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ORIGINAL ARTICLE

Importance of Serum Osteocalcin as Early Biomarker for Osteopenia in Preterm Neonates Receiving Total Parenteral Nutrition

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ABSTRACT

Background: Prematurity osteopenia is known by bone mineral content reduction, which occurs especially due to decreased mineral stores in premature infants that may be excessive by increased mineral demands in the neonatal period. Thus, this study aimed to detect the significance of osteocalcin as an early biomarker for preterm neonates receiving total parenteral nutrition (T.P.N) with osteopenia.

Methods: A Cohort study on preterm neonates receiving TPN carried out in Neonatal Intensive Care Unit of Pediatric Department, Faculty of Medicine of Zagazig University Hospitals in the period from April 2016 to September 2018. This study included 30 preterm neonates receiving (TPN) of both sexes with birth weight ≤1500gm. Comprehensive serum osteocalcin, alkaline phosphatase (ALP), calcium and phosphorus were assessed before and after TPN.

Results: we found that serum levels of calcium and phosphorous showed a significant decrease after given TPN (P=0.045, P<0.001), while Serum levels of ALP and osteocalcin showed a significant decrease after given TPN (P<0.001). In addition, our study revealed a positive significant correlation between osteocalcin with ALP, while a negative significant correlation was found between osteocalcin with birth weight, calcium and phosphorus (P<0.05).

Conclusion: Alkaline phosphatase and osteocalcin the bone metabolism markers are considered to be indicators of bone turnover. So, they can be used for early identification of osteopenia.

Keywords: Osteopenia; Prematurity; Total Parenteral Nutrition (TPN); Osteocalcin; alkaline phosphatase.

INTRODUCTION

Prematurity indicates the neonates who are born at less than 37 weeks gestation. World Health Organization (WHO) expanded the range of full-term neonates to be 37-42 weeks' gestation. Preterm neonates are exposed to dangerous morbidities including respiratory distress syndrome (R.D.S), bronchopulmonary dysplasia, and prematurity osteopenia. Generally, 12% of neonates are born prematurely **[1]**.

Prematurity osteopenia is defined as the reduction in bone mineral stores in preterm neonates that may be excessive by increased mineral demands in the neonatal period. In normal conditions, calcium (Ca) and phosphorus (P) are obtained by the fetus during the last trimester of pregnancy, so premature infants are born with considerably decreased mineral stores in comparison with term babies[2,4,5]. Osteocalcin is produced by osteoblasts and is considered a bone formation marker. Meanwhile, it is implicated in the mineralization process rather than the production of the matrix. Circulating concentrations of osteocalcin are significantly associated with histological measures of the rate of bone formation **[3]**.

The present study was aimed to evaluate serum osteocalcin level as an early biomarker for osteopenia in premature infants receiving total parenteral nutrition.

METHODS

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The present study was carried out at Neonatal Intensive Care Unit (NICU) of Pediatric Department, Faculty of Medicine, Zagazig University Hospitals in the period from April 2016 to September 2018. The present study included 30 preterm neonates receiving (TPN) of both sexes with birth weight ≤1500gm. After excluding neonates with intrauterine growth retardation, known genetic malformations, dysmorphic features, neurologic disability, those with liver dysfunction or sepsis, skeletal deformities, severe congenital malformation chromosomal abnormalities and neonates born to osteopenic mothers.

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Patients were subjected to history taking regarding age and sex with special stress to family history and consanguinity and the prenatal and natal history: mode of delivery, need for resuscitation and congenital sepsis. Special attention was given to the onset of starting total parenteral nutrition and duration of receiving it and onset of starting enteral feeding and which type of milk is used: (expressed breast milk or artificial formula).

Laboratory tests: blood was collected for CBC, CRP levels, kidney and liver function tests CRP and serum alkaline phosphatase, calcium and phosphorus: before and after 2 weeks of taking TPN at the University Hospital laboratory. Determination Serum Osteocalcin was done using ELISA technique also before and after 2 weeks of taking TPN.

All patients were subjected to physical examination with specific attention to abnormalities of growth and skeletal abnormalities. Skeletal examination including inspection and palpation for all joints and long bones. Abdominal examination for hepatomegaly for exclusion of liver dysfunction or sepsis. Vital signs: temperature (axillary), heart rate, respiratory rate and blood pressure.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data. Independent T-test and Mann Whitney test were used to calculate the difference between quantitative variables in two groups for parametric and non-parametric variables respectively. All statistical comparisons were two-tailed with a significance level of P-value ≤ 0.05 indicates significance, p <0.001 indicates highly significant difference while P> 0.05 indicates Non-significant difference.

RESULTS

This study included 30 preterms (53.3%) of them were males and (46.7%) were females with gestational ages ranging from 28 to 32 weeks and with a mean birth weight was 1280.67 (±150.97) gm (as shown in table 1).

Serum levels of calcium and phosphorous showed a significant decrease after giving TPN, while serum levels of ALP and osteocalcin showed a significant decrease after giving TPN (as illustrated in table 2 and Figures 1 & 2).

Our study revealed a positive significant correlation between osteocalcin with ALP, while a negative significant correlation was found between osteocalcin with birth weight, calcium and phosphorus (Table 3).

