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Systematic Review

Reliability of salivary biomarkers as skeletal maturity indicators: A systematic review

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Cortisol/cortisone ratio
Dehydroepiandrosterone sulphate DHEA-S
Skeletal Maturity
Pubertal Stages

Summary

Objective > To assess the reliability of different salivary biomarkers as skeletal maturity indicators when compared with other methods of skeletal maturity assessment.
Methods > A comprehensive search was conducted on three electronic databases: PUBMED, Google scholar and Cochrane library for the articles published from 2000 to July 2021. Assessment of skeletal age on the basis of levels of different salivary biomarkers at different pubertal stages was considered as the primary outcome. Electronic search, data collection and risk of bias assessment were performed by two authors with conflict resolution by the third author.
Results > Total 158 articles were retrieved after screening of titles, abstracts and full texts of all articles, of which 15 articles were selected for qualitative synthesis. All these studies were cross-sectional in design. These studies compared the levels of different salivary biomarkers as Alkaline Phosphatase (ALP), Insulin-like Growth Factor - I (IGF–I), Insulin-like Growth Factor Binding Protein–3 (IGFBP–3), Cortisol, Indian Hedgehog (IHH) protein and Dehydroepiandrosterone sulphate (DHEAS) with other methods of skeletal age estimation. Out of these six biomarkers salivary IGF-1 is a reliable indicator for skeletal maturity assessment.
Conclusion > The current evidence suggests that salivary biomarkers can be used as an adjunct for growth prediction during orthodontic treatment planning along with other methods of skeletal maturation assessment. Still there is need for further research with longitudinal studies in this field.

Introduction

A sound diagnosis and optimum treatment planning are indispensable parts of orthodontic therapy. One can expect the desired possible outcome when every aspect of the treatment is planned in accordance with skeletal maturation status of the patient [1,2]. The different decisions like extraction or non-extraction treatment approach, functional appliance therapy, orthopaedic correction, expansion and orthognathic surgery depend upon individual's growth status [3]. When the mechanics are in harmony with growth physiology, there will be a better exploitation of the growth potential at the apt time.

A multitude of methods since decades are used for skeletal maturation assessment. These include chronologic age, dental age, physiological age (sexual maturation) and radiographic

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methods based on hand wrist radiograph, lateral cephalogram, IOPA of middle phalanx of third finger [4–6]. The chronologic age is not a reliable parameter to assess the skeletal maturity [7]. Radiographic methods have been used as a vital tool, but they are highly subjective methods based upon visual inspection of developing bones, their morphology, sequential ossification stages and related changes in shape and size [8]. They also suffer the hazardous demerit of radiation exposure.

Currently several biochemical methods are at disposal to assess the skeletal maturity. These bio-indicators are directly associated with bone metabolism and its regulation. Variety of hormones and enzymes are released in the body in different concentrations during various pubertal stages. They are secreted into body fluids like blood, saliva, GCF and urine. These fluids act as mirrors which project the levels of these biochemicals. Collection of serum is an invasive procedure [9,10] whereas, collection of GCF is technique-sensitive procedure requiring special armamentaria [11]. Saliva can be used as an important diagnostic channel because of a wide spectrum of biomolecules like proteins/peptides, nucleic acids, electrolytes, and hormones originating from multiple local and systemic sources in it [12,13]. Collection of saliva is a less invasive procedure than venepuncture and is even possible in children.

There is a dearth of studies commenting on the validity and reliability of various salivary biomarkers in assessing the skeletal maturation. The aim of this systematic review is to investigate the possible association between different salivary biomarkers when compared with other methods of skeletal maturity assessment at different pubertal stages.

Materials and methods

Protocol and registration

The present systematic review was registered at the national institute for health research prospero international prospective register of systematic reviews (Registration number: CRD42021250203). The research protocol is designed according to the PRISMA (preferred reporting items for systematic review and meta-analyses) guidelines 2020.

PECOS

Patients undergoing orthodontic treatment around puberty participated in the studies.

In vivo methodological studies were included with salivary indicator assessing skeletal maturity as an exposure. The primary outcome studied was skeletal maturity assessment based on levels of different salivary indicators. The levels of these biomarkers were correlated with different pubertal stages based upon conventional orthodontic skeletal maturity assessment methods.

Eligibility criteria

Inclusion criteria

The inclusion criteria included the following:

- cross-sectional studies on healthy orthodontic patients;
- studies comparing salivary indicators for skeletal age estimation with other methods of skeletal maturation assessment;
- publications in English language, with full text available;
- relevant studies published from 2000 to July 2022.

