pneumonia at admission and only realized at echocardiographic evaluation. In the study by Uysal none of the patients had signs of CHF despite the relatively low ejection.

Anaemia is a recognized cause of cardiac dysfunction. It affects the heart through tachycardia and a hyperdynamic state. There is initially left ventricular hypertrophy as a compensatory mechanism then later progressive chamber dilatation due to increased circulatory volume. Patients with severe anaemia were excluded from our study as this would have been a confounding factor. The mean haemoglobin of our study patients was 10.6g/dl with an IQR 9.8-11.4g/dl.

Five (2.8%) patients died during the study period, four of them had moderate to severe left ventricular systolic dysfunction, and one had normal ejection fraction. All the patients

who died had low calcium levels with reported severe rickets in 4 and early rickets in 1. Of the patients who died, four had been started on antifailure medication while one died before initiation of treatment.

From the logistic regression model low calcium levels and severe radiological rickets were independently associated with development of left ventricular systolic dysfunction. Therefore, both may be used independently as screening tools to evaluate patients who are more likely to have cardiac dysfunction among patients diagnosed with rickets.

This study has demonstrated that echocardiographic abnormalities especially left ventricular systolic dysfunction occur in children with rickets; particularly so in severe disease. The most important approach would be to start treatment of rickets early before the patients develop cardiac dysfunction. From these results we would recommend echocardiographic evaluation for children with severe radiological rickets and severe hypocalcaemia as these are independent predictors of presence of cardiac dysfunction.

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Strengths of the study

1. Echocardiographs were done in the wards, therefore we were able to capture even the very sick children who otherwise would have been lost from the study if they were to be transported to the cardiology unit where echocardiographs are routinely done.

Limitations of the study

1. The study was done in very sick patients admitted to the wards; the relatively well children seen at outpatient and discharged were not included. The study therefore missed to capture the echocardiographic profiles of such children.

Conclusion

1. There is a high prevalence of echocardiographic abnormalities (59%) in patients with rickets at KNH.
2. Low calcium due to rickets is associated with left ventricular systolic dysfunction, the lower the calcium the higher the odds of having LVSD.
3. Severe rickets is associated with left ventricular systolic dysfunction
4. There was no difference between patients who had pneumonia and those without pneumonia with regards to cardiac dysfunction..

Recommendation

1. There is need for more aggressive search and early treatment of rickets in order to avoid the cardiovascular consequences.
2. Echocardiographic evaluation should be done in patients with severe radiological rickets and /or severe hypocalcaemia so that appropriate treatment of the cardiac dysfunction can be instituted.

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APPENDIX I

CONSENT EXPLANATION AND FORM

Study Title: Evaluation of the Echocardiographic features of children presenting with rickets at KNH.

Study Team

Investigator: Dr G.A.Odhiambo –Tel 0722666481

Supervisors: Dr Jowi - Tel 0722293454 or department of paediatrics (UON)

Dr Wamalwa – Tel 0721239493 or department of paediatrics (UON).

Dr Amayo – Tel 0733617678 or department of human pathology (UON).

Researcher statement

Rickets is a disease of growing children affecting bone, it is mainly due to lack of vitamin D because of lack of exposure to sunlight or diet deficiency or due to lack of calcium in the diet. It leads to weakness in the muscles which contribute to the development of recurrent pneumonia (infection of the lungs) and other infections. It can also affect the heart leading to abnormalities in the function of the heart.

Purpose of the study

The aim of this study is to find out what types of abnormalities occur in the heart due to rickets. This will guide the clinicians on how to investigate for these abnormalities early in order for treatment to be started in good time so as to reduce the number of times that the baby would be sick and admitted.

Procedures /Risks

This is a voluntary study, if you give permission for your child to be included you will be expected to fill a questionnaire and your child to undergo a clinical examination, the following tests will then be done;(a) a wrist X-ray to confirm the presence of rickets which means he/she will be exposed to irradiation, caution will be taken to avoid

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This is a voluntary study, if you give permission for your child to be included you will be expected to fill a questionnaire and your child to undergo a clinical examination, the following tests will then be done;(a) a wrist X-ray to confirm the presence of rickets which means he/she will be exposed to irradiation, caution will be taken to avoid

unnecessary dangerous irradiation. The procedure is not painful though may be a little uncomfortable.

