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ORIGINAL ARTICLE

Relationship between Vitamin D3 deficiency and sternebrae fusion age in young females: A cross-sectional study.

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ABSTRACT… Objective: To assess the relationship of vitamin D3 on the timing of sternebrae fusion in females aged 18 to 25. **Study Design:** Cross-sectional study. **Setting:** Azra Naheed Medical and Dental College, Lahore. **Period:** Aug 2023 to Jan 2024. **Methods:** A total of 400 females, aged 18-25, were categorized into four age groups within this cross-sectional study. Investigative modalities included serum vitamin d3 levels and lateral chest x-rays to determine the fusion status of sternebrae 2^{nd}-3^{rd}, 3^{rd}-4^{th}, 4^{th}-5^{th}. Statistical analysis, such as chi-square tests and regression models, were carried out to determine link between Vitamin D3 levels, age, and fusion status. **Results:** While vitamin D3 was deficient in 60.2% of the participants, fusion of the sternebrae was nearly complete in all age groups (97.5% to 99.2%). Besides this, statistical analysis revealed no significant association between vitamin D3 status and the fusion of any sternebrae segment ($p > 0.05$). Furthermore, regression analysis also showed no predictive value of vitamin D3 levels on the age of fusion. **Conclusion:** Vitamin D3 seems to have no significant influence on the fusion rate of the sternebrae; complete fusion occurred by 18 years of age in the majority of females. While Vitamin D3 itself remains an important nutrient, further research is necessary to determine what other systemic factors have a role in the timeline of sternebrae fusion.

Key words: Female Students, Sternebrae, Sternum/Growth and Development, Vitamin D3 Deficiency.

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INTRODUCTION

The determination of age forms a critical component of any proceeding that requires identity verification.[1] Under the medical sphere, age estimation is an indispensable step in forensic examination.[2,3,4] One anatomical marker deserves to be highlighted: the sternum, which has the potential to be an indicator of biological age.[5] The manner in which this can be fulfilled is via the scrutiny of the sternebrae.

The ossification centers within the sternal body typically fuse in a caudal-to-cranial direction, completing by early adulthood according to current evidence.[6] What literature fails to explain is the high variability in the timing of the sternal fusion. In a recently conducted research on the Bengali population, age 25 was stated to be the completion point for sternebral fusion.[7] Overall, there is a lack of availability of research data regarding the exact age of the fusion of sternebrae.

Vitamin D3 deficiency is a widespread health concern, particularly in young females living in areas with limited access to sunlight. A 2019 research in clinical orthopedics and trauma found that around one billion people worldwide had this deficiency.[8] Furthermore, 50-80% of the population in India, Brazil, and the Middle East are affected.[9] In 2021, Uzma Shamsi et al., stated up to 73% of youngfemales from Lahore haddeficiency at one point.[10] While it is well established Vitamin D3 plays a role in bone health, the relationship between it and sternal fusion remains poorly understood. The rationale being that since Vitamin D3 is a regulator of calcium metabolism, it should guide sternal fusion; however, the question remains on whether it works on all levels of fusion. Thus, it is the goal of this study to determine whether deficient levels of vitamin D3 within the young females from the south Asian population, lead to a delayed fusion of the sternebrae.

METHODS

This was a cross-sectional, analytical study that looked at a total of 400 medical students, aged 18 to 25 years, from Azra Naheed Medical &

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Dental College, Lahore. Ethical Approval was taken from Superior University, Lahore (ERB/ BMS/02/013/2023). Study was conducted in 6 months (August 2023 to January 2024). Initially, a sample size of 385 was established by using 95% confidence level and a 5% margin of error with expected frequency of sternebrae fusion rate among 18- 25 years females as 50% (conservative approach). However, it was difficult to divide 385 subjects equally so for this study a total of 400 subjects were considered, with 100 subjects in each group. All participants were enrolled in the study through convenience sampling.

Inclusion Criteria

Any female medical students between the ages of 18 and 25 years studying in the same college were included in this study.

