### A Comparative Study for the Evaluation of Serum Calcium as A Biological Growth Indicator

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#### **Keywords**

Serum calcium, Growth, Skeletal maturity indicator, Bio-marker, Growth Spurt.

#### Abstract

Introduction: Calcium is the most abundant mineral in the body. Adequacy of calcium is essential during childhood and adolescence, as this is the period of rapid skeletal growth. Skeletal maturity of subjects are assessed in various stages of puberty by evaluating serum calcium levels in this study. Aim of this study is to check any change in serum calcium level in adolescents. Material and methods: Two groups of 90 boys and 90 girls were made from selected 180 participants. They are further divided in to subgroups according to age (8-10 yrs. 11-12 yrs and 13-15yrs). Serum calcium of all these participants were performed. Lateral cephalogram, orthopantogram and hand-wrist radiographs were taken of these participants. Results: The result shows that CVMI, SMI and Dental Calcification) showed a strong positive correlation between the specified age groups with Serum Calcium Levels, with a strong correlation for Group A (Boys Group) Sub-Group III and Group B (Girls Group). In study sample, differences in serum calcium, skeletal age and dental calcification were found to be statistically significant. 13-15 yrs. of subjects of Group A show higher serum calcium level than serum calcium level of subjects of Group B. Conclusion: :In addition to other skeletal and dental maturity indicators, serum calcium can be used as an effective biological marker for identification and assessment of growth status of individuals.

#### 1. Introduction

Growth is a feature in the life of a child that distinguishes the child from an adult. Growth occurs in an individual starting from the time of conception till the child grows into a fully mature adult. <sup>1</sup>

Timing, direction and amount of growth is very important in orthodontic assessment. If children are having growth pattern normal than children will undergo a pubertal growth spurt. During this period magnitude, speed, onset and time period of growth shows variation in different individuals. Timing of this growth spurt is very important in prediction of the growth. This can be measured by various techniques like dental age, skeletal age, and skeletal maturity indices on radiographs, height and secondary sexual characteristics of individual etc.<sup>2</sup> Various radiographic methods are available for assessment of skeletal maturity of individual which helps to predict time, amount, speed of pubertal growth. Skeletal maturation is one of the most reliable biological marker. It is performed by taking radiographs of one or more parts of body. Comparison of the patient's hand-wrist bones with those in published atlases is one of the most reliable method for evaluation of skeletal maturity. To avoid radiographic exposure of other areas of body, cervical vertebra maturation (CVM) method is considered the best method for skeletal age evaluation of the patient.

Orthodontists use different methods to predict growth; none of them gives an accurate idea about the sequential biological events going on in the body during a particular time. In order to assess the biological events taking place in the body at particular intervals, biomarkers have proven to be beneficial. Predicting the growth and assessing the biological events are mandatory in orthodontics for planning growth modification.

Calcium is mainly deposited in bone. Calcium balance depends on absorption (intestinal) excretion (sweat gland, renal) intestinal, and sweat gland

excretion. Any disturbance in this may affect bone calcium. Bone resoprtion and deposition can affect this balance. Calcium balance is very important especially during rapid skeletal growth especially in childhood and adolescence. Individuals also comes for active orthodontic treatment in this age only.<sup>4</sup>

The aim of the study was to assess whether serum calcium levels can be used as important biological marker as skeletal maturity indicator to determine "peak pubertal period".

#### 2. Materials And Methods

This study was approved by the ethical committee and the ethical committee clearance certificate was obtained as per the norms set in by them. Written informed consent was obtained from the subjects and their parents following an explanation of the test procedure orally and through a bilingual patient information sheet.

#### 3. Methodology

Two groups of 90 boys and 90 girls were made from selected 180 participants. They are further divided in to subgroups according to age (8-10 yrs. 11-12 yrs and 13-15yrs).

General information like name, date of birth, contact detail were obtained from the subjects for correspondence related to study. Girls and Boys patients ranging from 8-15 years who presented for dental checkup were included in the study.

Participant undergoing treatment for any serious medical illness that may affect the results, Girls and Boys patients below or above the specified age and those with genetic syndromes or major congenital malformations which may affect the results were excluded from the study.