(b) Once the diagnosis of rickets is confirmed your child will have 3ml of blood drawn from a vein in a sterile manner to avoid risk of infection at the venepuncture site. The procedure may cause a little discomfort to the child and bleeding may occur, a dressing will be applied immediately to the site to minimize bleeding. The sample will be taken to the UON laboratory for processing to estimate the levels of calcium, phosphorus, alkaline phosphatase and albumin. No other tests will be performed on the blood sample without your consent. (c) Echocardiograph will then be done to assess the heart function –this is a harmless procedure involving use of electrical sound.

Potential benefits

You and the primary clinician will be provided with the results as soon as they are obtained to facilitate prompt institution of treatment of any abnormalities discovered. The tests will be done at no cost to you.

Participation in this study is voluntary. You may withdraw from the study at anytime; withdrawal will not affect the treatment your child is receiving. Confidentiality will be maintained at all times, the names on the questionnaire are only to assist in the delivery of the results.

If you have any question regarding the study you can contact the above named persons or Kenyatta National Hospital Ethics and scientific committee chairperson. Tel 2726300 Ext 44102.

Declaration

I, ----- of ----- has understood the study aim and procedures and do hereby give permission for my child to participate in this study.

Signed -----

Relationship to child (parent/guardian) -----

Signed (Witness) -----

APPENDIX II

QUESTIONNAIRE

1. Study No. [] IP No-----

2. Name -----

3. Sex Male-1 Female -2 []

4. Age (mo) []

5. Weight (Kg) []

6. Height (cm) []

7. BSA/m² []

8. Nutritional status Normal [] Underweight []

9. Birth Term [] Preterm []

10. Exclusive Breastfeeding months []

11. Housing Single [] Flats []

12. Family Size -----

13. Exposure to sunlight Yes [] No [] kind of outdoor dressing -----
Duration ----- Frequency -----

14. Milestones appropriate [] delayed []

15. Diagnosis at admission

- (a)Pneumonia []
- (b)Gastroenteritis []
- (c)Convulsions []
- (d)Others (specify) -----

16. Cardiac evaluation

Clinical/physical examination

(a) General examination

- Feeding difficulties Yes [] No []
- Excessive sweating Yes [] No []
- Cyanosis Yes [] No []
- Oedema Yes [] No []
- Finger Clubbing Yes [] No []

(b)Respiratory system

- Respiratory rate -----
- Crepitations Yes [] No []

(c) Cardiovascular system

- Pulse rate -----
- Peripheral pulses Normal [] Low volume [] High volume []
- Apex beat -displaced Yes [] No [] position -----
- Precordial heave Yes [] No []
- Gallop Yes [] No []
- Murmur Yes [] No []

(a) Location-----

(b)Type-----

- Loud P2 Yes [] No []

(d) Abdominal

Hepatomegally Yes [] No []

(e) Congestive Heart Failure Yes [] No [] (see appendix IV for
Criteria of diagnosis of CHF)

17. Laboratory Work Up

Parameter	levels	Reference Values
Ca ²⁺		See Appendix V
PO ₄ ⁻		See Appendix V
ALP		See Appendix V
Alb		See Appendix V
Hb		See Appendix V
cCa ²⁺		See Appendix V

18. Radiological Findings

- (a) Widening of the epiphyseal plate Yes[] No[]
- (b) Cupping Yes [] No []
- (c) Splaying, Fraying Yes [] No []
- (d) Osteopenia Yes [] No []

APPENDIX III

ECHOCARDIOGRAPHY REPORT

(A) M-MODE

LVDd -----LVDs----- FS-----%
EDV -----ESV -----EF-----%
HR ----- SV-----CO-----
IVST -----LVPWT ----- I/L ratio-----

(B) 2-DIMENSIONAL AND COLOR DOPPLER

Chamber size

(a)LV	Dilated []	Normal []	Small []
(b)RV	Dilated []	Normal []	Small []
(c)LA	Dilated []	Normal []	Small []
(d)RA	Dilated []	Normal []	Small []

Contractility Poor [] Normal []

Valves	Morphology	Function
Mitral	-----	-----
Aortic	-----	-----
Tricuspid	-----	-----
Pulmonary	-----	-----

Wall Motion Normal [] Abnormal []

Other abnormalities -----

(C) CONTINUOUS DOPPLER

Tricuspid regurgitation -----
Estimated Pulmonary artery pressure -----mmHg

(D) PULSED WAVE DOPPLER

Diastolic Function Normal []

Restrictive []

Pseudonormalized []

E/A ratio -----

Echocardiography done by-----

Assisted by-----

APPENDIX IV

NORMAL RESPIRATORY RATES

AGE	RATE (breaths/min)
3 -12 months	<50
>12months	<40

NORMAL HEART RATES

AGE	RATE (beats per min)
3 -12 months	90 -135
>12 months	85 -115

FRAMINGHAM CRITERIA OF HEART FAILURE DIAGNOSIS FOR INFANTS

Diagnosis of heart failure is made if the patient meets three major or two major and two minor criteria. The minor criteria should not be attributable to any other condition.

