

Development and Validation of Algorithm for Cervical Vertebral Bone Age Assessment in Maharashtra Population: A Cross-sectional Study

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ABSTRACT

Introduction: The skeletal maturity of individuals has been shown to vary over age, gender, ethnicity, etc. The commonly used methods for Cervical Vertebral Bone Age (CVBA) assessments are subjective and prone to inter-operator bias. This has led to the quest for establishing objective techniques. One such objective method for bone age estimation using multiple regression formulae for males and females has been established by the authors for the population of Maharashtra. However, the practical use of the equations is questionable, encouraging us to develop an automated technique for more practicality.

Aim: To establish and validate an algorithm for the quantitative assessment of CVBA in the population of Maharashtra.

Materials and Methods: This cross-sectional study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Terna Dental College and Hospital, Navi Mumbai, Maharashtra, India over a period of January 2024 to June 2025. This research developed an Artificial Intelligence (AI) algorithm for the objective estimation of CVBA. The research utilised 1000 radiographs from healthy individuals of Maharashtra origin (ages 9-20 years) to train a You Only Look Once (YOLO) v11 object detection algorithm, identifying C3 and C4 vertebral landmarks

after data augmentation. A Python program later assessed bone age using these detections and gender-specific methodologies based on pre-existing Maharashtrian formulae, which are stepwise multiple linear regression formulae. The algorithm was tested with 150 independent lateral cephalograms and validated by comparing unused 150 lateral cephalograms with hand-wrist radiographs using the Tanner-Whitehouse (TW3) hand-wrist method (gold standard), employing statistical analyses such as the Mann-Whitney U test, Spearman's rank correlation, and Intraclass Correlation Coefficient (ICC).

Results: The mean age estimated using AI technology and hand wrist age was 13.04 ± 1.95 and 12.78 ± 1.82 years, respectively and indicated a negligible difference in the age estimated by the AI and hand wrist age (TW3 method). There was an almost perfect agreement in the age estimated using AI and hand-wrist age (TW3 method) ($ICC=0.994$). There was an excellent positive correlation in the age estimated using AI and hand-wrist age (TW3 method) (Spearman's $\rho=0.974$).

Conclusion: The average age of AI algorithm was very close to TW3's hand-wrist method average estimated age. This was consistent across the genders. Thus, the algorithm that was built was dependable as it was closely related to the gold standard.

Keywords: Artificial intelligence, Bone age determination, Computer-assisted growth and development, Image processing, Machine learning, Validation studies

INTRODUCTION

Reliable evaluation of skeletal maturity is foundational to several critical medical and scientific disciplines, including paediatrics, orthodontics, and forensic science. This assessment provides essential information regarding an individual's growth potential, which is crucial for determining the optimal timing for various clinical interventions, such as interceptive orthopaedic therapies, and for monitoring developmental milestones [1]. Accurate determination of bone age allows clinicians to tailor treatment plans, predict final stature, and manage conditions influenced by skeletal growth, thereby significantly impacting patient care outcomes.

Historically, the assessment of skeletal maturity has relied on various radiological methods, with the hand-wrist radiography, particularly the Tanner-Whitehouse (TW) methods, widely recognised as a gold standard [2]. While highly effective, these methods necessitate a dedicated radiograph, adding to radiation exposure and sometimes incurring additional costs or logistical challenges. As an alternative, morphological examination of cervical vertebrae from lateral cephalograms, routinely taken in orthodontic practice, has gained traction as an essential aid for estimating skeletal maturity without

additional radiation exposure [3]. The pubertal growth spurt is reliably predicted from C3 and C4 vertebrae, which exhibit consistent shape changes with growth—an established fact and also because they are centrally oriented to lie on a standard lateral cephalogram (unlike most of the vertebrae, which are obscured by the shoulders or thyroid collars) [4]. However, the conventional qualitative staging methods for CVBA evaluation, though informative, are inherently prone to subjectivity and inconsistency amongst observers [5]. This inter-observer variability can compromise the reliability and reproducibility of bone age estimations, creating a significant challenge for consistent clinical application and accurate longitudinal monitoring.