Table (1): Demographic & clinical data distribution of the all patients.

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	Studied patients (N=30)
Gestational Age (weeks) Mean ± SD	30.40 ± 1.35
Female, n (%)	14 (46.7)
Birth weight (gm) Mean ± SD	1280.67 ± 150.97
Female, n (%)	14 (46.7)
TPN Duration (weeks) Mean ± SD	2 ± 0
Start of Enteral feeding	
7^{th} day of life, n (%)	1 (3.3)
8^{th} day of life, n (%)	3 (10)
9^{th} day of life, n (%)	4 (13.3)
10^{th} day of life, n (%)	3 (10)
11^{th} day of life, n (%)	5 (16.7)
12^{th} day of life, n (%)	7 (23.3)
13^{th} day of life, n (%)	5 (16.7)
14^{th} day of life, n (%)	2 (6.7)
Hemoglobin (g/dl) Mean ± SD	13.44 ± 1.29
TLC (10^3 /µL) Mean ± SD	7.69 ± 2.34
PLT (10^3 /µL) Mean ± SD	258.17 ± 84.15
Lymphocyte (%) Mean ± SD	35.58 ± 16.55
Neutrophil (%) Mean ± SD	50.06 ± 17.48
Serum creatinine (mg/dl) Mean ± SD	.493 ± .199
Albumin (g/dl) Mean ± SD	2.77 ± .285
AST (U/L) Mean ± SD	45.07 ± 13.94
ALT (U/L) Mean ± SD	42 ± 13.08
CRP (U/L) Mean ± SD	0.5 ± 0.629

TLC, Total leucocyte count; PLT, Platelets; AST, aspartate transaminase; ALT, alanine transaminase; CRP, C-reactive protein.

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Table (2): Mineral parameters before and after TPN of the studied patients

Mineral parameters	Before TPN	After TPN	P
Calcium (mg/dl) Mean ± SD	8.55 ± .614	8.27 ± .443	**.045**
Phosphorous (mg/dl) Mean ± SD	5.12 ± .232	4.87 ± .202	**.001**
Alkaline phosphatase (IU/L) Mean ± SD	243.44 ± 35.92	468.18 ± 75.87	**.001**
Osteocalcin (ng/ml) Mean ± SD	15.57 ± 6.84	186.14 ± 97.61	**.001**

Table (3): Osteocalcin Correlation with different parameters in all studied patients.

		Osteocalcin
Gestational Age	r	**-.460**
	p	**.011**
Birth weight	r	-.057
	p	.764
Hemoglobin	r	.063
	p	.742
Albumin	r	.127
	p	.503
Creatinine	r	.012
	p	.950
CRP	r	.065
	p	.735
Calcium	r	**-.365**
	p	**.038**
Phosphorous	r	**-.404**
	p	**.027**
ALP	r	**.419**
	p	**.026**

CRP, C-reactive protein; ALP, Alkaline phosphatase

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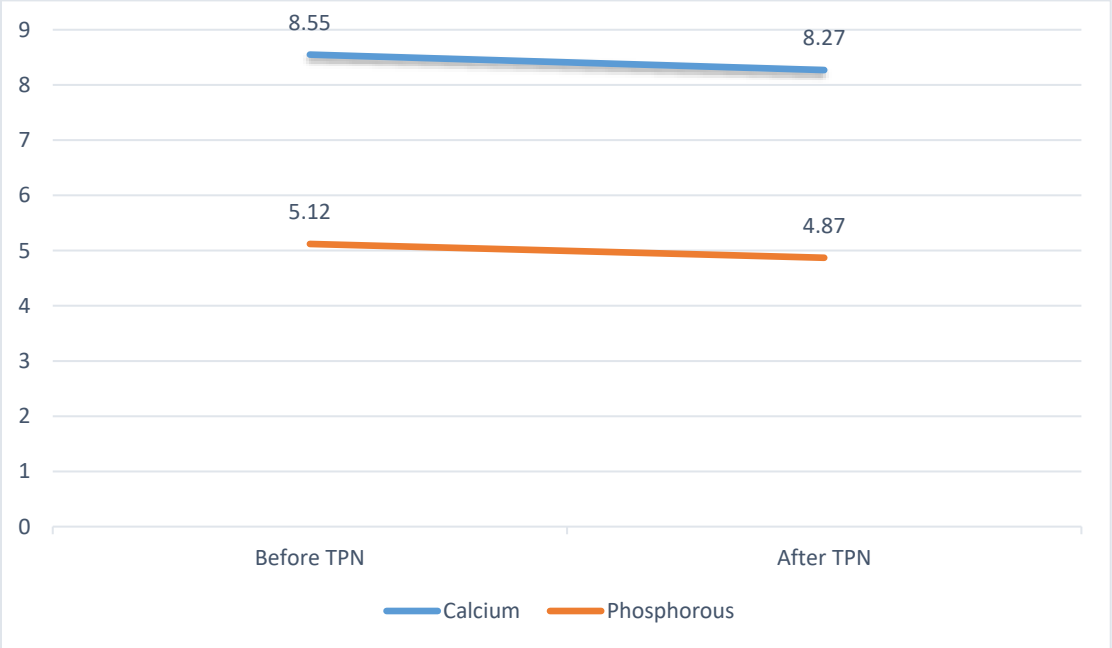

Figure (1): Calcium and phosphorous levels before and after receiving TPN in all patients.

