



## Review Article

# Alkaline Phosphatase Activity as Individual Skeletal Maturity Indicator: A Systematic Review

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### Abstract

**Background:** The contemporary skeletal maturity indicators identify the stages of growth by visual inspection of morphology of developing bone, but the radiation exposure and subjective outcome are its limitations. However, biomarkers as the alkaline phosphatase with different biochemical medium like serum, urine, saliva and gingival crevicular fluid can be used as safe and the best method to assess the individual skeletal maturity indicators. **Aim:** This systematic review aimed to elucidate the question: is alkaline phosphatase a reliable biomarker to assess skeletal maturity during growth. **Objective:** To collect, compile and synthesis the evidence on the levels of ALP activity and compare its reliability with contemporary maturity indicators in growing children. **Design, data sources, and methods:** Without language restrictions, an electronic search was performed to identify relevant data through PubMed, Medline, Embase, PsycINFO, Scopus, Ebsco, Hinari, bibliographies of included articles and the manual search performed in the department library for Grey Literature. Two reviewers independently retrieved data and risk bias was assessed using checklist provided by the clarity group at McMaster University for cross-sectional studies (MGUCCs). The BIOCROSS Scale was applied for quality assessment. **Results:** A sum of 1150 relevant titles was redeemed from various medical, dental and orthodontic journals. After thorough scrutiny, meticulous screenings of abstracts and on duplicate removal eight articles were finalized for qualitative synthesis, of these eight studies three studies compared ALP activity with CVMS, three with CVMI, and two studies compared with hand wrist radiographic method, and chronologic age. 80% of these studies has low risk bias and are of high quality (BIOCROSS Scale). **Conclusion:** As per MGUCCs seven out of eight were cross sectional and one longitudinal study, in which 80% of study has low risk bias and high quality. All the studies reported that levels of ALP activity follows growth curve.

**Keywords:** Biomarkers, Alkaline Phosphatase, Maturity Indicators, Growth Phase, Enzyme.

### Introduction

Skeletal maturity estimation plays a vital role in orthodontic diagnosis and treatment planning, as it potentially, influences the efficiency and effectiveness of orthodontic treatment, especially in growing children [1]. Precise prediction of individual growth spurts imparts critical clinical advantage to modify the growth of individual jawbones, as assessment of initial growth phase for maxillary growth modification, peak growth spurts for mandibular growth modification; and the end of the growth spurt assists

clinicians for orthognathic surgery [1, 2].

The inception of utilizing hidden growth as a primary objective of orthopedic treatment is fundamentally based on assessing skeletal age [1-3].

The assessment of individual skeletal maturity (skeletal age) can be determined by using various growth indicators such as chronological age, dental age and skeletal age [4]. The chronological age is not reliable entity as variability in individual growth pattern. Dental age assessment identifies precise phases of the growth spurt, but the individual teeth eruption sequence is unreliable [1-5].

One of the most accepted classic methods of assessing pubertal growth phase is skeletal age (skeletal maturity indicator) based on radiographs; it comprises pictorial examination of the developing bone and their initial appearance, sequential ossification and related changes in shape and size [1-7]. Thus, although it identifies pinpoint assessment of different stage of growth, but its evaluation is based on morphology of developing bone, requires additional radiation exposure, and subjective outcomes are few major limitations. Identifying morphologic stages of bone is mainly under the influence of local factors but the developing bone is under influence of both local and systemic factors [6,7]. Hence, the assessment of growth phases must represent the agents who are involved directly in bone growth and remodeling is devoid in skeletal age analysis [8].

To date, biomarkers have provided a new possibility to represent the agents who are involved in direct bone growth and remodeling [9,24]. The cellular process of bone growth discharges biochemical molecules into the body fluids like blood; urine and saliva are known as bone metabolism biomarkers. Investigating the levels of biochemical molecules provides accurate individual skeletal maturity stage as it being related to the physiology of the patient [1-6,7,9,10].

Traditionally, bone metabolism biomarkers were measured in urine as a safe, non-invasive and economic and pinpoints determinant of growth phase [8-10]. This method holds useful in adolescent to carry out the instructions for obtaining second void fasting urine [10]. In growing children sample collection is difficult to due holding of secondary urine is difficult. Hence, the measurement of bone markers in serum is preferred [10-12, 23].

