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Linking vertebral trabecular structure to growth rate: clinical and developmental insights

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Abstract

Objectives To evaluate the effectiveness of chronological age (CA), sex, and fractal dimension (FD) values in classifying skeletal maturation stages using multinomial logistic regression analysis.

Materials and methods Lateral cephalometric radiographs and hand-wrist radiographs (HWRs) of 923 individuals (409 boys and 514 girls) aged 7–18 years were analyzed. Nine regions of interest located in the C2, C3, and C4 vertebral bodies were selected for the fractal analysis. Skeletal maturation stages were determined using Fishman's skeletal maturity indicator (SMI) method and categorized into accelerating ($n=201$; 141 boys and 60 girls), high ($n=334$; 165 boys and 169 girls), and decreasing ($n=388$; 103 boys and 285 girls) growth velocity groups. Multinomial logistic regression was used to evaluate the associations of CA, sex, and FD values with maturation group membership. Relative risk ratios (RRRs) and 95% confidence intervals (CIs) were calculated to assess the effects of CA, sex, and FD values on maturation stage classification.

Results The full model was statistically significant compared with the intercept-only model and achieved an overall apparent classification accuracy of 77.8%. The age-and-sex-only model had a Nagelkerke R^2 of 0.768 and an accuracy of 77.2%, whereas the full model including FD variables had a Nagelkerke R^2 of 0.779 and an accuracy of 77.8%. These results indicate that FD measurements provided a modest additional contribution beyond CA and sex. CA and sex were the most stable predictors. Among FD variables, C2 anterior FD showed a significant overall effect, while C4 medial and C4 posterior FD showed more limited, category-specific associations.

Conclusion CA and sex were the primary predictors of maturation group membership. FD values from selected cervical vertebral regions may provide supplementary information, but their contribution was modest and should be interpreted cautiously until externally validated.

Clinical relevance Cervical vertebral fractal analysis may provide supplementary information for skeletal maturation assessment when combined with chronological age and sex; however, external validation is required.

Keywords Algorithms, Fractals, Growth and development, Machine learning, Orthodontics, Vertebrae

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Introduction

Assessing growth potential during pre-adolescence and adolescence is crucial and various indicators such as body height and weight, sexual maturation, chronological age (CA), dental development and skeletal development can be used to identify growth stages. The identification of an individual's growth and development stage has a significant impact on the diagnosis, treatment planning and treatment outcome of orthodontic treatment [1].

Hand-wrist radiographs (HWRs) are the gold standard and are widely regarded as the most reliable approach for determining skeletal maturity in orthodontics. Fishman established an eleven-stage system for evaluating skeletal maturation based on "Skeletal maturity indicators" (SMIs). This system spans the entire pubertal growth period by assessing epiphyseal and diaphyseal ossification through hand-wrist radiographs (HWRs) [2, 3].

Another commonly used method is cervical vertebral maturation (CVM), which involves examining the maturation stage of the cervical vertebrae [4, 5]. Radiographic exposure should always be minimized. HWRs in orthodontics should be used when the information provided is crucial for treatment planning and cannot be obtained through other methods [6]. Previous studies have shown a significant correlation between hand-wrist maturation (HWM) and CVM stages [7, 8]. It has also been reported that CVM is reliable in predicting pubertal development and correlates strongly with the HWM method in predicting skeletal maturation throughout adolescence [9, 10].

Fractal dimension (FD) analysis simplifies the complex structure of an image into an FD value using several image processing techniques. FD analysis can evaluate changes in the trabecular bone. The FD increases as the complexity of the bone pattern increases, and decreases as the complexity of the bone pattern decreases [11]. Bone development involves changes in mineralization, size, shape, and degree. Morphological changes in the cervical vertebrae are indicative of skeletal maturation [7, 12]. In addition to these changes, FD analysis has been employed to assess pubertal growth and maturation stages [7, 12, 13]. In this study, FD analysis was to measure the complexity of trabecular bone. This approach was chosen because it is cost-effective and does not require additional irradiation time. Few orthodontic studies have used FD analysis to determine bone maturation stages on lateral cephalometric radiographs (LCRs) [7, 12].

Although previous studies have investigated the relationship between FD analysis and skeletal maturation and reported associations between cervical vertebral FD measurements and maturation stages, most have primarily focused on correlations between FD values and maturation stages [7, 12]. The literature on predictive models based on cervical vertebral trabecular structures obtained from lateral cephalometric radiographs is limited. More importantly, there is insufficient evidence on whether regional cervical vertebral FD measurements provide additional predictive information beyond established demographic variables such as CA and sex. To our knowledge, no previous study has evaluated the combined use of CA, sex, and regional cervical vertebral FD values within a multinomial logistic regression framework to classify skeletal maturation stages according to growth velocity patterns.

Therefore, this study aimed to develop and evaluate a multinomial logistic regression model using CA, sex, and cervical vertebral FD measurements to classify skeletal maturation stages according to growth velocity patterns. We hypothesized that regional cervical vertebral FD measurements would vary across maturation stages and provide additional information beyond CA and sex for skeletal maturation stage classification.

