METABOLIC BONE DISEASE IN EXCLUSIVELY BREAST FED VERY LOW BIRTH WEIGHT INFANTS

BY

MARTIN M. MSOMEKELA, MD (UDSM)

MUHIMBILI MEDICAL UNIVERSITY
LIBRARY
DSM.

METABOLIC BONE DISEASE IN EXCLUSIVELY BREAST FED VERY LOW BIRTH WEIGHT INFANTS

BY

MARTIN M. MSOMEKELA, MD (UDSM)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH IN THE UNIVERSITY OF DAR ES SALAAM

1996

Supervisors:

1. Prof. Mbise R.L., M.B.Ch.B., M.Med, M.Sc.
2. Dr. Manji K.P., MBBS, M.Med

ABSTRACT

Metabolic bone disease (MBD) or rickets is frequent in very low birth weight infants. A descriptive cross-sectional hospital based study was carried out from April to June 1995 to find out the magnitude, contributory factors, morbidity and suitable biochemical diagnostic tests for MBD.

The outcome of this study was expected to show whether calcium and phosphate supplementation was indicated in exclusively breast fed VLBW infants. 100 infants at the postnatal age of 6-12 weeks with birth weight of 1500g or less were studied.

The health status of the infants was assessed by thorough history and physical examination. Double view radiography of left wrist was done on all infants and serum calcium, phosphate and alkaline phosphatase were determined. The radiography was used to identify presence or absence of MBD.

33 out of 100 (33%) infants, 16 males and 17 females were diagnosed to have metabolic bone disease radiographically. The mean gestational age of the infants with metabolic bone

disease was 30.4 weeks (SD=2.7 weeks) while that of the infants without metabolic bone disease was 32.4 weeks (SD=3 weeks) (P value=0.003).

There was no significant difference in birth weight between infants with MBD and infants without MBD. However, the postnatal weight was significantly different. Infants with MBD weighed 2955.5g to 5660g, while infants without MBD weighed 1170g to 3700g (P=0.008).

The mean postnatal occipito-frontal circumference was 35.6cm (SD=2.8) in infants with metabolic bone disease and 34.2cm (SD=3.4) in infants without metabolic bone disease(P value=0.053).

The mean length was 47.7cm (SD=5) and 45.0cm (SD=4.3) in infants with metabolic bone disease and in infants without metabolic bone disease respectively (P value=0.006). No significant difference in serum calcium and phosphate was observed between infants with MBD and those without MBD. The mean serum alkaline phosphatase was 1052.9iu/L (SD=493.3iu/L) in infants with metabolic bone disease and 766.8iu/L (SD=301.7iu/L) in infants without metabolic bone disease (P value=0.006).

MBD was not affected by parity or multiple delivery. It is concluded that metabolic bone disease is common in very low birth weight infants. Hence calcium and phosphate supplementation intervention study should be done to find out its effect on MBD incidence.

CONTENTS

Abstract ii

Acknowledgement vi

Declaration vii

Copyright viii

Abbreviatiations ix

Introduction and Review of Literature 1

Study objectives 11

Rationale of the study 12

Patients and methods 12

Results 19

Discussion 34

References 44

Appendix I: Questionnaire 53

Appendix II: Illustration of X-ray of normal bone . 55

Appendix III: Illustration of grade I metabolic bone change 56

Appendix IV: Illustration of grade II metabolic bone change 57

ACKNOWLEDGEMENTS

First of all I would like to express my sincere gratitude to Prof. R.L. Mbise and Dr. K. Manji who supervised this work. Their dedicated efforts, constructive criticism and encouragement enabled the production of this dissertation.

Immeasurable thanks to Dr. R. Kazema - head of Radiology Department for reviewing all the X-ray films in the study; Dr. L. Chuwa, Department of Clinical Chemsitry for allowing the biochemical analysis of the specimens; Mr. C. Makwaya for statistical analysis assistance, Mr. E. Mfinanga and Mr. C. Msuya for serum specimen handling and Miss. Jane Lubulira for manuscript type-writing.

I also wish to thank all mothers and their infants who participated in the study and all staff in Ward 36 for their co-operation.

Lastly but not the least; sincere gratitude to my wife Flora for her tireless encouragement and creation of conducive atmosphere for my work; my children Fidelis, Delphine and Erick for their calmness observed at home while I worked on this manuscript.

DECLARATION

I declare that this Dissertation is my original work and has never been submitted for a degree in any other University.

..... Date 08.10.1996

M. M. MSOMEKELA

..... Date 10-10-96

R.L. MBISE

..... Date 10.10.1996

K.P. MANJI

COPYRIGHT

All rights reserved. No parts of this work may be reproduced, stored in any retrieval system or transmitted in any form or by any means, mechanical, photocopying, electronics or otherwise without prior written permission of the author or the University of Dar es Salaam on his behalf.

ABBREVIATIONS

ALP	Alkaline phosphatase
Bwt	Birth weight
B	Coeffient
cm	Centimetres
CPL	Central Pathology Laboratory
GA	Gestational age.
g	grammes
IQ	Intellingence quotient
i.u/L	International units per litre.
MBD	Metabolic Bone Disease
MCH	Maternal and Child Health
MMC	Muhimbili Medical Centre
mmol/L	Millimoles per litre.
OFC	Occipito-frontal circumference
SD	Standard deviation
SE	Standard error
+ve	Positive
-ve	Negative
VLBW	Very low birth weight

INTRODUCTION AND REVIEW OF LITERATURE

Human breast milk has been recommended as an ideal food for infants. It offers immunological protection against infection, encourages maternal-infant bonding and may protect against necrotizing enterocolitis (1).

Although human breast milk may be an ideal nutrient for term infants, there is doubt about its adequacy in providing correct nutrition to preterm infants. Premature infants grow more rapidly than term infants in order to catch-up and this fast growth may unmask nutritional inadequacy of human milk which are not apparent at slower growth rates (2,3).

Studies on neuro-developmental outcome in preterm infants fed breast milk show that these infants fair better and have higher intelligence quotient (IQ) than those fed formula (4). Breast milk contains various factors for neurodevelopment. This has been substantiated by the fact that it contains long chain lipids (docosahexanoic acid 22:6 w-3) which accumulates in large amounts in the developing brain and retina (5). Human milk also contains numerous hormones and trophic factors (5,6).

