Correlation of Acetylcholinesterase activity and bone biochemical markers in premature and full-term neonates

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Original Article

Correlation of Acetylcholinesterase Activity and Bone Biochemical Markers in Premature and Full-term Neonates

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Abstract

Background: Prematurity lowers body weight and bone mineral density in nearly 30% of preterm newborns. Premature newborns are not screened for metabolic bone disease, hence bone biochemical markers should be investigated. Acetylcholinesterase mediates bone remodeling in recent investigations. Bone biochemical indicators and acetylcholinesterase (AChE) levels in preterm newborns may be linked.

Objective: The aim of our study is to evaluate the probable correlation of AChE levels with multiple bone biomarkers in full-term and premature neonates.

Subjects and methods: This study was designed as a case-control study. We examined 80 newborns (40 preterm with gestational age less than 37 weeks, 40 full-term). Gender, gestational week, and weight were recorded. ALP, Ca, P, Mg, PTH, and 25-hydroxycholecalciferol were directly measured in serum. We also measured the AChE level.

Results: Premature neonates had reduced Ca, P, and AChE levels but greater ALP and PTH. Gestational age increases body weight, Ca, and AChE levels. Gestational age correlated negatively with ALP and PTH.

Conclusion: The results of the current study revealed that there was a gestational age-related increase in AChE level. There were significant relationships between AChE levels with P, Ca, PTH, 25OHD and ALP in preterm and full-term groups.

Keywords: Acetylcholinesterase, Metabolic bone disease of prematurity, Premature

1. Introduction

Hypophosphatemia, hyperphosphatasemia, and late radiographic bone demineralization characterize the metabolic bone disease (MBD) of preterm.1 Bone strength is lower during infancy compared to adolescents and adults.2 No single ideal biomarker for the diagnosis of MBD exists.3 Acetylcholinesterase (AChE) hydrolyzes acetylcholine to inhibit neuronal cholinergic transmission. Fewer details are known regarding the non-classical activity of AChE, despite the fact that its functional involvement in neurite development and neural network is well documented. One of the many aliases given to AChE is “the protein with many faces.” Bone mineral density (BMD) loss, impaired learning and memory, and neurodevelopmental deficits were all linked to low AChE activity.4 AChE is able to modulate postnatal bone homeostasis via its classic enzymatic role.5 The protein AChE is well-known for its role in the enzymatic breakdown of acetylcholine in the central nervous system. As was just discussed, however, this enzymatic function is not unique to the nervous system, but is also seen in non-neural systems like bone. Remarkably, in addition to its typical hydrolytic actions, its wide variety of molecular structures and cholinergic binding sites also suggests nonenzymatic roles.6 In osteoblasts, AChE was found to be present in a tetrameric, amphiphilic form (AChET) that is
coupled with a proline-rich membrane-anchoring protein. In addition to its supposed enzymatic role, AChE is thought to play an adhesion role in post-natal bone development.

2. Subjects and methods

This study was a case-control study carried out on 80 neonates who were admitted or attended a neonatal out clinic in Al-Azhar University hospital during the time interval of the study. These neonates were divided according to their gestational age into 2 groups; Forty full-term neonates (37–40 weeks) were included in Group I, while 40 preterm neonates (<37 weeks) were included in Group II.

2.1. Inclusion criteria

Age (0–10) days, inborn and outborn neonates.

2.2. Exclusion criteria

The following neonates were excluded from the study:

Neonates with a family history of heritable bone disorders or conditions affecting acetylcholine such as myasthenia gravis. Neonates with maternal smoking, alcohol consumption or drug addiction. Neonates with prenatal asphyxia, endocrinopathies, cholestasis, congenital malformations, sepsis or critically ill neonates. Infants of diabetic mothers. Neonates after parenteral exchange transfusion, Ca infusion, diuretics, corticosteroids, phototherapy or medications that can interfere with laboratory results.