Exclusion criteria

The exclusion criteria included the following:

- descriptive studies, review articles, opinion articles, online published final dissertations;
- studies dealing with medically compromised patients;
- studies assessing serum or GCF indicators of skeletal maturity;
- studies on salivary metabolomic enzyme assays;
- studies correlating salivary biomarkers with other methods of pubertal staging like Tanner stages (based on secondary sexual characteristics) which are not routinely used in orthodontic practice;
- studies with an outcome to assess salivary enzyme levels in periodontal conditions;
- studies involving participants with periodontal problems, liver and bone diseases, growth abnormality, endocrine disorders and participants with any drug history.

Information sources and search strategy

A comprehensive search was conducted on electronic databases, additionally by manual search, to spot all relevant studies associated with salivary indicators for skeletal maturation assessment. Three electronic databases: PubMed, Google scholar, Cochrane library were searched by 2 reviewers using the key words "(skeletal maturity OR pubertal growth spurt OR skeletal age assessment) AND (salivary biomarkers OR salivary indicators OR salivary alkaline phosphatase OR salivary IGF-I OR salivary IGFBP-3 OR salivary cortisol OR cortisol/cortisone ratio OR salivary dehydroepiandrosterone sulphate OR salivary Indian hedgehog protein)".

The search lined all articles published from 2000 to July 2022. Duplicate records were removed.

Study selection

Two reviewers independently screened titles and abstracts of relevant studies retrieved by the electronic database searches. Additionally, all references of the selected studies were manually screened for potentially relevant additional studies. Any possible discrepancies encountered during selection process were resolved by discussion between two reviewers; and judgment of a third reviewer was considered decisive in case of persisting disagreement.

Data collection and data items extracted

Characteristics of included studies and numerical data were extracted in duplicate by two reviewers using predetermined and piloted data extraction forms. Information on authors' names, year of publications, study design, sample, age and gender of participants, different salivary indicators compared with other methods of skeletal maturation assessments and

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result was independently extracted by two reviewers based on a previously defined protocol.

Risk of bias within studies

The risk of bias was assessed for cross-sectional studies using Newcastle-Ottawa Scale [14] by two independent reviewers and discrepancies were resolved by discussion and appropriate consultation with a third reviewer.

Results

Study selection

The study selection process is summarized in *figure 1* following the PRISMA guidelines. The total number of hits was 158: 58 in PubMed, 46 in Cochrane Library, 54 in Google Scholar search resources. After removing the duplicates, 56 hits were scrutinized for inclusion in the study. Fifteen articles were selected for qualitative synthesis after title, abstract and full text screening

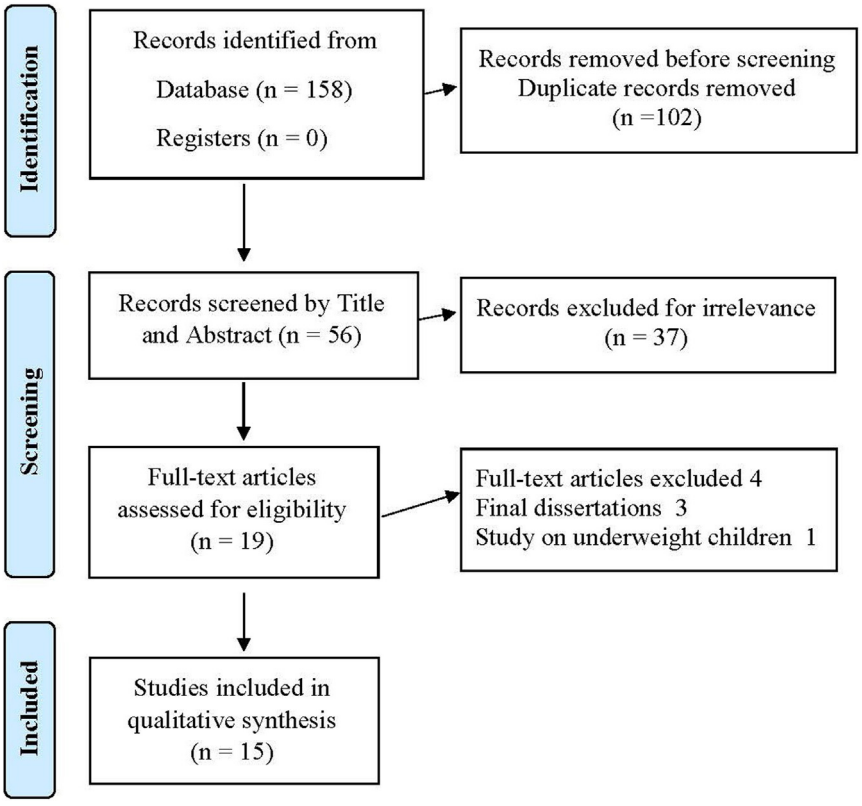

FIGURE 1

Prisma flow diagram showing the studies exclusion and final inclusion with reasons

TABLE I

Studies excluded with reasons.

