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Research Article

Salivary Alkaline Phosphatase – A Biomarker for Growth Prediction in Orthodontics

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Abstract

Introduction: *Precise assessment of an individual's growth status and a thorough forecast of remaining growth estimation is mandatory for success of treating malocclusions with skeletal etiology in growing individuals. In the recent past, tremendous concomitant research activities are taking place in the field of bio markers. ALP is the biomarker that has been utilized traditionally and increase levels in growing subjects is one of the indications of bone mineralization.This study aimed to compare mean alkaline phosphatase (ALP) in saliva at different stages of skeletal maturity using cervical maturity indicators and hand-wrist radiographs to determine whether salivary ALP can be used as a non-invasive biomarker for skeletal maturity assessment in patients undergoing orthodontic treatment.*

Materials and Method: *Subjects were selected from the age group of 8-18 years. The study sample was further distributed into 3 groups as Pre-pubertal, Pubertal and Post-pubertal. Salivary samples were collected and Alkaline Phosphatase activity was analyzed colorimetrically. It was then correlated with Fishman skeletal maturity indicators and cervical maturation method described by Hassel & Farman.*

Results: *The data collected was analysed by ANOVA (Analysis of Variance) for intergroup comparison. Mean salivary ALP activity was found to be highest in Group 2 (Peak) and lowest in Group 1 (pre peak) & 3 (Post peak) which is in accordance with Fishman's SMI and CVM stages. The intergroup comparison between groups were statistically significant.*

Conclusion: *From this study reports it can be concluded that the activity of Salivary Alkaline Phosphatase was in correlation with the cervical vertebral maturation and Fishman's SMI stages showing peak values at the pubertal stages. Hence Salivary Alkaline Phosphatase can be used as a non-invasive biochemical marker for identification of skeletal maturation.*

Keywords: *Alkaline phosphatase (ALP), saliva, biomarker, cervical vertebral maturation (CVM), hand wrist radiographs , non-invasive, skeletal maturity*

Introduction

Patients in their preadolescent and adolescent years seeking orthodontic treatment still have some craniofacial development to complete. Orthodontists should consider using the remaining growth to create efficient treatment strategies. Skeletal age and chronological age can be compared more readily to evaluate the maturity progress of an individual.[1]

Skeletal maturity assessment is crucial because it has significant clinical ramifications, particularly for orthodontic treatment of growing individuals.[2] For instance, when the functional appliance is utilized during the adolescent growth spurt, it can dramatically increase mandibular development in class II skeletal pattern and mandibular retrognathic patients. On the other hand, treating class III malocclusion orthopedically and rapid maxillary expansion (RME) during the preadolescent growth phase yields more benefits. As a result, accurately determining the subject's growth state is crucial for accurate orthodontic diagnosis and treatment planning. Skeletal maturity evaluation can be done with the help of various maturational indices.3 Among these indices, the most common are the radiographic assessment of bone maturation which includes cervical vertebrae maturation index (CVMI) and hand-wrist radiographic analysis. [4]

Novel prospects might be provided by biological markers as they are non-invasive and prevents radiation exposure.[5] Agents that directly stimulate bone development and remodelling are known as Biomarkers. ALP, Serum osteocalcin, and Propeptide puridinoline cross link of type 1 collagen are utilized as bone biomarkers.[6] The production of the enzyme Alkaline Phosphatase (ALP) will rise in tandem with the increase in osteoblastic activity during bone formation.[7] Thus, ALP is the biomarker for bone production that has been utilized traditionally.

Since whole saliva is a mirror of the body and reflects its diseased and normal state, it can be used as a diagnostic fluid over serum. Moreover, salivary collection is a non-invasive procedure easy to perform and well accepted by patients. Individuals with minimal training, including the patient, can obtain whole saliva non-invasively.[8]

Therefore, this study's aim was to compare the Alkaline Phosphatase (ALP) levels in saliva at distinct phases of skeletal maturity using a Hand-wrist radiograph and Cervical Vertebrae Maturity indicators and to analyse if ALP levels can be utilized as a non-invasive biomarker for evaluation of skeletal maturity in patients undergoing orthodontic treatment.