Exclusion Criteria

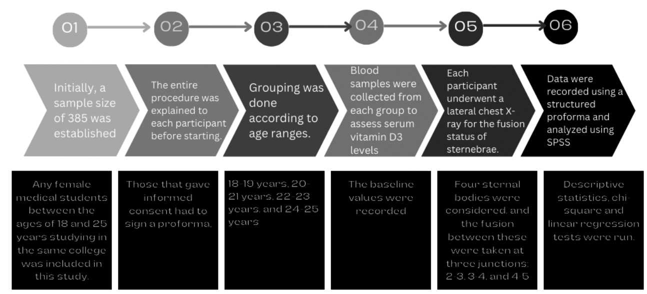
Any female student that was either pregnant, lactating, or had post traumatic or post-surgical architecture distortion of sternum. Besides this, any female with a history of active systemic disease or endocrine disorders was considered unfit for participation.

The entire procedure was explained to each participant before commencement, including what data would be shared. Those that gave informed consent had to sign a proforma, after which they were then grouped according to age ranges: 18–19 years, 20–21 years, 22–23 years, and 24–25 years. Blood samples were collected from each group to assess serum vitamin D3 levels and the baseline values recorded.A level below 30 ng/mL was considered deficient, while levels equal to or above 30 ng/mL were classified as normal. Additionally, each participant underwent a lateral chest X-ray, which was evaluated for the fusion status of sternebrae. Four sternal bodies were considered (number 2 to 5), and the fusion between these was taken at three junctions: 2-3, 3-4, and 4-5. For the purpose of evaluation, two experienced radiologists, blinded to the trial and participants, were enlisted to assess the x-ray films.

Data were recorded using a structured performa and analyzed using SPSS version 29. Furthermore, descriptive statistics were calculated for age, vitamin D levels, and fusion status. To compare the groups with each other, the Chi-square test was chosen. Lastly, linear regression analysis was conducted to explore the predictive value of vitamin D levels on fusion of the sternebrae. A p-value of less than 0.05 was taken as statistically significantthroughout this experiment. All phases of the study were followed in a strict sequence (Figure-1).

FIGURE-1

Visual flowchart showing steps in completion of the study



RESULTS

Amongst the 400 participants, analysis of serum vitamin D3 levels indicated that 241 people (60.2%) exhibited deficiency, whereas 159 persons (39.8%) shown normal levels (Table-I). Regarding radiographic evaluation, sternebrae fusion was practically ubiquitous amongst all individuals. Specifically, fusion was noted insternebrae 2-3, 3-4, and 4-5 in 97.5% (n=390), 98.8% (n=395), and 99.2% (n=397) of subjects respectively (Figure-2) (Table-II). However, comparisons among groups revealed no significant differences in fusion status across the four age categories ($p > 0.05$). The same held true for the connection between vitamin D3 levels and fusion of sternebrae, as both the vitamin D-deficient and vitamin D-normal cohorts demonstrated comparably elevated fusion rates across all segments ($p > 0.05$). Lastly, regression analysis indicated that the fusion of sternebrae 2-3, 3-4, or 4-5, as well as vitamin D levels, did not substantially predict chronological age in this cohort ($p > 0.05$) (Table-III).

TABLE-I	
Baseline parameters of the participants	
Baseline Parameter	**Value**
Mean Age (years)	21.5 ± 2.3
Age Range (years)	18 – 25
Mean Vitamin D Level (ng/mL)	26.8 ± 9.7
Vitamin D Range (ng/mL)	6.4 - 60.0
Vitamin D Deficient (<30 ng/mL)	241 (60.2%)
Vitamin D Normal (>30 ng/mL)	159 (39.8%)

TABLE-II

Fusion of sternebrae based on Vit D3 level

Sternebrae Segment	Fused - Normal Vit D3 (n, %)	Unfused - Normal Vit D3 (n, %)	Fused - Low Vit D3 (n, %)	Unfused - Low Vit D3 (n, %)
2-3	158 (99.4%)	1 (0.6%)	232 (96.3%)	9 (3.7%)
3-4	159 (100%)	0 (0%)	236 (97.9%)	5 (2.1%)
4-5	159 (100%)	0 (0%)	238 (98.8%)	3 (1.2%)