Study	Participants	Salivary Indicator	Reason for Exclusion
Sangeeth K. et al. 2017, India	66 9-18 years	DHEA	Online published final dissertation
Sharmilaa S. 2017, India	90 6-22 years	IGF-1, VEGF	Online published final dissertation
Anbuselvan P. 2020, India	60 8-18 years	ALP	Online published final dissertation
Harraa S. Mohammed-Salih 2017, Iraq	84 14-19 years	ALP	Study on underweight children (affected nutrition)

DHEA: Dehydroepiandrosterone; IGF-1: Insulin like growth factor –1; VEGF: Vascular endothelial growth factor; ALP: Alkaline Phosphatase.

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of the relevant articles. Excluded studies and reasoning for exclusion are mentioned in *table I*.

Study characteristics

The studies were conducted from 2000–July 2022. All the studies were cross-sectional in designs conducted on patients of age 6–25 years. Total 1271 patients were included in 15 studies. The gender predilection showed 607 females and 439 males in 12 studies with total 1046 participants. In three studies on 90, 80 and 55 children [15–17] gender predilection was not mentioned. Healthy patients without any systemic illness, growth abnormality or bone disorders were included. Out of 15 studies, 6 studies were conducted in India, 4 studies in Indonesia, 3 studies in Saudi Arabia, 1 study in USA and 1 study in Iran.

Most of the studies included in this review collected the salivary samples with passive drool method and the salivary indicators were analysed using Auto analyser, Electrochemiluminescence (ECL) immunoassay, Spectrophotometer, Colorimetric assay, ELISA, Enzymatic method. The subjects were informed in majority of the studies about the collection protocol. The timing of sample collection to avoid circadian effect was taken into consideration in 11 studies. Mostly saliva was collected during morning hours from 7 to 12 o'clock. Patients were instructed to avoid tooth brushing, food and fluid ingestion or chewing gum prior to sample collection for 20 minutes to 2 hours according to different authors.

Details of the participants like age, gender, number, region along with salivary indicator used and method of its assessment are mentioned in *table II*, comparing method of skeletal maturation, description of levels of different salivary indicators in different pubertal stages and conclusion are given.

Table II

Details of the Studies: population, salivary indicators used and its method of assessment, methods of comparison of skeletal maturation and description of the levels of different salivary indicators in the different pubertal stages and conclusion.

Study	Population (Number, Sex, Age)	Salivary indicator and assessment method	Methods of comparison	Description	Conclusion
Nayak et al. 2014, [18] India	45 (M – 21, F – 24) 7-23 years	IGF-1 IRMA	QCVM by Chen et al.	Salivary IGF-I levels were lowest in QCVM I (2.1 ng/ml). Gradual rise was seen later to a peak at the QCVM II (4.3 ng/ml) and then levels mildly decreased through QCVM III (3.5 ng/ml) to QCVM IV (2.6 ng/ml).	Salivary IGF-I can be considered as an additional diagnostic tool to assess the skeletal maturation.
Tarvade et al. 2015, [19] India	120 (M – 60, F – 60) 10-15 years	ALP Enzymatic method	MP3 Staging by Hagg and Taranger	The salivary ALP levels raised from MP3- F stage (M-1376.4 IU/L, F-1343.9 IU/L) to FG stage (M-1400.95 IU/L, F-1378.34 IU/L). Then a sudden ascent was observed during G stage (M- 2537.31 IU/L, F-1513.4 IU/L). Following the G stage, the values decreased through H stage (M- 1906.92 IU/L, F- 1016.48 IU/L) to I stage (M-1019.1 IU/L, F-563.4 IU/L).	Exploitation of growth potential during orthodontic treatment planning can be expected with salivary ALP used as biomarker for pubertal growth prediction.
Sowmya et al. 2016, [3] India	27 (F – 27) 9-16 years	Cortisol ECL Immunoassay	CVM staging by Baccetti et al.	Prepubertal group showed the lowest salivary Cortisol levels (0.28±0.05 µg/dl). Instant rise was seen from pre pubertal to pubertal group (0.50 ±0.11 µg/dl). The levels still increased in post pubertal group (0.61 ±0.13 µg/dl).	Salivary Cortisol can be used to predict peak growth velocity but the longitudinal studies with larger sample size were further required for validation of the results obtained from this study.

