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Hypophosphatemia in very low birth weight infants receiving parenteral nutrition

Rowjee, Vikesh

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Hypophosphatemia in Very Low Birth Weight Infants Receiving Parenteral Nutrition

Authors:

Dr V Rowjee

Student no. 0401956M

MBBch, FCPaed (SA)

General Pediatrics Chris Hani Baragwanath Academic Hospital

University of the Witwatersrand

Email: vikeshrowjee@gmail.com, Mobile Cell no. +27741845374

Prof Kebashni Thandrayen

MBBCh, FCPaed (SA), MMed, Certificate in Endocrinology and Metabolism (PAEDS), PhD

Pediatric Endocrinology Chris Hani Baragwanth Academic Hospital

University of the Witwatersrand

Dr Firdose Lambey Nakwa

MBBCh, FCPaed (SA), Certificate in Neonatology, Certificate in Paediatric Neurology

Head of the Neonatal unit, Chris Hani Baragwanath Academic Hospital

University of the Witwatersrand

DECLARATION

I, VIKESH Rowjee, declare that this research report is my original work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university. The submissible format of the research report will be submitted to the Journal of Perinatology, author guidelines provided in the appendix. I declare no conflict of interest.



Dr V Rowjee **Date: 05 August 2020**

I Prof Kebashni Thandrayen declare that this research report was supervised 50% by myself. I declare no conflict of interest.



Prof Kebashni Thandrayen **Date: 05 August 2020**

I Dr Firdose Lambey Nakwa declare that this research report was supervised 50% by myself. I declare no conflict of interest.



Dr Firdose Lambey Nakwa **Date: 05 August 2020**

DEDICATION

I dedicate this work to my late mother Charmaine Michelle Rowjee who has thought me the price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.

PRESENTATIONS ARISING FROM THIS STUDY

1. Poster presentation at the USANA 2017 conference, Tsogo Sun OR Tambo Hotel, Johannesburg, 15–17 September 2017.
2. Oral presentation at the University of the Witwatersrand, Department of Paediatrics Research Day, Marie Curie lecture theatre, Medical School, 1st December 2017.

ABSTRACT

Objective

To retrospectively assess serum bone mineral (BM) biochemistry in preterm (PT) infants with a birth weight (BW) ≤1500g that had received at least 5 days of parenteral nutrition (PN).

Study design

One hundred and nine patient files between July 2013 and June 2014 at Chris Hani Baragwanath Hospital (CHBAH) were assessed for volumes of nutritional support received, and BM biochemistry performed.

Results

Median gestational age was 29 weeks (IQR 28-30 weeks). Median BW was 1065g (IQR 935-1210g). Majority of the BM levels were obtained in weeks 3-5 of life. The median PO_4 was 1.71mmol/L (IQR 1.43-1.92mmol/L). There was a negative correlation between PO_4 and increasing volumes of PN ($p = 0.005$, $r = -0.2416$) and a positive correlation between PO_4 and increasing volumes of formula feeds, ($p = 0.042$, $r = 0.3153$).

Conclusion

Preterm infants weighing ≤1500g requiring PN for ≥5 days are susceptible to developing hypophosphatemia.

ACKNOWLEDGEMENTS

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They always provided me with heartfelt support and guidance and has given me invaluable inspiration and suggestions. Without their guidance, this research project would not have been possible and I shall eternally be grateful to them for their assistance.

Most importantly, I wish to thank my loving and supportive wife, Jivani who has provided me with unending inspiration.

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LIST OF ABBREVIATIONS

AA: Amino acids

AAP: American Academy of Paediatrics

Alb: Albumin

ALP: Alkaline phosphatase

BM: Bone minerals

BW: Birth weight

Ca: Corrected serum calcium

CHBAH: Chris Hani Baragwanath Academic Hospital

EBM: Expressed breast milk

ELBW: Extremely Low Birth Weight

ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition

IQR: Interquartile range

Mg: Magnesium

NHLS: National Health Laboratory Services

NNL: Neonatalyte® Adcock Ingram

PN: Parenteral nutrition

PO4: Phosphate

PT: Pre-term

VLBW: Very Low Birth Weight

INTRODUCTION

Advances in neonatal care such as antenatal steroids to mothers in preterm labour, surfactant for respiratory distress syndrome and newer, gentler modes of mechanical ventilation (both invasive and non-invasive) has improved survival rates in preterm neonates.[1] A preterm (PT) infant that receives suboptimal nutritional support in the immediate postnatal period is at risk of developing both postnatal growth failure and abnormal neurological development.[1]

Ideally, the PT infant nutritional support should mimic the intrauterine nutritional support that the infant would have received if it was carried to term.[2] Trying to mimic the natural intrauterine lifeline is a difficult task, and this is further complicated by the PT infants' immature and underdeveloped body systems.