Aim and Objectives of Study

The aim of this study was to determine the validity of salivary Alkaline Phosphatase as a non-invasive biomarker to assess skeletal maturation.

For this purpose, the following objectives were undertaken:

Compare and correlate the amount of Salivary Alkaline Phosphatase as a skeletal maturity indicator with SMI and CVMI stages

Materials and Method

Study Population

- This pilot study consisted of 15 patients who had reported to the Department of Orthodontics and Dentofacial Orthopaedics at Adesh Institute of Dental Sciences & Research (AIDSR) for orthodontic treatment with no previous history.

Inclusion Criteria

- Patients between the age Group - 8 to 18 years.
- Patients who had reported to OPD of Department of Orthodontics and Dentofacial Orthopaedics

Exclusion Criteria

- Any significant health issue or conditions as per medical history review (i.e. cancer, hepatitis, TB, Syndromes etc.)
- Medical conditions that could affect growth (i.e. acromegaly, dwarfism, hyper or hypothyroidism, achondroplasia, etc.)
- Use of medication or condition that could have effect on salivary production (i.e. antidepressants, Calcium channel blockers etc.)

Materials and Method

Sample:

The study's total sample size was 15. Sample were divided into 3 groups. Groups were selected based on the skeletal age of the patients. The 15 patients were divided into 3 groups based on the assessment method proposed by Fishman skeletal maturity indicator methodology – morphological analysis of hand wrist radiographs.

GROUPS	PUBERTAL STATUS	FISHMAN'S STAGE	CVMI STAGE
GROUP 1	PREADOLESCENT (before pubertal growth spurt) (pre-peak)	SMI STAGE 1, 2 , 3	CVM I & CVM II
GROUP 2	ADOLESCENT (during pubertal growth spurt) (peak)	SMI STAGE 4,5,6,7	CVM III & CVM IV
GROUP 3	POST ADOLESCENT (after pubertal growth spurt) (post-peak)	SMI STAGE 8,9,10,11	CVM V & CVM VI

The staging technique as described by Fishman skeletal maturity indicator was used. The staging technique is described as below:

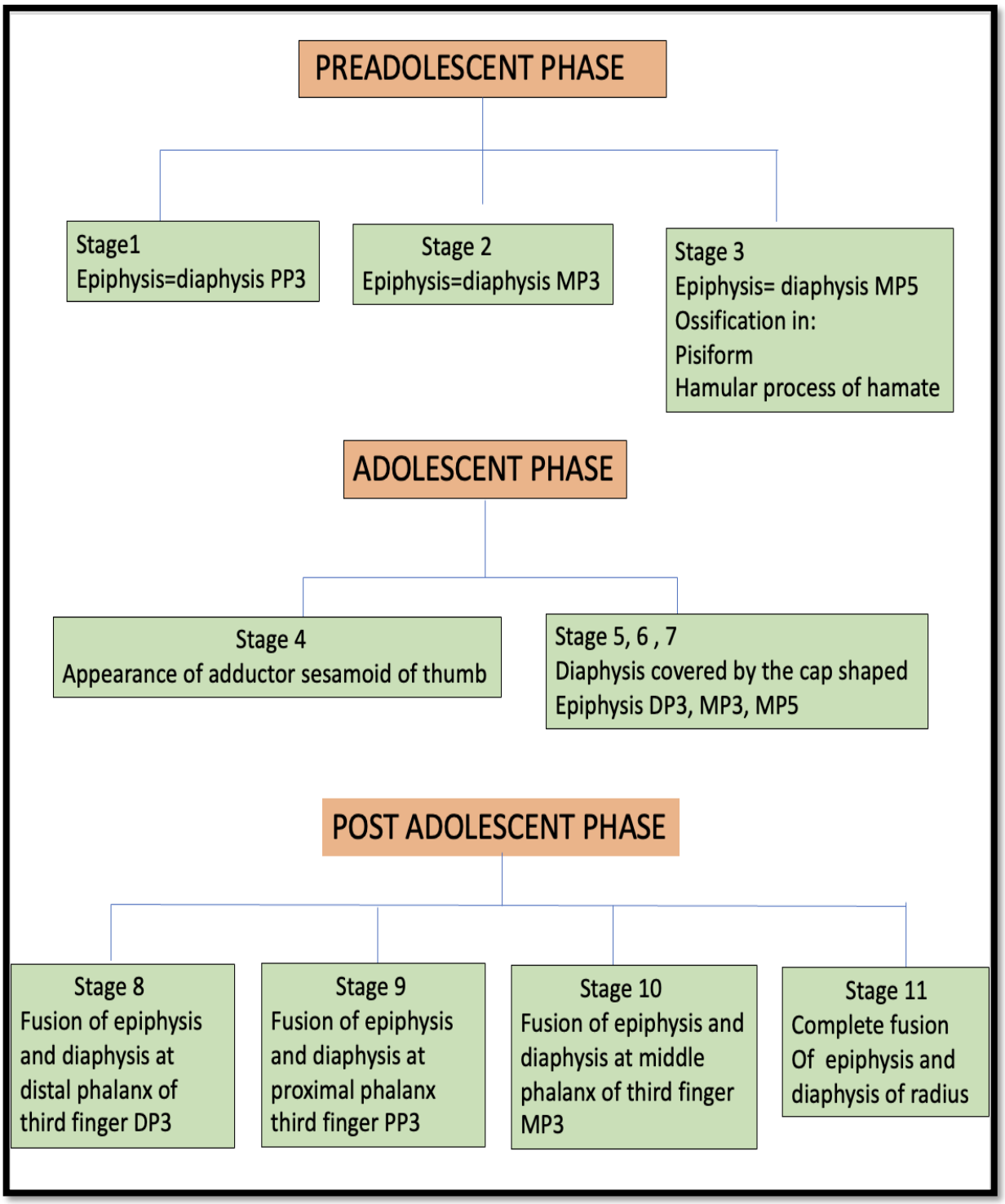

Fig 1a: Phases of fishman's skeletal maturity indicators

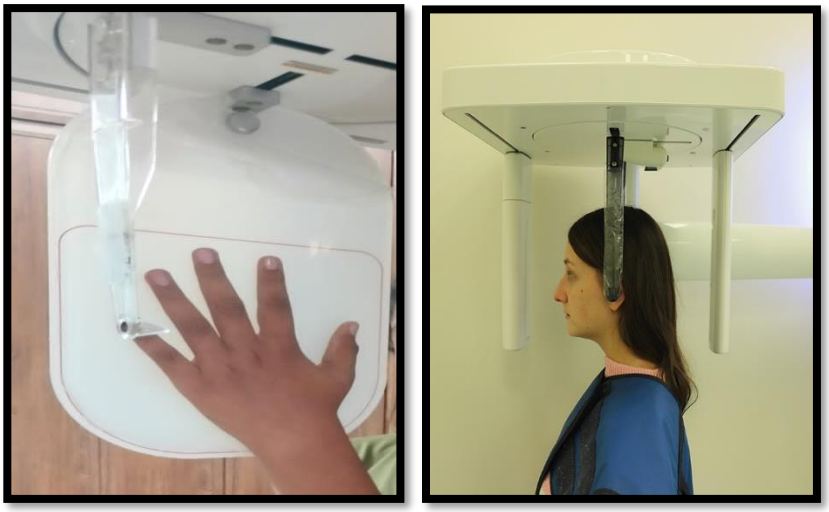

Fig.1b: Hand wrist and lateral cephalogram of the patient

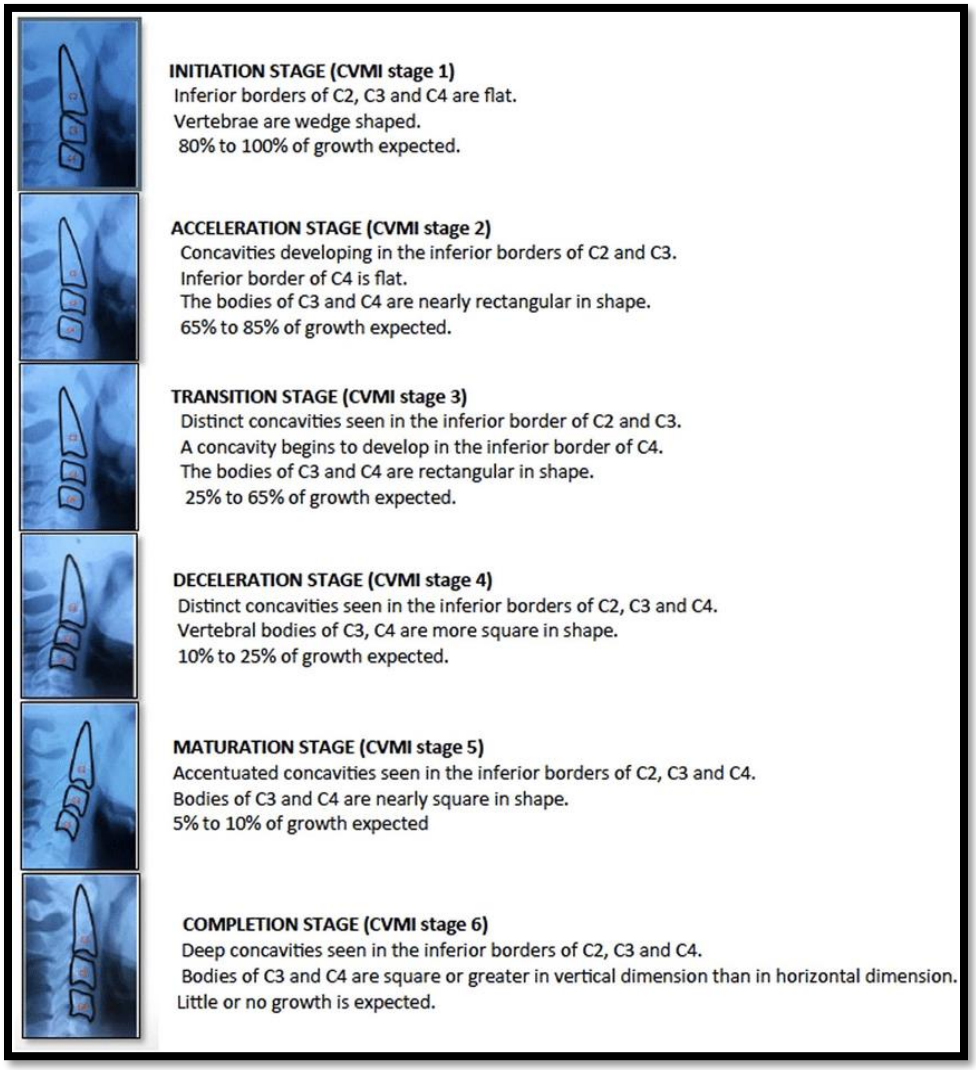

Fig.2: Cervical vertebrae maturation index Stages

Growth estimation was done using the CVM method on lateral cephalogram described by Hassel and Farman, the modifications of Lamparski's criteria were used. Briefly, these were defined as above:

Saliva Collection Procedure:

The patients were scheduled for enrollment at their first clinical examination. The saliva collection protocol was explained to each participant, including timing of collection of sample to prevent the circadian effect, omission of tooth brushing before collecting the sample, avoidance of consumption of food, liquids, or chewing gum for at least 30 minutes before sample collection. Saliva collection was done 10 minutes after rinsing using the passive drooling method.

In order to determine their growth status and assign them to various groups, lateral cephalograms and hand-wrist radiographs were all taken on the same day along with standard orthodontic diagnostic data.