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TABLE II **(Continued).**

Study	Population (Number, Sex, Age)	Salivary indicator and assessment method	Methods of comparison	Description	Conclusion
Wijaya et al. 2017, [20] Indonesia	136 (M – 64, F – 72) 8-18 years	BALP ELISA	CVM staging by Baccetti et al.	The highest level of salivary BALP was found during prepubertal growth phase (931.90±643.14 pg/ml) and later level declined during pubertal (782.28±451.10 pg/ml) and post pubertal growth phase (777.58±499.52 pg/ml).	Though BALP was a non- invasive biomarker from saliva but its practical applicability to assess the skeletal maturation was questionable.
Irham et al. 2018, [21] Indonesia	57 (F – 57) 8- 15 years	ALP Spectrophotometry	CVM staging by Hassel and Farman	Moderate levels were seen in prepubertal stage (192,87 ± 69,02 IU/L) with sudden hike during pubertal stage (233,39 ± 106,29 IU/L). The lowest levels were observed during post pubertal stage (79,20 ± 31,41 IU/L).	Salivary ALP when used as biomarker for skeletal age estimation provides an additional information during pubertal growth prediction.
Hegde et al. 2018, [15] India	90 6-19 years	BALP ELISA	Hagg and Taranger Method of hand wrist radiograph.	Increase in BALP level was seen from prepubertal (131.79 U/l) to onset of pubertal stage (173.43 U/l). A sudden hike was seen during peak of pubertal stage (181.16 U/l). The values then declined during pubertal deceleration (157.74 U/l) and growth completion (122.17 U/l) stages.	Along with previously used methods of skeletal maturity assessment, salivary BALP can be used as an additional tool for orthodontic treatment planning.
Alhazmi et al. 2019, [22] USA	79 (M – 31, F – 48) 7-23 years	ALP Colorimetric Assay	CVM staging by Baccetti et al.	Highest values were found in prepubertal growth phase coinciding with CVMS I (0.8 mU/mg). During CVMS II the values declined to 0.44 mU/mg. Again, the graph curled upward during CVMS III (0.48 mU/mg) and CVMS IV (0.53 mU/mg). CVMS V stage showed declining value of 0.47 mU/mg.	Salivary ALP activity when combined with chronological age may provide an additional data for skeletal age estimation.
Khan et al. 2019, [23] India	90 (M –42, F – 48) 8-20 years	IHH protein ELISA	CVM staging by Hassel and Farman	Salivary IHH activities during CVMS 1 and CVMS 2 were 171.01 ng/ml and 178.67 ng/ml respectively. A bell shape curve with highest levels of salivary IHH during CVMS 3 (209.13 ng/ml) and CVMS 4 (233.79 ng/ml) was seen. During the post pubertal stages CVMS 5 and 6, the salivary IHH activity declined to 224.31 ng/ml to 172.80 ng/ml respectively.	Salivary IHH can be used for skeletal maturation assessment to take the advantage of pubertal growth spurt during orthodontic treatment planning.

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Table II **(Continued).**

Study	Population (Number, Sex, Age)	Salivary indicator and assessment method	Methods of comparison	Description	Conclusion
Wijaya et al. 2019, [20] Indonesia	136 (M – 64, F – 72) 8-18 years	BALP ELISA	CVM staging by Baccetti et al.	The highest level of BALP was found in prepubertal stage (1001.71 pg/ml). Later BALP level showed inverse proportion with advancing pubertal phase (834.80 pg/ml) to post pubertal phase (691.16 pg/ml).	Along with chronologic age and BMI percentile salivary BALP can be used for the prediction of pubertal growth phases.
Nelwan et al. 2021, [24] Indonesia	20 (M – 10, F – 10) 8-14 years	ALP Autoanalyzer	Chronologic and dental age by Demirjian's tooth mineralisation stages using OPG	Negative correlation was found between dental age, chronologic age in comparison with salivary ALP levels. The median ALP concentration was 267.45 pg/mL. The values declined from prepubertal to post pubertal phase as 458.9 pg/mL to 95.26 pg/ml.	Combination of different methods like chronologic age, dental age along with salivary ALP increased the accuracy of predicting skeletal maturity levels.
Sultana et al. 2021, [16] India	80 8-14 years	DHEAS ELISA	CVM staging by Hassel and Farman/MP3 Staging by Hagg and Taranger modified by Liete et al.	Prepubertal groups with CVMS 1 and 2 showed DHEAS levels of 4.21 ± 0.30 ng/ml and 4.46 ± 0.30 ng/ml respectively. During the pubertal stages of CVMS 3 and 4, the values hiked up as 5.60 ± 0.45 ng/ml and 5.88 ± 0.24 ng/ml respectively. Same pattern of increased activity from prepubertal to pubertal stage was observed with MP3 stages also.	Significant difference between different groups was observed but use of salivary DHEAS as skeletal maturity indicator was not clearly mentioned.
Sookhakian et al. 2022, [17] Iran	55 7 20 years	1. IGF-1 - ELISA 2. ALP - Human ALP kit with Autoanalyzer	CVM staging by Baccetti et al.	Multinomial logistic regressions with different combinations of salivary IGF-1, alkaline phosphatase and chronologic age were utilized to predict the growth phase. Out of 7 regression models, CA+IGF-1+ALP model was having maximum growth phase correct classification rate of 89.1%. This model can predict the prepubertal, pubertal and post pubertal growth phases with correct classification rates of 95%, 80% and 90% respectively.	A method combining salivary IGF-1, alkaline phosphatase and chronologic age can be successfully used for growth prediction.
Almalki et al. 2022, [25] Saudi Arabia	90 (M – 34, F – 56) 6 – 25 years	IGF - 1 and IGFBP - 3 ELISA	MP3 Staging by Hagg and Taranger	1. IGF-1 – In males, IGF-1 level at prepubertal stage was 0.93 ng/ml. This value increased to 2.52 ng/ml at the pubertal onset and reached to 2.58	Salivary IGF-1, IGFBP-3 and IGF/IGFBP-3 ratio can serve as potential biomarkers for