A commonly used serum marker of bone formation is a bone-specific alkaline phosphatase (BALP), which is released at different stages of osteoblast proliferation and differentiation. Its levels follow a normal growth curve. But obtaining ethical approval is the issue as subjects were left untreated once a blood sample obtains from them [9-13].

Saliva is an oral fluid composed of enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products along with volatile compounds [14]. Enzyme ALP is a membrane-bound glycoprotein synthesized in polymorph nuclear leukocytes, osteoblasts, macrophages and releases into saliva, periodontium and gingival crevice. Various researchers (Suchita M. Tarvade, Harryanto Wijaya, Nora Alhazmi, Fadhline Irham) have reported that level of alkaline phosphatase secretion into saliva follows periodic growth curve [5, 11, 15-17, 26, 27].

This systematic review is aim to accentuate the bone specific property of ALP as individual skeletal maturity indicator and to report its use as a reliable method to identify different growth phases.

**Objective:** The objective of this systematic review is to collect, compile and review the accessible evidence on use of alkaline phosphatase activity in growing children and comparing its reliability with existing growth indicators.

**Focused question:** Is alkaline phosphatase a reliable biomarker to assess skeletal maturity during growth?

## Material and Method

### Literature Search

An extensive search was performed in English language through the following databases to smudge appropriate literature: PubMed and Embase, Web of Science, Google Scholar, Science Citation Index and manual search was performed to spot unpublished research reported in the grey literature using College and departmental library to search for a range of relevant databases, including library dissertations and thesis abstracts. Further search was performed via examining the reference lists from the selected articles using the ‘cited by’ function in Google Scholar. The article explored was followed a principle of saturation to stop scavenging when no additional relevant articles were found. Design of search word permutations was deceptive as there were no precise indexing terms to target alkaline phosphatase as skeletal maturity indicator. An appropriate articles were analyzed using a combination of search terms as ‘alkaline phosphatase OR growth markers OR biomarkers as a skeletal maturity indicators, combined used words and full electronic search approach for each article are illustrated in table 1 and 2.

### Eligibility Criteria

#### Inclusion criteria:

1. Articles published until December 2020.
2. Articles providing information of the levels of alkaline phosphatase and skeletal maturation.
3. Study setting should be hospital based and on growing volunteer children.
4. Participant should not have undergone orthodontic treatment.
5. All articles should be in English.
6. Full text articles.

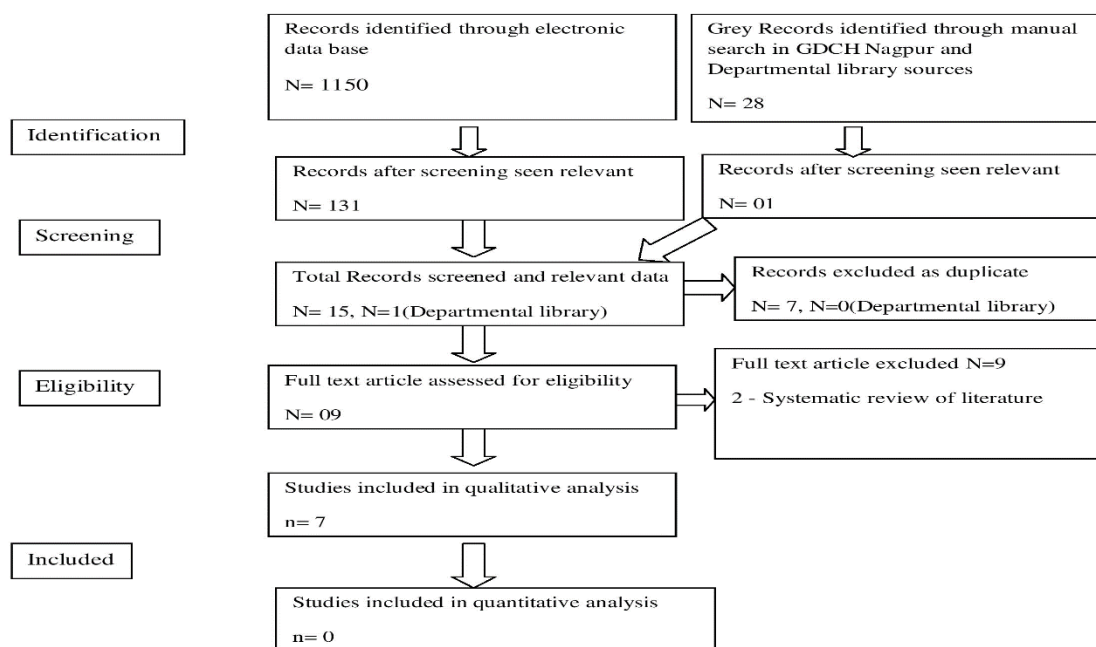
#### Exclusion criteria:

1. Articles that are narrative reviews, case reports, abstracts, letters to editorials, editorials, and animal studies.
2. Articles those are unclear about the levels of alkaline phosphatase and skeletal maturation.

## Study Selection

Objective of this systematic review was to select the articles, which provided valid information about the effect of skeletal maturation on the levels of enzyme alkaline phosphatase activity in growing children. Various electronic databases were explored using different search approach constituting the above-mentioned keywords with their permutations and combinations to spot relevant articles.

The titles and abstracts of the results of the search for desirable articles based on the PICO strategy were assessed by two review authors (SS and WB) independently (Figure 1). The wrangling among authors was settled through discussion and inappropriate articles were expelled; and the motive for their elimination is mentioned in Table 1.



**Figure 1:** PRISMA flow diagram of the included and excluded records

Primary Keywords	Secondary Keywords
Alkaline phosphatase as skeletal maturity indicator	Enzyme alkaline phosphatase levels in growing children
Serum Alkaline phosphatase as skeletal maturity indicator	Alkaline phosphatase as a growth marker
Salivary Alkaline phosphatase as skeletal maturity indicator	Levels of alkaline phosphatase in malnourished children
Bone specific Alkaline phosphatase as skeletal maturity indicator	Sources of alkaline phosphatase secretion in growing children
Urine Alkaline phosphatase as skeletal maturity indicator	Role of alkaline phosphatase as biomarker in determining innate growth in growing children

**Table 1:** Primary and Secondary keywords

Full texts of the articles were selected based on the abstracts/titles that congregate the preliminary selection criteria and a sum of 1122 and 28 articles were found through electronic database search and manual search grey literature respectively. After meticulous scrutinizing the titles, 132 articles legitimated most relevance; among which duplicate and unclear articles were removed. Full texts of 8 articles were potentially suitable for qualitative assessment. The allocation of the journals in which these articles are published is illustrated in (Table 2).

S. No	Key Words	No. of articles searched	No. article selected	Reason for exclusion
	Alkaline phosphatase as skeletal maturity indicator	03	01	Unclear about changes in levels of alkaline phosphatase , duplicate
	Enzyme alkaline phosphatase levels in growing children	28	00	Case reports, Not compared with skeletal maturity indicator
	Serum Alkaline phosphatase as skeletal maturity indicator	13	02	Phosphatase assay unclear, Unclear about changes in levels of alkaline phosphatase , duplicate
	Alkaline phosphatase as a growth marker	31	00	Case reports , Pathologic diagnosis
	Salivary Alkaline phosphatase as skeletal maturity indicator	09	03	Unclear about changes in levels of alkaline phosphatase , duplicate
	Levels of alkaline phosphatase in malnourished children	41	00	Case reports , Levels compared before and after protein supplements
	Bone specific Alkaline phosphatase as skeletal maturity indicator	03	01	Unclear about changes in levels of alkaline phosphatase , duplicate
	Sources of alkaline phosphatase secretion in growing children	103	00	Case reports , physiologic and Pathologic diagnosis
	Urine Alkaline phosphatase as skeletal maturity indicator	213	00	Case reports , Kidney diagnosis and its prognostic , Not compared with skeletal maturity indicator
	Role of alkaline phosphatase as biomarker in determining innate growth in growing children	678	00	Case reports , Compared the levels before and after treating malnourished children

**Table 2:** Electronic Search approach for Each Database

### Quality Assessment

The manuscripts of the entire selected article were assessed taking existing texts and checklist provided by BIOCROSS quality assessing tool for cross-sectional studies using biomarker data. It includes the assessment of Study design, consecutive or random enrollment of subjects with validated device, ascertainment of a biochemical assay of biomarker, circadian relation between flow and enzyme activity and control for confounding factors. For each item, a specific score was designated as score “0” for no description, score “1” for partial description and score “2” for complete and clear description. Later, based upon numbers of highest scores for every item in each study counted and the entire studies were randomly categorized into high or low quality studies. The studies reviewed were designated “stars” based on appropriate scores.