Assessement of ALP:

Saliva samples were collected and stored at a temperature of -20 0 C before sending it to the biochemistry laboratory, centrifugation was done to separate the precipitates in saliva. The samples were then analysed using an alkaline phosphatase kit that was commercially available following manufacturer's instructions and assessed using colorimeter at 400nm. Each saliva sample was centrifuged at 4000rpm for 15 minutes followed by addition of the reagents to supernatant sample and the value of ALP estimated in U/L. Salivary ALP levels were then correlated to CVMI stages and Fishman's skeletal maturity indicator stages.

Results

Group I was deemed to be Pre-peak because individual's tissue growth is relatively slower during this period. Average ALP value was found to be 7.6 U/L, indicating lesser individual growth.

Group II was Peak because there is increased growth during the puberty. Average ALP value was found to be 11.6 U/L, indicating higher individual growth.

Group III was Post-peak since the growth is accomplished at its highest. Average ALP value was found to be 4.6 U/L which indicated completed growth of the individual.

Study's results revealed that mean salivary ALP activity was found to be more in Group 2 (Peak) and lower in Group 1 (pre peak) & 3 (Post peak) which is in accordance with MP3 stages and CVM stages. This indicated that there is positive correlation between the skeletal maturation (MP3, CVM) and salivary alkaline phosphatase.

The results obtained were tabulated and the statistical analysis was performed. The data were analysed by ANOVA (Analysis of Variance) for intergroup comparison.

Bar graphs in Figure 3 depict the increased ALP levels during stage 3-4 of CVMI followed by stage 1-2 and 5-6. Mean ALP levels were seen more in pubertal stage (3-4) followed by pre-pubertal stage (1-2) and post-pubertal stage (5-6).

Bar graph in Figure 4 depicts the increased ALP levels at stage 5-7 of fishman's skeletal maturity indicator followed by stage 2-3,9,10,11. Mean ALP levels were seen more in pubertal stage (5-7) > pre-pubertal (2-3) > post- pubertal stage (9-11).