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TABLE II **(Continued).**

Study	Population (Number, Sex, Age)	Salivary indicator and assessment method	Methods of comparison	Description	Conclusion
				ng/ml at pubertal peak. Then the values declined to 1.67 ng/ml to 1.09 ng/ml during post pubertal stages. Similar curve was observed in females with prepubertal value of 0.57 ng/ml which increased to 0.87 ng/ml during pubertal onset. A sudden hike up to 2.22 ng/ml to 2.32 ng/ml was seen during peak pubertal stages. Lateron during post pubertal stage the value declined to 1.47 ng/ml. 2. IGFBP-3 – In males, during prepubertal stage IGFBP-3 level was 3.74 ng/ml. Then it raised to 3.96 ng/ml and 4.29 ng/ml during onset and peak of pubertal stage respectively. During deceleration phase the value declined to 3.60 ng/ml and again raised to 4.25 ng/ml at growth completion stage. Females showed prepubertal IGFBP-3 value of 3.23 ng/ml which hiked up to 3.31 ng/ml and 4.31 ng/ml during onset and peak of pubertal stage respectively. The value then declined to 4.06 ng/ml to 3.36 ng/ml during post pubertal stage. 3. IGF-1/IGFBP-3 Ratio – It showed a strong positive correlation with increasing stages of skeletal maturity.	skeletal age assessment.
Almalki et al. 2022, [26] Saudi Arabia	105 (M – 43, F – 62) 6 – 25 years	IGF - 1 and IGFBP - 3 ELISA	CVM staging by Baccetti et al.	1. IGF-1- During prepubertal stage, males showed IGF-1 level of 1.22 ng/ml. This value increased during pubertal stage up to 2.57 ng/ml and declined to 1.54 ng/ml during post pubertal stage. Females showed a rise in IGF-1 from 0.85 ng/ml at prepubertal stage to 1.57 ng/ml at pubertal stage. Then the level declined to 1.47 ng/ml during post pubertal stage. 2. IGFBP3 – Males showed IGFBP3 levels of 3.84 ng/ml,	Salivary IGF-1 and IGF-1/IGFBP3 ratio can be used along with other skeletal maturity indicators during orthodontic treatment planning.

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Table II **(Continued).**

Study	Population (Number, Sex, Age)	Salivary indicator and assessment method	Methods of comparison	Description	Conclusion
				4.15 ng/ml and 3.89 ng/ml in prepubertal, pubertal and post pubertal respectively. Same pattern with increase in IGFBP3 level from prepubertal (3.21ng/ml) to pubertal (3.67 ng/ml) and then decline during post pubertal stage (3.50 ng/ml) was seen in females. 3. IGF-1/IGFBP3 Ratio – IGF-1/ IGFBP3 ratios in males were 1.18, 2.31 and 1.47 during prepubertal, pubertal and post pubertal stages respectively. Females showed the ratios of 0.96, 1.62 and 1.60 during prepubertal, pubertal and post pubertal stages.	
Al Meshari et al. 2022, [27] Saudi Arabia	141 (M – 70, F – 71) 7 – 25 years	DHEA-S ELISA	CVM staging by Baccetti et al.	The values of DHEA-S during prepubertal stages CVMS 1 and 2 were 2.2 ng/ml and 2.8 ng/ml respectively. The value increased to 6 ng/ml and then to 6.9 ng/ml during CVMS 3 and 4 respectively. Post pubertal stages CVMS 5 and 6 showed the values of 8.6 ng/ml and 9.2 ng/ml.	There was rise in DHEA-S levels with progressing skeletal maturity stages. It can be used as non-invasive indicator to detect the growth phase.

