Inside the "*fragile*" infant: pathophysiology, molecular background, risk factors and investigation of neonatal osteopenia

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Summary

Current research in bone mineral metabolism reveals many aspects of osteopenia occurred in premature infants. This review examines not only the pathophysiological and molecular mechanisms of newborn osteopenia but also the risk factors and investigation. Osteopenia of premature infants has increased incidence among other diseases of prematurity. Identification of risk factors is essential for monitoring of osteopenia. Some of the risk factors include low birth weight, prematurity, long term administration of drugs such as corticosteroids, methyloxanthines, furosemide, abnormalities in vitamin D metabolism, poor maternal nutritional and mineral uptake etc. Neonatologists, pediatricians and endocrinologists should investigate premature, low birth weight infants that have high serum alkaline phosphatase and have at least one risk factor.

KEY WORDS: premature infants; osteopenia; bone metabolism; low birth weight; vitamin D metabolism.

Introduction

The study of bone mineral density (BMD) in infants is of great interest not only to neonatologists but also pediatricians and children endocrinologist specialists (1-6). During the last decade more studies focus on bone mineral content (BMC) and associated disorders in molecular level. Important determinants of skeletal strength and, therefore, risk of pathological fractures include size, structure and density of the bone (2-4). Low BMD (osteopenia) is an important fracture risk factor and concerns not only neonates but also adults. In neonates, especially those born prematurely or of very low

birth weight (VLBW), osteopenia is a common cause of pathological fractures.

Decreased BMD can be a result of either decreased bone mineralization or increased bone reabsorption. The imbalance of bone mineralization and reabsorption is not only located in the early years of life but also in latter ages. Many factors contribute to the increased risk of osteopenia in neonates, such as reduced opportunity for transplacental mineral delivery in premature infants, poor nutritional intake in vulnerable VLBW infants and excessive mineral loss after birth. The incidence of neonatal osteopenia is inversely associated with gestational age and body weight. As many as 30% of infants born with a birth weight less than 1000 g were reported to be osteopenic and it is especially frequent in babies under 28 weeks of gestation (2,3). Purpose of this review is to investigate the available data concerning neonatal osteopenia, the molecular and pathophysiological basis, the risk factors, monitoring and investigation. Therefore by elucidating neonatal osteopenia recommendations can be drawn to help specialists like neonatologists, orthopedics and endocrinologists to identify high risk group of neonates.

Pathophysiological and molecular mechanisms

Development of the fetal skeleton requires large amounts of energy, protein and minerals. Minerals, such as calcium (Ca) and phosphorus (P), are actively acquired by the fetus from the mother. By the 2nd semester of pregnancy, fetal serum Ca and P concentrations are ~20% higher than maternal serum concentrations. Bone mineralization occurs predominantly during the 3rd semester. If the increased fetal demand in minerals is not met, then inadequate fetal bone mineralization may result (7). There is evidence that mothers increase Ca supply during pregnancy, e.g. by increased intestinal absorption of Ca and increased skeletal mineral mobilization. The importance of maternal Ca consumption is suggested by the improvement of adverse effects of severe maternal dietary restriction by Ca supplementation. Notice that the supplementation of Ca may have important adverse effects for the mother. From the early studies in osteopenic premature infants, vitamin D was considered to be an important factor associated with the pathophysiology of osteopenia. Vitamin D is transferred transplacentally predominantly as 25-hydroxyvitamin D and subsequently converted to 1,25-dihydroxyvitamin D in the fetal kidney. Although the exact role of 1,25- dihydroxyvitamin D in fetal bone mineralization is unclear, it has been shown that chronic maternal vitamin D deficiency can adversely affect fetal skeletal development (7-11). The role of vitamin D and its biotransformation in placenta supports the theory of the serious involvement of placenta in BMC. Hence many factors may directly or indirectly affect Ca absorption including maternal vitamin D status, solubility and bioavailability of Ca salts, quality and quantity of the mineral, amount and type of lipids and gut function (7, 8).