Major Criteria

- ❖ Cardiomegally
- ❖ Acute pulmonary oedema
- ❖ S3 gallop
- ❖ Tender Hepatomegally
- ❖ Hepatojugular reflex
- ❖ Neck vein distension

Minor criteria

- ❖ Peripheral and facial oedema
- ❖ Tachycardia (adjusted for age)
- ❖ Raised jugular venous pressure
- ❖ Crepitations
- ❖ Tachypnoea

UNIVERSITY OF NAIROBI
MEDICAL LIBRARY

CALCIUM liquicolor

Photometric Test for Calcium CPC Method

Package Size

REF	10011	200 ml	Complete test kit
IVD			

Method ^{1,2}

Calcium ions react with o-cresolphthalein-complexone in an alkaline medium to form a purple coloured complex. The absorbance of this complex is proportional to the calcium concentration in the sample.

Contents

BUF	100 ml Buffer Solution	
	Lysine buffer (pH 11.1)	0.2 mol/l
	Sodium azide	0.095 %
RGT	100 ml Colour Reagent	
	8-Hydroxyquinoline	14 mmol/l
	o-Cresolphthalein complexone	0.1 mmol/l
	Hydrochloric acid	40 mmol/l
STD	3 ml Standard	
	Calcium (II)	8 mg/dl or 2 mmol/l
	Sodium azide	0.095 %

Reagent Preparation

Add **RGT** to **BUF** in equal volumes as required, mix and allow to stand for 30 minutes at room temperature before use.

Reagent Stability

The reagents and the standard are stable even after opening up to the stated expiry date when stored at 2...25°C. Contamination must be avoided.

The working reagent is stable for 7 days at 2...8°C and for 3 days at 15...25°C.

Specimen

Serum, heparinised plasma

Stability in serum: 10 days at 2...25°C.

Assay

Wavelength: 570 nm, Hg 578 nm

Optical path: 1 cm

Temperature: 20...25°C

Measurement: Against reagent blank. Only one reagent blank per series is required.

Pipetting Scheme

Pipette into cuvettes	Reagent blank	Sample or STD
Sample or STD	---	20 µl
Working reagent	1000 µl	1000 µl

Mix and measure the absorbance of sample (ΔA_{sample}) and standard (ΔA_{STD}) against the reagent blank within 5 to 30 minutes.

Calculation of the Calcium Concentration

$$c = 8 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \text{ (mg/dl)}$$

or

$$c = 2 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{STD}}} \text{ (mmol/l)}$$

Performance Characteristics

Linearity

The test is linear up to a calcium concentration of 15 mg/dl (3.75 mmol/l). Samples with a higher concentration have to be diluted 1 + 1 with distilled water.

Repeat the assay and multiply the results by 2.

Typical performance data can be found in the Verification Report accessible via

www.human.de/data/gb/vr/ey-ca.pdf or

www.human-de.com/data/gb/vr/ey-ca.pdf

Normal values

Serum/plasma: 8.1 - 10.4 mg/dl or 2.02 - 2.60 mmol/l

Quality Control

All control sera with calcium values determined by this method be employed.

We recommend to use our animal serum based HUMATROL quality control sera or our human serum based SERODOS.

Automation

Proposals to apply the reagents on analysers are available upon request. Each laboratory has to validate the application in its own responsibility.

Notes

- Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.
- The test is not influenced by hemoglobin up to 200 mg/dl or bilirubin up to 20 mg/dl.
- Lipemic and hemolytic samples require a sample blank. Using the same pipetting scheme mix 20 µl of sample with 1000 µl of distilled water and measure the absorbance (against sample blank) against distilled water. ΔA sample blank has to be subtracted from ΔA sample.
- BUF** and **STD** contain sodium azide (0.095%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes!