IGF-1: Insulin like Growth factor-1; IRMA: Immunoradiometric Assay; ALP: Alkaline Phosphatase; BALP: Bone specific Alkaline Phosphatase; ECL: Immunoassay - Electrochemi-luminescence Immunoassay; ELISA: Enzyme-linked immunosorbent assay; IHH Protein: Indian Hedgehog Protein; DHEAS: Dehydroepiandrosterone sulphate; IGFBP3: Insulin like growth factor binding protein; QCVM: Quantitative cervical vertebral maturation (QCVM) stages; CVMS: Cervical vertebral maturation stages; MP3: Middle Phalanx of third finger; M: Males; F: Females; CA: Chronologic age.

Risk of bias within studies

The risk of bias was assessed for cross-sectional studies using Newcastle-Ottawa Scale [14]. Risk of bias for cross-sectional studies included in the review was assessed by two independent reviewers with consultation with third reviewer whenever required. The domains for risk assessment were graded as stars, based on selection, comparability and outcome. Thus, the overall risk for individual studies were assessed as low, moderate or high risk based on the domains and criteria. The study was assessed to have a low overall risk only if all domains were found to have low risk and high overall risk if one or more of the seven domains were found to be at high risk. A moderate risk assessment was provided to the studies when one or more domains were found to be uncertain, with none at high risk.

The domains and criteria for assessment of risk of bias included selection, outcome and comparability as discussed in *table III*. Based on these domains all the involved studies were graded with stars and cumulative total score out of 10 was given to each study as described in *table IV*. Out of 15 studies, 9 studies got score of 10 and 6 studies got score of 9. The quality of all studies was very good. All the studies conducted showed low risk for cross sectional study design and had methodology that could be followed in future studies.

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TABLE III

Domains and criteria for risk of bias assessment for cross sectional studies.

Selection (Maximum 5 stars *)	
Representativeness of the sample: Truly representative of the average in the target population. * (All subjects or random sampling) Somewhat representative of the average in the target population. * (non- random sampling) Selected group of people. No description of the sampling strategy. Sample size: Justified and satisfactory. * Not justified. Non-respondents: Comparability between respondents and non-respondents' characteristics is established, and the response rate is satisfactory. * The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. No description of the response rate or the characteristics of the responders and the non-responders. Assessment of the exposure (risk factor): Validated measurement tool. ** Non-validated measurement tool, but the tool is available or described. * No description of the measurement tool.	
Comparability: (Maximum 2 stars)	Outcome: (Maximum 3 stars)
1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled. The study controls for the most important factor (select one). * The study control for any additional factor. * (Hence, Max. two ** - i.e. a+b) 2) Non comparable outcome groups.	Assessment of the outcome: Independent blind assessment. ** Record linkage. ** Self-report. * No description. Statistical test: The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (*P* value). * The statistical test is not appropriate, not described or incomplete.
The quality of the study can be assessed based upon the following criteria. Very Good studies: 9-10 stars. Good studies:7-8 stars. Satisfactory studies: 5-6 stars.	

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Table IV
Risk of bias within studies.

Studies	Selection				Comparability	Outcome		Total
	1	2	3	4	1	1	2	
Nayak et al. [18]	*	*	*	**	**	**	*	10
Tarvade et al. [19]	*	*	*	*	*	**	*	9
Sowmya et al. [3]	*	*	*	**	**	**	*	10
Wijaya et al. [20]	*	*	*	**	**	**	*	10
Irham et al. [21]	*	*	*	**	**	**	*	10
Hegde et al. [15]	*	*	*	**	*	**	*	9
Alhazmi et al. [22]	*	*	*	**	**	**	*	10
Khan et al. [23]	*	*	*	**	*	**	*	9
Wijaya et al. [20]	*	*	*	**	**	**	*	10
Nelwan et al. [24]	*	*	*	**	*	**	*	9
Sultana et al. [16]	*	*	*	**	*	**	*	9
Sookhakian et al. [17]	*	*	*	**	*	**	*	9
Almalki et al. [25]	*	*	*	**	**	**	*	9
Almalki et al. [26]	*	*	*	**	**	**	*	10
Al Meshari et al. [27]	*	*	*	**	*	**	*	9

Discussion

A famous quote by Geoffrey Chaucer says "Time waits for no one". Same appeals to pubertal growth spurt in orthodontic diagnosis and treatment planning. The importance of growth assessment in orthodontics is the utmost incentive to hunt for the most accurate and least invasive method of tracking the pubertal growth spurt [25]. Less invasive method of skeletal age assessment with biochemical markers is getting popularity nowadays. Numerous biomarkers are secreted in varying amounts which are replicated in body fluids like serum, saliva and GCF correlating with different stages of pubertal growth. These biomarkers are associated with bone metabolism, cartilage development and production of signals stimulating the growth of craniofacial skeleton [28]. A study conducted by Tripathi et al. [9] showed the reliability of serum ALP in assessing the skeletal maturity. Perinetti et al. [11] used GCF to evaluate the levels of ALP and correlated with different pubertal stages. However, collection of serum is invasive and that of GCF is technique sensitive. Hence, saliva offers various advantages over serum and GCF. The chairside collection of saliva requires less of armamentaria and can be routinely performed in clinical practice [29]. Different biomarkers from saliva reflect the rate of bone metabolism and can be used to predict the growth.