Preterm infants differ from term infants in a number of aspects. Some of the intrinsic difference with important nutritional implication in the preterm include:- poor

storage of glycogen, fat, calcium, phosphorous and iron (7). For this reason, during the period of rapid growth, clinical conditions like aneamia of prematurity and rickets of prematurity have been seen to affect the preterm infant (2-8).

With the renewed interest in breast feeding and the discussion on nutritional advantages of breast milk versus milk formulars, there is an increased desire among mothers giving birth prematurely to provide expressed breast milk. Harvey et al analysed milk from 10 mothers giving birth at 38-40 weeks gestation and other 17 mothers giving birth at 26-33 weeks gestation for energy and macronutrients. Except for protein, which was 10-20% higher in mothers giving birth to preterm babies, the concentrations of the other nutrients measured (sodium, pottasium, chloride, phosphorous and magnesium) were similar in both groups. This study indicates that preterm milk is suitable for the preterm infant (9). However, a meta-analysis of 10 studies by various authors indicate that the levels of calcium and phosphorous are either equal or less than those obtained from milk of mothers delivering at full term (10). This implies that the rapid catch-up growth of the preterm infant is not adequately met with these levels of calcium and phosphate.

METABOLIC BONE DISEASE (RICKETS OF PREMATURITY)

Metabolic bone disease in extreme preterm infants is now a well recognised problem (11-13). It is also known as rickets of prematurity or osteopenia. Decreased mineralisation or osteopenia is frequent in preterm infants, usually sub-clinical and diagnosed only after development of severe complications such as fractures or overt rickets (14-17). While osteopenia implies only under-mineralisation, rickets implies a radiological and clinical disease at the growing end of the long bones (14-17).

The pathogenesis of rickets include vitamin D deficiency and subtrate deficiency of calcium and phosphorous. Although vitamin D intakes of 800 to 1000 IU per day may be required to ensure adequate hepatic 25-hydroxylation, there is little evidence that this is the primary cause of metabolic bone disease (18). The main cause of metabolic bone disease of prematurity is substrate deficiency.

Infants weighing less than 1500gms have large requirements for calcium and phosphate. The quantity of calcium and phosphorous retained by the fetus during the last trimester is also inadequate. It is evident that unsupplemented breast milk can supply only a fraction of these to meet the rapid catch-up growth of these infants with poor stores of calcium and phosphate (2,19,20).

Although calcium absorption by the preterm infant is usually poor while phosphate is well absorbed, there is evidence that phosphorous deficiency is an important factor in metabolic bone disease of prematurity. Infants fed breast milk may have extremely low plasma concentration of phosphate, but normal or even higher levels of calcium (21,22) and a raised urinary calcium excretion (18). Human breast milk contains 15mg phosphorous per 100 ml in contrast to term formulas (about 30mg per 100ml) and formulas designed for preterm infants (about 40 mg). When phosphorous intake is inadequate, plasma reserves fall and phosphorous is withdrawn from the skeleton in order to maintain protoplasmic function. Calcium cannot be used for bone growth in the abscence of phosphate and so it is lost in the urine (23). In a multicentre study, plasma phosphate concentration was low and alkaline phosphatase activity was higher in infants fed breast milk than those fed formula (24).

It is not possible at present to define an independent role of calcium deficiency in bone disease of prematurity, though some studies suggest that breast fed preterm infants may benefit from supplementing both calcium and phosphate (25).

Information is lacking on the influence of prenatal nutrition on the risk of developing metabolic bone disease, but it is possible that poor placental transfer of calcium and phosphorous may contribute since it has been found to be more common after deliveries complicated by pre-eclampsia (26). Likewise copper and other minerals have been suspected to cause rachitic like bone changes but the importance of this is uncertain (16,27).

Antenatal factors related to neonatal hypocalcaemic convulsions occuring in infants at the end of the 1st week of life are:- increasing maternal age, higher parity and lower social class (28). Cases of neonatal rickets associated with asymptomatic hypocalceamia have been reported (29).

A study done by Hillman et al in 110 third trimester pregnant women showed no correlation of calcium and 25 hydroxyvitamin D serum levels, social class and child spacing with maternal age, parity or gestation (30). It is not known whether maternal age or parity can influence the occurence of metabolic bone disease (MBD) in the newborn, especially in preterm infants who are more affected by the disease.

Gestational age on the other hand has been found to be

associated with MBD. Koo et al in their study in 19 very low birth weight infants (<1500 gms), 6 infants with MBD had mean gestational age of 26.8 ± 0.8 (weeks) vs 13 infants without MBD with mean gestational age of 29.2 ± 2.5 (weeks). The gestational age difference in the two groups was statistically significant (P value <0.001) (31). In the same study infants with MBD had lower birth weight than in infants without MBD (P = 0.05).

Litte is known about parity or multiple delivery in conjuction with MBD. However, Kovar et al has reported one case of MBD developing in the 2nd twin at 6 weeks of life. The twins were born at 30 weeks gestation after a normal pregnancy to a 23 year - old well nourished primigravida (32).

INCIDENCE

Different incidences have been reported from different centres. Studies done in the developed countries show that the incidence of metabolic bone disease ranges between 13-32% (11,12). It is has been found most often and in its severest form in infants less than 1000 gms birth weight, in whom radiological rickets has been described in up to 57% of cases (14). Local data are missing as regards the incidence and prevalence of metabolic bone disease amongst surviving preterms in Tanzania.

DIAGNOSIS

The 3 diagnostic approaches to MBD are: clinical, biochemical and radiological.

CLINICAL DIAGNOSIS.

Early signs of metabolic bone disease of infants include:- craniotabes, thickening of wrists and ankles and palpable enlargement of costochondral junctions (rickets rosary) (33). Rachitic infants may present with irritability, pain and tendeness of affected bones. In severe cases fractures and respiratory distress may occur (34,35).

In 19 infants studied, 6 of them were diagnosed to have radiological metabolic bone disease and showed evidence of craniotabes (31).

BIOCHEMICAL DIAGNOSIS:

Metabolic bone disease of prematurity is associated with raised plasma alkaline phosphatase activity (36). This has been useful in epidemiological studies and those with grade II and III changes have activities greater than 1000 IU/l. However the normal values overlap with minor grades. Thus it remains useful in confirming severe cases (37). In the same study it was shown that alkaline phosphatase activity of five times the upper limit of the normal adult reference range is acceptable in the preterm infant but an activity

higher than this may suggest rickets. Levels above 7.5 times higher are associated with active bone disease (38).

Raised alkaline activity could be detected as early as 5 weeks of life in 6 infants with metabolic bone disease out of 19 infants with less than 1500g birth weights studied (31).