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04-2004-15



human

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PHOSPHORUS liquirapid

Photometric UV Test for the Determination of Phosphorus

Package Size

[REF] 10027 2 x 100 ml Complete test kit
[IVD]

Method 1,2

Phosphate reacts with molybdate in strong acidic medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration.

Reaction Principle (simplified)



Contents

[RGT] 2 x 100 ml Reagent
Ammoniumheptamolybdate 0.3 mmol/l
Sulphuric acid (pH < 1.0) 160 mmol/l
Detergent 1 %
Activators and stabilisers
1 x 5 ml Standard
Phosphorus 10 mg/dl or 3.2 mmol/l

Reagent Preparation

[RGT] and **[STD]** are ready for use.

Reagent Stability

The reagents are stable, even after opening, up to the stated expiry date when stored at 2...25°C. Avoid contamination!

Specimen

Serum.

Plasma must not be used. Anticoagulants may cause false low results.

Stability in serum: 7 days at +4°C
2 days at 20...25°C

Assay

Wavelength: 340 nm, Hg 334 nm
Optical path: 1 cm
Temperature: 20...25°C
Measurement: against reagent blank; one reagent blank per series is required

Setting Scheme

	Reagent blank	Sample or [STD]
Sample/ [STD]	—	10 µl
[RGT]	1000 µl	1000 µl

Mix, incubate at least 1 minute at room temperature. Measure the absorbance of the sample and the **[STD]** against the reagent blank within 60 minutes (ΔA).

Calculation of the Phosphorus Concentration

$$C = 10 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{[STD]}}} \quad (\text{mg/dl})$$

or

$$C = 3.2 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{[STD]}}} \quad (\text{mmol/l})$$

Performance Characteristics

Linearity

The test is linear up to a phosphorus concentration of 20 mg/dl or 6.4 mmol/l. Dilute samples with a higher concentration 1 + 1 with distilled water.

Multiply the result by 2.

Typical performance data can be found in the Verification Report, accessible via

www.human.de/data/gb/vr/su-phos.pdf and ../su-phos717.pdf

www.human-de.com/data/gb/vr/su-phos.pdf and ../su-phos717.pdf

Normal values

Inorganic phosphorus
Adults: 2.5-5.0 mg/dl 0.81-1.62 mmol/l
Children: 4.0-7.0 mg/dl 1.30-2.26 mmol/l

Quality Control

All control sera with values determined by this method can be used.

We recommend the use of our HUMATROL quality control serum based on animal serum or our SERODOS based on human serum.

Automation

Special applications for automatic analyzers are available on request

Notes

- Icteric and slightly lipemic samples require a sample blank. By using the same pipetting scheme, mix 10 µl sample with 1000 µl distilled water and measure the absorbance against distilled water. The absorbance $\Delta A_{\text{sample blank}}$ has to be subtracted from ΔA_{sample} .
- Strong lipemic and hemolytic sera should not be used.
- Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.
- [RGT]** contains sulphuric acid. If skin or mucous membranes come into contact with the reagent wash thoroughly with water and consult a doctor.

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HUMAN

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ALKALINE PHOSPHATASE liquicolor

DEA Buffer, DGKC

Orthophosphoric Monoester Phosphohydrolase
(Alkaline Optimum) (EC 3.1.3.1)

Package Size

[REF]	12217	16 x 5 ml	Complete M-Test Kit
	12017	10 x 10 ml	Complete Test Kit
	12027	8 x 50 ml	Complete Test Kit
	12037	4 x 250 ml	Complete Test Kit

[IVD]

Method³

"Optimised standard method" according to the recommendations of the German Clinical Chemistry Association (Deutsche Gesellschaft für Klinische Chemie).

Reaction Principle



Contents

[REF]	12217	12017	12027	12037
[BUF]	16 x 4 ml	10 x 8 ml	8 x 40 ml	4 x 200 ml
[SUB]	1 x 16 ml	2 x 10 ml	8 x 10 ml	4 x 50 ml
[BUF]	Buffer			
	Diethanolamine buffer (pH 10.35 ± 0.2)		1.25 mol/l	
	Magnesium chloride		0.625 mmol/l	
[SUB]	Substrate			
	p-Nitrophenyl phosphate		55 mmol/l	

Reagent Preparation and Stability

Procedure 1 with reagent start

The reagents are ready for use.

The reagents are stable even after opening up to the stated expiry date when stored at 2...8°C. Contamination of the reagents must be avoided.