The aim of this study was to investigate the possible association between different salivary biomarkers when compared with other methods of skeletal maturity assessment. The salivary biomarkers used as growth indicators include alkaline phosphatase (ALP), insulin like growth factor I (IGF-I), insulin like growth factor binding protein 3 (IGFBP3), Cortisol, indian hedgehog protein (IHH), dehydroepiandrosterone sulphate (DHEAS).

Salivary alkaline phosphatase (ALP)

Out of 15 studies, 8 studies assessed reliability of salivary ALP as skeletal maturity indicator. In humans, however, different isoforms of ALP are found that include tissue non-specific (liver, bone and kidney), intestinal and placental ALP isoforms [15]. These all the different isoforms contribute to total ALP activity and hence the fractionation of these ALP isoforms is required before interpretation of any result. Separation of the intestinal and placental ALPs is easy, but distinguishing BALP from liver ALP is difficult. The possible reason is these two isoforms are the products of a single gene and only small difference occurs during post-translational glycosylation [20]. BALP is an important

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product of osteoblasts during bone mineralization and its levels can be detected in saliva separately. Out of 8 studies, 3 studies [15,20,30] evaluated the levels of salivary BALP and remaining 5 studies evaluated the levels of total salivary ALP (without fractionating the isoforms) [17,19,21,22,24].

In 3 studies, normalization of salivary ALP and salivary BALP was done by taking the ratios of specific proteins to total proteins to examine the changes in overall protein secretion because, stimulation or alteration of salivary flow can affect the concentrations of salivary proteins [20,22,30].

According to two studies by Wijaya et al. in 2017 [20] and 2019 [30], where salivary BALP levels were correlated with CVM staging by Baccetti et al. peak levels of salivary BALP were seen during prepubertal stage and then levels declined during pubertal and post pubertal stages. Similar results were observed by Alhazami et al. [22] and Nelwan et al. [24]. However, Hegde et al. compared salivary BALP levels with CVM staging by Hassel and Farman and found peak BALP levels during the pubertal stage. The prepubertal group showed lower BALP level than the post pubertal group [15]. These results were similar to study conducted by Tarvade et al. correlating salivary ALP levels with MP3 Staging by Hagg and Taranger [19].

Irham et al. also found peak salivary ALP levels during pubertal stage, however, lowest salivary ALP levels were encountered during post pubertal stage [21]. A study conducted by Sookhakian et al. attempted to provide regression models to assess the skeletal maturity based upon salivary IGF-1, salivary alkaline phosphatase and chronologic age [17].

Out of 7 different combination models of these 3 parameters, a model combining levels of salivary IGF-1, ALP and chronologic age all together showed the highest rate of skeletal maturity prediction.

Numerous studies had studied the levels of salivary ALP during orthodontic tooth movement. Local changes in bone metabolism due to orthodontic forces alter the levels of salivary enzymes and ALP is one of such biomarkers associated with bone remodelling [29,31,32]. Other local factors like oral health, caries status of child, gingival and periodontal conditions also affect the levels of salivary ALP [33,34]. Additionally diurnal variation influences salivary ALP levels [28]. Along with growth changes during pubertal phase, these all-other parameters also add up to the resultant ALP activity. Though, according to most of the authors, salivary ALP can be used as diagnostic tool for skeletal maturation assessment as an adjunct to other methods, the current evidence is scarce to assess the reliability of salivary ALP in skeletal age estimation. This warrants the necessity of further research in this topic.

Insulin-like Growth Factor–I (IGF–I)

IGF-1, a polypeptide hormone synthesized in liver is a mediator of Growth hormone (GH) function and plays an essential role in growth and development of almost every organ [35]. The diurnal fluctuation is not seen in IGF-1 levels that adds to its diagnostic value in determining GH status of an individual [36]. Study conducted by Nayak et al. [18] correlated salivary IGF-1 levels with QCVM staging given by Chen et al. [37]. The sharp rise was seen in salivary IGF-1 levels from prepubertal to pubertal stage and then decline during post pubertal stage. Same results were encountered in studies conducted by Almalki et al. where salivary IGF-1 levels were correlated with CVM staging by Baccetti et al. and MP3 staging by Hagg and Taranger [25,26]. In these studies, males demonstrated higher salivary IGF-1 levels than females during pubertal onset. The possible reason is stimulatory action of Testosterone and inhibitory action of Estrogen on GH function [38,39].