In another study, a significantly higher alkaline phosphatase activity was noted starting from 2 - 4 weeks of age in 37 out of 157 infants studied by Callenbach et al. Radiological rickets was later confirmed in these 37 infants (12).

While levels of phosphates are constantly low in metabolic bone disease, calcium levels can be normal or even raised. In most of the infants with clinical evidence of metabolic bone disease proven by raised alkaline phosphatase and radiological evidence, the serum calcium levels were normal (18,19). This did not mean that they had adequate calcium levels and these were in fact found to poorly correlate with the grade of the disease.

Infants fed breast milk may have extremely low levels of plasma phosphate, but normal or even high concertrations of plasma calcium and raised urinary excretion of calcium

(18,19). Infants with grade II and III bone changes have a significantly lower phosphate level than those infants without serious bone disease(38).

RADIOLOGICAL DIAGNOSIS:

Despite its limitations in quantifying the severity of the disease and the fact that it may miss minor changes, it remains an important diagnostic tool and is reliable for the diagnosis of marked generalised under-mineralisation and bone end changes of all grades of metabolic bone disease of prematurity. The following classification has been suggested and proves to be satisfactory in practice(31).

Grade 0- normal.

Grade I- rarefaction only.

Grade II- bone end changes (fraying and cupping of the metaphysis, subperiosteal new bone formation).

Grade III-the changes in Grade I and II, with fractures.

Radiography of the wrist is the best for early diagnosis since characteristic bone changes of the ulna and radius occur at an early stage (33).

PROGNOSIS OF METABOLIC BONE DISEASE

Some of the infants with biochemical and radiological evidence of osteopenia have a self limiting course. However some of them do develop rib fractures and respiratory

distress. Some infants develop acute metabolic disturbance comprising of profound hypophosphataemia and hypercalceamia. Biochemically evident rickets is associated with short term delay in linear growth. The long term consequences, however, remain to be investigated(39).

HYPOTHESES:

1. The prevalence of Metabolic bone disease is high in very low birth weight infants.
2. Exclusively breast fed very low birth weight infants are prone to develop metabolic bone disease.
3. MBD is related to low gestational age, parity, postnatal growth and multiple delivery.

STUDY OBJECTIVES:

BROAD OBJECTIVE:

To determine the magnitude of metabolic bone disease in preterm infants of birth weight of 1500g or less who are exclusively breast fed.

SPECIFIC OBJECTIVES:

1. To determine the prevalence of metabolic bone disease in exclusively breast fed preterm infants.

2. To determine the association between metabolic bone disease and mean length at 6-12 weeks postnatal age in very low birth weight preterm infants.

3. To determine the relationship between the gestational age of the infant and metabolic bone disease.

4. To determine whether infant factors like multiple deliveries such as twin, triplets, quadriplets influence the development of metabolic bone disease.

5. To determine whether maternal factors such as parity, influence the development of metabolic bone disease.

6. To find out serum levels of calcium, phosphorus and alkaline phosphatase in infants with MBD and compare them with those of infants without MBD.

RATIONALE OF THE STUDY:

In Tanzania exclusive breast-feeding is advocated for 4 - 6 months of life except for orphans who do not have a first degree relative to foster. The incidence of low birth weight in Tanzania is 16% and nearly 40% of these are less than 1500g (40). Among the low birth weight infants surviving through the critical neonatal period the post-neonatal mortality is nearly 20% (40). There are no studies that have looked into the growth patterns of LBW infants who are exclusively breast fed and whether metabolic bone disease of the preterm is a major problem.

The outcome of this study will indicate if metabolic bone disease is common among the very low birth weight infants. The findings will lead to consideration of supplementation of calcium and phosphates in these infants.

PATIENTS AND METHODS:

The study was a descriptive cross sectional and hospital based. The study period extended from 15th April to 30th June 1995. The target population consisted of 100 exclusively breast fed infants of 1500g or less birth weight who were aged between 6 and 12 weeks.

SAMPLE SIZE - Based on the prevalence of metabolic bone disease of 13 - 32% as reported by other studies from

different countries the estimate of the prevalence (P) of metabolic bone disease in this study was assumed to be 22.5% and the margin of error (E) to be 9.5%. By using the formula:-

$$n = \frac{(1.96)^2 . \ P \ (100-P)}{E^2}$$

for sample size calculation for cross - sectional studies(41), where n = Sample size, P = rough estimate of prevalence and E = margin of error, the minimum required sample size for this study was found to be 75 infants. However, 100 infants were studied so as to increase the power of the study.

INCLUSION CRETERIA INCLUDED:

Infants with birth weight of 1500g or less.

Age between 6 - 12 weeks

Exclusive breast feeding

Absence of major congenital abnormality

Parents' informed consent granted.

EXCLUSION CRETERIA:

1. Clinical diagnosis or suscipicion of the following conditions:-
   - Renal disease
   - Hepatic disease
   - G.T.I. problems e.g. steatorrhoea, diarrhoea

. Severe malnutrition

. Thyroid disease

2. Infants with insufficient perinatal records.

STUDY POPULATION:

The study was carried out at MMC postnatal follow up clinic. The clinic caters for all infants who were admitted into the neonatal unit for various reasons including preterm infants weighing 1500g or less at birth. The usual first follow up postnatal visit was between 4 and 6 weeks which coincided with the time for diphtheria, pertussis, tetanus and oral polio immunization.

DATA COLLECTION:

During the first follow up clinic attendance, informed consent regarding the study was sought from the mothers of all eligible infants. Enrolment of infants was done as they came. A structured questionnaire was filled with the required information of the infants and mothers (see appendix I).

Medical documents such as discharge forms of the infants and mothers and MCH cards were used to obtain the relevant information required in the questionnaire. When sufficient information was not available infants were excluded from the study.

Health status of infants was determined by thorough history and physical examination. Infants discovered to have conditions which could interfere with calcium and phosphate homeostasis (see exclusion creteria) were excluded from the study.

RADIOLOGICAL EXAMINATION:

For all infants recruited in the study, anteroposterior and lateral view X-rays of the left wrist were done to identify those with metabolic bone disease. The X-ray findings were reported by the same radiologist.