Figure (2): ALP and osteocalcin levels before and after receiving TPN in all patients.

DISCUSSION

Most of preterm neonates need total parenteral nutrition for a period of time until sufficient nutritional intake enterally or orally become tolerated [6]. Total parenteral nutrition occasionally pointed out as hyperalimentation in preterm infants [7]. In general, infants started hyperalimentation earlier than adults did. Thus, the younger the neonate is, the sooner he needs proper nutritional intake. Subsequently, TPN

should be started as early as possible in preterm neonates even from the 1st day of birth [8].

In our study, all patients included in the study started receiving TPN from the first day of birth and continued for 2 weeks. Administration of TPN has become a standard method in the nutritionally deprived and critically ill patients' management, such as low birth weight premature neonates [9]. Moreover, Mehta and Compherm, [10] proven that TPN solutions should have the following: protein as crystalline amino acids, fats as lipids, carbohydrates as glucose, electrolytes, like sodium, potassium, chloride, calcium and magnesium, metals/trace elements.

Osteocalcin (OC) is, the bony matrix non-collagenous protein, also it is a specific osteoblastic activity and bone formation biomarker. It is synthesized by osteoblast, its serum concentrations are increased whenever bone turnover is elevated, making it a possible useful agent to diagnose osteopenia [11]. Osteopenia is documented to be as high as 30 % in babies studies less than 1,500 grams at birth especially those with primarily propped with long time TPN without appreciable enteral feedings [12].

Elevated levels of ALP indicate the increase of bone turnover and when ALP exceed 700-750 IU/L with decreased serum phosphorus concentration is correlated with osteopenia of prematurity diagnosis even if it is still asymptomatic [13].

In our study, there were 47% of the patients were females and 53% were males with mean GA 30.40 weeks and mean birth weight 1280.67 gm. Czech-Kowalska et al. [14] reported that low gestational age and birth weight increase the bone mineral disease (BMD) risk. Unquestionably, the BMD frequency is negatively linked with gestational age and birth weight as documented by Viswanathan et al. [15] and Figueras-Aloy et al. [16].

In our study, we found that 100% of the patients started TPN at 1st day of life. Perinel et al. [17] documented comparable results, and reported that TPN should be started as early as possible in preterm neonates.

In line with our findings, Visser et al. [18] observed an elevated serum alkaline phosphatase with decreased serum phosphate after receiving TPN and this is positively linked with the metabolic bone disease incidence. In addition, D'Ascenzo et al. [19] studied preterm infants with birth weights ranging 500–1249 g. They also reported no significant differences in serum alkaline phosphatase levels between or within groups of overall quality assessment in samples collected on days 7 and 14 of TPN.

In contrast to our results, Skouroliakou et al. [20] found no significant differences in final serum alkaline phosphatase or phosphate. On the other hand, Papandreou et al. [21] reported significant increase in Ca, and P in neonates receiving TPN. They also documented that the change in serum Ca was directly associated with the OC changes.

Czech-Kowalska et al. [14] reported that osteocalcin reached significance in MBD preterm infants. Pastore et al. [22] have measured bone turnover markers in children on PN. They reported an increase in OC concentration following pamidronate infusion, for treatment of MBD. In the contrary, Epstein et al. [23] reported a normal osteocalcin level was noted in patients receiving TPN.

We found significant direct correlation of osteocalcin with ALP in neonates receiving TPN, while a negative correlations were found between osteocalcin, calcium and phosphorous. Similar to our finding, Singh et al. [24] documented a positive correlation between osteocalcin and ALP. Although important, these results from adult studies may not be representation of bone metabolism in children [25].

Figueras-Aloy et al. [16] reported that metabolic bone disease in very preterm infants was positively correlated with the duration of TPN. In consistence with these findings, Czech-

Kowalska et al. [14] also reported that OC appear to be very specific reduced bone mass indicator in preterm infants at 3 months corrected age. Their data indicated that OC was the best MBD predictor from evaluated markers of bone metabolism.

To detect the early asymptomatic phases of impaired bone mineralization and allow early intervention, all neonates at high risk of MBD, insufficient intake of minerals and abnormal bone turnover biochemical markers should be regularly monitored. The strength of our study is the fact that it is the first to explore the OC as an early marker of bone turnover in preterm neonates. Limitations of our study included the wide range of the biomarker value, in addition to the relatively low numbers of the study population which underpowered our results.

CONCLUSION

Osteopenia can be silent until pathological fractures occurred. Therefore, it is important to be detected early. Preterm neonates receiving TPN need to be regularly evaluated for osteopenia by a rapid, readily available and easily followed bedside measurement. Serum alkaline phosphatase and serum osteocalcin are considered to be indicators of bone turnover. So, they can be used for early detection of osteopenia.

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Conflicts of Interest: The authors declare no conflict of interest.

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