Breastmilk feeds is the preferred nutritional support for a neonate.[2] Initiation of breast milk feeds of the PT infant soon after birth, even under ideal circumstances, is difficult. Even if feeds were to be gradually increased at 20ml/kg/day, optimal nutritional support would not be achieved until the neonate is on full enteral feeds.[1]

The foetus accumulates varying amounts of nutrients from the placenta depending on the gestational age. Parenteral nutrition tries to mimic this, by providing the appropriate energy, protein, lipid, carbohydrate, electrolyte and elemental requirements to the PT infant.[2] The provision of early parenteral nutritional support whilst enteral feeds are gradually increased aims to provide adequate nutritional and energy support to the PT infant.[2]

During foetal life, in the last trimester, there is a net active transport of phosphate across the placenta at a rate of 60-70mg/kg/day.[3] Seventy five percent of this is utilised for bone mineralisation and 25% in the remaining tissues.[3] The PT infant's phosphate stores are not

maximal at birth. There is an increased fractional excretion of phosphate and a higher risk for developing signs and symptoms of phosphate deficiency.[3]

A retrospective study by Dik et al[4] showed a significant association between early aggressive parenteral nutrition (defined as 4g/kg/day of intravenous protein delivery in the first week of life[5]) and hypophosphatemia in neonates with a birth weight less than 1250g.[4] The infants with hypophosphatemia in the study by Dik et al[4] presented more frequently with sepsis and the need for mechanical ventilation and vasoactive medication.[4]

In view of the hypophosphatemia phenomenon occurring with parenteral nutrition in PT infants with a VLBW[5, 6] ; is there a need to change current practices and should there be earlier supplementation and monitoring of serum minerals?

The aim for this study was to retrospectively assess bone minerals (BM) such as phosphate and calcium in preterm infants that are very low birth weight (VLBW) and had received at least five days of PN within their first few weeks of life and to assess BM biochemistry in relation to various other nutritional supports e.g. breast milk, supplemented breast milk and preterm formula that was received within the first few weeks of life.

METHODS

The objectives of the study were 1. To evaluate if any of the diagnosis affected the duration or need for parenteral nutrition; 2. To assess for other confounding factors that may affect phosphate levels such as the presence of confirmed sepsis and the use of pharmaceutical agents that affect the phosphate levels; 3. To assess calcium, magnesium, phosphate, albumin and alkaline phosphatase (ALP) levels in very low birth weight infants that received varying volumes of enteral and parenteral nutrition per week within the first ten weeks of life.

For the purposes of this study the following definitions were applied:

1. "Sepsis": an infant with a positive blood culture.
2. "Preterm infant": an infant born before 37 completed gestational weeks.
3. "Very low birth weight": an infant born with a birth weight ≤1500g.

Patients included in the study were PT infants with a birth weight ≤1500g admitted within the first 72 hours of life and that had survived beyond the first two weeks of life over a 12-month period (between July 2013 and June 2014) at the neonatal unit in Chris Hani Baragwanath Hospital (CHBAH). The PT infant should have received a minimum of 5 days of PN. These infants should have had at least one set of measurements of serum calcium, magnesium, phosphate levels during their admission. All calcium levels included in the study should be accompanied by a simultaneous serum albumin level to allow for the calculation of a corrected serum calcium level.

Neonates excluded from the study either had no medical records available for review; or demised before two completed weeks of life; or with confirmed endocrine and renal disorders affecting bone mineral homeostasis or born to mothers with confirmed endocrine disorders that will affect neonatal bone mineral homeostasis. Patients that had results with only a serum calcium level and no simultaneous albumin level was excluded from the study analysis. Sepsis, alveolar hyperventilation and many pharmaceutical drugs e.g. amphotericin B, diuretics and glucocorticoids are known to affect serum phosphate levels,[7, 8] thus tests requested on infants that met these criteria was excluded to control for any confounding factors. Tests that were too few to yield significant analyses was also excluded.

At CHBAH, during the study period, preterm neonates weighing ≤1500g were initiated on enteral feeds commenced at 20ml/kg/day, whilst the remainder of the fluid and caloric requirement was given intravenously. On day one of life, a potassium free neonatal maintenance solution with 10% dextrose is prescribed to meet the total fluid requirements. The potassium free neonatal maintenance solution is changed to Sabax Neonatalyte® Adcock Ingram (NNL), with

10% dextrose on day two of life once a normal serum potassium result is obtained. When breastmilk is available, it is initiated at 20ml/kg/day and increased at this daily rate until maximum enteral feeds are provided to the PT infant. If breast milk is not available, then PT infant formula is provided if the clinical condition allows. Only if the weight is ≤1200g will donor breast milk be provided depending on the availability.

The prescribed intravenous fluid volume is reduced accordingly as the enteral volume increases to match the total fluid and energy requirement. On day two of life, there is an attempt to initiate most if not all PT neonates weighing ≤1200g onto parenteral nutrition (PN) ITNBABY102 Fresenius Kabi®, provided this was allowed by the clinical condition. Parenteral nutrition provides intravenous calories in the form of protein, carbohydrates and lipids, and provides vitamins, trace elements and electrolytes. Parenteral nutrition is prescribed at increments of 50mls/kg/d to a maximum of 150mls/kg/d. When the volume of PN does not meet the total fluid requirements for the day, the shortfall is administered as NNL. A PT infant with any other medical and or surgical condition such as NEC that restricted enteral feeds for a prolonged period and thus were candidates for PN; which then allowed the study to include these patients with a BW ≤1500g.

As per unit protocol, in most circumstances; the calcium, magnesium, phosphate and ALP levels were routinely checked on day 14 of life or earlier if clinically indicated for all VLBW preterm infants. Thereafter, repeated after 14 days or at more frequent intervals if clinically indicated. All infants receiving PN, under ideal circumstances, would need serum urea, electrolytes, creatinine, calcium, phosphate, triglycerides and a full panel of liver function tests monitored every week if they weighed ≤1200g and every second week if they weighed ≥1200g.