The increasing demand for more precise, objective, and efficient diagnostic techniques has driven research towards automated solutions in bone age assessment. Recent progress in AI and machine learning offers prospective directions to overcome the limitations of manual evaluation. Research has shown that AI-based algorithms can offer accurate and efficient skeletal age assessments, often surpassing the efficiency of traditional manual methods while maintaining high levels of agreement [5,6]. For instance, fully automated algorithms have shown significantly higher

accuracy and efficiency compared to manual methods, reducing the time required for assessment and minimising human error [5]. Despite these global advancements, there remains a notable gap in the literature regarding population-specific diagnostic techniques, especially within diverse demographic settings like India. The varied genetic and environmental factors across different populations can influence growth patterns, underscoring the need for localised validation of skeletal maturity assessment tools.

Addressing these critical shortcomings, the present study focuses on the populace of Maharashtra, India, aiming to transition from a qualitative staging method to a quantitative, digital approach for CVBA assessment. The key focus of this project is to create and verify a programmed approach capable of objectively evaluating the age of cervical vertebral bones. This novel AI-based algorithm seeks to provide a reliable, consistent, and less subjective method for skeletal maturation analysis, tailored to the specific demographic characteristics of the Maharashtra region. The algorithm created is based on the formulae derived previously by Gaonkar P et al., and both pieces of research are a part of the larger project [7]. By developing and validating this automated system, this research contributes to bridging the existing literature gap by offering a population-specific tool and demonstrates the feasibility of integrating advanced AI technology into routine clinical practice for enhanced diagnostic accuracy and efficiency.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Terna Dental College and Hospital, Navi Mumbai, Maharashtra, India over a period of January 2024 to June 2025. The ethical clearance was provided by Terna Dental College Ethics Committee (TDC/EC/08/2022).

Inclusion criteria: The study subjects consisted of participants of Maharashtrian origin in the age range of 9-20 years with no history of any trauma and/or operation in the craniofacial or hand region, no history of orthodontic intervention, and no underlying systemic illness, bone development disorders, growth-developmental retardation, or physical defects.

Exclusion criteria: Individuals with a history of congenital physical defects in the craniofacial hand region, metabolic or endocrine disorders were excluded from the study. Radiographs with poor image quality or positioning errors were also omitted from the study.

Sample size: The study utilised a total of 1300 lateral cephalograms (1000 training; 150 testing, and 150 validation).

Study Procedure

In order to develop the algorithm, a sample of 1000 lateral cephalograms in the age span of 9-20 years was used to train the model. In the age range of 9-20 years, the data was divided as follows: 9-11 years, 12-15 years, 16-18 years and 19-20 years. The 1,000-image training set demonstrates that there is enough architectural complexity to allow the algorithm to learn the subtle morphological characteristics at each of the six Cervical Vertebral Maturation (CVM) stages, resulting in approximately 120-150 samples per stage to represent biological variance while minimising overfitting. Such a large volume aids in the use of transfer learning and also in data augmentation, thereby minimising the chance of overfitting and ensuring that the model generalises over varied patient demographics. Further, it was ensured that there was equal representation of male and female data for each CVM stage.

These lateral cephalograms belonged to males and females of Maharashtrian origin belonging to six administrative divisions of Maharashtra: namely Amravati division, Vidarbha (Nagpur Division), Marathwada (Aurangabad division), Konkan, Khandesh (Nashik division), and Western Maharashtra (Pune Division).

The testing data set of 150 lateral cephalograms was independent of the ones used for training the model to ensure unbiased validation. The testing sample size was adequate for statistical validation, as supported by previous studies [5,6,8]. The validation was performed by comparing these results of 150 unused lateral cephalograms with corresponding hand-wrist radiographs using the Tanner-Whitehouse 3 (TW3) method (gold standard) [2].

The present study focused on building a machine learning algorithm aimed at streamlining the process of estimating CVBA from C3 and C4 vertebrae with regression equations that are already recognised for the Maharashtra demographic [7]. The C3 and C4 vertebrae were selected as they undergo shape changes consistently as growth occurs and at the same time, they're not obscured with the thyroid collar unlike other vertebrae on the lateral cephalogram [4]. The stepwise multiple linear regression formula derived by Gaonkar P et al., was as follows [7]:

Regression formula for girls: $\text{Age} = 100.121 + (11.295 \times \text{MH3}/\text{AP3 width}) - (153.759 \times \text{PH3}/\text{AP3 width}) + (56.820 \times \text{AH3}/\text{PH3}) + (28.765 \times \text{DoC}/\text{MH C3}) + (194.916 \times \text{AH4}/\text{AP4 width}) - (181.686 \times \text{AH4}/\text{PH4}) - (11.520 \times \text{DoC}/\text{MH C4})$.