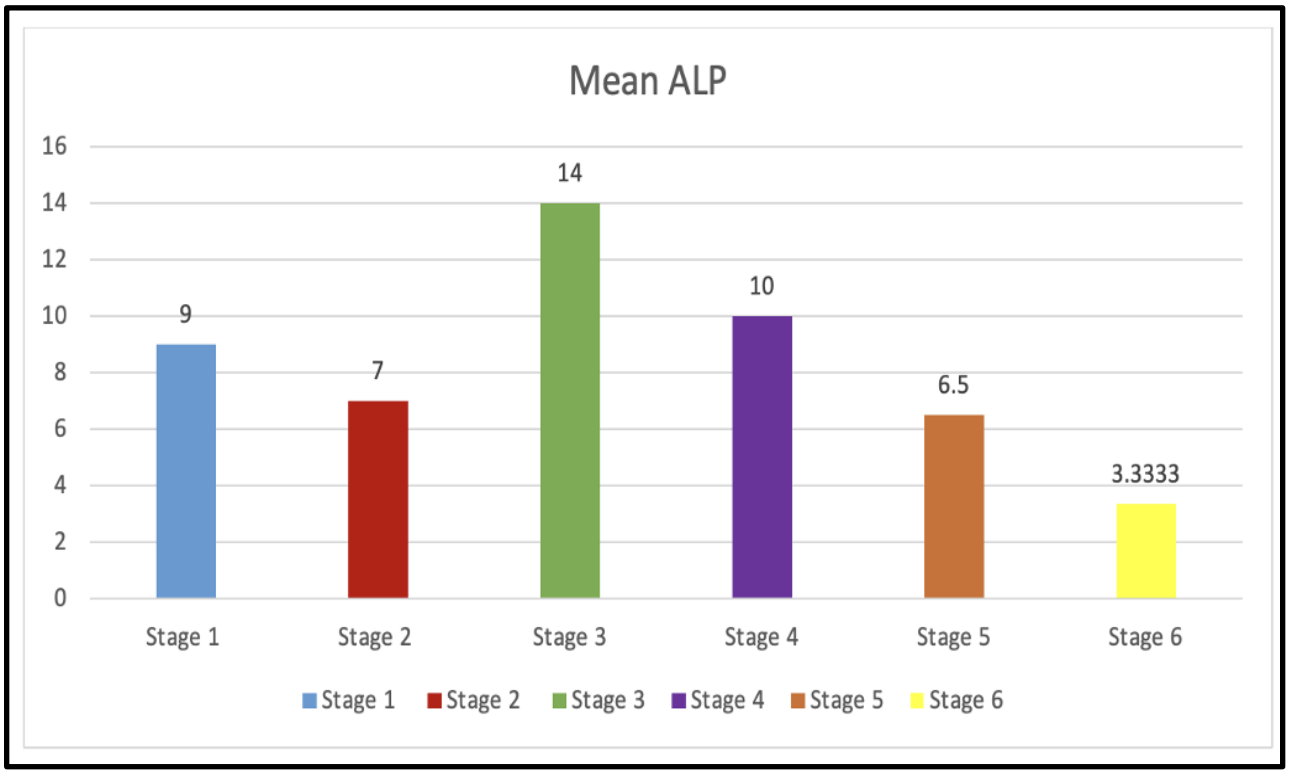

Fig 3. Bar Graph Depicting Mean Comparison of Alp Levels with Cvmi Stages

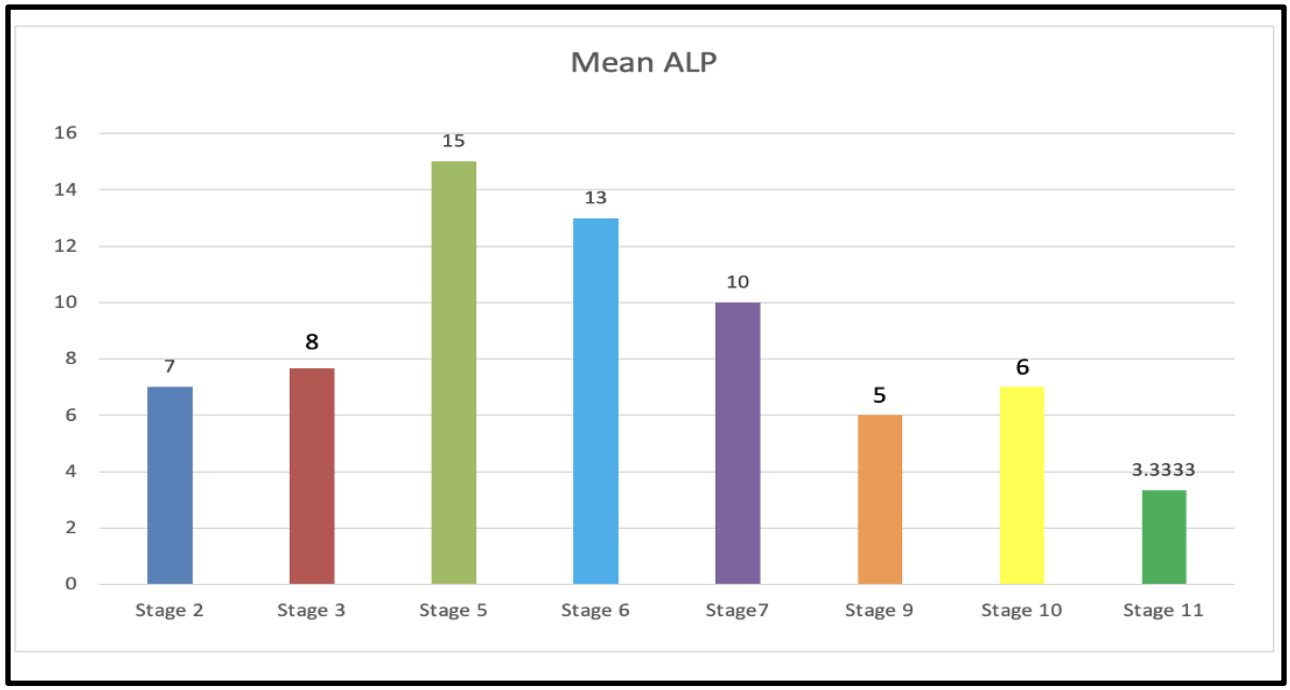

Fig 4. Bar graph depicting mean comparison of ALP levels with fishman's stages

Discussion

Accurate evaluation of the patient's development status and comprehensive prediction of residual growth are critical for the effective management of skeletal origin malocclusions in growing individuals. A range of techniques for evaluating growth have been put forth by different writers. Growth charts, dental development, skeletal maturation, appearance of secondary sexual features, and chronological age are frequently used in clinical orthodontic treatment to predict growth. [9]

The biological clock, also referred to as the hand-wrist radiograph, is a frequently employed diagnostic method for skeletal maturity evaluation.[10] Different methods of using hand-wrist radiographs to measure skeletal maturity and correlate growth status were introduced by Greulich and Pyle (1959), Bjork (1972), Fishman (1982),[11] Grave and Brown (1976)[12], and Hagg and Taranger (1980).[13] The interpretation of Fishman's skeletal maturity indicators through SMI stages, is the most well-established techniques used in the study to group the samples and assess its relationship with the other indications. The SMI stage was employed in the creation of the categories because of its simplicity, lack of complexity, and the literature's plenty of research supporting it.[14] Later cervical maturation stages were also used to assess the skeletal maturity; results showed that these methods were equally as accurate as hand-wrist radiographs. Hassel and Farman (1995) refined Lamparski's technique by utilizing only C2 to C4 morphological abnormalities to assess left outgrowth.[15] Baccetti created a modified method in 2002 to measure mandibular growth.[16]

Finding a substitute that would reduce inter-operator errors and also eliminate the need for hand-wrist or CVMI radiographs would greatly progress the diagnosis of growing patients in terms of skeletal maturity levels.

ALP activity has been used historically to measure osteoblastic activity in primary hyperparathyroidism, osteomalacia, rickets. It's a biochemical marker for bone production which is essential for bone mineralisation and proposed as a diagnostic aid in periodontology and orthodontics Almost every bodily fluid, including serum, GCF, and saliva, is a source of alkaline phosphatase.[17]