Both groups were subjected to the following:

1) Full medical history including personal data, medical, medicinal information, perinatal and family history.
2) Complete clinical examination including anthropometric parameters for all candidates.
3) Investigations were done as follows: venous blood samples were collected aseptically from studied groups, the blood was collected in a plain vacutainer tube and allowed to clot for 20 min in water bath at 37 °C, the serum was separated by centrifugation at 3000 rpm for 15 min The separated serum was divided and kept in 2 separate sterile plastic tubes. One tube was directly used for routine investigations and the second tube was kept frozen at −20 °C and was used for determination of AChE level.

An informed written consent was obtained from relatives of all participants. Approval for the study was obtained from The Medical Ethics Committee of Al-Azhar University. Our study was conducted in the Faculty of Medicine, Al-Azhar University for boys, medical biochemistry and pediatric departments, Cairo, Egypt. The study was conducted between January 2020 and January 2022.

Serum 25-hydroxycholecalciferol was measured by enzyme-linked immune sorbent assay (ELISA) using Qualisa 25 OH-vitamin D test kit.

Serum AChE activity and PTH were measured by enzyme-linked immune sorbent assay (ELISA) using kits supplied by Sunred Bio (Shanghai China).

Serum Ca, Mg, P and ALP levels were measured using Dimension Rxl max autoanalyzer provided by Siemens by using reagents supplied by Dimension® clinical chemistry system.

Statistical analysis of the data was coded, entered, and processed on a computer using a statistical package for Social Science (SPSS) (version 18). The results were represented in tabular and diagrammatic forms, then the interpreted value was considered significant, as the following:

* P > 0.05: Nonsignificant * P ≤ 0.05: Significant * P < 0.001: highly significant.

3. Results

3.1. Phosphorus

Table 1 and Fig. 1 show that there is a statistically significant difference in serum phosphorus level between studied groups (P<0.05).

3.2. Calcium

Table 2 and Fig. 2 show that there is a statistically highly significant difference in serum calcium level between studied groups (P<0.001).

3.3. Magnesium

Table 3 and Fig. 3 show that there is NO statistically significant difference in serum Mg level between studied groups (P > 0.05).

3.4. ALP

Table 4 and Fig. 4 show that there is a statistically highly significant difference in serum ALP level between studied groups (P<0.001).

Table 1. Mean ± S.D. of serum phosphorus in preterm and Full-term Groups.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>Mean ± S.D. (mg/dL)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>5.88 ± 0.93</td>
<td>2.056</td>
<td>0.04</td>
</tr>
<tr>
<td>Full term</td>
<td>6.21 ± 0.42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.5. PTH

Fig. 5 and Table 5 show that statistically there is highly significant difference in serum PTH level between studied groups (P<0.001).

3.6. 25OHD

Fig. 6 and Table 6 show that there is a statistically highly significant difference in serum 25OHD level between studied groups (P<0.001).

3.7. Acetylcholinesterase (AChE)

Table 7 and Fig. 7 show that there is a statistically highly significant difference in serum AChE level between studied groups (P<0.001) (Table 8).

Table 2. Mean ± S.D. of serum calcium in preterm and full-term groups.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>Mean ± S.D.</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>6.74 ± 0.94 (mg/dL)</td>
<td>9.672</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Full term</td>
<td>8.25 ± 0.28 (mg/dL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) There is a statistically significant positive correlation between AChE level with gestational age, weight and Ca.
(2) There is a statistically significant negative correlation between AChE levels with ALP and PTH.
(3) There is a statistically non-significant correlation between AChE levels with P, Mg and Vitamin D.

4. Discussion

Decreased bone mineral content, as seen by radiographic abnormalities in bone and high serum alkaline phosphatase (ALP), is a hallmark of Metabolic Bone Disease (MBD) of preterm.\(^1^4\)

There are multiple recommendations for MBD screening, and most recently the American Academy of Pediatrics endorsed routine bone mineral status evaluations for very low birth weight newborns (VLBW).\(^1^5\) Currently, there is no gold standard biomarker for the detection of MBD.\(^3\) Although ALP appears to be the most widely used screening method among U.S. neonatologists, it is not without its flaws.\(^1^6\) While there is currently no agreed-upon
cutoff value for ALP, multiple studies have demonstrated a link between high levels of ALP and MBD. However, screening using ALP alone may have low sensitivity and specificity, and serum phosphorous level integration into algorithms may enhance these metrics.17

