Original Research Paper

University J Dent Scie 2022; Vol. 8, Issue 2

Serum Alkaline Phosphatase: An Alternative to Skeletal Age Assessment.

Abstract:

Purpose- To assess the growth status of adolescents by cervical vertebra maturity indicators (CVMI) and its correlation with the level of serum alkaline phosphatase (ALP) and also to evaluate the diagnostic importance of serum alkaline phosphatase as an indicator of skeletal maturation.

Settings and Design: prospective cross sectional observational study

Methods and Materials : 150 subjects (75 males and 75 females) were selected in the age group 6-17 years of age.The study sample was distributed into three groups based on chronological age Group-I(Pre- Pubertal) consisting of sample between 6-9 yrs, Group-II(Pubertal) between 10-13 yrs & Group-III (Post- Pubertal)14-17yrs of age, with each group containing 25 males and females each. To appraise the cervical vertebral development by Hassel and Farman method lateral cephalograms were taken in natural head position. To evaluate the serum Alkaline phosphatase levels 2 ml of venous blood sample was collected and was subjected to biochemical assessment.

Statistical analysis used: paired sample T test, Pearson Correlation Coefficient , ANOVA analysis

Results : The mean alkaline phosphatase levels were evaluated in each group, Group II (Pubertal) had the highest alkaline phosphatase level which was statistically significant among all groups. The correlation between alkaline phosphatase and CVMI was assessed using Pearson Correlation Coefficient. A significant negative correlation between the two entities was found ($r = -0.630$, $p<0.001$). On evaluating the CVMI stages and alkaline phosphatase levels individually in males and females, males had higher levels which were statistically significant.

Conclusions : The study demonstrates age trends in the level of serum alkaline phosphatase levels that may be used to evaluate the skeletal maturation. The levels in males were higher than females which also are of diagnostic importance.

Key Words : skeletal age evaluation ,Cervical Vertebra Maturity Indicators, Serum alkaline phosphatase .

Introduction:

The evaluation of craniofacial growth and development is a critical component in orthodontics[1].A number of parameters have been proposed and assessed as probable markers of an individual's peak growth. Chronological age alone is a poor indicator of skeletal maturity which is due to the intra and inter individual growth variations[2]. Physical Markers such as increase in height and appearance of secondary sexual characteristics cannot be used to predict the growth as they are retrospective in nature[3]. Dental age as an indicator for maturity is a simple but not so accurate method because of wide variations in timing of eruption of teeth due to the influence of local and environmental factors[4].

Appraisal of skeletal maturation is essential in planning individual orthodontic treatment because of marked individual variations in timing, duration, and intensity of pubertal growth[5].

Access this article online

Website: www.ujds.in

Quick Response Code

DOI: https://doi.org/10.21276/ujds.2022.8.2.9

]Fishman[2],[6] used skeletal maturity indicators (SMI) in hand-wrist radiographs using four stages of bone maturation at six anatomic sites, which used to be amongst the most commonly used methods to assessment. Morphological

[1]ARBAB ANJUM, [2]PRADEEP RAGHAV, [3]SHISHIR SINGH, [4]MUNISH REDDY, [5]SAURABHBHALLA, [6]ARINA ARIF

[1]Dept. of Orthodontics, Dr. Ziauddin Ahmad Dental, College, Aligarh Muslim University, Aligarh

[2,4]Dept. of Orthodontics, Subharti Dental College, SVSU, Meerut

[3]NCR Medical College, Meerut

[5]Dept. of Orthodontics, Rungta Dental College, Bhilai, Chattisgarh

[6]Dept. of Conservative Dentistry & Endodontics, K.D. Dental College, Mathura

Address for Correspondence: Dr. Arbab Anjum
Assistant Professor
Dept of Orthodontics, Dr. Ziauddin Ahmad Dental College, Aligarh Muslim University, Aligarh
Email : arbab.anjum@gmail.com

Received : 31 March, 2022, **Published :** 30 June, 2022

How to cite this article: A Anjum, P Raghav, S Singh, M Reddy, S Bhalla, A Arif. (2022). Serum Alkaline Phosphatase: An Alternative To Skeletal Age Assessment. UNIVERSITY JOURNAL OF DENTAL SCIENCES, 8(2). 51-57

changes in the cervical vertebrae of adolescents have gained rising interests. The cervical vertebrae seen in normal routine lateral cephalograms is as clinically reliable in assessing skeletal age as hand wrist[7]. This was further confirmed by Hassel and Farman[8], Garcia Fernandez[9]. The Cervical Vertebra Maturity Indicator CVMI method eliminates radiation exposure as it utilizes the lateral cephalogram, which is routinely used in orthodontics[10].