## Results

### Study Selection

The article search was executed depend on the title admissible to the systematic review. A sum of 1150 relevant titles were redeemed from various medical and dental journals, out of which 132 and 28 relevant articles were obtained via electronic database and manual hand search, respectively; after thorough scrutiny 131 studies were shortlisted. On duplicate removal and meticulous screening of abstracts, 16 full-text articles were finalized to determine their compliance with the eligibility. Finally, 8 articles satisfied the selection criteria and were selected for qualitative synthesis of the systematic review. This comprised 48.7% of the total articles obtained pertaining to the data search. The present study was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA), the outline of which is illustrated in (Figure 1).

### Characteristics of included studies

The Characteristics of included studies are summarized in Table 3. The number of included children varies, ranging from 59 to 123. The mean age of these participants ranges from 8 to 18 years. The data were sourced from hospital, institutes where new patients visited for orthodontic treatment. The majority of studies collected information on subjects visiting to the department with skeletal malocclusion at the age of 8-18 years.

### Quality Assessment

Eighty per cent of studies had a low risk of bias with regards to the adequacy of representativeness and random sample selection. All the studies were cross-sectional and only one study was longitudinal. Thus, BIOCROSS Scale was applied for quality assessment. Methodological evaluations of the quality of the included studies were reviewed using the BIOCROSS Scale. The scores vary between 0, 1 and 2, indicating that the quality of the studies was low to moderate to high. The detailed quality assessment of all studies is tabulated in Table 4. The evaluation checklist for risk of bias quality assessment from Clarity group McMaster University for cross-sectional studies (CCs) are shown in Table 5, demonstrating that the CCs pertained a low risk of bias.

S. No.	Name of the journal	No. articles
01	Scientific Reports	01
02	Journal Of Orthodontics,	01
03	Angle Orthodontist,	01
04	Advances In Health Science Research,	01
05	Asian J Pharm Clin Res	01
06	Journal Of International Dental And Medical Research	01
07	Indian Journal of Dental Research	01

**Table 3:** Distribution of the journals in which the articles are published

The various methods of biological maturity indicators included in eight studies which employed hand wrist radiographic method, cervical vertebral maturation [19], modified cervical vertebral maturation and chronological age to assess the levels of alkaline phosphatase activity in growing children as individual skeletal maturity indicator. Hand wrist method was used out in 1 study, cervical vertebral maturation method (CVMS) in 3 studies, modified (CVMI) in 3 study and chronologic age in 1 study.

Issue consideration	Study design	Sample size	Radiographic method	Biomarker assay	Mean value of GCF ALP activity		
					Pre	pubertal	Post-pubertal
Suchita Tarvade 2015	Cross- sectional	120	MP3	Colorimetric analyzer	1400.95	2537.31	1343.9
Sandesh et al 2018	Cross-sectional	90	Hand wrist	ELISA	1317	1734	1577
Tulika Tripathi 2017	Cross-sectional	150	CVMI	ELISA	1650.7	2001.1	1450.0

Fadhlina Irham 2018	Cross-sectional	57	CVMI	Spectrophotometer	1628.7	2333.9	1432.5
Harryanto et al 2019	Cross-sectional	136	CVMS	ELISA	1513.2	1834.7	1691.6
Tulika Tripathi 2019	Cross-sectional	63	CVMI	ELISA	1439	1955	1323
Nora Alhazmia; 2019	Cross-sectional	76	CVMS	Colorimetric analyzer	1379.3	2002.4	1463.1
Wi jaya et al. 2017	Cross-sectional	136	CVMS	ELISA	1391.9	1782.8	1677.9

**Table 4:** Summary of selected articles

	Domains Assessed for Quality Evaluation																				
	Is the source population representative of the population of interest?				Is the response rate adequate?				Is there little missing data?				Is the survey clinically sensible?				Is there any evidence for the reliability and validity of the survey instrument				
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
Wi jaya et al. 2017	*				*					*				*				*			
Sandesh et al 2018			*			*				*				*				*			
Tulika Tripathi 2018	*				*					*				*				*			
Fadhlina Irham 2018	*				*					*					*			*			
Harryanto et al 2019			*		*					*				*				*			
Tulika Tripathi 2019	*				*					*				*				*			
Nora Alhazmia; 2019	*				*					*					*			*			
Suchita Tarvade 2015			*			*					*					*					*

A=Definitely yes (low risk of bias), B=Probably yes, C=Probably NO, D=Definitely no (high risk of bias)

**Table 5:** Items and Criteria for Quality Assessment with the Clarity Group at McMaster University for cross-sectional study.