Radiographic and Blood Profile investigations were conducted on patients. Hand Wrist x-ray , radiograph of Cervical Vertebrae and OPG were taken in this study (Fig 1, 2, 3)





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Figure: 1 Lateral cephalogram of 12 yr old girl

Figure: 2 Orthopantomogram of 12 yr old girl



Figure: 3 Hand wrist Radiograph

Serum calcium of all these participants were performed. Comparison of levels of Serum calcium to Skeletal Maturity Indicator of Fishman and Hassel and Farman Schour and Massler along with Dental Calcification Stages.<sup>5-7</sup>

Digital lateral cephalograms, OPGs and hand wrist radiographs were taken using Newton software by a single operator. The radiographs like lateral cephalogram, OPGs and Hand wrist radiographs taken from a single operator were evaluated by two postgraduate students of the department of orthodontics. Hand wrist radiograph and cervical vertebrae maturation were evaluated by Fishman's Skeletal Maturity Indicator method and Hassel and Farman Skeletal Maturity Indicator method respectively. Schour and Massler's Dental Calcification method used for dental was calcification stage evaluation from OPGs.

### 4. SERUM CALCIUM LEVEL DETEMINATION

Blood was collected in a plain vacuette from the children of all the groups with the consent of their parents, explaining the details of the study. The samples of collected blood were sent to white pathology laboratory for determination of calcium levels in blood serum.

Calcium with Arsenazo III (1, 8-Dihydroxy-3, 6disulpho-2, 7-naphthalene-bis (azo) di benzene arsonic acid), at neutral pH, yields a blue colored complex. The intensity of the color formed is proportional to the calcium concentration in the sample. Normal reference values =8.7-11.0mg/dl. Table: 1 Correlation between CVMI, SMI, DC and serum calcium in boys (among all specified boys)

Correlations <sup>a</sup>					
					serum
			SMI	dental	calcium
		CVMI stage	stage	calcification	mg/dl
CVMI stage	Pearson	1	.892**	.731**	.577**
	Correlation				
	P VALUE		.000	.000	.000
	N	90	90	90	90
SMI stage	Pearson	.892**	1	.777**	.522**
	Correlation				
	P VALUE	.000		.000	.000
	N	90	90	90	90
dental	Pearson	.731**	.777**	1	.467**
calcification	Correlation				
	P VALUE	.000	.000		.001
	N	90	90	90	90
serum calcium	Pearson	.577**	.522**	.467**	1
mg/dl	Correlation				
	P VALUE	.000	.000	.001	
	N	90	90	90	90
**. Correlation	is significant at the	e 0.01 level (2-tailed).			

#### 5. Results

The results showed that CVMI (Cervical Vertebrae Maturation Index), SMI (Fishman's Skeletal Maturation Index) and DC (Dental Calcification) had a strong positive correlation between the specified age groups with Serum Calcium Levels. A strong correlation was observed for Group A (Boys Group) and Sub-Group III (Table-1). CVMI (Cervical Vertebrae Maturation Index), SMI (Fishman's Skeletal Maturation Index) and DC (Dental Calcification) showed a strong positive correlation between the specified age groups with Serum Calcium Levels, with a high statistical significance for Group B (Girls Group), Sub-Group III (Table-2).

In study sample, differences in serum calcium, skeletal age and dental calcification were found to be statistically significant. 13-15 yrs. of subjects of Group A show higher serum calcium level than serum calcium level of subjects of Group B. The "Peak Activity Period" of Serum Calcium was observed first in females in the Group B, Sub-Group

II (11-12 years) as compared to the males in Group A and Sub -Group III. Serum Calcium Level continued to increase until puberty after which it remained stable in boys (Table: 3, 4)

#### 6. Discussion

In the current era, the orthodontic intervention involves growth modification, the information regarding patient's growth potential has become mandatory. The clinical decisions with regarding the use of the extraoral force, functional appliances, treatment without extractions and the Orthographic surgery is based on the considerations of the growth, for this reason, the prediction of the quantity of active growth, mainly in the craniofacial complex, are useful to the Orthodontists.<sup>8</sup>

Physical development can be measured by skeletal age as development of facial growth is similar to general skeletal growth as maximum peak of both comes close to each other- only 6 to 8 months difference.<sup>9,10</sup> With the help of this knowledge, estimation of acceleration or decelerating of growth can be estimated which plays a crucial role in orthodontic diagnosis and treatment.<sup>11</sup>