When it comes to sample collection, taking a sample from serum is intrusive, while taking a sample from GCF is challenging and time-consuming. On the other hand, Salivary ALP was chosen in this study due its easy collection method, non-invasiveness, well-acceptance by patients and easy transportation.

In the study, total of 15 samples were collected, and the samples were grouped in accordance to the SMI in hand wrist X-rays and the CVMI as Pre peak, Peak, and Post peak. After collecting salivary samples the activity of alkaline phosphatase was measured. It was then correlated with Fishman skeletal maturity indicators and cervical maturation method described by Hassel & Farman. Study's results revealed that salivary ALP activity was seen to be more in Group 2 (Peak) and lower in Group 1(pre peak) & 3(Post peak). Thus a strong relation of ALP with growth spurt was established. Results of the present study are in correlation with the studies done by Tobiume et al. (1997), Perinetti et al. (2011)[18] and Tarvade et al. (2015).[19]

This indicates that there is positive correlation between skeletal maturation (Fishman's SMI, CVMI) and salivary alkaline phosphatase. Given that ALP is a measure of osteoblastic activity, developing children have higher amounts than adults. Peak ALP levels are found during childhood's fast-growing stages, like infancy and puberty. Therefore, Salivary alkaline phosphatase can definitely be considered as an indicator of skeletal maturation based on the current investigation. This approach maintains the validity of the targeted goals while providing new opportunities for the assessment of development maturation through the use of a much simpler and noninvasive procedure that causes minimal discomfort to the patient.

Conclusion

From this study reports, it can be concluded that:

The activity of Salivary alkaline phosphatase was in correlation with the cervical vertebral maturation and Fishman's SMI stages showing peak values at the pubertal stages. Hence salivary alkaline phosphatase can be used as a biochemical marker for identification of skeletal maturation.

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