Thus, it is common for clinicians to evaluate serum bone biomarkers such as serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, calcium and phosphate excretion in the urine. However, their routine application as diagnostic tools is a matter of debate.18

However, the function of AChE produced by osteoblasts in bone development and remodeling remains unclear. Researchers have found evidence that suggests AChE is a previously unrecognized bone matrix protein involved in the bone remodeling process.19

The current study revealed that premature healthy infants had serum ALP levels higher than that of full-term newborns. These results agreed with Dokos et al., whose results showed significant higher ALP in premature healthy infants compared with full-term newborns.20

As regards serum Ca there was a significant low Ca level combined with a low P level in preterm newborns compared with full-term infants.

It is common knowledge that metabolic bone disease of prematurity (MBDP) is linked to lower levels of calcium and phosphorus in the serum.21

Levels of serum calcium are susceptible to changes in diseases such as hypophosphatemia. Therefore, it is safe to infer that the sole factor that accurately reflects the amount of phosphorus in the bone is the serum phosphorus concentration.22

Table 3. Mean ± S.D. of serum magnesium in preterm and full-term groups.

<table>
<thead>
<tr>
<th>Studied group</th>
<th>Mean ± S.D.</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>2.33 ± 0.37 (mg/dL)</td>
<td>0.741</td>
<td>0.461</td>
</tr>
<tr>
<td>Full term</td>
<td>2.28 ± 0.26 (mg/dL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the present study, there was significant positive correlation between AChE level and serum Ca. These results agreed with those obtained by Dokos et al. who showed a positive correlation between AChE activity with Ca.  

As regards serum P there was a non-significant correlation between AChE level and Phosphorus, but in contrary to our results, Dokos et al., showed a significant negative correlation between AChE activity and serum P.  

As regards serum Mg there was no difference between the study groups and there was no significant correlation between AChE level and serum Mg. These results agreed with those obtained by Dokos et al., which did not find a correlation between AChE activity with Mg.  

As regards AChE level, our study found a significant increase in AChE level with gestational age. A significant increase in AChE activity has been observed from the 40th gestational week until the first year of life.  

Also, these results agreed with those obtained by Dokos et al., which showed a significant increase in AChE activity with gestational age.  

Little research has looked into AChE’s potential role as a new bone mediator in osteoblastogenesis, a process in which it does not act in a cholinergic fashion. However, the majority of research on AChE’s nonenzymatic roles in bone has focused on the osteoblastic lineage. AChE is expressed by osteoblasts both as a membrane protein and a protein of the bone matrix.

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| Table 4. Mean ± S.D. of serum alp in preterm and full-term groups. |
|------------------------|----------------|---------|----------|
| Studied group          | Mean ± S.D. (U/L) | t-test  | P value  |
| Preterm                | 491.8 ± 93.75 | 16.671  | <0.001   |
| Full term              | 218.27 ± 44.466 |         |          |
These findings corroborated those of Sato et al., who demonstrated the indirect involvement of AChE in osteogenesis via a reduction in ALP activity in osteoblastic culture.19

Significant changes in osteoblast differentiation were also observed throughout limb development in chickens when Spieker et al. inhibited acetylcholinesterase.23

Also, these results go in hand with the results of Inkson et al., who reported that AChE has been found to be essential for osteogenesis as well as chondrogenesis.24

In contrary to our results, Sato et al., showed that while genetic knock-out of AChE impacts the systemic cholinergic system, it may have non-neuronal functions, such as bone production. The AChE inhibitor donepezil also reversed the bone loss in mice. AChE also affects bone development and resorption in vitro. A rise in the expression of acetylcholinesterase (AChE) was discovered during the differentiation of bone marrow macrophages into osteoclasts. Through the use of siRNA, AChE was genetically inhibited, which boosted osteoclast differentiation. On the other hand, recombinant AChE protein delivery increased receptor expression in bone marrow macrophages, leading to increased osteoclast production. Last but not least, donepezil, an AChE inhibitor, may also directly suppress osteoclastogenesis in vitro. Thus, decreasing AChE levels boosts bone formation while simultaneously decreasing bone resorption. Results from the study were also connected to a rise in cholinergic activity.25