BLOOD COLLECTION:

All infants (33 of them) with radiological bone changes of rickets, and other 33 randomly selected unmatched infants with normal x-ray results, had blood specimen taken from them. Using non-reusable (disposal) syringes and needles, femoral venepuncture was done after thoroughly cleaning the venepuncture site with a swab soaked in 70% alcohol. About 5 mls of blood was drawn and put into empty sterile bottles labelled with serial numbers of the infants. Serum was separated from the blood specimens by a qualified technician and put into similar serially labelled aliquot containers. Serum in these aliquot containers was stored under - 20°C and was analysed for calcium and phosphorus levels and for alkaline phosphatase activities after one to two weeks.

ANTHROPOMENTRY:

WEIGHT:

This was taken while the child was nude using a standard beam balance (Seca). The weight was recorded to the nearest 10g.

LENGTH:

Lenght boards were used to measure the lengths. The infant was placed lying supine on a flat surface. One observer held the child's head in contact with the board at the top of the table, while the investigator straightened the legs and turned the feet upwards at right angles to the legs and the sliding foot board was brought in contact with the infant's heels. Then length was determined and recorded to the nearest 0.1cm.

OCCIPITO-FRONTAL CIRCUMFERENCE:

This was measured in cm using a non-stretchable tape from the occiput to pass just above the eyebrows.

BIOCHEMICAL DETERMINATION:

Biochemical tests were done at the Department of Clinical Chemistry, CPL, Muhimbili Medical Centre. Calcium and phosphate serum levels were estimated using Sackler-Anal and Hohenwallner-Wimmer Brock methods (42) respectively. Serum alkaline phosphatase activity was determined using Bassay Lowry Brock method (42).

STATISTICAL ANALYSIS:

The data was analysed in the Institute of Public Health,

Muhimbili University College of Health Sciences (MUCHS). Data Processing (entry and cleaning) was done using DBASE IV computer programme. Statistical analysis packages of Epi-Info and SPSS were used in the major part of data analysis. Statistical significance testing of differences between means and proportions were done using student t-test and X^2 tests respectively at a significance level of 0.05. That is, a P-value of <0.05 indicated a significant association or difference that was being tested.

The outcome variable (MBD) as measured by radiological bone changes was treated as a binary response. That is, the infant was either normal (Code=0) or has metabolic bone disease (Code=1). Multiple logistic linear regression was used to examine effects of alkaline phosphatase, gestational age, birth weight, postnatal age and current weight on the outcome variable. These five explanatory variables were picked on the basis of their medical importance in explaining radiological bone changes.

A computer program LOGXACT was used to fit the logistic regression model of the form:- Logit p=Bo + B1x1 + B2X2 + B3X3 + B3X3 + B4X4 + B5X5; where p is the probability that an infant has the disease given the values of the explanatory variables, X's are the explanatory variables and B's are the regression coefficients.

Using a backward selection procedure the final model was obtained as:- LOGIT P=9.84 + 0.002 SERUM ALKALINE PHOSPHATASE - 0.36 GESTATIONAL AGE. This means that the probability (p) of an infant having MBD is high when raised (+) serum ALP is found. Similarly it means that an infant of lower (-) gestational age has high probability of being MBD case.

LIMITATION:

For cultural reasons mothers may not practice exclusive breast feeding and some form of supplementation may be given at home. Verbal response was the source of information of whether the mother exclusively breast fed her infant or not and this information may not always be correct.

RESULTS

1. Characteristics of the study population:

During the period 15th April to 30th June 1995, a total of 100 exclusively breast fed VLBW infants aged 6-12 weeks attending MMC postnatal clinic with their mothers were recruited into the study. The exclusively breast fed VLBW infants studied consisted of 45 males and 55 females, thus giving us a male:female ratio of 1:1.2.

The characteristics of the study population by gestational age, weight at birth, at 6 and 12 weeks postnatally and currently are presented in table 1. The gestational age of the infants ranged from 26 weeks to 37 weeks, the mean gestational age being 31.5 weeks (SD 3.0 weeks). Birth weight ranged from 800g to 1490g, mean birth weight was 1337.2g (SD 16.1g). Postnatal age ranged from 6 weeks to 12 weeks, mean postnatal age was 8.5 weeks (SD 2.8 weeks). The weight of infants at the time of study (current weight) ranged between 1170g and 5660g, the mean current weight being 3079g (SD 1153.2g).

Table 1: Characteristics of study population by gestational age, birth, postnatal and current weights.

	Minimum	Maxmum	Mean	SD
Birth weight (g)	800	1490	1337.2	16.1
Gestational age (weeks)	26	37	31.5	3
Postnatal age (weeks)	6	12	8.5	2.8
Current weight (g)	1170	5660	3079	1153.2

2. Prevalence of Metabolic bone disease (MBD):

The prevalence of metabolic bone disease is shown in table 2. 33 (33%) out of 100 infants were radiologically diagnosed to have metabolic bone disease. 16 (35.6%) out of the 45 males and 17 (30.9%) out of of the 55 females were found to have MBD. The difference in the incidence of MBD between the two sexes was not statistically significant (Chi-square value =0.24, P value = 0.622).

Table 2: Prevalence of MBD by sex:

MBD	Males (%)	Females (%)	Total
+ve	16 (35.6)	17 (30.9)	33
-ve	29 (64.4)	38 (69.1)	67
Total	45	55	100

Chi-square value = 0.24, P value = 0.622

3. Distribution of Grades of radiological MBD in the study population:

The radiological bone changes are normally graded as follows: Grade 0 : Normal (no bone changes).

Grade 1 : Rarefaction.

Grade 2 : (a) Cupping or fraying of bone ends

(b) Periosteal reaction

(c) Combination of (a) and (b).

Grade 3 : Grade 1 and 2 plus fractures.

Grade 1-3 radiological bone changes indicate MBD. Appendix II, III and IV show typical X-ray appearances of grade 0, 1, and 2 radiological bone changes in that order.

The radiological grades of MBD and its distribution is described in table 3. Out of 100 infants, 67(67%) had grade 0, 10(10%) had grade 1 and 23(23%) had grade 2 bone changes. There were no infants with grade 3 bone changes.

MEDICAL UNIVERSITY COLLEGE LIBRARY DSM.

Table 3: Distribution of various radiological grades of MBD in the study population:

Radiological grades of MBD	Infants No	(%)
0	67	(67)
1	10	(10)
2	23	(23)
Total	100	(100)

4. Gestational age:

Table 4 shows a comparison of mean gastational age between infants with MBD and those without MBD. The mean gestational age in infants without metabolic bone disease (MBD) was 32.4 weeks (SD 3.0 weeks), while that of infants with MBD was 30.4 weeks (SD 2.7 weeks), giving a difference of 2 weeks between the two groups of infants. This difference was found to be statistically significant (t value= 3.1, on 98 degrees of freedom and P value= 0.003).