## Risk of bias assessment

Checklist Provided By the Clarity Group at McMaster University for cross-sectional studies was applied to assess the risk bias and is illustrated in table 4. Seven out of the eight included cross-sectional studies and one longitudinal study satisfactorily addressed the method of stratified random sampling. For blinding, in these studies, only exposure bias was possible and performance bias not possible as subjects and operators were well explained about the clinical procedures.

## Synthesis of results

Three out of the eight studies investigated levels of ALP activity and compared with CVMS, another three studies compared with CVMI stage and remained two studies compared with hand wrist radiographic method, and chronologic age in growing children. These studies had different measurement of ALP assay. Five studies used ELISA; two used colorimeter and one study used spectrophotometer for ALP assessment. Detail illustration is given in table 5.

## Discussion

### Synthesis of evidence

The methodological inequality was observed in all these studies as some researchers have used distinct devices to measure the levels of ALP activity as well as compared it with different skeletal maturity indicators. It additionally noted that the medium advocated for biomarkers and biochemical assay was heterogenic. Some of the studies have used saliva as a medium for biomarkers with p-nitro phenyl phosphate for ALP and colorimeter, while others have used serum and urine with ELISA and spectrophotometer for their analysis.

In 2015, Suchita M. Tarvade et al [5] investigated the salivary levels of ALP activity and compared it with the MP3 staging method. This study was conducted on 120 growing children with mean age of 14 years, range 9-16 years. A radiograph of the middle phalanx of third finger for everyone was taken to detect the MP3 stage of each radiograph as described by Hagg and Taranger (MP3 F stage to MP3 I Stage). Saliva samples were collected and centrifuged to separate the precipitates in saliva. The enzymatic activity of these samples was analyzed using a colorimetric analyzer at 409nm wave length, and then the outcome was subsequently correlated to MP3 stages. The results from the study have shown that there was a gradual increase in salivary ALP levels was seen from F stage (1343.9 IU/L) to FG stage (1378.34 IU/L) of MP3. A shootout was seen in G stage (1513.4 IU/L) then followed by a sudden decline in ALP levels from H stage (1016.48IU/L) to I stage (563.4 IU/L). The study has an overall high risk of bias and is low to medium quality study, as the author has not mentioned

any method for concealment of allocation sequence (table 1). In addition, researchers have not clarified a research hypothesis, sampling method, compounding factor, biochemical assay, study limitation and biomarker data modeling (table 2).

**Harryanto wijaya et al (2017)** [11] conducted a cross-sectional study with 136 subjects (64 boys and 72 girls) with mean age 13 years, range of 8-18 years. The study was aimed to determine the levels of salivary ALP activity and correlate it with cervical vertebral maturity indicators. The registered subjects underwent a professional scaling (supra-gingival and sub-gingival) prior to sample collection. Passive drooling saliva collection method was used and immediately sent for lateral cephalogram. Level of salivary ALP activity was investigated with help of Bradford's assay kit (Thermo Fisher Scientific, USA) following the manufacturer's instructions. Obtained results were co-related with CVM stages described by Baccetti. The authors found that peak levels of salivary ALP activity with pubertal phase and declined in other two phases (pre, post).

Overall impression of this study was, it is a low risk bias and is a high quality study as the authors have clearly mentioned the research hypothesis, cofounding and biomarker assay (table 4). Furthermore, it has noted that sampling, its source and clinical implications (table 5).