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As the postnatal growth of an infant's bone marrow cavity is faster than the increase in the cross-sectional area of the bony cortex, over the first 6 months of life, the long bone density can decrease almost 30%. It is thought that these alterations may reflect differences between postnatal and prenatal hormonal profiles and patterns of mechanical forces exerted through the skeleton (12, 13). The hormonal status is altered by a significant reduction of maternal estrogens. Also it is noticed a postnatal increase of parathyroid hormone (PTH) level due to a reduction of the Ca supply by the placenta. The fall of serum Ca level in the first day, stimulates the PTH secretion that continues 48 hours after birth. At this point we have the maximum increase of serum Ca, and stabilization of the mineral level.

An important cofactor that must be taken in account is mechanical force pattern, for example fetal movements such as kicking against the uterine wall, which may stimulate cortical bone growth (14). Therefore preterm infants may have less cortical growth with a consequent decrease in bone strength. These mechanical factors accompanied with decreased opportunity for transplacental mineral accretion place premature infants at high risk for neonatal osteopenia (13). In addition the mineralization process is determined by synthesis of organic bone matrix by osteoblasts with deposits of Ca and P salts. However less is known about the precise molecular mechanisms underlying osteopenia in infants in bone tissue level.

Risk factors

The major risk factors concerning neonatal osteopenia are summarized in Table 1. According to current literature the most important risk factors that are thoroughly discussed are prematurity of neonates, lack of mechanical stimulation, administration of specific drugs and pathologic conditions such as bronchopulmonary dysplasia.

Prematurity
Our increased understanding of the pathophysiology and molecular background of neonatal osteopenia has raised awareness among specialists of the need for early monitoring, prevention and treatment of this condition in high risk infants. As

Table 1 - Major risk and aetiological factors of neonatal osteopenia.

Factors of neonatal osteopenia
Bronchopulmonary dysplasia
Enterocolitis
Sex hormones and prostaglandins
Delay in establishing full enteral feeding
Prolonged parental nutrition
Methylxanthines administration
Diuretics administration (e.g. furosemide)
Dexamethasone administration
Prematurity
Lack of mechanical stimulation
Very low birth weight
Hormonal imbalance and vitamin D metabolical alterations
Poor nutritional intake by mother

mentioned above, prematurity is a very important risk factor, because transplacental Ca and P delivery is greatest after 24th gestation week. Almost 66% of the fetal accretion of Ca is occurring during this period. Generally, it is estimated that 80% of mineral accretion occurs in the 3rd semester of pregnancy (15). As a result, premature infants have depleted bone mineral stores at birth that may not be adequate for the rapid bony growth that occurs during the postnatal period. From that week and afterwards, the fetus gains ~30 g per day which requires approximately 310 mg Ca and 170 mg P per day (14, 16). It seems that the amounts of minerals required for bone regeneration are widely different depending on the age of the neonates. The period of greater skeletal development during intrauterine life requires not only minerals but also a great amount of proteins (14-16).

Lack of mechanical stimulation
Bone development is strongly influenced by forces that are exerted upon the bones therefore preterm infants are vulnerable due to lack of mechanical stimulation. It has been shown in an *in vitro* study that osteoblastic activity increases with mechanical loading (17). Furthermore the lack of mechanical stimulation may lead to increased bone resorption, decreased bone mass and increased urinary Ca loss (18). The skeletal structure remodels according to the prevalent forces, leading to increased bone strength at areas where this is most needed. Lack of mechanical stimulation in preterm infants places them at increased risk of osteopenia. Through the current bibliography there is a strong link between skeletal development and nervous system. Mechanical factors are also thought to contribute to inadequate bony growth in infants born with hypotonic muscular disorders. The association between decreased bone mineral density and reduced spontaneous movements has also been demonstrated in a study using quantitative ultrasound measurement (QUS) in subjects with cerebral pathology. Therefore infants with decreased levels of physical activities and movements against resistance, such as preterm ones are at high risk of developing osteopenia (19-22).