Table 4: Comparison of mean gestational age between infants with MBD and without MBD.

MBD	No.	Mean GA (Weeks)	SD
+ve	33	30.4	2.7
-ve	67	32.4	3.0

t value = 3.1, P value = 0.003

5. Birth weight:

Comparison of mean birth weight of infants with MBD and those without MBD is shown in table 5. The mean birth weight was 1313.6 g (SD=163.7 g) in infants with MBD and 1348.8 g (SD = 159.8 g) in infants without metabolic bone disease. The mean birth weight of infants with metabolic bone disease was thus lower by 35g than that of infants without metabolic bone disease. However, the difference in birth weight between the two groups of infants was not statistically significant (t value = 1.026 on 98 degrees of freedom and P value = 0.308).

Table 5: Comparison of mean birth weight (Bwt) between infants with metabolic bone disease (MBD) and infants without MBD.

MBD	No.	Mean Bwt	SD
+ve	33	1313.6	163.7
-ve	67	1348.8	159.8

t value = 1.026, P value = 0.308

6: Current weight (Cwt):

Table 6 presents results of mean current weight (postnatal weight at the time of study). The mean current weight in infants with metabolic bone disease was 3269 g (SD = 1325.5 g), while that of infants without metabolic bone disease was 2955.5 g (SD 1111.3g). Comparison between the two categories of infants means shows higher mean current weight in infants with MBD by 313.5g. Statistical analysis using student t test showed that there was a significant difference between the two categories of infants in the current weight means(t value = 2.8, on 98 degrees of freedom and P value= 0.008).

Table 6: Comparison of mean current weight values of study infants by metabolic bone disease status.

MBD	No.	Mean cwt	SD
+ve	33	3269	1325.5
-ve	67	2955.5	111.3

t value = 2.8, P value = 0.008

7. Occipitofrontal circumference (OFC):

Table 7 shows a comparison between mean OFC in infants with MBD and those without MBD. The mean occipito-circumference was 35.6cm (SD=2.8 weeks) and 34.2cm (SD=3.4 weeks) in infants with MBD and those without metabolic bone disease respectively. The mean OFC was higher in infants with MBD by 1.4cm when compared with infants without MBD. However, the difference was not statistically significant (t value = 1.932, on 98 degrees of freedom and P value = 0.053).

Table 7: Comparison between mean OFC in infants with MBD and infants without MBD.

MBD	No.	Mean OFC	SD
+ve	33	35.6	2.8
-ve	67	34.2	3.4

t value = 1.932, P value = 0.053

8. Length:

Table 8 shows a comparison of mean body length between infants with MBD and those without MBD. Infants with metabolic bone disease had mean body length of 47.7cm (SD=5 cm) compared to 45.0cm(SD = 4.3cm) in those without MBD. Thus the mean body length in infants with MBD was 2.7cm longer than that of infants without MBD and this was statistically significant (t value = 2.801 on 98 degrees of freedom, P value = 0.006).

Table 8: Comparison between mean body length in infants with MBD and infants without MBD.

MBD	No.	Mean Length	SD
+ve	33	47.7	5.0
-ve	67	45.0	4.3

t value = 2.801, P value = 0.006

9. Serum calcium:

The mean serum calcium was 2.21mmol/L (range 1.89-3.43 mmol/L; SD=0.18 mmol/L) in infants with MBD and 2.08mmol/L (range 1.53-2.33 mmol/L; SD=0.28 mmol/L) in those without MBD (see table 9). The difference in the mean serum calcium between the two groups of infants was not statistically significant (t value=2.4, on 64 degrees of freedom and P value=0.051).

Table 9: Comparison of mean Serum calcium in infants with MBD and infants without MBD.

MBD	No.	Mean serum calcium (mmol/L)	SD
+ve	33	2.21	0.18
-ve	33	2.08	0.28

t value = 2.4, P value = 0.051

9.1 Serum phosphorus:

Serum phosphorus ranged between 0.540 mmol/L to 2.650 mmol/L in infants without MBD and between 0.080 mmol/L to 3.160 mmol/L in infants with MBD (see table 10). The mean serum phosphorus was 1.58 mmol/L (SD=0.57 mmol/L) in infants with MBD, and 1.62 mmol/L (SD=0.45 mmol/L) in those without MBD.

Thus the mean serum phosphorus was lower by 0.04mmol/L in infants with MBD, when compared with those without MBD, but the difference was not statistically significant (t value =0.286, on 64 degrees of freedom and P value=0.773).

Table 10: Comparison of mean serum phosphorus in infants with MBD and infants without MBD (n = 66)

MBD	No.	Mean phosphorus (mmol/L)	SD
+ve	33	1.58	0.57
-ve	33	1.62	0.45

t value = 0.286, P value = 0.773

9.2 Serum Alkaline phosphatase:

Serum alkaline phosphatase ranged between 252 iu/L to 1342 iu/L in infants without MBD and 256 iu/L to 2216 iu/L in infants with MBD (see table 11).

The mean serum alkaline phosphatase was 1052.9 iu/L (SD=493.3 iu/L) in infants with metabolic bone disease and 766.8 iu/L (SD=301.7 iu/L) in those without MBD. The mean serum alkaline phosphatase was higher in infants with MBD when compared to those without MBD. This was found to be statistically significant (t value =2.843, on 64 degrees of freedom and P value = 0.006).

Table 11: Comparison of mean serum alkaline phosphatase in infants with MBD and infants without MBD.

MBD	NO.	Mean serum alkaline phosphatase (iu/L)	SD
+ve	33	1052.9	493.3
-ve	33	766.8	301.7

t value = 2.843, P value = 0.006

10. Parity:

18 out of 33 infants with MBD were first born, while 15 were second born or of higher order. There was no significant difference in incidence of MBD between the two groups when parity is considered (Chi-square value =0.13, P value =0.722).

Table 12: Metabolic bone disease distribution in infants according to parity of their mothers.

Parity	MBD		Total
	+ve (%)	-ve (%)	
1	18(34.6)	34(65.4)	52
2+	15(31.3)	33(68.7)	48
Total	33	67	100

Chi-square value = 0.13, P value = 0.722

11. Type of delivery:

Study infants were grouped under singleton or multiple delivery (twins, triplets etc). As shown in table 13; 23 (34.3%) out of 67 singleton delivery infants had MBD and 10 (30.3%) out of 33 multiple delivery infants had MBD. There was no statistical significant difference in incidence of MBD (Chi-square value =0.16, P value =0.687).