Materials and methods

Study design

This retrospective study was approved by the Research Ethics Committee of Recep Tayyip Erdoğan University (decision date and number: 24.04.2024/78) and involved LCRs and HWRs of patients referred for orthodontic treatment at the Department of Orthodontics, Faculty of Dentistry, Recep Tayyip Erdoğan University.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments [14]. At the beginning of treatment, consent forms explicitly stating the use of patient data in scientific investigations were checked. The study included patients who met specific criteria: Turkish ethnicity, aged between 7 and 18 years, with high-quality LCRs and HWRs, normal growth and development, no systemic disease, no congenital deformities, no bone syndromes, no previous hand-wrist or cervical region injuries, and good nutrition without serious illness. LCRs and HWRs were taken on the same day, and all LCRs with a clear view of the cervical spine (C2-C4) and sufficient quality were included in the study.

LCRs and HWRs were obtained using a Planmeca Promax 2D S2 imaging unit (Planmeca Oy, Helsinki, Finland) with specific exposure settings (66 kVp, 10 mA, 10.5 s for LCRs, and 60 kVp, 4 mA, and 10.5 s for HWRs). For LCR acquisition, ear rods and nasal support were used to stabilize the head. The Frankfort horizontal plane was aligned parallel to the floor. The HWRs were obtained using a standardized method, with the left hand in contact with the sensor and the long axis of the middle finger aligned parallel to the forearm. The fingers were spread apart, and the thumb was positioned at an angle

of approximately 30° to the forefinger. The X-ray tube was positioned at the center of the third metacarpal.

We examined the LCRs and HWRs of 1383 patients and excluded the radiographs of 460 patients who did not meet the study requirements. We analyzed 923 LCRs and HWRs of untreated patients (409 boys and 514 girls). A detailed patient selection flowchart illustrating the eligibility assessment, exclusion reasons, and final study inclusion is presented in Fig. 1.

Study group

The target variables were identified using Fishman's 11-stage SMI method, with only the left HWRs included in the analysis [3]. The SMI stages are as follows:

- The width of the epiphysis is equal to the width of the diaphysis in the following: (1) proximal phalanx of the third finger (PP3), (2) middle phalanx of the third finger (MP3), and (3) middle phalanx of the fifth finger (MP5).
- (4) Ossification of the adductor sesamoid bone in the thumb.
- Epiphyseal capping: (5) capping of the distal phalanx of the third finger (DP3), (6) MP3 capping, and (7) MP5 capping.
- Fusion of the epiphysis and diaphysis: (8) DP3 U, (9) PP3 U, (10) MP3 U, and (11) radius.

In this study, the target variable was divided into three distinct groups, defined as follows:

SMI stages 1–3 represent the group with accelerating growth velocity (Group 1), SMI stages 4–7 represent the group with high growth velocity (Group 2), and SMI stages 8–11 represent the group with decreasing velocity