STATISTICAL ANALYSIS

Data was extracted from the patient's hospital files and entered onto a data collection sheet. A study number was assigned to each patient and the data collected was entered onto an excel worksheet to create a database for analysis.

The laboratory data such as biochemical findings were obtained from the National Health Laboratory Services (NHLS). The data with missing information and erroneous or suspicious entries were removed from analysis.

There is a paucity of data on the appropriate lower limit of normal cut off value to define hypophosphatemia in a PT infant. A lower limit cut off value of 1.6mmol/L was used in the recent 2018 European Society for Paediatric Gastroenterology, Hepatology and Nutrition Guidelines (ESPGHAN) 2018[9] and a lower limit cut off value of 2mmol/L was used by Hellstern et al in their study.[10] Both lower limit cut off values were utilised in the data analysis for this study.

All information from the data collection sheet was entered into Microsoft Excel spreadsheets and then imported into Statistica TIBCO Software Inc. (2018) (data analysis software system), version 13. Standard statistical measures were applied. Continuous variables were described using medians and interquartile (IQR) ranges. Categorical variables were described using frequencies and percentages. Comparisons between serum phosphate, magnesium, ALP and corrected calcium levels to the various nutritional provisions per week were made with Spearmen's correlations. The one-way analysis of variance (ANOVA) was used to determine whether there was any statistical difference between the weeks for the serum BM biochemistry.

RESULTS

Demographic data

A total of 833 PT patient files with a VLBW were reviewed. During the study period, one hundred and forty individual patient files that had received at least 5 days of PN were reviewed. Thirty-one patient files were excluded as the files were incomplete, Figure 1.

There was a total of one hundred and nine PT infants included in the study of which 36% (n = 39) were males. The median birth weight and gestational age was 1065g (IQR 935–1210g) and 29 weeks (IQR 28-30 weeks) respectively.

Infant diagnoses

The three most frequent diagnoses were respiratory distress syndrome (RDS) 94% (n = 102), neonatal jaundice (NNJ) 66% (n = 72) and necrotising enterocolitis (NEC) 55% (n = 60) of which 42% presented with stage one NEC (n = 46) and 13% with stage two NEC (n = 14).

Indications for parenteral nutrition and total days exposed to parenteral nutrition

The primary indication for PN was for nutritional support (83% (n=90)) as the enteral feeds were advanced. Fifty five percent (n = 60) of infants received parenteral nutrition as they developed NEC during the advancement of enteral feeds.

Infants with a diagnosis of RDS and NNJ received 10 days (IQR 6–16 days) and 11 days (IQR 7–16 days) of PN respectively. Patients with confirmed sepsis received 12 days (IQR 7–17 days)

of PN. Infants with a diagnosis of NEC received 14 days (IQR 9–19 days) of PN. There were no differences between the median duration or number of days on PN and the different types of diagnoses (data not shown).

Frequency of serum calcium, magnesium, phosphate, albumin and alkaline phosphatase tests requested

Serum calcium, magnesium, phosphate, albumin and ALP tests were performed from week one to week ten of life. Deviation from unit protocol for tests performed in week one (88, 8%) was for clinical indications. A collective total of 1074 serum BM biochemistry tests were performed over the ten weeks. The highest percent of tests requested were during weeks three (n=210, 20%), four (n=179, 17%) and five (n=169, 16%). Analysis was done on the test results of weeks three to five as the other weeks had a lower percentage of total tests requested and thus the number of tests were too small for further meaningful analyses. The results for the serum BM biochemistry (weeks three to five) is illustrated in Table 1. In this study; there was a higher prevalence of hypophosphatemia, average of 80%, when using 2mmol/L as a lower limit cut off value to define hypophosphatemia as illustrated in Table 2.

Relationship between serum bone mineral biochemistry levels and volumes of nutritional support received

There was a positive correlation between serum phosphate levels and increasing volumes of PT infant formula ($r = 0.315$; $p = 0.042$), Figure 2A. A positive correlation between serum albumin levels and increasing volumes of PT formula was also found with a mean albumin of 28.47g/L, ($p = 0.023$, $r = 0.378$), Figure 2C.

There was a negative correlation between serum phosphate levels and increasing volumes of parenteral nutrition received ($r = -0.242$; $p = 0.005$), Figure 2B. In addition, a negative correlation between serum albumin levels and increasing volumes of PN ($p = 0.00$, $r = -0.3583$) and NNL ($p = 0.013$, $r = -0.236$) was shown in Figure 2D-E.

Figure 2F shows a gradual increase of serum phosphate between weeks three to five ($p = 0.023$). There was a more significant increase between weeks three and five ($p = 0.01$) and between weeks four and five ($p = 0.02$).

There was no correlation between serum phosphate, calcium, magnesium, ALP and albumin levels with the volumes of the other types of nutritional support (expressed breast milk, supplemented and mixed feeds) received by the infants as shown in Table 2.

Phosphate supplementation

During weeks three to five, only 4% (n=13) of the patients had received phosphate supplementation.

Analysis of serum phosphate, sepsis, pharmaceutical agents and ventilatory requirements

During weeks three to five, there was only 0.96% (n=1) of serum phosphate tests requested during sepsis; and 2.88% (n=3) of serum phosphate tests requested during amphotericin B treatment and ventilation. These serum phosphate tests requested during this period were too small to yield any significant results.