Table 13: Comparison of distribution of MBD among singleton and multiple delivery infants.

Type of delivery	MBD +ve(%)	MBD -ve (%)	Total
Singleton	23(34.3)	44(65.7)	67
Multiple	10(30.3)	23(69.7)	33

Chi-square value = 0.16, P value = 0.687

12. Effect of alkaline phosphatase, gestational age, birth weight, postnatal age and current weight on metabolic bone disease (MBD):

As mentioned earlier, multiple logistic linear regression analysis was done for these factors. It was found that gestational age and alkaline phosphatase were the 2 most important predictive factors in the diagnosis of MBD. Thus high alkaline phosphatase values were significantly associated with MBD and large gestational age values tended to reduce the risk of an infant having a metabolic bone disease. A summary of the results of logistic regression analysis is given in table 14.

Table 14: A summary of regression coefficient estimate together with their standard errors.

Variable	B	SE(B)	t-value (B/SE)	P-value
Constant	9.844	3.35	2.9	0.003
Serum alkaline phosphatase	0.0019	0.0008	2.4	0.02*
Gestational age	0.3615	0.1067	3.3	0.007*
Postnatal age	0.0321	0.15	0.2	0.83
Birth weight	0.003	0.0025	1.2	0.28
Current weight	0.0003	0.0004	0.75	0.48

* Significant.

DISCUSSION

In the present study metabolic bone disease (MBD) was seen in 33/100 (33%) of the exclusively breast fed very low birth weight infants. Callenbach et al(12) and Koo et al(30) reported incidences of 32% and 31.5% respectively. Thus the incindence in these latter studies both of which were done in developed countries are comparable to that of the present study. The slightly higher MBD incidence in this study probably explains a socio-economic variation of the study population. This variation may affect the calcium and phosphorus milk content of the mothers. Local data or data from any other developing countries are not available for comparison.

The incidence of MBD in this study may be under-represented for several reasons. Firstly, although a birth weight of 1500g was the cut-off point in this study, infants of 1500g to 2000g birth weight may also be affected with MBD. Secondly, extreme VLBW preterm infants might have died earlier before the study was started. Some of them might have had MBD. Thirdly, infants who did not have birth details were also excluded. Some of these infants might have had metabolic bone changes as well. Fourthly, infants who were given feeds other than breast milk were excluded. These infants might be more prone to MBD especially knowing that most weaning foods are inadequate in quality.

Probably higher rates of metabolic bone disease might have been found if infants supplemented with feeds other than breast feeding were not excluded from the study. Nutritional balance studies show that preterm infants fed exclusively on breast milk or formula designed for term infants are liable to develop metabolic bone disease (43). On the other hand breast milk supplemented with calcium and phosphate or preterm designed infant formula fortified with calcium phosphate have been shown to prevent metabolic bone disease (44,45).

It is known from experience that preterm infants in our environment are given various natural feeds or formulas which may differ from one infant to another. Calcium and phosphorus contents in such supplementation feeds may differ. Depending on the nature of dietary components in the feeds absorption of calcium and phosphorus may be affected. For example while lactose and other sugars enhance calcium and phosphorus absorption, fat can decrease the absorption of these minerals (46,47). This means that even when supplemented with other feeds, preterm infants may develop metabolic bone disease. However, this speculation needs evaluation by further studies in our environment.

Fifthly, the diagnostic criteria of MBD was based on

radiology which was correlated to alkaline phosphatase activity. The limitation of radiology in the detection of minimal or early bone demineralization is well known.

Other techniques for measuring bone mineral content include:- CT scan, photon absorptiometry, radiogrammetory, photodensitometry and X-ray absorptiometry. Some of these techniques e.g. CT scan are said to be precise and sensitive for measuring bone mineral content (48). However, these techniques require highly trained staff and highly specialized equipment which is very expensive. Because of these reasons, such techniques are not available in many centres including ours. Moreover their use in infants especially is still being researched on (49).

Similar other studies had used radiology for detecting or confirming metabolic bone changes (11,12,31,50).

As seen in this study X-ray was able to screen infants with metabolic bone changes of grade 1 and above. Being relatively cheap and available in many centres, this study has found x-ray to be a useful tool for screening metabolic bone disease in infants, despite the limitations mentioned.

The high incidence of MBD found in this study based on radiology, shows that this problem is of public concern.

Shorter gestational age was significantly associated with metabolic bone disease in the present study. A study done by Koo et al showed that shorter gestational age of 26.8 ± 0.8 weeks was associated with metabolic bone disease (31)

Intrauterine calcium accretion has been shown to follow an exponential increase in the last trimester of pregnancy. The accretion rate increases from 130mg/kg/day at 28 weeks to 140mg/kg/day at 32 weeks and 150mg to 155mg/kg/day by 36 weeks to 38 weeks respectively (51,52). Thus the quantity of calcium and phosphate accretion rate is directly related to the gestational age of the infant (19,20). Hence, it is not surprising to find lower rates in infants with lower gestation age. Logistic regression analysis in the present study showed that lower GA increased the chance of an infant being MBD case. The implication is that GA is a good predictor of MBD in an infant.

By inference, VLBW preterm infants in the present study with shorter GA had much lower calcium and phosphate bone content at birth than VLBW preterm infants with relatively longer gestational age. Furthermore, poor postnatal mineral bone content in such infants might have been aggravated by the inadequacy of these minerals in their mothers' milk (53,54). This implies that VLBW infants with shorter GA were more exposed to MBD than VLBW infants with

relatively longer GA in the study.

Alkaline phosphatase is an enzyme for active bone growth. Kovan et al (35) in their study of 30 preterm infants found that serum alkaline phosphatase was raised well above childhood and adult reference values in all the VLBW infants with or without MBD. Similar pattern of alkaline phosphatase values was observed in the present study. However, much higher alkaline phosphatase activity was associated with active metabolic bone disease (P value 0.006). This is in accordance with reports from other studies in the literature (11,12,31,50).