The present day methods of skeletal maturity assessment like the hand-wrist radiographs or cervical vertebrae radiographs are expensive, require elaborate equipment and accounts for high radiation exposure, especially for growing children[11],[12]. Even though these skeletal maturity indicators are helpful but the exact nature of peak growth velocity cannot be estimated hence the use of biological indicators is advocated.

Many changes occur during puberty in an organism as a result of important hormonal changes, especially increase in the serum level of biochemical indicators and one such indicator is serum alkaline phosphatase (ALP). Alkaline phosphatase is a host enzyme that allows bone deposition by hydrolyzing inorganic pyrophosphate, which is a potent inhibitor of the mineralization process[13]

ALP has been extensively correlated with the rate of bone formation. The main source of alkaline phosphatase present in serum is mainly from the liver and intestine with a modest amount from bone. Its release into the blood is increased with high turnover of osteoblasts[14].The plasma concentration of alkaline phosphatase is one of the biochemical indicators of bone formation. Growing children have higher levels of serum alkaline than full-grown adults, as elevated levels are due to active bone formation. Increased serum alkaline phosphatase is also seen in bone, liver & gall bladder dysfunction[13],[14].

Most of the current diagnosis is based on radiographic parameters, but the inherent drawbacks of the radiographs are well documented in literature. However the use of biological indicators can give us a clearer picture of the growth status of the individual. Though serum alkaline phosphatase level estimation has commonly been used in pediatrics whereas, in orthodontics not much research has been done.

Therefore the present study was designed to assess the growth status of the children by CVMI and its correlation with the level of ALP levels and also to assess its diagnostic importance as an indicator of skeletal maturation[27].

Material and Methods:

The study was conducted on 150 subjects (75 males and 75 females) in the department of orthodontics.

Subjects were included after obtaining an informed and written consent from the patients and their guardians. The study was approved by the Institutional Ethical committee (D.No05/02/11SDC) .

Sample size calculation :

For sample size estimation ,software G*Power ver. 3.1.9.2 (Dusseldorf University, Dusseldorf, Germany) was used with p-values of < 0.05, a power of 80%, and an effect size of 0.3 from previous studies as 150.

The study sample was distributed into three groups based on chronological age Group-I (Pre-Pubertal) consisting of sample between 6-9 yrs, Group-II (Pubertal) between 10-13 yrs & Group-III (Post-Pubertal) 14-17yrs of age, with each group containing 25 males and females each[27]. A written informed consent was obtained from all the subjects or his/her parents as prescribed and the study was reviewed and approved by the ethical committee of the university.

Inclusion Criteria:

Subjects in the age group (6 – 17 yrs) with normal growth and development and good nutrition status without any serious illness (with special attention to bone and liver diseases).

Exclusion Criteria:

Subjects having any history of congenital or acquired malformations of the cervical vertebrae and/or developmental alterations due to medical syndromes or hormonal disorders, previous trauma or injury to the face and cervical vertebra region were excluded from the study[22].

The pretreatment lateral cephalograms were taken in natural head position by the same operator (PAX-400C VATECH, value added technology, Korea) with the film distance to the X-Ray tube fixed at 5 feet. All lateral cephalograms were evaluated by two orthodontists (A.A. and P.R.). Cervical vertebral development of the sample was evaluated by Hassel and Farman[8], modification of Lamparski's criteria, which assesses maturational changes of the second, third and fourth cervical vertebrae. The assessment of the cervical vertebrae maturation stages were done individually without the knowledge of the chronologic ages. The average of the two was considered as the vertebrae maturation stage.[22]

To evaluate the serum ALP levels 2 ml of venous blood sample was collected from the left hand of each volunteer by using sterile non-toxic plastic syringes into sterile containers. The sample was taken in the morning hours (10am-12am) .The colorimetric method was used with p-nitrophenyl phosphate as substrate and diethanolamine (DEA) buffer were used .this method is based on the action of alkaline phosphatase on the DEA buffer . The plasma fraction was separated by centrifugation at 1000g for 5 minutes using Vitros-250 centrifuge .[16]. Blood sample and radiographs were taken on the same day. The CVMI readings were then evaluated against serum ALP levels in order to assess the correlations.

Results:

Statistical analysis used:

The paired sample T test was used to determine measurement accuracy. Pearson Correlation Coefficient to assess the correlation between the variables (serum ALP and CVMI), ANOVA analysis to determine whether there are any significant association between the means of the independent groups .

Results:

To ascertain reliability, skeletal maturity was ascertained by cervical vertebra maturity by Hassel and Farman method[8], random samples were selected from each group and reassessed after three weeks . The paired sample T test was used to determine measurement accuracy. No statistically significant difference was found between the first and second measurements.($p>0.05$).

The mean serum ALP levels were evaluated in each group There was a statistically significant difference among the groups. Group II (10-13) had the highest alkaline phosphatase level, (Table 1)

Table-1 shows the mean alkaline phosphatase levels in each group.

