

**Original article:**

## Salivary alkaline phosphatase- a biochemical marker for growth prediction

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### Abstract:

**Objective:** To evaluate the salivary alkaline phosphatase (ALP) activity in growing subjects in relation to the stages of individual skeletal Maturation using middle phalanx of third finger MP3.

**Material and methods:** The study was conducted on 60 girls and 60 boys who were age group of 10yrs to 15yrs were selected using simple random sampling technique.

Salivary ALP were estimated by the enzymatic method using p- nitro phenyl phosphate as substrate and MP3 stages were assessed using Hagg and Taranger method .Mean salivary ALP and MP3 were compared by analysis of variance ANOVA test .

**Results:** Test showed highly significant differences between ALP and MP3 stages with p value 0.033. Salivary ALP levels showed good association with skeletal age in 13yrs in boys subjects with highest mean ALP and significant value p value of 0 .015 and in 12yrs girls and boys at 13yrs at G stage with maximum growth.

**Conclusions:** Salivary ALP levels can be used as an additional diagnostic tool to optimize orthodontic treatment timing

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### Introduction

The importance of age can be explained by the famous quote of Tom Stoppard “Age is a very high price to pay for maturity”.<sup>1</sup> Appropriate timing of the interception of a skeletal malocclusion is a key to success in dentofacial orthopaedics.<sup>2</sup> In Class II growing subjects, the amount of supplementary mandibular growth induced by functional appliances appear to be significantly greater when the functional treatment is preformed during the pubertal growth spurt. Maximum efficacy of orthopaedic treatment carried out during pre puberty growth phase in skeletal class II malocclusion<sup>4</sup> and rapid palatal expansion<sup>5</sup> was seen. Therefore identification of growth phase has major clinical implication with orthodontic treatment in growing subjects specially when there is skeletal disharmony

The assessment of growth potential is essential because of individual variation in timing, duration and velocity of growth. The classic method of assessing skeletal maturity is by the use of a hand wrist radiographs. Assessing the degree of cervical vertebrae maturation stage on lateral cephalometric radiograph<sup>6-14</sup> and recording MP3 Stages on periapical X ray films are also used to identify peak mandibular bone growth and skeletal maturity<sup>15,16,17,18</sup>. Radiographic methods which are highly subjective techniques involve radiation exposure.

If any of these methods do not show the exact stage, the additional biochemical methods have been proposed to be used to predict the maturation stage accurately.<sup>19</sup> Saliva is an oral fluid, and interest in it as a diagnostic medium in medicine and dentistry<sup>20,21</sup> has advanced exponentially in the last 10

years. Salivary components for any diagnosis include enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, fibroblasts and volatile compounds.<sup>22</sup>

The enzyme ALP plays a role in bone metabolism. It is a membrane-bound glycoprotein produced by many cells, such as polymorpho nuclear leukocytes, osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice.

ALP is essential for bone mineralisation and proposed as a diagnostic aid in periodontology and orthodontics. Christesen<sup>23</sup>, Takimoto<sup>24</sup>, Insoft<sup>25</sup> have shown increase in serum ALP levels during puberty. But as it is an invasive procedure, many times its objected by patients and parents. Baccetti and Perinetti<sup>26</sup> have shown increase in GCF ALP level during puberty. But collection of GCF is a tedious procedure. It can be hypothesized that there will be increases in salivary ALP during puberty. Salivary collection is a non-invasive procedure easy to perform.

The purpose of this study was to associate salivary ALP levels with stages of MP3 so as To assess if salivary ALP levels can be used as pubertal maturity indicator. Also to find the peak salivary ALP levels and its correspondence with MP3 stages in both the genders.

#### **Material and methods**

The study was conducted in the Department of Orthodontics, CSMSS Dental College, Aurangabad, Maharashtra, India

The study was conducted on 120 (60 boys and 60 girls) growing healthy children requiring orthodontic treatment. Children suffering from systemic illness, growth abnormality, and bone disorders were excluded. The Study was approved by the Institutional Ethical Committee. Parental/patients

informed consent was acquired from each individual before enrollment in the study.

#### **Procedure:**

Radiographs of middle phalanx of third finger for each individual was obtained from the radiology department. Observer was blinded about each Patients age, pubertal status and ALP levels. The same observer detected the MP3 stage of each radiograph after the gap of 15 days of taking the radiographs. The MP3 staging technique as described by Hagg and Taranger<sup>27</sup> (MP3 F stage to MP3 I Stage) was used.

Saliva samples were collected and stored in an ice box before sending to the biochemistry laboratory, where they were centrifuged to separate the precipitates in saliva. The samples were analyzed using enzymatic method and assessed using colorimetric analyzer at 409nm. Salivary ALP levels were then subsequently correlated to MP3 stages.