DISCUSSION

The main findings of this study were the inverse relationship shown in serum phosphate levels between increasing volumes of PN (negative correlation) and PT infant formula (positive correlation) received. This is important for the practicing paediatrician so phosphate levels can be closely monitored in the PT infant with a VLBW receiving PN; especially in the infants' first few weeks of life. This will facilitate prevention and early treatment of symptomatic hypophosphatemia. Breast milk feeds is the preferred option[2] however under circumstances where PT formula is the only option, it is reassuring to know that PT formula provides adequate phosphate supplementation.

Phosphorus is the main component of adenosine triphosphate (ATP), membrane phospholipids, nucleic acids and is deposited in bone matrix with calcium.[3, 11] Phosphorus utilisation is prioritised towards accretion of tissue, and with a relative deficiency, it is favoured towards cellular metabolism.[9] The bench mark for the optimal delivery of calcium and phosphorus to a PT infant is based on the average foetal weight gain of 17g/kg/d before 35 weeks of gestation with 3.4mmol/kg/day of calcium and 2.6mmol/kg/day of phosphorus.[9] The 3.4mmol/kg/day of calcium is utilised for bone mineralisation with corresponding utilisation of 2mmol/kg/day of phosphorus leaving 0.6mmol/kg/day of phosphorus for protein accretion and other cellular activities. An estimation of 0.3mmol/kg/day of phosphate is required for 1g/kg/day of protein accretion.[9] This means that the remaining 0.6mmol/kg/day of phosphorus will allow for 2g/kg/day of protein accretion.[9] Any protein supplied above 2g/kg/day will require extra phosphorus to be adequately accreted. PT infants have a high growth velocity and providing them with 3.5g/kg/day of protein each day promotes optimal extrauterine growth.[1, 5] This growing process involves high metabolism and utilisation of phosphorus and this negatively affects serum phosphorus levels.[5]

The PN provided to patients in this study at a maximum rate of 150ml/kg/day provides 1.5mmol/kg/day of phosphate and 3.11g/kg/day of protein. The amount of phosphate provided

alone is not enough for bone mineralisation. This supports the negative correlation between serum phosphate and increasing volumes of PN. The available phosphate supplied by the PN is prioritised for cellular metabolism and tissue accretion.[9] This causes a relative phosphate deficiency, where intracellular phosphate stores are mobilised to replete serum levels which may depict a normal serum phosphate level, however total body stores are low. This supports a higher limit for the lower cut off value to define hypophosphatemia.

In this cohort of patients, there was a negative correlation with increasing volumes of parenteral nutrition and serum albumin. The median serum albumin was 30g/L for weeks three to five. The larger volumes of parenteral nutrition received was associated with lower albumin levels, not necessarily hypoalbuminemia. There are limited studies on the effect of PN on serum albumin in the paediatric population. The protein provided may not be appropriately accreted due to inadequate supply of phosphate from the PN and this explains the negative correlation between serum albumin and higher volumes of PN. Albumin has a half-life of fourteen to twenty days.[12] Serum albumin levels is a good marker of nutritional support for the preceding three weeks. However, it is not a good marker for acute nutritional status in any clinical condition including during inflammation which causes protein loss.[12] In this study, the loss of protein from inflammation during NEC can also explain the inverse relationship between serum albumin and increasing volumes of PN.

There was also a negative correlation between increasing volumes of NNL and serum albumin levels. This can be explained by the absence of amino acids in the NNL formulation.

A case report by Mizumoto et al,[13] also described this phenomenon of hypophosphatemia in an ELBW infant who had received early aggressive parenteral nutrition and likened it to the refeeding syndrome.[13] Refeeding syndrome is when the distinctive biochemical abnormality of hypophosphatemia occurs three to four days after refeeding.[13] This was the motive for using a minimum of 5 days of PN nutrition as the cut-off point for inclusion into this study. Bosante et al[11] prospectively studied the influence of early parenteral nutrition on homeostasis of calcium

and phosphate and they also noted severe hypophosphatemia in PT infants receiving high intake of AA.[11]

This phenomenon of hypophosphatemia with early aggressive parenteral nutrition almost replicates the biochemical findings that occur in the refeeding syndrome. This can't simply be named refeeding syndrome. There is interrupted placental supply of nutrition when the infant is born preterm. By providing parenteral nutrition, the PT infant can receive carbohydrates, lipids and amino acids. However, parenteral nutrition has a limited ability in supplying high calcium and phosphate concentrations due to their inherited insoluble chemical properties.[1] Bosante et al[11] proposed this phenomenon be called "Placental incompletely restored feeding syndrome".[11] It could best be explained as hypophosphatemia in PT infants receiving parenteral nutrition where the parenteral nutrition does not provide adequate phosphate in relation to the protein provided for optimal extrauterine growth of the PT infant.

There was a positive correlation between increasing volumes of PT formula and serum phosphate and serum albumin levels. The PT formula provided at a volume of 180ml/kg per day allows for an approximate daily intake of 120mg/kg/day of phosphorus equivalent to 3.6mmol/kg/day of phosphorus and 4.14g/kg/day of protein. After considering the serum phosphorus utilised for bone mineralisation, there is 1.6mmol/kg/day of phosphorus that will allow for 5.3g/kg/day of protein accretion. PT formula at a volume of 180mls/kg/day exceeds the daily phosphate requirement for optimum growth and allows for the accretion of the supplied protein. This explains the positive correlation between PT infant formula and serum phosphate and albumin. As time progressed from weeks three to five, there was an increase in serum phosphate levels, this correlated with the increase volumes of PT formula over the same period.