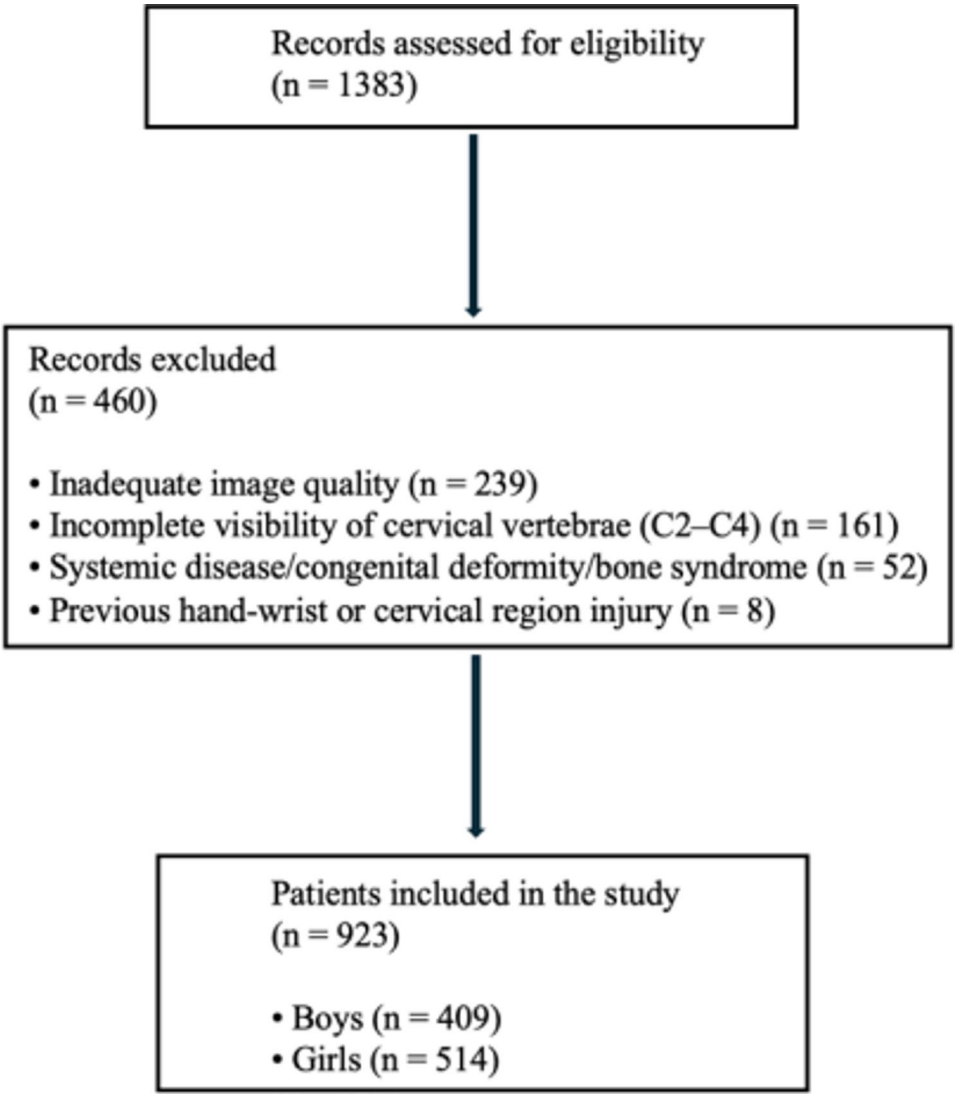


Fig. 1 Patient selection flowchart illustrating eligibility assessment, exclusion reasons, and final study inclusion

(Group 3). The 11 SMI stages were grouped into three growth velocity categories to represent clinically meaningful phases of skeletal maturation. This approach was adopted because the primary objective of the present study was not to predict individual SMI stages but to classify biologically relevant growth velocity patterns. Grouping the stages into accelerating, high, and decreasing growth velocity phases simplifies clinical interpretation while preserving the overall progression of skeletal maturation described by Fishman's method [2, 3]. An orthodontist with 10 years of clinical experience (MG) evaluated and determined SMI stages.

Measurement of fractal dimension

The LCRs in this study had an X-ray resolution of 2093 × 1937 pixels. A radiologist (DNG), blinded to age and sex, conducted FD analysis on the LCRs using the box-counting tool of ImageJ v1.52 software (National Institutes of Health, Bethesda, United States). Figure 2 shows all FD areas. Except for the cortical margins, nine square regions of interest (ROIs) (30 × 30 pixels) were selected for the FD analysis of the LCRs. Three ROIs were selected from each cervical vertebra, including the anterior, medial, and posterior trabecular regions of the C2, C3, and C4 vertebral bodies. ROI placement was standardized by using the same ROI size for all measurements and positioning each ROI within the trabecular bone area of the vertebral body. Cortical margins, cortical outlines, and visually dense cortical areas were avoided as much as possible.

The FD value of each area was calculated using the box-counting method of White and Rudolph [15]. The selection technique included rotating the LCRs to acquirely acquire the ROIs. The selected ROIs were duplicated and blurred using a Gaussian Blur filter (σ = 35 pixels). A Gaussian blur step was applied to reduce the high-frequency image noise and brightness variations while preserving the overall trabecular pattern. This preprocessing procedure improves the stability of FD calculations by minimizing the influence of nonstructural image artifacts. This method effectively reduced high and medium brightness variations caused by the variable thickness of the superficial soft tissues overlying the bone. The blurred image was subtracted from the original image, and a gray value of 128 was added to each pixel to normalize the grayscale distribution and enhance the contrast between the trabecular bone and marrow spaces. This step was performed prior to binarization to improve the consistency of the images across all radiographs. Subsequently, the "Threshold" tool was applied to convert the processed image into a binary (black-and-white) image, enabling a clear separation of trabecular bone structures and marrow spaces. The "Erode" option reduced image noise, while the "Dilate" option expanded existing structures and improved visibility. To make the trabecular bone stand out, the "Invert" option made the white areas darker and the black areas white. The trabecular structure was shown as skeleton lines and made ready for fractal analysis using the "Skeleton" option [15]. After completing image processing (Fig. 3), the FD values were calculated and used as input variables for the models. FD analysis was conducted by an oral and maxillofacial radiologist (DNG) with 8 years of experience who was blinded to patient information to ensure an unbiased assessment.



Fig. 2 Demonstration of standardized region of interest (ROI) selection on lateral cephalometric radiographs. The images show the original lateral cephalometric radiograph (LCR), rotated radiograph in which the cervical vertebrae were aligned perpendicular to the ground plane, and standardized ROI placement on the C2, C3, and C4 vertebral bodies. Three square ROIs measuring 30 × 30 pixels were placed on each vertebra, corresponding to the anterior, medial, and posterior trabecular regions of vertebrae. The cortical margins and visually dense cortical areas were avoided as much as possible during ROI placement. The anterior, medial, and posterior the ROIs are represented by orange, green, and blue, respectively