All the studies showed the homogenous results with peak salivary IGF-1 levels during pubertal growth spurt. Similar results were documented by Jain et al. [40] for serum IGF-1 and Sinha et al. [41] for serum and urine IGF-1 with peak levels during pubertal growth spurt. As IGF-1 reflects the underlying GH status of an individual without any diurnal variation, it is considered to be a good indicator of skeletal maturity assessment.

Insulin-like growth factor binding protein-3 (IGFBP-3)

Insulin growth factor binding proteins (IGFBPs) are the carrier proteins for IGFs. They control the release and prolong the half-life of IGFs [42]. Out of 6 different IGFBPs, IGFBP-3 is more accurate discriminator of GH dependent parameters due to its good reproducibility on repeated testing [43]. Studies conducted by Almalki et al. showed highest levels of IGFBP-3 during pubertal stage and greater values during post pubertal stage than prepubertal stage [25,26]. These studies also showed positive correlation between salivary IGF-1/IGFBP-3 ratios. There are studies analysing serum IGFBP-3 levels and ratio of IGF-1/IGFBP-3 during different growth stages [44,45] but available literature on salivary IGFBP-3 is very scarce to assess its reliability in predicting growth stages.

Cortisol

The studies conducted by Senaris et al. and Martinelli and Moreira found out the association between GH and cortisol [46,47]. Cortisol has an action to modulate the GH activity. Cortisol increases GH secretion by activating GH gene transcription and upregulates GH-Releasing Hormone (GHRH) receptor expression [23,48]. There is only one study found in the literature which was conducted by Sowmya et al. in 2016 comparing salivary cortisol levels with CVM staging by Baccetti et al. [3]. The highest levels were found during post pubertal stage. However, further longitudinal studies with larger sample size are warranted in this field to assess the reliability of salivary cortisol as skeletal maturity indicator.

Indian Hedgehog (IHH) protein

IHH is considered as one of the factors regulating the craniofacial growth. It is involved in chondrocytic activity and subsequent

endochondral bone growth. It regulates growth plate formation and secondary cartilage development at the condylar head during skeletal growth and development [49]. Khan et al. in 2019 conducted a study comparing salivary IHH levels with CVM staging by Hassel and Farman [27]. Peak IHH levels were observed during pubertal stage compared with the pre- and post-pubertal stages. Though this study claimed that salivary IHH can be used as an indicator of skeletal age estimation, a conclusion cannot be given regarding its reliability of skeletal maturity assessment which encourages the further research in this field.

Dehydroepiandrosterone sulphate (DHEAS)

DHEAS is an adrenal precursor essential for the steroid synthesis. It is a growth promoting hormone that increases the osteoblastic activity by cell proliferation [50]. The estimation of steroid levels in saliva can help to assess the skeletal changes during growth and development. Sultana et al. in 2021 correlated salivary DHEAS levels with CVM staging by Hassel and Farman and MP3 Staging by Hagg and Taranger with modifications by Liete et al. and observed higher DHEAS levels in pubertal group [16]. Another study by Meshari et al. attempted to discover the correlation between salivary DHEAS and CVMI stages by Baccetti et al. [51]. It showed that the prepubertal stage had lowest salivary DHEAS level which further increased during pubertal and post pubertal stages with highest activity during post pubertal stage. This is not in accordance with the results of the study by Sultana et al. where the pubertal stage showed the highest DHEAS activity. Based on these heterogeneous data, the reliability of salivary DHEAS in assessing skeletal age is questionable.

Strengths and limitations

There are relatively smaller number of studies included in this systematic review with substantial heterogeneity among the studies regarding the different indicators used, collection protocol considering the circadian effect, different assessment methods of salivary indicators with different units of measurements, variety of comparison methods of skeletal maturation assessment and unequal sex distribution in participants. A standard classification of normal ranges of numerous biomarkers in different pubertal stages is not possible because of genetic, ethnic, geographic variations along with disparity between nutrition and socioeconomic status.

In some studies, participants were simultaneously undergoing orthodontic treatment, which may affect the local bone metabolism associated with deposition and resorption, resulting in altered levels of various biomarkers such as ALP. The scope for meta-analysis was limited due to the heterogeneity of the data obtained. Despite all these limitations, this systematic review evaluated the reliability of different salivary indicators for the assessment of skeletal maturity.

Conclusion

There is scarce evidence available in this field considering the wide range of salivary biomarkers, their different assessment methods and ranges. Salivary biomarkers can be used as an adjunct for growth prediction during orthodontic treatment planning along with other methods of skeletal maturation assessment. Out of six different salivary biomarkers like ALP, IGF-1, IGFBP-3, Cortisol, IHH, DHEAS, salivary IGF-1 is a good reliable indicator for skeletal maturity assessment. Further longitudinal studies with larger sample size and standard protocol are warranted to provide an additional data on this topic to check the reliability of other salivary biomarkers in skeletal age estimation.

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