This study could not show a correlation between hypophosphatemia, any pharmaceutical agents and sepsis or ventilatory requirements due to the small number of tests performed and patients. However, associations between hypophosphatemia and a deleterious clinical condition in PT infants that had received PN has been reported.[4, 13] In a study by Cradock et al,[14] the increased

rate of sepsis for individuals receiving PN was attributed to acquired leukocyte dysfunction in chemotaxis and phagocytosis due to leukocyte adenosine triphosphate (ATP) depletion in the presence of hypophosphatemia.[14]

Due to this study being retrospective, there were several limitations. Data collected was dependant on adequate and accurate documentation from the practicing clinician. Supplementations, medications and nutritional support prescribed was captured directly from the clinicians' notes in the medical records as treatment charts were not always present or complete. Supplements such as vitamin D, multivitamins and folate is routinely prescribed once the PT infant is on full enteral feeds and was not consistently documented in the patients file. This study lacked the power to analyse the effect of these supplements on BM biochemistry due to the lack of its documentation in patient files.

CONCLUSION

It is evident that the PT infants exposed to larger volumes of parenteral nutrition; hence receiving a higher delivery of AA; are at risk of developing hypophosphatemia. It is also evident that PT infant formula provides an excess amount of phosphate.

This study can advocate for closer monitoring of serum phosphate and other bone minerals in the PT infants with a VLBW that receives parenteral nutrition. Further prospective studies will add more meaning to this phenomenon of hypophosphatemia and parenteral nutrition.

There is a need to have larger studies to help define the absolute accepted lower limit cut off value for hypophosphatemia in PT VLBW infants to optimise phosphate replacement and to prevent complications of hypophosphatemia.

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Figure 1: Study sample

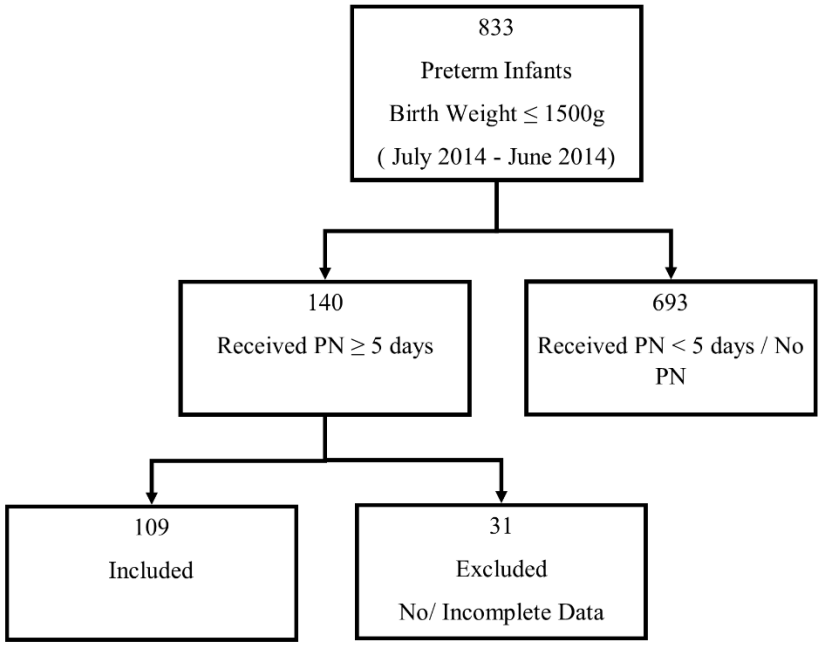
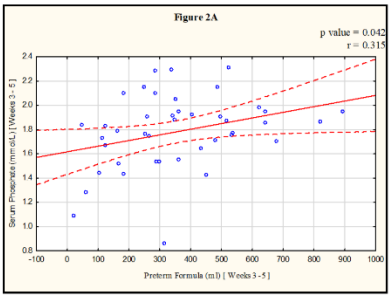
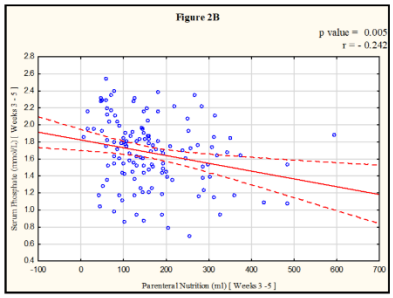
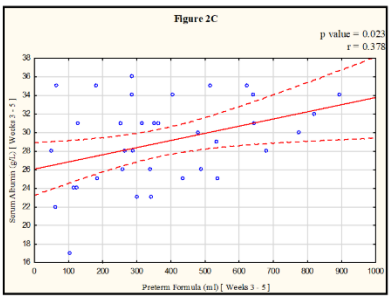
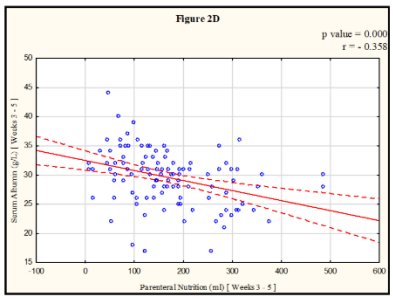