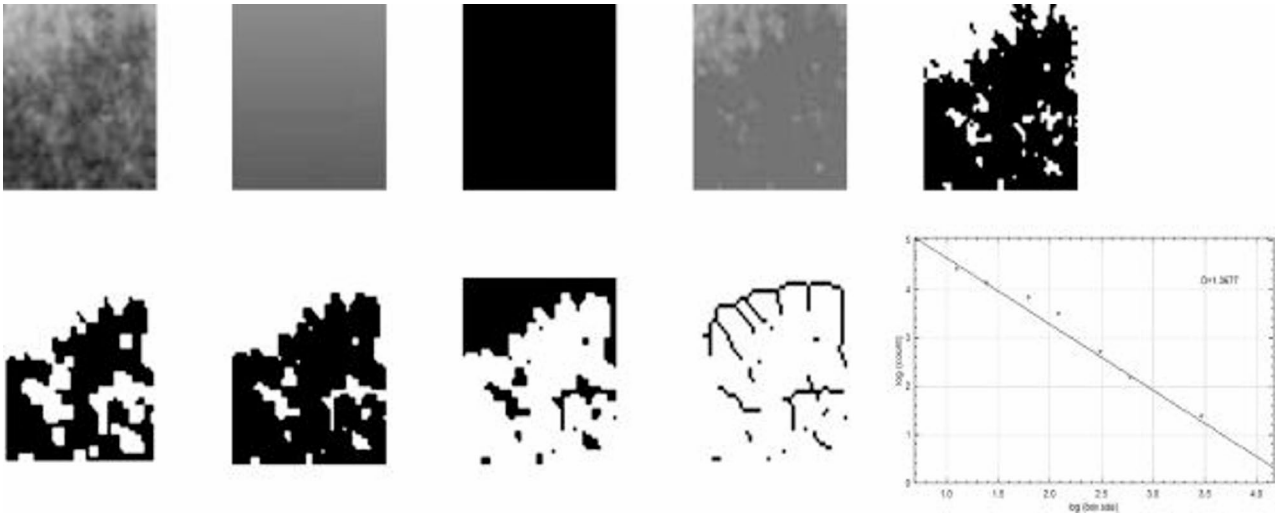


Fig. 3 Workflow of the fractal dimension (FD) analysis performed using ImageJ software on selected regions of interest (ROIs). The top row illustrates the image processing steps, including the duplicated image, Gaussian-blurred image, subtracted image, grayscale-normalized image (gray value of 128 added), and thresholded binary image. The bottom row shows the eroded, dilated, inverted, skeletonized, and the final fractal dimension calculated using the box-counting method

Measurement error

Measurements were repeated in 350 randomly selected patients one month after the initial evaluation. For intra-observer reliability assessment, SMI staging was repeated by the first orthodontist, and FD measurements were repeated by the oral and maxillofacial radiologist who performed the initial evaluations. For inter-observer reliability assessment, SMI stages were independently evaluated by the first orthodontist, a second orthodontist, and two oral and maxillofacial radiologists. FD measurements were independently evaluated by oral and maxillofacial radiologist and a second orthodontist. ICC values were calculated to assess both intra- and inter-observer agreement.

Statistical analysis

Sample size calculations were performed using G*Power software version 3.1.9.7. The calculation was based on the z-test family for logistic regression with a two-tailed test. Based on the reference study, the probability of membership in Group 1 was set to 0.311, and the odds ratio was set to 2.653. With an alpha error probability of 0.05 and statistical power of 0.95, the minimum required sample size was calculated as 235 participants [16]. The final study sample included 923 individuals, exceeding the minimum required sample size. A post-hoc power analysis based on the final sample size indicated a statistical power of 99.99%.

Statistical analyses were performed using IBM SPSS Statistics (version 25). Descriptive statistics were calculated for the CA and FD values according to maturation group and sex. Multinomial logistic regression was used to examine the associations of CA, sex, and FD measurements with maturation group membership [17–19]. The dependent variable consisted of three maturation groups: CA and FD measurements were entered as covariates, and sex was used as a categorical predictor. Female sex was used as the reference category, and Group 3 was used as the reference outcome category in SPSS output. Relative risk ratios (RRRs), 95% confidence intervals, and p-values were reported. Statistical significance was set at $p < 0.05$.

To evaluate the additional contribution of FD variables, a reduced model including only CA and sex was compared with the full model including CA, sex, and all FD variables. The model performance was evaluated using likelihood ratio tests, pseudo-R^2 values, and classification accuracy. Pearson and deviance goodness-of-fit tests were reported; however, these statistics were interpreted cautiously because sparse subpopulations may occur when continuous predictors are included.

Multicollinearity among the independent variables was assessed using tolerance and variance inflation factor (VIF) values. Ordinal logistic regression was performed as a sensitivity analysis to examine the consistency of the results with the ordinal structure of the dependent variable.

Results

The intra- and inter-observer reliability of the SMI stage were found as 0.948 (95% CI: 0.915 to 0.981) and 0.888 (95% CI: 0.854 to 0.922) with perfect reliability, respectively [20]. The intra- and inter-observer reliability of FD values were 0.975 (95% CI: 0.969 to 0.979) and 0.966 (95% CI: 0.959 to 0.973), respectively with perfect reliability [21].

Table 1 summarizes the CA and FD values according to maturation group and sex. CA increased across the maturation groups, as expected from SMI-based grouping. FD values showed smaller and region-specific differences across groups, suggesting that trabecular complexity may vary by vertebral region rather than following a uniform, linear pattern.

Table 2 presents the results of the multinomial logistic regression. The full model was statistically significant compared to the intercept-only model, indicating that the included predictors improved the explanation of maturation group membership. In the full sample, the model had a Nagelkerke R^2 of 0.779 and an overall apparent classification accuracy of 77.8%.