Drugs administration
Neonatologists and other specialists should be very careful in the prolonged administration of drugs. Use of various medications for neonatal diseases increases the risk of osteopenia in newborn infants. For example in preterm infants, the use of long term methylxanthines and diuretics such as furosemide, increase renal Ca excretion required for bony growth (23). Also, use of high dose systemic corticosteroids has been demonstrated to impair bony growth. An *in vitro* study showed inhibition of osteoblast function and DNA synthesis with high dose systemic steroids, while a clinical study showed a reversible reduction in serum bone-specific alkaline phosphatase (ALP) and osteocalcin (OC) after a 3 week course of systemic dexamethasone. VLBW infants with bronchopulmonary dysplasia are frequently exposed to such medications, further increasing their risk of developing osteopenia (24, 25). This problem is compounded by fluid restriction and relatively high energy requirements, limiting the supply of minerals and energy available for skeletal development.

Other pathological conditions
Despite a lack of alterations in bony biomarkers during infection, it has been shown that neonatal osteopenia is associated with infection. It is thought that this is related to the infant's

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catabolic state during infection period (26, 27). Sepsis, cerebral pathology, neuromuscular disorders may result in prolonged periods of immobility associated with poor bone mineralization. In addition chronic damage to placenta may alter the phosphate transport; therefore babies with intrauterine growth restriction may be osteopenic (14). Demineralization is observed also in mother with chorioamnionitis and placental infection.

Investigation and monitoring

Von Sydow noticed rickets in preterm infants back in 1946 for the first time. Since then the reported cases of rickets increased especially in VLBW infants (28). Until nowadays, a wide range of metabolic bone disorders in neonates have been reported, ranging from metaphyseal demineralization to generalized bone demineralization, periosteal reactions and fractures. It may hypothesize that rickets, bone demineralization, fractures and periosteal reactions are all part of the same disease with the same pathogenesis but with different clinicoradiological findings (29). However these entities may represent a different disease with its own pathogenesis and outcome.

Severe neonatal osteopenia can lead to serious complications, such as rickets and pathological fractures. Often, the earliest clinical features of osteopenia in neonates are these complications. High risk infants, such as VLBW infants or neonates received for long term medications such as diuretics should be regularly monitored for the possibility of osteopenia. This would allow the condition to be detected as early as possible so that appropriate management may avert the development of serious complications. Several modalities and surrogate markers for the measurement of BMC and BMD have been developed the latest years.

Radiological findings
Plain radiographs can sometimes show evidence of osteopenia such as previous fractures and cortical thinning (due to hypomineralization process). These alterations are often very late signs as a decrease in BMC of less than 30 - 40% is unlikely to be apparent on conventional radiographs (30). The most widely used modality to assess BMD in the adult literature is currently dual-energy X-ray absorptiometry (DEXA). DEXA has been shown to be superior to other methods of absorptiometry such as single photon absorptiometry, which although has been shown to correlate with BMC in infants, does not appear to correlate well with rickets or fracture risk. However DEXA has been shown to correlate well with fracture risk. Although DEXA has been widely used as a measure of BMD in adults, its use in paediatric patients in general and neonates in particular, is still limited (30-33).

A study by Rigo et al. (1) has shown that DEXA can be used to estimate BMC in both preterm and term infants. One of the main problems with the use of DEXA to measure BMD in non-adult patients is the "areal" nature of the measurement derived. As defined, the BMD measured by DEXA is BMC/Ap which is a two-dimensional measurement. The true density is a three-dimensional measure and should correctly be BMC divided by the volumetric measurement. The areal approximation can be accomplished in adult patients, but introduces systematic over estimation of BMD in larger patients (34, 35). This can be to some extent corrected by complex mathematical conversions based on assumptions of the skeletal structures of different bony regions. However, further studies are required to establish reliable neonatal, ethnic and sex specific normograms.

A portable and inexpensive method of investigating premature infant osteopenia is QUS. The speed of sound is analyzed to derive parameters that are correlated with BMD. It has been shown that QUS measurements are associated with bone density and structure (36), but not the thickness of the bony cortex. There are referenced values for both preterm and term infants for QUS. It has been shown that QUS parameters are associated with fracture risk in adult subjects independently of BMD, and QUS has been suggested to be a practical method of assessing for osteopenia in premature infants (16, 37-41). A recent study by Rack B, showed that preterm infants had significant lower QUS than term infants and a significant correlation of QUS with serum ALP, the supplementation with Ca, P, and vitamin D as well as risk factors for reduced BMD (42).