The high levels of alkaline phosphatase in association with metabolic bone disease in the present study were not as remarkable as those found by Kovan et al (35). Other studies associating raised alkaline phosphatase activity with metabolic bone disease such as that by Koo et al, have reported relatively lower values of the enzyme activity than that found in the present study (20). The variability in the actual serum alkaline phosphatase activity in these studies may be attributed to the different methods used for determination of the enzyme activity. It may as well be attributed to factors like drug therapy and gastrointestinal, hepatic and viral diseases which are known to affect alkaline phosphatase activity (35,55,56).

In the present study infants with clinical evidence of hepatic disease and gastro-intestinal diseases like diarrhoea and steatorrhoea were excluded from the study. Drug therapy and viral infections were not considered. No exclusion criteria was indicated in those previous studies reviewed (11,12,31,50) and this may be another explanation for variability of the enzyme activity.

Another factor related to alkaline phosphate (ALP) is zinc. Zinc deficiency may co-exist in exclusively breast fed infants and is known to be associated with a raised alkaline phosphatase activity (57). Determination of serum zinc was not done in the present study because of the high cost involved. Therefore the effect of serum zinc status to the raised alkaline phosphatase in this study was unknown. Logistic regression analysis done in the present study has shown that infants with raised ALP are more likely to be having MBD. Hence as a screening tool, alkaline phosphatase has been found to be very useful and sensitive indicator of deranged bone mineralization.

In the present study, serum calcium and phosphate levels did not correlate with the various radiological grades of metabolic bone disease. This finding has been noted in other studies (20,26), although some studies indicate low phosphate levels (2,11,12). Normal serum calcium and

phosphate may be maintained at the expense of bone demineralisation in infants with metabolic bone disease (18,19). Therefore, it was not suprising to find comparable serum calcium and phosphate levels in both groups of infants in this study.

It is known that calcium and phosphate is required for skeletal growth which is monitored by postnatal length. Body weight and occipito-frontal circumference of the infant are also a measure of its physical growth. Larger OFC was observed in infants with metabolic bone disease in the present study. However this was not statistically significant. Similarly, the postnatal length and weight was higher in infants with metabolic bone disease than in those without it. Perhaps their rapid growth exposed them more to metabolic bone changes as compared to less rapidly growing infants without metabolic bone disease.

Callenbach et al (12) in their study of preterm infants found no association of length with metabolic bone disease. But less postnatal weight was associated with metabolic bone disease in the same study. Contrary to this finding Kovar et al has reported shorter linear growth in one of the identical twins with rickets (30). Similarly, Bosyle et al (26) observed that 6 preterm infants with rickets were shorter when compared to 12 infants without rickets.

The reports by these studies including the present one as regards postnatal length and weight show some variability. These differences may be viewed on the general nutritional components in their mothers milk such as energy and zinc content. Zinc deficiency has been documented to impair growth (58). Other factors affecting growth of the infant include genetic and environmental effects such as temperature. Early neonatal hypothermia which is not uncommon in VLBW preterm infants impairs later postnatal growth (59). Post-conceptual age matching to the length of the infants which was not done in this study and by some of the previous studies, may also explain such differences. Further similar studies, therefore, should consider such confounding factors. Moreover, suitable designed study protocols are needed to assess the longterm effect of MBD on linear growth and other morbidities.

Parameters of parity and type of delivery were not associated with metabolic bone disease in the present study. No previous data is available which have shown an association of metabolic bone disease with parity and type of delivery.

CONCLUSIONS:

1. Metabolic bone disease is common in very low birth weight exclusively breast fed preterm infants.

2. The mean length was significantly related to MBD, being higher in infants with MBD than in those without it.

3. MBD affects VLBW infants of lower gestation.

4. Multiple delivery (twins, triplets and quadriplets) did not influence MBD.

5. Parity (order of birth) did not influence MBD.

6. Alkaline phosphatase activity is a reliable biochemical test which aids the diagnosis of metabolic bone disease in collaboration with radiography.

RECOMMENDATIONS:

1. Use of radiology, gestational age and alkaline phosphatase as indicators of metabolic bone disease in very low birth weight preterm infants. This should be done more often in order to detect this treatable condition.

2. Calcium and phosphate supplementation intervention study should be done to find out if the incidence of MBD would be reduced in otherwise exclusively breast fed VLBW infants.

3. Long-term study to follow-up infants with MBD.

REFERENCES:

1. Formon SJ, Ziegler EE, Vazquez HD. Human milk and small premature infant. Am J Dis Child 1977;131:463-467.

2. Rowe JC, Daniel HW, David MD. Nutritional hypophosphataemic rickets in a premature infant fed breast milk. N Eng J Med 1979; 300:293-296.

3. Forbes GB. Is human milk the best food for low birth weight babies? Pediatr Res 1978;12:434.

4. Lucas A, Morley R, Cole TJ. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992; 339:261-264.

5. Carey EM. The biochemistry of fetal brain developement and myelination. In: The biochemical development of fetus and neonate by Jones CT. New York; Elsevier Biomedical Press 1982: 287-336.

6. Read LC: Milk growth factors. In: Cocburn F, ed. Fetal and neonatal growth. Chichester: Wiley and sons, 1988:131-52.

7. Schaffer AJ, Raye JR. Disorders of vitamins and trace minerals. In: Avery ME, Taeusch HW. Schaffer's disease of the newborn 5th edition. WB Saunders, London 1984:800-812.

8. Levene MI. Special care of the sick newborn infant. In: Levene MI. Jolly's diseases of children. 6th edition. Blackwell scientific publications, Oxford 1990: 105-108.

9. Anderson GH, Atkinson SA, Bryan HB. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am J Clin Nutr 1981;34:258-265.

10. Pereira GR, Barbosa NMM. Controversies in neonatal nutrition. PCNA 1986;33:65-89.

11. Kulkarni PB, Hall RT, Rodes PG. Rickets in very low birth weight infants. J. Pediatr 1980; 96:249-52.

12. Callenbach JC, Sheehan MB, Abramsons SJ. Aetiological factors in rickets of very low birth weight infants. J. Pediatr 1981;98:800-805.

13. Seino YI, Shimotsuji T, Ishida M. Plasma active

vitamin D concentration in low birthweight infants with rickets and its response to vitamin D treatment. Arch Dis Child 1981;56:628-32.

14. McIntosh N, Livesly A, Brooke OG. Plasma 25 hydroxyvitamin D and rickets in infants of extremely low birth weight. Arch Dis Child 1982;57:848-50

15. Tulloch AL. Rickets in premature. Med J Aust 1974; 1:137-40.

16. Griscom NT, Craig JN, Newhauser EBD. Systemic bone disease developing in small premature infants. Pediatr 1971;48:883-5.