TABLE-III

Regression analysis of prediction of fusion based on Vit D3 level

Sternebrae Segment	Beta Coefficient (Î2)	Standard Error	P-Value
2-3	1.703	1.04	0.102
3-4	-0.7	1.93	0.718
4-5	-0.833	2.11	0.69

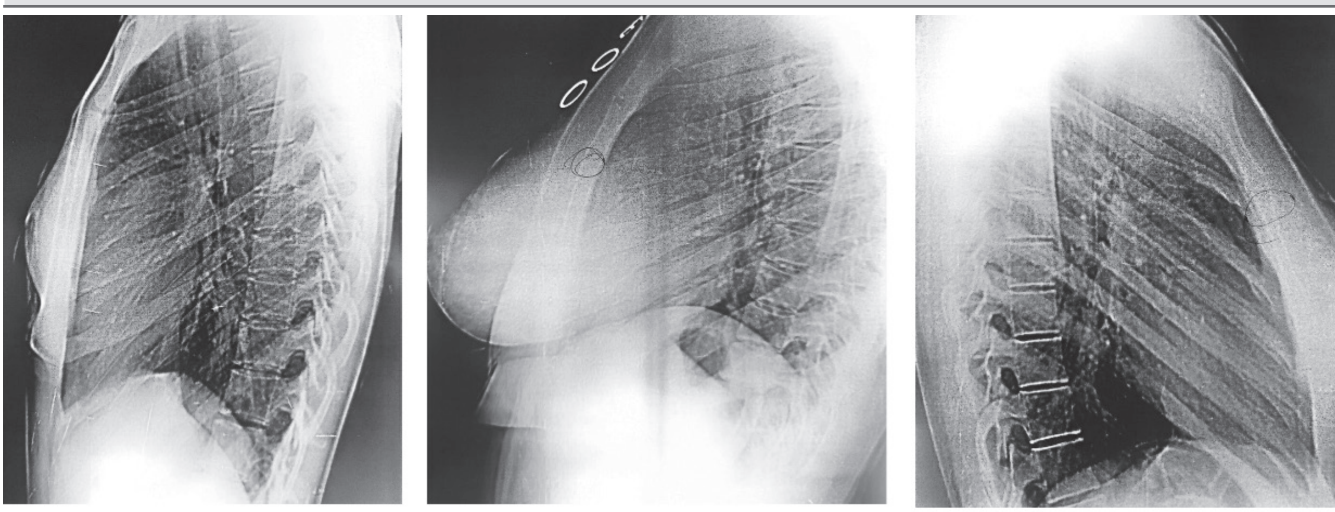
DISCUSSION

This is the first study that exclusively focuses on the influence of Vitamin D3 level on sternebrae fusion. Despite the numerous shortcomings of the study design and the overall lack of significant findings, the insights provided by the findings will serves to push the drive for further research on the subject matter. That said, a number of findings should be discussed.

Firstly, this study found how sternal body fusion was nearly complete by 18 years of age in over 97% of participants, regardless of their age subgroup. The x-ray films also did not show any morphological deformity in any of the participants (Figure-2). Other studies have reported similar findings; study is by Sahin et al. (2025) is relevant, despite the results highlighting age of ossification instead of complete fusion; this study reported that patterns of ossification in the sternal body began around 15 years, for both female and males.[11] The variability here does suggest how sternal fusion may be both population-specific and even gender-specific, though the authors Pankaj et al (2018) refuted the latter, as in a population of 770 individuals, the bisexual variation in the sternal body fusion was found to be inconclusive.[12] Clinically speaking, the value in this finding lies in the fact that it supports the use of sternebrae fusion as a confirmatory marker that an individual is atleast17 to18 years old.

FIGURE-2

Lateral x-rays showing fused sternal bodies in three of the participants. No morphological aberration was noted in any of the films.