Pearson and deviance goodness-of-fit tests did not indicate a statistically significant lack-of-fit. However, these results were not interpreted as evidence of a perfect model fit, particularly because sparse subpopulations were observed in the SPSS output. Therefore, model adequacy was evaluated together with likelihood ratio tests, pseudo-R^2 values, classification results, and sensitivity analyses. The Nagelkerke R^2 value for the full model was 0.779. In the likelihood ratio tests, CA and sex were the most stable predictors of maturation group membership. Among the FD variables, C2 anterior FD had a statistically significant overall effect. C4 medial and C4 posterior FD did not reach statistical significance as overall effects in the likelihood ratio tests but showed category-specific associations in the parameter estimates. Therefore, FD-related findings were interpreted as region-specific and supplementary, rather than as primary predictors.

To examine the additional contribution of FD variables, a reduced model including only CA and sex was compared with the full model including CA, sex, and FD measurements (Table 3). The age- and sex-only model had a Nagelkerke R^2 of 0.768 and an overall accuracy of 77.2%.

Table 1 Descriptive statistics of CA and FD values by group and sex

		Group 1 (n=201) (male=141, female=60)		Group 2 (n=334) (male=165, female=169)		Group 3 (n=388) (male=103, female=285)	
		mean	SD	mean	SD	mean	SD
CA	(male)	10.88	1.53	13.39	1.30	15.75	1.15
CA	(female)	9.65	1.27	11.58	0.94	14.29	1.73
CA	(total)	10.51	1.56	12.47	1.45	14.68	1.72
C2_anterior FD	(male)	1.343	0.084	1.348	0.087	1.378	0.081
C2_anterior FD	(female)	1.306	0.090	1.316	0.094	1.330	0.102
C2_anterior FD	(total)	1.332	0.088	1.332	0.092	1.343	0.099
C2_medial FD	(male)	1.302	0.082	1.295	0.086	1.297	0.085
C2_medial FD	(female)	1.299	0.090	1.278	0.088	1.250	0.093
C2_medial FD	(total)	1.301	0.084	1.286	0.087	1.262	0.093
C2_posterior FD	(male)	1.269	0.104	1.270	0.099	1.290	0.104
C2_posterior FD	(female)	1.242	0.089	1.259	0.100	1.243	0.096
C2_posterior FD	(total)	1.261	0.100	1.264	0.100	1.255	0.100
C3_anterior FD	(male)	1.377	0.067	1.382	0.064	1.372	0.076
C3_anterior FD	(female)	1.370	0.071	1.361	0.073	1.341	0.086
C3_anterior FD	(total)	1.375	0.068	1.371	0.070	1.349	0.085
C3_medial FD	(male)	1.333	0.083	1.313	0.083	1.325	0.072
C3_medial FD	(female)	1.304	0.099	1.307	0.089	1.302	0.088
C3_medial FD	(total)	1.324	0.089	1.310	0.086	1.308	0.085
C3_posterior FD	(male)	1.224	0.108	1.232	0.100	1.251	0.111
C3_posterior FD	(female)	1.230	0.094	1.247	0.098	1.239	0.101
C3_posterior FD	(total)	1.226	0.104	1.239	0.099	1.242	0.104
C4_anterior FD	(male)	1.308	0.102	1.324	0.095	1.338	0.086
C4_anterior FD	(female)	1.286	0.097	1.288	0.098	1.277	0.106
C4_anterior FD	(total)	1.302	0.101	1.306	0.098	1.293	0.105
C4_medial FD	(male)	1.311	0.089	1.320	0.077	1.299	0.081
C4_medial FD	(female)	1.310	0.086	1.297	0.082	1.280	0.087
C4_medial FD	(total)	1.311	0.088	1.309	0.080	1.285	0.086
C4_posterior FD	(male)	1.229	0.110	1.254	0.100	1.241	0.104
C4_posterior FD	(female)	1.225	0.103	1.236	0.100	1.228	0.103
C4_posterior FD	(total)	1.228	0.107	1.245	0.100	1.232	0.103

FD: fractal dimension; CA: chronological age; SD: standard deviation; Group 1 is defined as the accelerating velocity group; Group 2 as the high-growth velocity group; and Group 3 as the decreasing velocity group

Table 2 Multinomial logistic regression model summary and diagnostic results

Analysis component	Result
Final model vs. intercept-only model	$\chi^2 = 1068.827$, df = 22, $p < 0.001$
Full model Nagelkerke R^2	0.779
Overall apparent classification accuracy	77.8%
Group 1 accuracy	73.1%
Group 2 accuracy	74.9%
Group 3 accuracy	82.7%
Significant predictors in likelihood-ratio tests	Age, sex, C2 anterior FD
FD variables with category-specific associations	C4 medial FD, C4 posterior FD
VIF range	1.040–1.137
Tolerance range	0.879–0.961
Ordinal sensitivity analysis	Nagelkerke R^2 = 0.773; test of parallel lines $p = 0.204$

FD: fractal dimension; VIF: variance inflation factor. Classification accuracy refers to the apparent classification performance of the analytic sample. C4 medial FD and C4 posterior FD did not reach statistical significance as overall effects in the likelihood ratio tests but showed category-specific associations in the parameter estimates.