17. Minton SD, Steichen JJ,Tsang RC: Bone mineral content in term and preterm appropriate for gestational age infants. J Pediatr 1979;95:1037.

18. Senterre J, Salle B. Calcium and phosphorous economy of the preterm infant and its interaction with vitamin D and its metabolites. Acta Pediatr Scand 1982; suppl 296:85-92.

19. Davies DP; Hughes CA; Moor JR. Rickets in preterm infants. Arch Dis Child 1978; 53:88-89.

20. Koo SW, Fraser D, Reilly BJ. Rickets due to calcium deficiency. N Eng J Med 1977;297:1264-6.

21. Lyon AJ, McIntosh N, Wheeler K. Hypercalceamia in extremely low birth weight infants. Arch Dis Child 1984;59:1141-4.

22. Senterre J, Putet G, Salle B. Effects of vitamin D and phosphorous supplementation on calcium retention in preterm infants fed banked human milk. J Pediatr 1983; 103:305-7.

23. Senterre J, Salle B. Renal aspects of calcium and phosphorous metabolism in preterm infants. Biol Neonate 1988; 53:220-229.

24. Steichen JJ, Gratton TL, Tsang RC. Osteopenia of prematurity, the cause and possible treatment. J Pediatr 1980;96:528-34.

25. McIntosh N, Linesey A, Brooke OG. Plasma 25-OH vitamin D rickets in low birth weight babies. Arch Dis Child 1983;58:476-7.

26. Bosley ARJ, Verrier-Jones ER, Campbell MJ. Aetiological factors in rickets of prematutiry. Arch

Dis Child 1980;55:683-6.

27. Walravens PA. Nutritional importance of copper and zinc in neonates and infants. Cl Chem 1980; 26:185-89.

28. Cockburn F, Belton NR, Purvis RJ, et al. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. Br. Med J 1980; 281:11-14.

29. Brooke OG, Lucas A; Metabolic bone disease in preterm infants. Arch Dis Child 1985;60:682-685.

30. Hillman LS, Haddad JG; Perinatal vitamin D metabolism: III factors influencing late gestational human serum 25-hydroxyvitamin D. Am J. Obstet Gynecol 1976;125:196-200.

31. Koo WWK, Gupta Jm, Nayanar VV. Skeletal changes in preterm infants. Arch Dis Child 1982;57:447-52.

32. Kovar IZ, Mayne P, Walls J: Neonatal rickets in one of identical twins. Arch Dis Child 1982;57:792-4.

33. Barness LA. Nutritional disorders. In: Behrman RE, Vaughan VC. Nelson Text-book of Pediatrics 13th ed. Saunders, London 1987:149-153.

34. Keipert JA. Rickets with multiple fractured ribs in a premature infant. Med J Aust. 1970;1:672-5.

35. Glasgow JFT, Thomas PS. Rachitic respiratory distress in small preterm infants. Arch Dis Child 1977;52: 268-73.

36. Kovar I, Mayne P, Barlthrop D. Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. Lancet 1982; 1:308-10.

37. McIntosh N. Rickets of prematurity. Bone 1984;1:26-7

38. Kovar I, Mayne P. Alkaline phosphatase activity in preterm neonate. Acta Pediatr Scand 1981;70:501-506.

39. Lyon AJ, McIntosh N, Wheeler K. Hypercalceamia in extreme low birth weight infants. Arch Dis Child 1984:59: 1141-4

40. Manji KP, Low Birth Weight and Early Outcome. Dissertation. University of Dar-es-salaam, 1991.

41. Varkevisser C.M, Pathmanathan I, Brownlee. A designing and conducting systems research projects; health systems research training series. IDRC Ottawa

1991; 1:214.

42. Tietz NW. Electrolytes. In: Tietz NW. Fundamentals of clinical chemistry 2nd ed. WB Saunders, London 1982:908 and 915.

43. Growss SJ: Growth and biochemical response of preterm infants fed human milk or modified infant formula. N. Eng J Med 1983;308:237-241.

44. Greer FR, Steicken JJ, Tsang RC: Calcium and phosphorus supplements in breast milk related rickets. Am J Dis Child 1982;136:581-583.

45. American Academy of Paediatrics Committee on Nutrition. Nutritional needs of low-birthweight infants. Pediatrics 1977;60:519-530.

46. Ziegler EE, Fomon SJ: Lactose enhances mineral absorption in infancy. J. Pediatr Gaestroenterol Nutr 1983;2:288-294.

47. Southgate DAT, Widdownson EM. Smith BJ, et al: Absorption and excretion of calcium and fat by young infants. Lancet 1969;1:487-489.

48. Murray RO, Dennis JS. Skeletal disorders of metabolic

and endocrine origin. In: Sutton D. A textbook of radiology and imaging. 4th ed. Volume 1. Churchill Livingstone, London 1987:229-242.

49. Kazema RR: Radiologist and Head of Radiology Department-MMC. Personal Communication.

50. Chudley AE, Brown DR, Holzman IR, et al: Nutritional rickets in 2 very low birthweight infants with chronic lung diseases. Arch Dis Child, 1980;55:687-690.

51. Cameron JR and Sorenbon J: Measurements of bone minerals in vivo: an improved method. Science 1963;142:230.

52. Ziegler EE, O' Donnel Am. Nelson SE, et al: Body composition of the reference fetus. Growth 1976;40:329.

53. Lemons JA, Moye L, Hall D, et al: Differences in the composition of preterm human milk during early lactation. Pediatr Res 1982;16:113-117.

54. Anderson DM, Williams FH, Merhatz RB, et al: Length of gestation and nutritional composition of human milk. Am J Cl Nutr 1983; 37:810-814.

55. Nathan E: Transient hyperphosphatasemia of infancy Acta Pediatr Scand 1980;40:235-38.

56. Rosalki S, Foo Y: Transient hyperphosphatasaemia of infancy; four new cases and suggested aetiology: Clin Chem 1980;26:1109-10.

57. Atkinson SA, Whelan D, Whyte RK, et al: Abnormal zinc content in human milk: Risk for development of nutritional zinc deficiency in infants. Am J Dis child 1989;43:608-611.

58. Castillo-Duran C, Rodriguez A, Venegas G. et al. Zinc supplementation and growth of infants born small for gestational age. J. Pediatr. 1995;127:210-211.

59. WHO/FE/MSM. Thermal control of the new-born; a practical guide. Geneva: WHO/FHE/MSM/1992;93:4.