The second major finding was that vitamin D3 deficiency was highly prevalent, affecting 60.2% of participants, and this was consistent with reports from other South Asian populations. One systematic review of south-east Asian adults showed how more than 68% of the population had vitamin d3deficiency with a significant heterogeneity, with the highest prevalence was found in Pakistan (73%) followed by Bangladesh and India (67% for both).[13] In non-Asian populations, Hatun et al. discovered vitamin D deficiency in 11–18-year-old females.[14] Furthermore, the fact that Shakiba et al. and Moussavi et al. from Iran, as well as Saintonge et al. and Ford et al. from the US all found different rates of vitamin D insufficiency in teenage females shows that this problem is widespread across age groups and regions.[15,16,17,18] Moreover, amongst the affected population, females comprised the majority. This is reinforced by Rana et al. who found that females aged 10–19 had a greater frequency of deficit than boys.[19] Another review paper reported similar findings in children too, with over 50% of children having very severe vitamin d3 deficiency (<30nmol/L).[20]

Despite the high prevalence of deficiency, no significant association was observed between vitamin D3 levels and sternebrae fusion status within this cross-sectional study. One possible explanation is that while vitamin D3 is critical for bone mineralization, the ossification and fusion of the sternum may be more strongly influenced by other factors. The evidence of this lies in anolder research paper by Kaneriya et al. that mentioned how fusion of the sternebrae followed a predictable pattern for the population studied; with the third and fourth sternebrae fusing at puberty, while the first and second, and second and third sternebrae fuse between puberty and 25 years of age.[21]

Lastly, the lack of significant age prediction from sternebrae fusion in this sample downplays the potential role this marker may have in forensic utility beyond confirming legal adulthood. The cause can be attributed to the high degree of variations.[22] Vatzia et al concluded how sternal variations were not only frequent and asymptomatic but they were independent of factors such as nutritional status or environment.[23] In fact, more studies focus on the occurrences of morphological variations of the sternal body at different ages instead of the timing of the fusion.[24,25] Clinically and forensically, this reinforces the importance of using multiple skeletal and biochemical markers for more accurate age estimation, rather than relying on sternal fusion alone.

This study is not without limitations. Firstly, the study design itself carries with it a number of weaknesses, primarily how a causal relationship between vitamin D levels and sternal fusion cannot be established due to absence of longitudinal follow-ups to see changes over time. Secondly, the sample population was entirely young adult females from a single institution, and while this was by design, it prevents generalizations being made on the outcomes. Specifically, it is unknown how other age groups, males, or populations with different genetic, nutritional, or environmental backgrounds would have affected the results. Thirdly, as postulated, there may have been a number of other factors that interfered with the results and the study did not account for these. These factors that could have impacted bone development and ossification include genetic variability, dietary calcium intake, physical activity, socioeconomic status, or sun exposure patterns.

Future research should address these gaps by the following measures. Firstly, a larger and more diverse population across different age ranges and sexes should be included, but this would only be possible at multiple centers or atleast a hospital scale. Secondly, and more importantly, the study design should be longitudinal prospective, with checkpoints where the sternal fusion is evaluated alongside other laboratory parameters besides vitamin D3. Biochemical markers such as calcium or parathyroid hormone levels would also provide a more comprehensive understanding of skeletal maturation. Additionally, incorporating advanced imaging modalities would be crucial since these are the gold standard for bone assessments. There is also the option of applying more technologically advanced techniques such as machine learning, which has garnered more attention in recent years.[26]

CONCLUSION

Despite being a small-scale study, the findings from this paper bring several insights which hold value clinically. While sternebrae fusion is largely complete by 18 years of age in the majority of young females, there is no significant influence of vitamin D3 status on the timing of fusion, despite the increased prevalence of deficiency. Thus, sternebrae fusion may serve as a marker for confirming legal adulthood but has limited value in distinguishing between later age groups or in reflecting metabolic bone health. It is reiterated that further multi-center and longitudinal studies to explore additional factors that may influence sternal development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1	**Fatima Farooq:** Conception of idea, data analysis, manuscript writing.
2	**Rafea Tafweez:** Proof reading.