Table 3 Comparison of reduced and full multinomial logistic regression models

Model	Predictors	Nagelkerke R^2	Overall apparent accuracy
Reduced model	CA + sex	0.768	77.2%
Full model	CA + sex + FD variables	0.779	77.8%

CA: Chronological age; FD: Fractal dimension. The accuracy values represent the apparent classification performance of the analytic sample

After adding FD variables, Nagelkerke R^2 increased to 0.779, and the overall accuracy increased to 77.8%. This finding indicates that FD measurements provided a modest additional contribution beyond CA and sex.

The detailed multinomial logistic regression equations are presented in the Supplementary Material. The parameter estimates showed that chronological age and sex were the most consistent predictors of maturation group membership. As expected, older individuals were more likely to be classified into more advanced maturation group than younger individuals. Male patients were less likely than female patients to be in the advanced maturation groups, which is consistent with the earlier skeletal maturation pattern generally observed in females.

Among the FD variables, C2 anterior FD showed the clearest association with maturation group membership. C4 medial FD and C4 posterior FD also showed some category-specific associations, but these effects were more limited. Overall, the contribution of FD measurements was modest compared with chronological age and sex. Therefore, FD measurements should be considered supplementary indicators rather than primary predictors of skeletal maturation stage. Because FD values were distributed within a narrow numerical range and some FD-related confidence intervals were relatively wide, these effects were interpreted cautiously. Therefore, we placed greater emphasis on the direction and clinical significance of the associations rather than on the isolated interpretation of individual log-odds coefficients.

Multicollinearity diagnostics showed no evidence of problematic multicollinearity among the independent variables. The tolerance values ranged from 0.879 to 0.961, and the VIF values ranged from 1.040 to 1.137, indicating that multicollinearity was unlikely to materially affect the regression estimates.

Ordinal logistic regression was performed for sensitivity analysis. The model was statistically significant and showed comparable explanatory performance with a Nagelkerke R^2 of 0.773. The test of parallel lines was not significant ($p = 0.204$), indicating that the proportional odds assumption had not been violated. Nevertheless, multinomial logistic regression was retained as the primary analysis because it allowed category-specific interpretation of FD effects across maturation group comparisons.

Discussion

This study evaluated whether CA, sex, and cervical vertebral FD measurements could classify skeletal maturation stages. CA and sex were the primary and most stable predictors of maturation group membership, respectively. FD measurements provided only modest additional information, with C2 anterior FD showing the most consistent association and C4 medial and C4 posterior FD showing more limited region-specific effects. Clinically, these findings suggest that cervical vertebral FD analysis may be used as an adjunctive quantitative indicator of skeletal maturation but not as a replacement for established maturation assessment methods.

Differences in growth around puberty are more closely linked to the stage of development than to the CA. Changes in the timing and extent of growth are closely related to changes in the development stage. Understanding the growth of the oro-facial area is crucial for orthodontic treatment. Determining a person's developmental stage is important for making appropriate orthodontic treatment plans because people grow at different times, durations, and velocities. Orthodontic treatment must be chosen carefully, with the proper appliances prescribed and followed by the patient to modify growth. When doctors decide on extraoral pulling forces, useful tools, extraction vs. non-extraction treatments, or orthognathic surgery, they mostly base their decisions on the patient's growth [22, 23]. Many studies have reported that radiographic measurements of skeletal growth stages

may provide more accurate information than CA. Skeletal developmental stages may show a wide range of CAs [2, 24].

HWRs are the gold standard; however, CVM, which is based on determining the cervical vertebrae maturation stage, is another approach frequently employed to determine skeletal maturity in orthodontics [4, 5]. The CVM method is in good agreement with the hand-wrist method [25, 26], making it a reliable skeletal maturity indicator, especially when HWR images are not available [27]. The main limitations of morphologic CVM evaluations are subjectivity, inadequate validity, and reproducibility [28]. To make the evaluation more objective, evaluations were also made according to the vertebral length, angle, and ratio measurements [4, 5].

Along with growth and development, dimensional changes and alterations in the trabecular structure can occur [7, 29]. FD analysis quantifies the complexity of structures by transforming intricate fractal properties and data sizes into simplified numerical values using image processing techniques. A higher FD value signifies a more complex structure, whereas a lower FD reflects a simpler structure [11]. FD values provide critical insights into the architecture of bone trabeculae [30]. In such cases, FD analysis can provide more objective data as a quantitative method. However, the existing literature is limited in terms of investigations of the relationship between FD analysis applied to different bones of the body and skeletal maturation [7, 12, 13, 29].

In our study, the full model showed an acceptable apparent classification performance, with a Nagelkerke R^2 of 0.779 and an overall accuracy of 77.8%. The highest classification accuracy was observed for Group 3, whereas lower but comparable accuracy rates were observed for Groups 1 and 2. CA and sex were the most stable predictors of maturation group membership. The addition of FD variables resulted in only a modest improvement over the age- and sex-only model, suggesting that FD measurements may provide supplementary rather than primary predictive information.