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
1	50	238.46	45.951	6.498	225.40	251.52
2	50	296.52	55.167	7.802	280.84	312.20
3	50	160.70	78.256	11.067	138.46	182.94
Total	150	231.89	82.620	6.746	218.56	245.22

On evaluating the mean alkaline phosphatase levels with different CVMI groups. The peak value was seen in CVMI 3, with a mean value of 298 u/l, which gradually decreased to 115 u/l in the CVMI 6. ($p< 0.001$).

(Table 2)

Table-2 shows mean alkaline phosphatase levels in different CVMI groups

CVMI STAGES	N	Mean ALP	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
1	22	219.60	47.74	12.26	195.08	244.12
2	30	248.95	63.28	11.83	225.29	272.61
3	26	298.54	63.21	10.18	278.18	318.9
4	23	235.33	57.23	12.78	209.77	260.89
5	25	193.10	51.83	14.11	164.88	221.32
6	24	115.63	38.07	8.47	98.69	132.57
Total	150	231.89	53.18	10.75	210.39	253.39

The correlation between serum ALP and CVMI was assessed using Pearson Correlation Coefficient (Table-3).

Table 3 shows correlation between alkaline phosphatase and CVMI .

*($p<0.001$).

		ALP	CVMI
	Pearson Correlation	1	**-.630(**)**
	Sig. (2-tailed)		.000
	N	150	150
	Pearson Correlation	**-.630(**)**	1
	Sig. (2-tailed)	.000	
	N	150	150

A significant negative correlation between the two entities was found ($r=-0.630$, $p<0.001$).

On evaluating the CVMI stages and alkaline phosphatase levels individually in males the mean levels were highest (285 u/l) in the CVMI 3 group which reduced gradually to (139 u/l) CVMI 6, but was not significant on ANOVA analysis (Table-4/Fig 1)

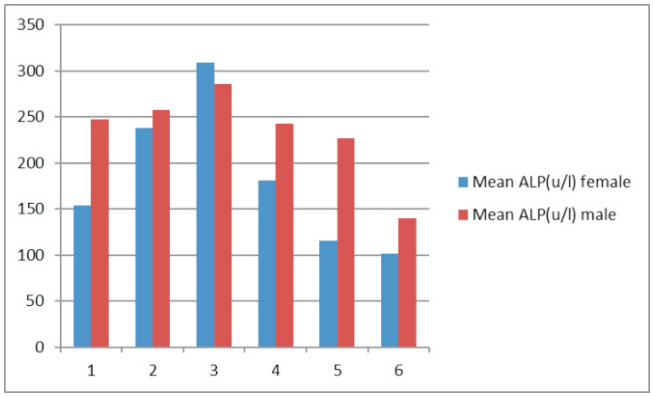
Table 4 relates CVMI stages with alkaline phosphatase levels in males.

CVMI STAGES	N	Mean ALP(u/l)	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
1	13	247.57	64.29	14.75	218.07	277.07
2	16	258	51.34	10.21	237.58	278.42
3	14	285.29	63.34	15.66	253.97	316.61
4	11	243	56.12	12.49	218.02	267.98
5	12	226.43	59.48	12.36	201.71	251.15
6	9	139.6	42.26	14.55	110.5	168.7
Total	75	245.61	60.27	11.23	223.15	268.07

Whereas for females, the mean levels were highest (308 u/l) in the CVMI 3group, which reduced gradually to (101 u/l) CVMI 6 ,which was significant on ANOVA analysis.(Table-5/ Fig 1)

CVMI STAGES	N	Mean ALP (u/l)	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
1	9	154.33	67.45	16.69	120.95	187.71
2	14	238.29	53.87	11.95	214.39	262.19
3	12	308.77	63.22	13.59	281.59	335.95
4	12	181.5	43.28	15.78	149.94	213.06
5	13	115.33	40.67	21.09	73.15	157.51
6	15	101.53	13.96	3.38	94.77	108.29
Total	75	218.17	54.35	12.88	192.41	243.93

Figure 1 CVMI stages with alkaline phosphatase levels in males and females



Discussion:

The present study was designed to evaluate the growth status of the children by routine radiographic methods and its correlation with the level of serum alkaline phosphatase in the North Indian population. A sample of 150 subjects (75 males and 75 females), an equal proportion of males and females was taken because an ideal research design for a comparative study should have matching groups for comparison of the characteristics[21].

The sample was divided into different age groups because every individual shows variations in timing, duration and velocity of growth. Hence the samples were distributed in three groups with an age range in between 6-17yrs.Group-I (pre- pubertal)consisting of sample between 6-9 yrs, Group-II(pubertal) between 10-13 yrs & Group-III(post-pubertal) 14-17yrs of age, with each group containing 25 subjects each.