Bone remodeling, bone resoprtion and inflammation in bone are few tissue changes which can be accurately predict and diagnosed by biological markers which can be collected from various tissues. They may draw our attention to some pathologic process. Abnormal values of these biomarkers may be as a result of due to therapeutic intervention or it may be reflecting normal biologic process. <sup>12,13</sup>

Many factors can affect orthodontic tooth movement. These factors may be age, diet, genetics, hormones, serum calcium level.<sup>14</sup> for this reason, this study was carried out to investigate the relationships among chronologic age, CVMI and SMI, dental calcification and serum calcium levels among Indian school children for identifying the pubertal status of an individual. The purpose was merely to investigate whether the serum calcium could be used to assess skeletal age in case of dispute.

Roman et al<sup>15</sup> indicated that the Due to many reasons Hassel and Farman classification is used to measure skeletal maturity. Unlike Lamparski classification which is accurate in males only, Hassel and Farman classification can be used in both the sexes. Hassel and Farman classification needs to assess less number of vertebral bodies.

In 1941, Schour and Masseler<sup>16</sup> published developmental charts for deciduous and permanent teeth with 21 chronological steps from 4 months to 21 years of age updated and published them in 1982 by The American Dental Association, to compare the calcification stages of teeth on radiographs with the standards. We have used this parameter devised by Schour and Masseler to assess the dental calcification stage in this study.

Orthodontist must consider few factors like overall health of individual, good calcium metabolism, diet in selection process, risk assessment, diagnosis and treatment planning.<sup>17</sup> Disturbance in calcium metabolism may lead to <sup>failure</sup> in maintaining good serum calcium level due to one reason or may be more than one reason may lead to inadequate bone mass. In such cases it is not possible for orthodontist to perform Orthodontics and Orthognathic surgery. <sup>18</sup>

In order to use Serum Calcium level as a biological marker and as an adjunctive diagnostic tool for determining the "peak pubertal status" of an individual, the present study was carried out. We described age- and sex-specific Serum Calcium levels for ages 8 to 15 years. The level of serum calcium had been correlated to CVMI, SMI and DC status individually for each subject in the specified age group by Arsenazo III method. (This method is preferred over other biological markers as it is compatible to patients and cost-effective. No special reagents or tests are required and Calcium level can be found during routine hematological investigation). 180 healthy individuals of appropriate height and weight, excluding malnourishment and over nourishment were selected to avoid bias. The WHO Criteria of height and weight correlation with serum calcium was followed.

In boys, serum calcium levels showed the highest levels for group A, Sub-group-III (13-15). Spearman rank-order correlation coefficient between serum calcium and CVMI, SMI, and DC were 0.393,0349 and 0.448 respectively, which was also statistical significance (p <0.001) for this age group. The serum calcium levels also showed a statistically

significant correlation between CMI, SMI and DC (p <0.005) for the other two age groups-Group-A, Sub-Group-I and Sub-Group-II.

In girls, serum calcium levels showed somewhat different values as compared to males. The serum calcium levels for the Group-B, Sub-Group-II (11-12 years) were recorded highest amongst the three Sub-groups. Spearman rank-order correlation coefficient between serum calcium levels and CVMI, SMI, and DC were -0.133, 0.271, and -047, respectively. The serum calcium levels also showed a statistically significant correlation between CVMI, SMI, and DC (p<0.005) for the other two age groups-Group –B, sub-group-I and Sub-group-III.

In the present study, the peak activity period of serum calcium levels was highest among the 13-15 age group of boys and for girls 11-12years (Graph – 1). The above result was in accordance with a study conducted by Donald Baily et al<sup>19</sup>. An experimental study was conducted by Engstrom et all<sup>20</sup> on Rats. According to that study, a change in serum calcium level is a determining factor for bone resorption and formation along with parathyroid hormones.