**In 2018, Sandesh s Hegde et al** [25] attempted to evaluate the levels of salivary ALP and compare it with different stage of hand wrist radiograph method. The study design was an observational and cross-sectional with 90 growing children range from 6-19-year age (mean age 13 years). The sample distribution ration was 1:1:1 into five subgroups based on-hand wrist radiograph as per Hagg and Taranger method. Saliva sample and hand wrist radiographs were collected on a same day. The level of salivary ALP activity was analyzed using enzyme-linked immunosorbent assay (ELISA) kit. Individual skeletal maturation stages were determined by manual tracings of the hand-wrist radiographs into five the predetermined subgroups as Subgroup S0 (Prepubertal), Subgroup S (Pubertal onset), Subgroup MP3-G (Peak pubertal), Subgroup DP3 (Pubertal deceleration) and Subgroup R-J (Growth completion). The outcome of study illustrated that the salivary ALP levels were significantly higher in subgroup 3 (MP3) (181.16 UI) as compared to subgroup 1 (So) (131.79 UI) and subgroup 5 (R-J) (122.17 UI). The overall study has high-risk bias (table 4) and is low quality research, as authors could not explain biomarker assay, cofounding factors (table 5).

**Fadhlina Irham, Siti Bahirrah (2017)** [22] an observational analytic study with cross-sectional design conducted on 57 growing female patients with mean age 12.5 year (range of 8-15 years). The study was aimed to determine the level of salivary ALP activity and compare it with cervical vertebral maturity indicator

(CVM1-CVM6) as described by Hassel and Farman. Prior to sample collection patients were instructed for not to eat/ drink at least one hour before sample collection. Passive whole saliva was collected and centrifuged for 2 minutes to remove precipitate from saliva. ALP assay was done with help of commercial available kit (BioAssay Systems-USA) under spectrophotometer at 405 nm in room temperature. Using later cephalogram subjects were divided into three groups according to their growth phases i.e. prepubertal (CVM 1 and CVM 2), pubertal (CVM 3 and CVM 4), and post-pubertal (CVM 5 and CVM 6).

Authors noted that the peak of salivary ALP levels was observed in the pubertal growth phase (233,  $39 \pm 106$ , 29 IU/L,) whereas gradual declined in pre-pubertal phase (192,  $87 \pm 69$ , 02 IU/L) and in post pubertal growth phase (79,  $20 \pm 31$ , 41 IU/L). The overall study has low risk bias (table 5) and is medium to high-quality study (table 4).

**Tulika Tripathi et al (2017)** [28] performed a cross-sectional study on 150 growing children with mean age group was 13.5 years, range from 8 to 20 years. They used stratified random sampling method for sample distribution. The study aimed to investigate levels of serum ALP activity and compare it with vertebral maturation index (CVMI) stages. A peripheral venous blood sample collected, centrifuged and serum ALP activity assessed using ELISA (Quidel Corporation, CA, and USA) kits. The variations in the levels of serum ALP has correlated with individual skeletal maturity indicator as defined and described by Hassle and Farman. This study reported that the level of serum ALP activity demonstrated a progressive pattern in the growth curve with a peak at CVMI 3 ( $236 \text{ ng mL}^{-1}$ ), CVMI 2 ( $186 \text{ ng mL}^{-1}$ ), and CVMI 6 ( $117 \text{ ng mL}^{-1}$ ). The overall study has low risk bias (table 5) and is medium to high-quality study (table 4).

### The study Limitations

The evidence obtained for this study was limited (eight studies). The outcome of these studies were heterogenic due use of different devices. Most of the studies could not clarify the source of data, sampling method. Seven out of eight studies were cross-sectional without study duration. Eight out of eight studies have used different methods, medium for biomarkers and biochemical assay. These were the major limitations and future scope for the study.

### The study strength

All the included studies confirm that the levels of alkaline phosphatase activity follow growth curve. A multiple electronic database (pubmed, medline, ebsco, hinary, embase, Psycinfo), grey literature and references were searched. Risk bias was analyzed using Checklist Provided By the Clarity Group at McMaster University for cross-sectional studies. Quality assessment was

executed using BIOCROSS tool. BIOCROSS tool is the most reliable tool for quality assessment of cross sectional studies with biomarker database.

## Conclusion

This systematic review was conducted to answer the question “: is the level of ALP activity reliable in determining skeletal maturity indicator in growing children? Based on the obtained evidence from eight retrieved studies, we have concluded that: ‘Yes,’ the levels ALP activity is a reliable determinant to evaluate individual skeletal maturity. However, this study warns about future scope and limitations of the study published rather than meticulous use.

**Authors’ Contributions:** Dr. Siddharth Sonwane: Writing, Design, Compile, Data collection, Analysis.

Dr. Wasundhara Bhad: Concept, Supervision, Grammar.

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