Regression formula for boys: $\text{Age} = 122.732 - (6.185 \times \text{PH3}/\text{AP3 width}) + (78.166 \times \text{AH3}/\text{PH3}) - (0.781 \times \text{AH3}/\text{MH3}) + (43.570 \times \text{DoC}/\text{MH C3}) + (234.269 \times \text{AH4}/\text{AP4 width}) - (214.673 \times \text{MH4}/\text{AP4 width}) + (19.423 \times \text{PH4}/\text{AP4 width}) - (17.892 \times \text{AH4}/\text{PH4}) - (179.700 \times \text{AH4}/\text{MH4}) - (16.789 \times \text{DoC}/\text{MH C4}) - (19.963 \times \text{AH3}/\text{AP3 width}) + (16.670 \times \text{MH3}/\text{AP3 width})$.

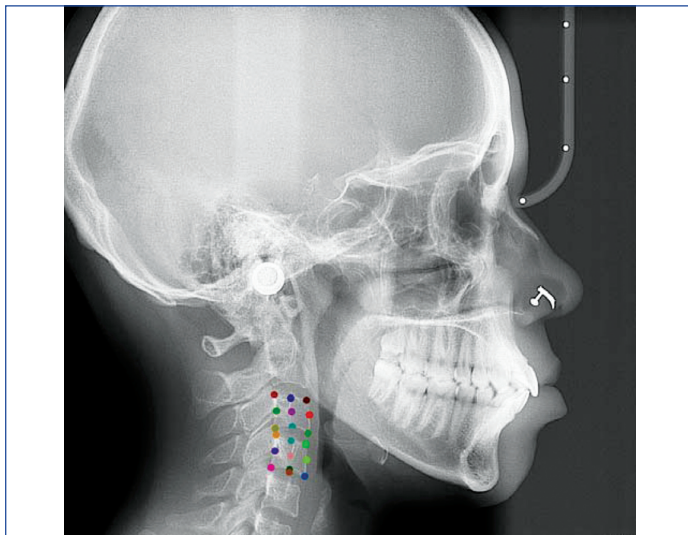
All the images were standardised to a size of 648×648 pixels so that the neural network could have consistent input [Table/Fig-1]. This particular resolution was picked to enhance the detection of small vertebral details while also keeping things efficient on the computational side.



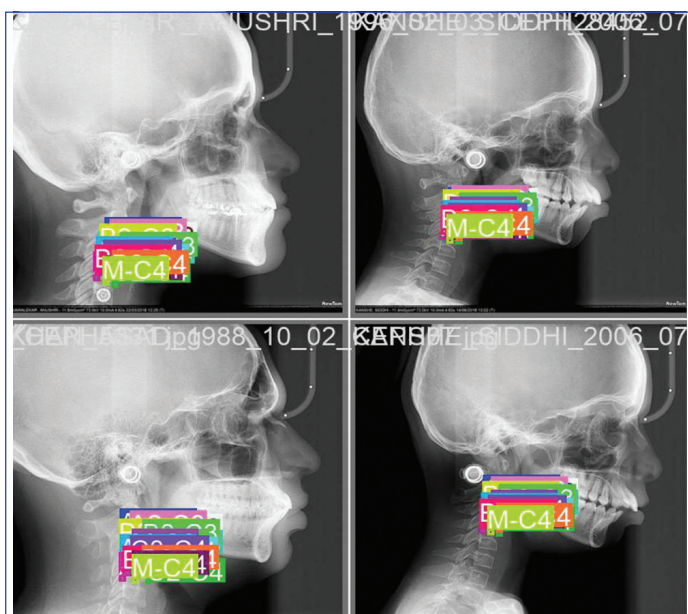
[Table/Fig-1]: Standardisation and model training sample.