Orthodontic literature suggests a correlation between the CA and CVM; however, the strength of this correlation varies across studies. Magat reported a positive association between CA and CVM stages [31]. Similarly, another study found a significant relationship between skeletal age and CA, although the correlation was relatively low, underscoring the limitations of using CA as a standalone predictor [32]. In contrast, a different study reported a much higher correlation coefficient ($r = 0.864$) between the CA and CVM stages [33]. In the present study, the maturation stage progressed as CA increased.

Research indicates that sex significantly influences the timing and nature of skeletal maturation. For instance, studies have shown that females generally reach skeletal maturity earlier than males, which can affect the timing of orthodontic interventions [34]. Pradopo et al. indicated significant differences in the timing and duration of growth spurts between sexes, with girls experiencing a faster growth spurt duration than boys, as evidenced by the shorter time required to reach skeletal maturity [35]. This earlier maturation in females suggests that orthodontic treatment may need to be initiated sooner in female patients to capitalize on their growth potential [36].

In this study, being male consistently reduced the likelihood of belonging to more advanced maturation stages than females. Specifically, males were significantly less likely to be in Groups 2 than Group 1, 3 than Group 2, and in Group 3 than Group 1, indicating a negative impact of being male on progression through the maturation stages.

Previous studies examining the relationship between FD values and skeletal maturation stages produced variable results. Some studies have reported increases, decreases, or no changes in FD values as maturation progresses. These inconsistencies may be attributed to the use of different diagnostic tools, such as HWR, LCR, and cone beam computed tomography (CBCT), as well as variations in the selection of ROIs and racial differences [7, 12, 37–39].

For instance, Akbulut et al. demonstrated that the fractal analysis of the middle finger phalanxes (DP3 and MP3) and radius could provide important insights into predicting the success of rapid palatal expansion (RPE). Their findings revealed that patients with successful RPE had lower FD values, whereas those with failed RPE had higher FD values. Additionally, they reported a positive correlation between HWM stages, CA, and FD values in the radius bone [29]. However, another study using HWRs found that FD analysis alone could not predict maturation stages in growing patients. This study suggested that FD analysis might be a growth rate prediction method when combined with Chapman sesamoid stages, CA, and sex [13].

Furthermore, a study using CBCT images found a strong negative correlation between cervical vertebral maturation (CVM) stages and FD values in the midpalatal suture and reported that FA could be a valuable objective tool for assessing midpalatal suture maturation [38]. Another study that assessed skeletal maturation using the HWM method reported a correlation between CA, HWM stages, and FD values of the midpalatal suture in CBCT images, showing that as age increased, SMI values increased but FD values decreased [39].

Pamukcu et al. similarly highlighted the negative correlations between C4 FD values and the divided HWM and CVM stages, indicating that the fractal analysis of cervical vertebrae, particularly C4, could be useful for

evaluating the pubertal growth spurt [7]. In contrast, Eninanc and Bayraktar found moderate positive correlations between HWM stages and FD values of the radius and weaker but significant correlations with FD values of the proximal phalanx, medial phalanx, and C2 and C3 [12].

In addition to biological variability, methodological factors may substantially influence FD measurements, contributing to inconsistencies among studies. The ROI size is particularly important because larger ROIs may encompass more heterogeneous trabecular structures, whereas smaller ROIs may be more sensitive to localized architectural variations. Image quality, radiographic resolution, exposure conditions, and projection geometry may also affect FD calculations by altering trabecular visibility [15]. Furthermore, differences in age range, sample distribution across maturation stages, and image-processing protocols—including Gaussian blur settings, thresholding procedures, erosion–dilation steps, skeletonization methods, and FD calculation algorithms—may significantly influence the FD values [11, 15].

Previous studies investigating cervical vertebral FD measurements have reported stronger associations between FD values and skeletal maturation stages than those observed in this study. Pamukcu et al. reported significant FD differences among maturation groups using 40 × 40-pixel ROIs, whereas Eninanç and Büyükbayraktar observed significant associations using 60 × 60-pixel ROIs. In contrast, the present study identified only a limited number of region-specific associations. These discrepancies may be related not only to differences in study populations and maturation stage distributions but also to methodological variations in ROI selection and image acquisition [7, 12]. Therefore, discrepancies among FD studies likely reflect a combination of biological and methodological factors, rather than true biological contradictions. Consequently, direct comparisons among FD studies should be interpreted with caution.

Future investigations would benefit from the greater standardization of ROI selection, image acquisition protocols, and image-processing workflows. Such standardization may improve inter-study comparability and facilitate the clinical applicability of FD analysis as a supplementary tool for skeletal maturation assessments.

In our study, as maturation progressed, specifically from Group 1 to Group 2, there was an increase in the posterior C4 value, a decrease in the medial C4 value from Group 2 to Group 3, and an increase in the anterior C2 value. Our study focused on determining the critical parameters for maturation stages and performing regression analysis rather than simply evaluating correlations because comparing it with other FD maturation studies using LCR is difficult. Additionally, our study groups were structured based on the three stages of maturation defined by growth velocity rather than directly aligning with each HWM stage.