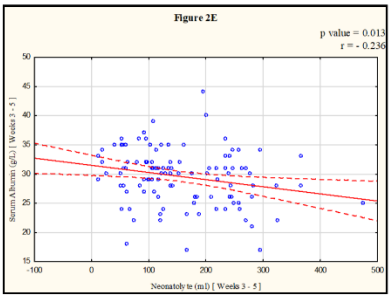
Figure 2: (A) Correlation between serum phosphate levels and increasing volumes of PT infant formula, (B) Correlation between serum phosphate levels and increasing volume of parenteral nutrition received (C) Correlation between serum albumin levels and increasing volumes of PT formula, (D) Correlation between serum albumin levels and increasing volumes of PN, (E) Correlation between serum albumin levels and increasing volumes of neonatal maintenance, (F) Phosphate levels for weeks 3-5











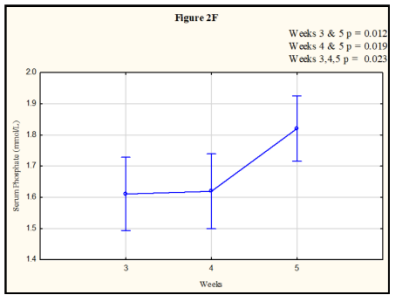


Table 1: Serum BM biochemistry measurements for weeks three to five

	n	Week 3 Median (IQR)	n	Week 4 Median (IQR)	n	Week 5 Median (IQR)	n	Weeks 3-5 Median (IQR)
Corrected serum calcium (mmol/L)	39	2.35 (2.27-2.48)	32	2.46 (2.31-2.61)	33	2.42 (2.35-2.49)	104	2.40 (2.31-2.52)
Serum magnesium (mmol/L)	49	0.89 (0.83-0.97)	41	0.86 (0.8-0.93)	41	0.88 (0.82-0.96)	131	0.88 (0.81-0.96)
Serum albumin (g/L)	43	31 (27-33)	33	30 (26-31)	35	30 (27-32)	111	30 (26-32)
Serum ALP (U/L)	24	329 (219-414)	21	374 (330-446)	22	356 (281-445)	67	351 (274-445)
Serum phosphate (mmol/L)	51	1.55 (1.28-1.83)	44	1.6 (1.37-1.96)	38	1.85 (1.75-1.95)	133	1.71 (1.43-1.92)
Serum phosphate < 1.6 mmol/L (%)*	28	53	23	50	5	13	56	39
Serum phosphate < 2.0 mmol/L (%)**	44	83	37	80	29	76	110	80

*Phosphate < 1.6 mmol/L as per ESPHGAN guidelines,[9] ** Phosphate < 2 mmol/L used by Hellstern et al.[10]

Table 2: Correlations of serum BM biochemistry and volume of nutritional support

	Parenteral nutrition	Neonatal maintenance	Expressed breast milk	Supplemented Feeds	Preterm formula	Mixed feeds
Corrected serum calcium						
Correlation co-efficient (r)	0.07	-0.05	-0.09	0.27	0.16	-0.51
p value	0.48	0.60	0.47	0.46	0.4	0.11
Serum magnesium						
Correlation co-efficient (r)	0.10	-0.04	0.10	-0.27	-0.04	0.33
p value	0.24	0.65	0.37	0.42	0.8	0.27
Serum phosphate						
Correlation co-efficient (r)	**-0.24**	0.03	0.14	0.54	**0.32**	0.44
p value	**0.005**	0.78	0.20	0.08	**0.04**	0.10
Serum albumin						
Correlation co-efficient (r)	**-0.36**	**-0.24**	0.02	0.15	**0.38**	0.15
p value	**0.00**	**0.01**	0.84	0.72	**0.02**	0.65
Serum ALP						
Correlation co-efficient (r)	0.07	-0.01	-0.11	-0.94	-0.00	-3.26
p value	0.52	0.95	0.92	0.71	1.00	0.33

APPENDIX 1





Hypophosphatemia in VLBW infants receiving parenteral nutrition

Candidate: **Dr V Rowjee**

Student No: **0401956M**

Protocol Submitted for degree: MMed (Paed)

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1. Glossary:

ALP	alkaline phosphatase
AA	amino acid
PTH	parathyroid hormone
CMP	calcium, magnesium, phosphate
PCT	proximal convoluted tubule
DCT	distal convoluted tubule
GFR	glomerular filtration rate
Calcitriol	1,25 dihydroxy-vitamin D 3
NaPi2A/ NaPi2B/ NaPi2C	sodium dependent inorganic phosphate transporter
PT	preterm less than 37 weeks of completed gestational age
VLBW	very low birth weight
ELBW	extremely low birth weight
ATP	adenosine triphosphate

2. Introduction:

Due to the advances in biomedical sciences such as administration of antenatal steroids, utilisation of exogenous surfactant and mechanical ventilator methods (invasive and non-invasive), the survival rates of preterm very low birth weight (VLBW) infants are on arise.

Postnatally, it is difficult to mimic normal intrauterine nutritional support of premature infants till 37 completed weeks of gestation. During the vulnerable period of prematurity, these VLBW preterm infants are at greater risk for major morbidities. This is further compounded by their response to extra uterine adaptation due to immature and underdeveloped body systems.

Preterm neonates that receive suboptimal nutrition postnatally are at high risk of developing postnatal growth failure and in the long term this also hinders neurological development.[1] The preterm VLBW infant has a higher energy requirement in comparison to a term neonate. This higher energy expenditure is required for the maturation of their underdeveloped systems, to keep up with the expected weight gain of at least 15g/kg/day and for adaptation to the extrauterine environment.[2] In addition to this, due to the short intrauterine life, the preterm neonate has not accumulated sufficient energy stores.