The You Only Look Once (YOLO) v11 architecture was utilised as the core element of the detection system [9]. Accurate ground-truth data was generated through the annotation of the vertices and margins of the C3 and C4 vertebrae using the LabelME software [Table/Fig-2] [10]. Landmark accuracy was guaranteed by a double check of two different researchers; any discrepancies in the coordinates were settled after a consensus-based review

with a senior researcher acting as the “gold-standard” truth. ICC analysis was conducted to quantitatively verify this process. To strengthen the model and mitigate overfitting, data augmentation methods—including random rotations, shearing, and modifications to luminance and contrast—were employed on the training dataset. Model refinement was conducted using a Stochastic Gradient Descent (SGD) optimiser, initialised with a learning rate set to 0.01, momentum as 0.937, and weight decay=0.0005 to ensure stable convergence and avoid overfitting of the network. For improved noise robustness, the training pipeline included a number of data augmentation parameters: rotations of $\pm 10^\circ$ to simulate pose changes and shearing of $\pm 2.0^\circ$ to introduce perspective effects. In addition, brightness and contrast were randomly changed up to 50% to simulate the fluctuation of kVp and mAs settings in X-ray exposure, while a horizontal flip (with probability 0.5) was performed to obtain symmetry invariance. These technical choices lead to a model that is both very accurate and robust against the variations met in clinical practice. During the training phase, the network’s internal parameters were methodically refined to reliably predict bounding boxes with high confidence scores, facilitating precise localisation of the spatial coordinates (x, y) and dimensions (w, h) of vertebral landmarks [Table/Fig-3].



[Table/Fig-2]: Landmark annotations on LabelMe software.



[Table/Fig-3]: Localisation of the spatial coordinates and dimensions of vertebral landmarks.

The computational framework was implemented in Python, using the Ultralytics engine to unify data preparation and model training. The pipeline started with a user-defined LabelME 2 YOLO script,

which processed JSON (JavaScript Object Notation) metadata of LabelME programmatically into standardised YOLO TXT. This script carried out the deterministic 1,000:150:150 dataset split and ensured that class maps for vertebral landmarks were consistent across subsets. After preparing the data, the program initialised training with the PyTorch-based YOLOv11 framework using the default hyperparameters of the pre-trained model included in Ultralytics documentation. Since the weights were pre-trained and already had strong feature-extraction capability, these default settings (such as the SGD optimiser, standard training anchors, etc.) achieved perfect convergence without manual experimental modification.

After detection, the output of the model was sent to a post-process module for automatic linear measurements and ratios [Table/Fig-4]. After the midpoints of the found bounding boxes were calculated, using these as landmarks, it was possible to use Euclidean distance formulas to calculate vertebral body height, width, and depth of concavity. These quantitative measurements were then entered into gender-adjusted regression models to determine bone age. The algorithm was then tested with 150 independent lateral cephalograms.

Ratios	Parameters assessed
AH/AP	Anterior height/anteroposterior width
MH/AP	Middle height/anteroposterior width
PH/AP	Posterior height/anteroposterior width
AH/PH	Anterior height/posterior height
AH/MH	Anterior height/middle height
MH/PH	Middle height/posterior height
CONC/MH	Depth of concavity/middle height

[Table/Fig-4]: Ratios calculated.

Ultimately, the validation was obtained by comparing 150 unseen unique lateral cephalograms to the TW3 hand-wrist method, which is considered the established benchmark [3].

STATISTICAL ANALYSIS

The data was subjected to statistical analysis in Statistical Package for the Social Sciences (SPSS) v23.0. Since the data demonstrated a non normal distribution, non parametric and parametric statistical techniques- including the Mann-Whitney U test, ICC, and Spearman’s rank correlation were employed to assess the model’s reliability and precision. The level of significance was set below 0.05.

RESULTS

The mean age estimated using AI technology and hand wrist age for the entire sample was 13.04 ± 1.95 and 12.78 ± 1.82 years, respectively, and there was a non significant difference in the age estimated by the two methods. Specifically, it revealed no statistically significant difference between the mean estimated age provided by the AI and calculated based on the widely accepted TW3 manual method ($p=0.393$ for males and $p=0.656$ for females), confirming that CVBA formula showed objective reliability in both genders [Table/Fig-5]. These results are also consistent with expected sexual dimorphism in cross-sectional growth studies and reflect a lower mean age for females who consistently reached maturation markers earlier than their male counterparts. On examination of the outliers, it was discovered that the circumpubertal CS3 to CS4 period had provided the highest challenge, as anatomical transitions occurred at different rates; this age range equated approximately to 10.5-13 years for girls and 12-14.5 years for boys.