The accelerating and high growth velocity groups represent the prepubertal growth spurt (PGS), and the decreasing velocity group reflects the post-PGS phase. The distribution of individuals within the pre- and post-PGS groups in Pamukcu et al.'s study [7] and their alignment with specific growth stages were also significant for our study comparison. For instance, it is important to determine whether the distribution of individuals in the pre-PGS group in their study corresponds more closely to Groups 1 and 2 in our study. This issue further limited the direct comparability of the two studies.

The morphological development of the cervical vertebral bodies is closely associated with key biomechanical functions, including spinal cord protection, load bearing, and head movement. Furthermore, vertebral morphology is intricately connected to various biomechanical capacities, such as different locomotion modes [40]. Miller et al. highlighted that the growth in depth and height of the cervical vertebrae supported a close relationship between the morphologic development of the cervical vertebral bodies and the functional requirements for enhanced stability during head and neck movements. Furthermore, distinct growth patterns were identified for the C2 and C3-C7 vertebrae, which were documented and classified accordingly [41]. Considering the growth and biomechanical functions together, it may be more accurate to interpret alterations in the trabecular structure as inherent 'variability' rather than classifying them strictly as increases or decreases. The absence of a clear linear pattern in the FD values observed in our study may reflect this intrinsic variability.

The biological interpretation of FD changes in the cervical vertebrae is complex because trabecular bone remodeling is influenced not only by skeletal maturation but also by local biomechanical demands. Cervical vertebrae undergo continuous growth and remodeling during adolescence, including changes in bone deposition, mineralization, and trabecular organization [40, 41]. The C2 vertebra possesses unique structural characteristics associated with the odontoid process, which enables a wide range of rotational movements at the atlantoaxial joint, whereas the lower cervical vertebrae contribute more prominently to load transmission and postural support [40]. In the present study, significant associations were observed only in the anterior region of C2 and the medial and posterior regions of C4, whereas no significant associations were observed in C3. These findings suggest that trabecular adaptations related to skeletal maturation may vary across the cervical vertebral regions and be influenced by local biological and biomechanical factors. Therefore, FD measurements may reflect localized

remodeling responses rather than a generalized vertebral responses to skeletal maturation.

These region-specific findings may also be related to the developmental morphology of cervical vertebrae. During maturation, the cervical vertebral bodies undergo characteristic shape changes, progressing from relatively wedge-shaped forms to more rectangular configurations as skeletal maturation advances [24]. Because these developmental changes are not necessarily uniform throughout the vertebral body, trabecular adaptation may vary among the anterior, middle, and posterior regions. This biological variability may partly explain why significant FD associations were observed in specific regions rather than uniformly across all evaluated cervical vertebral areas.

These findings suggest that FD analysis may provide important insights into growth and maturation. However, its predictive accuracy may vary depending on the ROI size and diagnostic method. Functional demands that influence changes in the cervical vertebrae may play a significant role in these outcomes. Research incorporating larger and more diverse sample sizes, encompassing a range of ethnicities, and using longitudinal data is necessary.

Several limitations should be considered when interpreting the findings of this study: First, this was a retrospective single-center study that included only individuals of Turkish ethnicity, which may limit the generalizability of the results to other populations. Second, although the model demonstrated acceptable apparent classification performance, no external validation cohort was available; therefore, the reported accuracy should not be interpreted as a definitive predictive performance. Third, FD measurements were obtained from two-dimensional lateral cephalometric radiographs and may not fully capture the three-dimensional complexity of the trabecular bone architecture. In addition, FD values were measured within a relatively narrow numerical range, and some FD-related relative risk ratios were associated with wide confidence intervals; therefore, the magnitude of FD-related effects should be interpreted caution. Furthermore, although multicollinearity diagnostics did not indicate problematic multicollinearity, the stability of FD-related predictors should be confirmed in future studies. Finally, because of the cross-sectional retrospective design, causal relationships between FD changes and skeletal maturation could not be inferred, and potential interaction effects between age, sex, and FD measurements were not evaluated.

Conclusions

From a statistical perspective, CA and sex were the primary and most consistent predictors of maturation group membership. FD measurements from selected cervical vertebral regions provided modest supplementary information beyond age and sex. However, because FD-related effects are region-specific and the model has not been externally validated, FD analysis should be considered an adjunctive quantitative indicator rather than a standalone predictor. Future studies incorporating independent validation cohorts, standardized FD assessment protocols, longitudinal designs, and more diverse populations are needed to determine the clinical applicability and generalizability of FD-based skeletal maturation assessments.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12880-026-02583-x.

Supplementary Material 1

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Author contributions
MG: Conceptualization, methodology, acquisition of the data, data analysis, writing original draft preparation, reviewing, and editing. MFS: Methodology, data analysis, supervision, writing- reviewing, and editing. DNG: Acquisition of the data, writing-reviewing, and editing. TEK: Acquisition of the data, writing-reviewing, and editing. BBG: Acquisition of the data, writing-reviewing, and editing. SU: Acquisition of the data, writing-reviewing, and editing.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments. The Institutional Review Board and Clinical Ethics Committee of Recep Tayyip Erdoğan University Medicine Faculty approved this retrospective clinical study (decision date and number: 24.04.2024/78). For participants under 16 years of age, detailed information about this research was provided to their parents or legal guardians before the study, and an informed consent form was obtained. For participants aged 16 and older, informed consent was obtained directly from the individuals themselves.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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