In our study we found a significant negative correlation between AChE level and alkaline phosphatase. These results were in agreement with those obtained by Sato et al., which showed in osteoblastic culture the indirect involvement of AChE in osteogenesis with a decrease of ALP activity.19

In agreement with the present results, Dokos et al. did find a negative correlation between AChE

Fig. 4. The mean ± SD of serum ALP in preterm and full-term groups.
activity with ALP, the main bone metabolic disease biomarker.\textsuperscript{20}

As regards serum PTH, the present study showed a statistically significant higher PTH level in premature infants compared with full-term newborns. These results were in agreement with those obtained by Papantoniou et al., which showed suppression of PTH in premature infants as early as the 19th gestational week.\textsuperscript{26}

Also, these results go in hand with the results of Dokos et al. who showed a statistically significant higher PTH level in premature healthy infants compared with full-term newborns.\textsuperscript{20}

In the present study, there was significant negative correlation between AChE level and PTH. These findings corroborated the findings of Dokos et al., who discovered a strong relationship between PTH and physical activity, but no other research has been conducted to shed light on this phenomenon. Different molecular forms of acetylcholinesterase (AChE) are thought to have various physiological roles, not only in the cholinergic system but also in bone metabolism, which is assumed to be supported by the enzyme's complicated structural polymorphism.\textsuperscript{20}

According to our results, there was a significant low 25-Hydroxycholecalciferol serum level in preterm newborns compared to full-term ones. These results were in agreement with those obtained by Tung and Kelly who showed that sub-optimal 25OHD was more common among infants born \( \geq 28\) weeks, while elevated 25OHD was more common in infants born <28 weeks and preterm infants in our study were more than 28 weeks.\textsuperscript{27}

Also, these results go in hand with the results of Matejek et al., that showed preterm newborns

| Table 5. Mean ± S.D. of serum PTH in preterm and full-term groups. |
|-----------------|-----------------|-----------------|-----------------|
| Studied group   | Mean ± S.D.     | \(t\)-test      | \(P\) value     |
| Preterm         | 128.37 ± 30.57 (pg/ml) | 4.846            | <0.001          |
| Full term       | 96.67 ± 27.87 (pg/ml)  | 12.547           | <0.001          |

Fig. 5. The mean ± SD of serum PTH in preterm and full-term groups.
vitamin D deficiency occurred in 75% at birth and in 30% in the 36th gestational week.28
In contrary to our results, normal levels of 25OHD have been reported in both premature infants with and without rickets and 25(OH)D level has not been shown to be a useful screen for MBD in preterm infants.27
In the present study, there was no significant correlation between AChE level and serum 25-Hydroxycholecalciferol.

![Fig. 6. The mean ± SD of serum 25OHD in preterm and full-term groups.](image-url)

**Table 6. Mean ± S.D. of serum 25OHD in preterm and full-term groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± S.D. (ng/ml)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>7.91 ± 4.64</td>
<td>4.916</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Full term</td>
<td>16.31 ± 9.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 7. Mean ± S.D. of serum AChE level in preterm and full-term groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± S.D. (ng/ml)</th>
<th>t-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>10.25 ± 2.54</td>
<td>3.756</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Full term</td>
<td>15.56 ± 8.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1. Conclusion

We concluded that AChE enzyme has a role in bone metabolism in addition to its neuronal function. Also, there was a gestational age-related increase in AChE levels.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article.

Sources of funding

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Conflict of interest

There are no conflicts of interest.

Table 8. Shows a correlation between serum Acetylcholinesterase levels and other parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson Correlation (R)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td>0.283</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight</td>
<td>0.327</td>
<td>0.003</td>
</tr>
<tr>
<td>Ca</td>
<td>0.292</td>
<td>0.009</td>
</tr>
<tr>
<td>Mg</td>
<td>0.178</td>
<td>0.114</td>
</tr>
<tr>
<td>Al. Ph</td>
<td>-0.364</td>
<td>0.001</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.266</td>
<td>0.017</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.134</td>
<td>0.235</td>
</tr>
</tbody>
</table>
References