In our methods of analyzing maturation, it was found that females are early matures compared to males. These results coincide with studies carried out by Grave and Townsend and, Demirjian et al., who found that females mature earlier than males, which becomes an important consideration in the general evaluation of an orthodontist patient. From our study, it is evident that serum calcium level can be used as a biological marker in identifying the pubertal status of an individual along with other skeletal maturity indicators. Also, in planning for orthodontic treatment (early intervention for correction of class II or III malocclusion), the pubertal status of the individual should be taken in to account. Difference in serum calcium levels in puberty, before and after puberty, positive correlation of chronological age, CVMI, SMI and DC and serum calcium level suggest that along with other maturity indicators, serum calcium can be used as reliable biomarker to estimate growth of individuals. Few more prospective studies with a large number of sample size is required to confirm the effectiveness and accuracy of this method in assessment of intensity and time of growth spurt.

#### 7. Conclusion

- There exists a statistically significant positive correlation between CVMI, SMI, DC and Serum calcium levels which suggests that serum calcium is an important marker in assessing the status of growth of an individual in addition to other skeletal and dental maturity indicators.
- Serum calcium activity is an applicant biomarker of individual skeletal maturation. Serum calcium can be used to confirm whether an individual has any growth potential remaining to intercept the developing malocclusion.
- Serum calcium can be used to identify "the peak activity period (puberty age) commence orthodontic treatment.

#### References

- Todd TW. Atlas of skeletal maturation. 1st ed. St. Louis: The CV Mosby Company, 1937.
- [2] Greulich, WW. & Pyle, SI. Radiographic Atlas of Skeletal Development of the Hand and Wrist. 2nd ed. London, England: Oxford University Press, 1959.
- [3] Midgett RJ, Shaye R, Fruge Jr JF. The effect of altered bone metabolism on orthodontic tooth movement. Am J Orthod. 1981;80 (3):256-262.
- [4] Rhodes WF, Pflanzer R. Human Physiology. Philadelphia: WB Saunders,1989.
- [5] Fishman LS. Radiographic evaluation of skeletal maturation. A Clinically Oriented method based on hand-wrist films. Angle Orthod. 1982; 52:88-112.
- [6] Hassel B., Farman A. Skeletal maturation evaluation using cervical vertebrae. Am J Orthod Dentofacial Orthop. 1995; 107:58-66.
- [7] Schour I, Massler M. Development of Human Dentition. J Am Dent Assoc 1941; 20:379-427.
- [8] Toledo, V. Orthognathic surgeon simplification of the treatment of surgical orthodontic in adults, Amorca caracas, Venezuela,2004.
- [9] Haag and Turanger: Maturation indicators and the pubertal growth spurt. Am J Orthod 1982;82(4):299-309.



- [10] Haag and Turanger. Skeletal stages of the hand and wrist as indicators of the pubertal growth spurt. Acta odontol scand 1980, 38:187 – 200.
- [11] Himanshu Trivedi et al. Growth and growth studies in orthodontics a review. Journal of dentistry and oral care 2016;2(2):65-69.
- [12] D. Nagarajan et al. Biomarkers in orthodontics. International Journal of Oral Health and Medical research 2015,2(3):88-90.
- [13] Adel M Alhadlaq, biomarkers of orthodontic tooth movement in gingival crevicular fluid – a systemic review, journal of contemporary dental practice 2015;16(7):578-587.
- [14] Nanda RS. Cephalometric study of the human face from serial roentgenogram. Ergeb d Anat d Untwicklungs-Geschichte; 1956; 35:358-419.
- [15] Paloma San Roman JCP, M Dolores Pteo, Esther Nevado. Skeletal maturation determined by cervical vertebrae development

.European journal of orthodontics 2002; 24:303-11.

- [16] Panchbhai AS,Dental radiographic indicators, a key to age estimation. Dentomaxillofacial Radiology (2011) 40, 199–212.
- [17] Lee W. Graber et al, Orthodontics current principles and techniques, Elsevier, St. Louis, Missouri, 2017.
- [18] Jones SJ: Secretory territories and rate of matrix production of osteoblasts. Calc Tiss Res 14:309-314, 1974.
- [19] Donald A. Bailey et al, Calcium accretion in girls and boys during puberty: a longitudinal analysis. Journal of bone and research 2000;15(11):2245-2250.
- [20] Engstrom C, Granstrom G, Thilander B. effect of orthodontic force on periodontal tissue metabolism, Am J Orthod Dentofacial Orthop 1988; 93:486-495.