#### **Statistical analysis:**

Analysis of variance (ANOVA), post HOC test and TUCKEY test were used to compare salivary ALP levels and MP3 maturational stages. ANNOVA test revealed a significant difference between MP3 stages with *p* value of 0.0367 [*f*=2.65 with 121% of freedom] 5% level of significance was used. According to Newman Keuls multiple comparison test ( post HOC test, TUCKEY test), the difference between MP3 for the stage G and stage I was significant with *p* value 0.015.

#### **Results**

A strong correlation of ALP levels in girls when correlated with MP3 stages. [Table no 1]. 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). The peak levels of salivary ALP thus were seen

correlating with G stage of MP3. This was followed by a sudden decline in ALP levels from H stage (1016.48 IU/L) to I stage (563.4 IU/L) in girls.

In boys [Table no 2] similar to girls there is a steady rise in salivary ALP levels from F (1376.4 IU/L) to FG stage (1400.95 IU/L). The levels were seen at its peak levels (2537.31 IU/L) in the G stage of MP3. There was a steady decline in the ALP levels in H stage (1906.92 IU/L) which continued even in I stage (1019.1 IU/L)

### **Discussion**

The present study was done to evaluate salivary ALP levels at MP3 stages, since MP3 stages are clinically more relevant. Traditionally, total serum ALP activity has been used as a biochemical marker for bone formation to assess osteoblastic activity in primary hyperparathyroidism, Rickets, osteomalacia and Paget's disease. Since ALP is a marker for osteoblastic activity, growing children have higher levels than fully grown individuals. Highest levels of ALP are detected during the rapid growth phases of childhood such as infancy and puberty.<sup>28,29,30,31</sup>

Study was done to correlate the ALP levels with MP3 stages. Hagg and Taranger<sup>27</sup> stages of ossification of middle phalanx of the third finger of the hand were used. FG stage indicates acceleration of the curve of pubertal growth spurt and G stage with maximum point of pubertal growth spurt and declines at H stages deceleration of curve of pubertal growth spurt. As per Gruelie and Pyle<sup>32</sup> et al, the G stage denoting maximum pubertal growth is attained by girls at the age of 12 years where as in boys at the age of 13 years. Our study showed similar findings.

When ALP salivary levels were correlated with MP3 maturational stages, [Table 6] it was found that peak

levels of salivary ALP levels in girls as well as in boys correlated with G stage of MP3 at the age of 13. Thus a strong correlation of salivary ALP levels with growth spurt was established. This is in accordance with studies carried out by Schiele F et al<sup>28</sup>, Croftan et al<sup>29</sup>, Fleisher et al<sup>30</sup> and Penttila et al<sup>31</sup>. Their studies have shown highest levels of serum ALP during the rapid growth phases of childhood such as infancy and puberty.

### **Conclusion:**

The identification of the individual skeletal maturation stages is crucial for the success of functional and orthopedic treatment. Radiographic methods for the analysis of SMI have been conventionally used along with its drawbacks. Hand and wrist SMI is the most frequently used indicator for pubertal growth spurt. Garn et al<sup>33</sup> stated that the ossification sequence and timing of the skeletal maturity within the MP3 and other indicators show polymorphism and sexual dimorphism which can limit their clinical predictive use. Since these methods are mainly morphological, while new possibilities might be offered by biochemical markers. Biomarkers avoid radiographic exposure, and they represent agents that are involved directly in bone growth and remodeling.

Saliva has been used as a diagnostic fluid in medicine and dentistry. Collection of saliva for analysis is an easy, non-invasive procedure and requiring routine armamentarium. The present study have shown that salivary ALP levels can be used as strong chemical biomarkers for identification of skeletal maturational stages.

Table No. 1

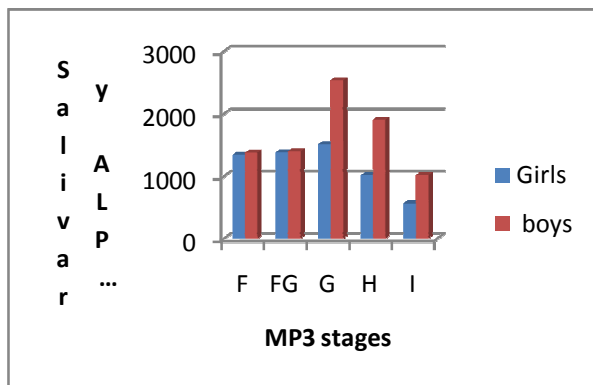
Sr. No	MP3 stage	ALP levels
1	F	1343.9
2	FG	1378.34
3	G	1513.4
4	H	1016.48
5	I	563.4

Table no 1 showing correlation of MP3 stages with salivary ALP levels in girls.

Table No. 2

Sr. No	MP3 stage	ALP levels
1	F	1376.4
2	FG	1400.95
4	G	2537.31
5	H	1906.92
6	I	1019.1

Table no 2 showing correlation of MP3 stages with salivary ALP levels in boys.



Graph showing salivary alkaline phosphatase levels in girls and boys at various MP3 stages.

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