Early nutritional support to mimic intrauterine nutritional support and appropriate management of extra uterine adaptation of these babies are crucial in reducing morbidities and improving long term outcomes.

By nature, the best nutritional support to a neonate is through breast milk feeds. . Initiation of breast milk feeds of the VLBW neonate soon after birth, even under ideal circumstances, is

difficult. Even if feeds were to be gradually increased at 20ml/kg/day, optimal nutritional support would not be achieved until the neonate is on full enteral feeds.

Naturally the foetus accumulates varying amounts of nutrients from the placenta depending on the gestational age. Parenteral nutrition tries to mimic this, by providing the appropriate energy, protein, lipid, carbohydrate, electrolyte and elemental requirements. Early parenteral nutritional support is given as enteral feeds are gradually increased to mimic intrauterine growth and to provide adequate nutritional and energy support to the VLBW preterm neonate. Early aggressive parenteral nutrition is when a total of 4g/kg/day of amino acid is administered via standardised parenteral nutrition to neonates over the first week of life.[3]

The estimated energy requirement based on the resting metabolic rate, new tissue synthesis and an estimated weight gain of 15g/kg/day is estimated to be between 120-130kcal/kg/day. Based on the gestational age the estimated protein requirement ranges from 2.5-4mg/kg/day, carbohydrates 8.6-11.5g/kg/day and lipids 2.5-3.5g/kg/day. In a growing preterm infant especially after the first week of life sodium requirement is 3-5mmol/kg/day and potassium 2-3mmol/kg/day.[1]

Mineral requirements are calculated from the estimated placental accretion during the last trimester. Apart from the estimated mineral accretion, the homeostatic balance between calcium, magnesium and phosphorus is intricate and complex. The complexity is greater in a preterm neonate due to underdeveloped organ systems and lack of adequate stores.

During the foetal stage, calcium is actively transported across the placenta. The normal foetus should accumulate a net total of 25 to 30 grams of calcium by the end of gestation, and 80% of this accumulation occurs in the third trimester[4]. The preterm infant will not accumulate the 25-30 grams of total body calcium.[4]

During foetal life especially in the last trimester, there is a net active transport of phosphate across the placenta at a rate of 60 to 75 mg/kg/day. 75% of this is utilised for bone mineralisation and the remaining 25% in remaining tissues. The preterm infants' phosphate stores are not maximal at birth. They have an increase in fractional excretion of phosphate and are at a higher risk for developing signs and symptoms of phosphate deficiency[4].

Ultimately the foetal accretion of calcium and phosphorus from the placenta is 120mg/kg/day and 70mg/kg/day respectively. Due to their chemical properties, these minerals have limited solubility in formulated parenteral nutrition. Calcium to phosphorus ratio by weight of 1.7:1 and 1.3:1 is recommended for optimal bone mineralisation. The recommended elemental calcium and phosphate concentration in parenteral nutrition is 12.5mmol/l and 13-15mmol/l respectively. With these concentrations, parenteral nutrition given at a rate of 150ml/kg/day would provide 75-95mg/kg/day of calcium and 60-70mg/kg/day of phosphorus.[1] This clearly shows the difficulty of maintaining these calcium and phosphorus ratios and thus duplicating intrauterine accretion rates of these minerals.

In addition, early parenteral nutrition in VLBW infants also has an impact on calcium and phosphorus. A retrospective study by Dik et al[5] involving early parenteral nutrition to VLBW infants weighing less than 1250 grams, showed that there was an association of hypophosphatemia and hypercalcaemia in the first week of life.[5] The severe hypophosphataemic infants had a more deleterious clinical outcome requiring vasoactive drugs and mechanical ventilation, 43% of these infants had IUGR. This study did not report any symptoms of hypophosphatemia but reports an association of sepsis with hypophosphatemia, however there were other confounding factors involved. Another review also indicated potential acquired leukocyte dysfunction in chemotaxis and phagocytosis due to leukocyte adenosine triphosphate (ATP) depletion in the presence of

hypophosphatemia.[5] Law et al also reported a similar phenomenon of hypophosphatemia in their cohort of patients receiving early aggressive parenteral nutrition.[3]

Phosphorus is the main component of ATP, membrane phospholipids and nucleic acids. Extremely low birth weight (ELBW) infants have the highest growth velocity and providing them with adequate amounts of amino acids (AA) promotes extrauterine growth. This growing process involves high metabolism and utilisation of phosphorus and this may affect serum phosphorus levels.[3]

A recent case report by Mizumoto et al[6], also described this phenomenon of hypophosphatemia in an ELBW infant who had received early aggressive parenteral nutrition and likened it to refeeding syndrome.[6] Refeeding syndrome is when the distinctive biochemical abnormality of hypophosphatemia occurs three to four days after refeeding.[6]

Bosante et al[7] prospectively studied the influence of early parenteral nutrition on homeostasis of calcium and phosphate and recommended an equation for the adequate amount of phosphate that should be added to parenteral nutrition per the amount of AA administered to the neonate.[7] They also noted severe hypophosphatemia in the patients receiving high intake of AA.[7]

This phenomenon of hypophosphatemia with early aggressive parenteral nutrition almost replicates the biochemical findings that occur in refeeding syndrome. But this can't simply be named refeeding syndrome because there is interrupted placental supply of nutrition and inability to mimic this nutritional supply by providing adequate calories and protein in the form of AA, but not supplying adequate phosphate. Bosante et al[7] proposed this phenomenon be called "Placental incompletely restored feeding syndrome". It could best be explained as hypophosphatemia syndrome in suboptimal early parenteral nutrition of the preterm.