Procedure 2 with Sample Start

[REF] 12037 and 12027: Pour the entire contents of one bottle [SUB] into one bottle [BUF], mix thoroughly.

[REF] 12217: Pipette 1 ml from bottle [SUB] into one bottle [BUF], mix thoroughly.

[REF] 12017: Pipette 2 ml from bottle [SUB] into one bottle [BUF], mix thoroughly.

The working reagent is stable for 4 weeks at 2...8°C, for 5 days at 15...25°C. The working reagent must be kept light protected.

Specimen

Serum or heparinised plasma.

Avoid hemolysis

Loss of activity within 7 days: 0% at 4°C, 10% at 20...25°C.

Assay

Wavelength: Hg 405 nm (400 - 420 nm)

Optical path: 1 cm

Temperature: 25°C, 30°C or 37°C

Measurement: Read against air (increasing absorbance)

Warm the reagent and the cuvette to the desired temperature.

Temperature must be kept constant (± 0.5°C) for the duration of the test

Procedure 1 with Reagent Start*

Pipette into cuvettes	25°C, 30°C, 37°C
Sample	20 µl
[BUF]	1000 µl
Mix, incubate for 1 min. at the desired temperature.	
[SUB]	250 µl
Mix, read the absorbance after 1 min. and at the same time start the stopwatch. Read the absorbance again exactly after 1, 2 and 3 minutes.	

Procedure 2 with Sample Start*

Pipette into cuvettes	25°C, 30°C, 37°C
Sample	20 µl
Working reagent	1000 µl
Mix, read the absorbance after 1 min. and at the same time start the stopwatch. Read the absorbance again exactly after 1, 2 and 3 minutes.	

* semi-micro method; for macro methods multiply volumes by 2

Calculation

From the readings calculate the mean absorbance change per minute ($\Delta A/\text{min.}$).

Calculate the alkaline phosphatase activity in the sample using the following factor:

$$\text{U/l} = \Delta A/\text{min.} \times 3433 \text{ (procedure 1 with reagent start)}$$

$$= \Delta A/\text{min.} \times 2757 \text{ (procedure 2 with sample start)}$$

Conversion factor from traditional units (U/l) in SI-units (kat/l):

$$1 \text{ U/l} = 16,67 \times 10^{-3} \mu\text{kat/l}$$

$$1 \mu\text{kat/l} = 60 \text{ U/l}$$

Performance Characteristics

Linearity

If the absorbance change per minute ($\Delta A/\text{min.}$) exceeds 0.250 dilute 0.1 ml of the sample with 0.5 ml physiological saline (0.9%) and repeat the assay using this dilution. Multiply the result by 6.

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/en-ap-li.pdf

www.human-de.com/data/gb/vr/en-ap-li.pdf

Reference Values

Temperature	25°C U/l	30°C U/l	37°C U/l
Women ¹	40-190	49-232	64-306
Men ¹	50-190	61-232	80-306
Children up to 15 years ²	up to 400	up to 488	up to 644
Children up to 17 years ²	up to 300	up to 366	up to 482

Quality Control

All control sera with alkaline phosphatase values determined by this method can be employed.

We recommend to use our animal serum based HUMATROL quality control sera or our human serum based SERODOS.

Automation

Proposals to apply the reagents on analysers are available on request. Each laboratory has to validate the application in its own responsibility.

Notes

- [BUF] and [SUB] contain sodium azide (0.095%) as preservative. Do not swallow. Avoid contact with skin and mucous membranes!
- During the reaction p-nitrophenol is produced. This substance is poisonous when inhaled, swallowed or when absorbed through the skin. If the reaction mixture comes into contact with skin or mucous membranes, wash copiously with water and consult a doctor.

References

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7th January 2008

Ref: KNH-ERC/ 01/ 29

Dr. Odhiambo Grace Akinyi
Dept. of Paediatrics & Child Health
School of Medicine
University of Nairobi

Dear Dr. Odhiambo

**RESEARCH PROPOSAL: "EVALUATION OF THE CARDIOVASCULAR STATUS OF CHILDREN
PRESENTING WITH CLINICAL RICKETS AT KENYATTA N. HOSPITAL" (P329/11/2007)**

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your above cited research proposal for the period 7th January 2008 – 6th January 2009.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

- c.c. Prof. K.M.Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept, of Paediatrics & Child Health, UON
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