The consensus was analysed between age estimated from AI and hand-wrist age (TW3 method) for males and females [Table/Fig-6]. The agreement between the male, female, and total measurements was excellent, with an ICC of 0.988 (0.978-0.994) (males), 0.995

Method	Mean	Range	Difference	p-value
AI-based age - (Overall)	13.04±1.95	9-20	0.26	0.929
Hand wrist age (TW3 method)- (Overall)	12.78±1.82	9-19.80		
AI-based age-Males	13.52±2.14	9.0-20.0	0.18	0.393
Hand wrist age (TW3 method)- Males	13.34±2.02	9.4-19.8		
AI-based age-Females	12.18±1.86	9.0-20.0	0.09	0.656
Hand wrist age (TW3 method)- Females	12.09±1.74	9.0-19.5		

[Table/Fig-5]: Comparison of the age (in years) estimated by two methods using the Mann-Whitney U test.

(0.988-0.998) (females), and for all subjects together, 0.994 (95% CI: 0.990 to 0.996).

Method	ICC value	Interpretation
Male	0.988 (0.978-0.994)	Almost perfect agreement
Female	0.995 (0.988-0.998)	Almost perfect agreement
AI-based age vs Hand wrist age (TW3 method)	0.994 (0.990-0.996)	Almost perfect agreement

[Table/Fig-6]: Assessment of the agreement between the age estimated by two methods using Intraclass Correlation Coefficient (ICC).

The factor determining correlation between AI-based estimated age and hand-wrist age (TW3 method) is analysed in [Table/Fig-7]. Spearman's rank correlation coefficient demonstrated a very strong statistically significant positive association between AI-estimated age and TW3 hand-wrist age in males ($p=0.936$), females ($p=0.987$), and overall ($p=0.974$) with p-values <0.001 . This means that as the skeletal age increases based on the TW3 method, the AI-estimated age will also consistently increase.

Correlation	Spearman's rho	p-value
Male	0.936	$<0.001^*$
Female	0.987	$<0.001^*$
AI-based age vs Hand wrist age (TW3 method)	0.974	$<0.001^*$

[Table/Fig-7]: Assessment of the correlation between the age estimated by two methods using Spearman rank correlation test.

Taken together, the results corroborate that the AI model shows extremely good reliability (agreement) and validity (correlation) compared to existing TW3 hand-wrist age determination method, indicating its probable clinical applicability for age estimation in both genders.

DISCUSSION

The average age of AI technology's age estimate (13.04 ± 1.95 years) was very close to TW3's hand-wrist method average estimated age (12.78 ± 1.82 years). This was consistent across the genders, where there were also no statistically significant differences between AI-derived age for males (13.52 ± 2.14) and for females (12.18 ± 1.86) compared to TW3 age estimates for males (13.34 ± 2.02 , $p=0.393$) as well as females (12.09 ± 1.74 , $p=0.656$). This commonality of means helps to corroborate the viability of the AI algorithm as a consistent measure for bone age. The AI system does not systematically over- or underestimate age relative to the gold standard, gives us confidence in its average ability to predict. The parity between the AI's average estimates and the manual gold standard is consistent with findings by Larson DB et al., who demonstrated that deep learning neural networks can achieve accuracy levels comparable to, or exceeding, senior paediatric radiologists when assessing skeletal age [11]. Their research highlighted that automated models effectively eliminate the systematic bias often found in human observation, providing a standardised output that remains stable across varied datasets. Similarly, Kim JR et al., noted that automated systems derived from validated methods like TW3 provide a high

degree of confidence in average ability to predict age because they are anchored in biologically sound maturation stages rather than chronological estimations [12].

Further bolstering the reliability of the AI algorithm, an almost perfect agreement between AI-estimated age and TW3 hand-wrist age was observed, as evidenced by an ICC of 0.994. This ICC of around 1.0 indicates that not only the AI method yields similar average values but also assesses participants in a very consistent order at which, with respect to maturational status, they stand next to each other as does the TW3 method by doing so [13]. This "almost perfect agreement" is important for a single person use of the AI system as substitute to TW3 method. Consistent with this this study presents a very good positive correlation between the two methods (0.974; Spearman's rho). Such strong correlation confirms the high stability and predictability of the AI model.