3 Purpose of study

At Chris Hani Baragwanath Academic Hospital, preterm neonates weighing ≤ 1500gare initiated on enteral feeds which is commenced at 20ml/kg/day, with the remainder of the fluid and caloric requirement given intravenously. On Day one of life, a potassium free 10% dextrose solution is prescribed to meet the total fluid requirements, and when breastmilk is available, it is initiated at 20ml/kg/day and increased at this daily rate until maximum enteral feeds are obtained. If breast milk is not available, then a premature infant formula is provided and only if the weight is ≤ 1200g and dependant on the availability will donor breast milk be provided. The prescribed intravenous fluid volume is reduced accordingly as the enteral volume increases to match the total fluid requirement. On day two of life there is an attempt to initiate most if not all preterm neonates weighing ≤1500g onto parenteral nutrition provided the clinical condition allows this. In most circumstances the calcium, magnesium and phosphate levels are checked on day 14 of life, thereafter repeated two weekly or at more frequent intervals if clinically indicated. In view of this hypophosphatemia phenomenon occurring with early aggressive parenteral nutrition, is there a need to change current practices in this unit and should these mineral levels be checked much earlier?

3.1 Aim and objectives:

The aim of this study is to retrospectively assess the phosphate levels in very low birth weight infants that received early aggressive parenteral nutrition over a one year period at CHBAH. VLBW infants are defined as infants born with a weight less than or equivalent to 1500 grams. Preterm birth is defined as being born before 37 completed weeks of gestation.

Objectives:

1. To assess calcium, magnesium and phosphate levels in very low birth weight infants that received early aggressive parenteral nutrition.
2. To describe the baseline characteristics including gestational age, gender, and birth weight of these infants.
3. To evaluate for trends of enteral feeds, parenteral nutrition and supplementation as these may affect phosphate levels.
4. To assess for other confounding factors affecting phosphate levels such as presence of suspected or confirmed sepsis, the need for ventilation and the use of pharmaceutical agents affecting the phosphate levels.

3.2 Study Population

The study population will include all preterm neonates with very low birth weight (≤1500g) born over a one-year period (between June 2013 and May 2014) that was admitted within the first 72 hours of life at the neonatal unit in Chris Hani Baragwanath Academic Hospital.

3.3 Inclusion Criteria

VLBW infants that survived beyond the first two weeks of life.

VLBW infants that had at least one set of measurements of serum calcium, magnesium, phosphate levels within the two-week period.

3.4 Exclusion criteria

Patients with no medical records available for review.

Patients that demised before two completed weeks of life.

Neonates with confirmed endocrine disorders affecting bone mineral homeostasis. Neonates born to mothers with confirmed endocrine disorders that will affect neonatal bone mineral homeostasis.

3.5 Data Collection Sheet

Sex	GA	BW	Week	Diagnosis apart from prematurity	Ca	Mg	PO4	ALP	%EF- Formula	%EF– Breast milk	%Parenteral nutrition	Sepsis	Ventilation	Ca, Mg, PO4 Replacement	Supplements	Medication
			0-1													
			1-2													
			2-4													
			4-6													
			6-8													
			8-10													

3.6 Data collection

Data will be collected using patient clinical hospital files from CHBAH. The data collected will be used to create a database that will be analyzed. The data collected will only be accessible by the researcher. Information will be de-identified to maintain confidentiality and entered onto Microsoft Excel. Datasheets will be assigned a study number. The study numbers will allow for the matching of datasheets to the patients and will only be accessible to myself, the primary researcher.

Information such as biochemical findings that is required to complete the database and is not available from patient records will be obtained from the National Health Laboratory Services. The data will be scrutinised and assessed for missing information and erroneous or suspicious entries. These entries will be verified with the original patient records if necessary. Database will then be exported to STATISTICA for analysis.

4. Data analysis

For descriptive purposes, means and standard deviations (SD) or median (interquartile range) will be reported for continuous variables depending on the normality of distribution. All categorical data will be reported as frequencies (percentages). Calcium, magnesium and phosphate variables will subsequently be compared to a set of normative data appropriate for the study group. Any deviation from the normative values will also be correlated with presence of suspected or confirmed sepsis and the need for ventilation.

5. Limitation

The hospital archive system is not complete or computerised. Reliable information will not be easily obtainable from files, as some files may not be complete, or the writing maybe illegible. Gestational age is determined by the attending clinicians and is open to inter-variability between clinicians, so the use of gestational age will only be complimentary, however the birth weight will be the deciding factor for inclusion.

6. Cost

The cost involved in this study will be for stationary. Printing of protocol and data collection sheets and binding of final documents should cost near R1500 and will be borne by the researcher.

7. Time-lines

Year	2017	2017	2017	2017	2017	2017	2017	2017
Month		Feb	March	April	May	June	July	Aug
Activity	Protocol							
	Ethics							
	Data Collection							
	Data Analysis							
	Write Up							

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APPENDIX 2

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Vikesh Rowjee

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170253

NAME: (Principal Investigator)	Dr Vikesh Rowjee
DEPARTMENT:	Paediatrics Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Hypophosphataemia in very low birth weight infants receiving parenteral nutrition
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	Title change 20/05/2019
SUPERVISOR:	Dr Firdose Nakwa and Dr Kebashini Thandrayen
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	13/03/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application November invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 3

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APPENDIX 4

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