Serum biomarkers of bone metabolism
Serum biochemical markers such as Ca, P, ALP and OC have been used to detect the development of neonatal osteopenia in premature infants (3). There are several limitations to the use of these biomarkers. For example, while serum P concentration reflects the bony P levels well (persistently depressed concentrations reflect inadequate P levels and increased risk of osteopenia), serum Ca concentration is stringently controlled at the expense of bone Ca content. Further, serum Ca is affected by conditions that may not be related to neonatal osteopenia, such as hypophosphataemia (43, 44). Serum total ALP concentration has been used as a marker of bony turnover. Concentrations are elevated with increased bone cellular activity. It has been shown that concentrations > 750 IU/L, are associated with neonatal osteopenia and may precede clinical features of osteopenia of prematurity.

The literature regarding total ALP is conflicting, with poor associations reported in other studies. Bone-specific ALP, a more specific biomarker that is located on osteoblast surfaces may present a more accurate picture of bone turnover, and may be considered in cases with high levels of total ALP to increase diagnostic value (45-49). In addition the results of two recent studies of our study group involving preterm normal and osteopenic infants have been shown a significant increase of serum ALP compared with full term infants (50,51). ALP level is negatively associated with both body weight and gestational age in preterm infants (50). Beyers et al. in a large scale study indicate that serum ALP and high urinary hydroxyproline indicated increase bone turnover. Bone resorption may be more important than bone formation in preterm infants (52). Also Mitchell et al. in a recent study propose not only a radiograph of the wrist and/or knee to evaluate rickets, but also multiple measurements of ALP (>800 IU/L) (53).

Another biomarker of osteoblastic activity is OC, a non-collagenous protein of the bony matrix. It is synthesized by osteoblasts regulated partly by 1,25-dihydroxyvitamin D partly. Circulating concentrations of OC are elevated during periods of increased bone turnover. Despite its specificity, no correlation between serum OC and BMC has been shown during the first 4 months of age (48, 53-55).

Urine analysis
There is a large debate concerning urine analysis of Ca and P excretion as biomarkers of postnatal bone mineralization. It

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is known that infants with excertion of Ca and P greater than 1.2 mmol/L and 0.4 mmol/L respectively have the highest bone mineral accretion (56). A study by Hellstern G et al. (57) confirm that extremely preterm infants (23rd-25th gestation week) have a much lower threshold than any other preterm infants, leading to urinary P excretion even in low P levels. The best proposed biomarker is the percent tubular reabsorption of P (TRP) because P is not binding to plasma. TRP > 95% shows inadequate supplementation, however there is a strong relationship of inadequate Ca intake, increase PTH and hence tubular leak of P (58). In addition the use of urinary mineral to creatine ratios may seem to be appropriate in this case. Reference ranges of these rations in preterm infants have been reported (59). However results are needed careful interpretation because drug administration such as furosemide and theophylline lead to significance increase in the urinary Ca creatinine ratio (60).

Guidelines – Conclusions

Conclusively infants born prematurely may have a predisposition to osteopenia and related bone metabolic disorders. Neonatologists, paediatricians and endocrinologists specialists should monitor babies for bone disease if they have:
• birth weight <1500 g (14, 16, 50, 52)
• gestational week ≤28 weeks (14, 50, 52)
• total parental nutrition for more than 4 weeks (14, 16)
• long term course of diuretics and steroids (14, 16).
Therefore each week it is important to have a full biochemical bone profile (Ca, P, ALP) (50,52,53). Notice that if P is <1.8 mmol/L and ALP is > 500 IU/L, it must be examined the contribution of urinary tubular P reabsorption (16). Also the administrated medications must be reviewed and if appropriate to stop diuretics and steroids. The key for management of newborn osteopenia is monitoring and regular screening to identify high risk infants.

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