These findings are supported by the work of Spampinato C et al., who argued that for an AI tool to be clinically useful, it must not only produce similar mean values but must also correctly rank-order participants according to their biological maturational status [14]. Similar results are crucial for verifying each new tool and approach to skeletal age assessment, as they demonstrate that the biological maturity captured by the AI algorithm is consistent with that of weight bearing regions in human bones, which is measured using the traditional method [15-17]. The potential utility of AI to objectively determine bone age with such a high degree of agreement and correlation, further translates in time saving and reduction in observer variability associated with manual methods [17].

In orthodontic AI research, the increasing focus on the morphological characteristics of the C3 and C4 vertebrae has a theoretical basis that is consistent with our algorithm decision. As an illustration, Sadeghi TS et al., trained deep learning arms (VGG16 specifically) to automate cervical maturity staging; the study showcased that AI could reach overlapping precision with experienced clinicians while significantly reducing subjectivity that accompanies human visual inspection [18]. This objective view is further evidenced by Makaremi M et al., who proved that Convolutional Neural Networks (CNNs) had high reliability in accurately classifying maturation stages on lateral cephalograms [19].

The feasibility of these automated measurement protocols is well-established and was shown by K  k H et al., that complex AI architectures performed better than conventional techniques in terms of processing time and inter-rater reliability [20]. These outcomes are consistent with those in our study, where automation of CVBA estimation markedly reduced observer variability. Moreover, the multi-factorial approach proposed by Guo M et al., incorporating different parameters of maturity, correlates well with our approach, where biological sex was used as an important variable to improve vertebral assessments predicting overall age [21]. Together, these studies provide evidence validating our approach of employing targeted vertebral data (C3 and C4) to yield high-resolution bone age estimates that are statistically comparable to standard validated hand-wrist reference standards.

This AI-powered CVBA algorithm provides clinical measurable application to orthodontic diagnosis with its high precision and radiation efficiency, as the skeletal maturity problem can be detected from standard lateral cephalograms directly on C3 and C4 vertebrae. Providing a consistent, uncomplicated measure of bone age, the system allows clinicians to identify the best biological window for growth modulation and functional appliance therapy without increasing the amount of ionising radiation associated with hand-wrist X-ray images. Moreover, the strong agreement with the established gold standard ensures that diagnostic decisions are objective and standardised, eliminating both inter-observer subjectivity as well as nudging time-consuming manual staging to a background task. Moving to this automated, metric-based

skeletal assessment approach enables more effective and efficient longitudinal monitoring as well as having a better estimate of treatment outcome during daily orthodontic practice.

The current study represents a foundational phase of an extensive, multistage project focused on establishing the reliability of automated measurement protocols against the TW3 gold standard. While the primary objective was to validate our integrated Python-based framework and gender-linked regression models, this focused scope ensures the internal precision of the algorithm before expanding to broader comparative analyses with other contemporary AI architectures.

Limitation(s)

A primary limitation is the lack of direct benchmarking against other AI systems, as this initial phase of an extensive project focused solely on validating the novel framework against the TW3 gold standard. The use of a localised dataset and 2D lateral cephalograms introduces constraints related to ethnic generalisability and geometric “noise” from radiographic positioning. Furthermore, the circumpubertal period (CS3 to CS4) presented a specific mathematical complexity due to rapid morphological changes, making precise age estimation more challenging than in stable adult stages.

CONCLUSION(S)

The present study shows that fully automatic algorithm is a highly efficient and standardised method to determine CVBA in clinical settings. In addition, the proposed framework serves as basis for future research of CVBA estimation methods that can be extrapolated to diverse global populations. Providing a highly accurate tool to clinicians, such technologies can potentially improve the accuracy of growth modulation interventions and increase overall diagnostic reliability.

Future research must focus on its validation in various ethnic population samples and different demographic factors to validate its global application; variable parameters such as different skeletal maturation patterns can also be tested. Moreover, longitudinal studies can be performed to assess the performance of this algorithm in monitoring actual growth changes throughout orthodontic treatment. Combining this vertebral assessment with additional automated signatures of bio-age diagnosis, such as enamel mineralisation stages or AI-based soft tissue assessments, can support a more comprehensive, multi-faceted biological profile. Finally, further research could investigate how this framework can be integrated into cloud-based orthodontic software, thus leading the integration of the system for a transition to be considered as a standard real-time chairside diagnostic tool.

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