The methods employed in the study were skeletal maturation as assessed by cervical vertebrae maturation and serum alkaline phosphatase level. The serum ALP levels were evaluated by taking venous blood sample. Even though the method should also be non invasive, but the objective of the study was to develop a safe and economical method, which can be easily used in day to day orthodontic practice and also in places with no elaborate armamentarium.

In our study, the Hassel and Farman [8] modification of Lamparski method was used. It can be used on both sexes accurately, whereas the Lamparski classification has been found to be more effective in females[12] ,Also it uses less number of vertebrae and the evaluation is more detailed [22].

The serum ALP levels were estimated by the colorimetric method, using p-nitrophenyl phosphate as substrate and diethanolamine (DEA) as buffer as DEA buffer shows increased sensitivity as compared to the AMP buffer. According to Tietz [20], this increased sensitivity allows the use of small sample volumes, thus the higher buffer concentrations on proteins and especially enzymes could be easily demonstrated using DEA.

The results were analyzed using "Statistical Package for Social Sciences" (SPSS) for Windows (version 21.0). A negative correlation between serum ALP and CVMI was

found. As the CVMI stages increases, the level of alkaline phosphatase decreases. ($r=-0.63$, $p<0.001$).

The mean serum ALP level for adults is 33-96 u/l and can vary from as low as 20 u/l to as high as 140u/l[23].The prepubertal group had a mean value of 238 u/l. The highest mean alkaline phosphatase value was seen in the pubertal group was 296 u/l whereas in the postpubertal group, a mean alkaline phosphatase level of 160 u/l was found.

The pubertal group i.e. Group II had the highest serum ALP level which was statistically significant among all groups. The results of the study are in accordance with Fleischer [14] , who found the highest levels in the pubertal group. Turran [16] also stated that higher ALP levels were noted at ages 10-11 years in girls (587 u/l) whereas serum ALP levels start to decline after 12-13 years of age in boys (559 u/l).

The relationship between alkaline phosphatase and CVMI showed a significant negative correlation between the two entities ($r =-0.630$, $p<0.001$). The levels of alkaline phosphatase increased upto CVMI 3 which had the maximum level and then gradually decreased to CVMI 6.

Bjork and Helm [3] found that the MP3cap stage is an indicator of the peak (PHV) of pubertal growth spurt, which also corresponds to stage 3 of the CVMI [17] Fishman [24] , Soegiharto [1],[28]. In our study the peak value of alkaline phosphatase was seen in CVMI stage 3.

According to Pancherz [25] and Franchi [26] , the prepubertal stage is the phase when growth modification should be planned in cases of maxillary deficiency for e.g. in a case of skeletal Class III with deficient maxilla where protraction headgear is being considered [28].

In males, the highest serum ALP was seen in CVMI 3 (286 u/l) which however on ANOVA was statistically not significant among all the groups. The pre-pubertal levels were high (247 u/l) and gradually increased, with the highest levels during the pubertal period (286 u/l) which than decreased and the lowest values were seen in the post pubertal period(139 u/l) .

In females, the highest serum ALP was seen in CVMI 3(307 u/l) which on ANOVA is statistically significant among all the groups. The pre-pubertal levels were (154 u/l) and increased, with the highest levels during the pubertal period (308 u/l) which then decreased and the lowest values were seen in the post pubertal period (101 u/l).

On the overall comparison between males and females, in pre pubertal group the males showed higher levels of alkaline phosphatase than females which was statistically significant, whereas in the pubertal group, the females showed higher levels of serum ALP than males but this was also not statistically significant. Fleischer [14] also found that the highest levels were found in the pubertal group.

The puberty peak activity occurs in girls between 11-12 years, in boys between 13-14 years. Turran [16] also found that serum ALP levels tend to decline after 12 years of age in girls and after 14 years of age in boys.

The alkaline phosphatase levels in females were found to be lower than males in the group 14-17yrs (post pubertal) which was statistically significant .In females the puberty finishes before their male counterparts, so does the active bone formation. This finding is also in accordance with Turran[16].They found that the upper ranges of adults earlier in girls but not in boys until they reach the ages of 16 to 18 years.

Shortcomings:

The present study is cross-sectional with the amount of variation in the results quite high, hence a need for a long term longitudinal study with larger sample size is required to reduce the degree of variation and to increase the reliability.

Conclusions:

In determining the relationships among cervical vertebrae and serum alkaline phosphatase levels of 150 subjects, the following conclusions were drawn:

1. There was a statistically significant correlation between chronological age, maturation of cervical vertebrae and serum alkaline phosphatase levels.
2. The highest Serum ALP levels were seen in Group II(

Pubertal) and the peak ALP value was seen in CVMI 3 with a mean value of 298 u/l , indicating the peak growth rate at this stage.

3. Serum alkaline phosphatase level estimation might be useful as an adjunct if not as an alternative and further studies are required to assess its reliability.

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