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Original Article

Reliability of Biological Marker, Insulin Like Growth Factor-1 (IGF-1) as an Indicator in Assessing Skeletal Maturity Using Blood Sample By ELISA Technique

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***ABSTRACT*:**
Background: Growth plays major rolein treatment planning and retention in orthodontics and it has been under scrutiny for years. This research introduces a new method of growth prediction with serum Insulin- like growth factor- 1 and its correlation with established methods of growth prediction. **Method**: 150 subjects were divided into 5 groups according to 5 stage CVMI stages. 1ml of serum was obtained from 2.5 ml of blood sample collected from each subject using minimally invasive technique. The blood was centrifuged at 3000 rpm for 5 minutes. After centrifuging the serum was separated and stored in different vials. The serum IGF-1 levels determination was done using IGF-1 600 ELISA kit (96 wells) using ELISA method.**Results**: IGF-1 levels were seen to start low in the prepubertal stages (CVMI 1 and MP3 - F) with a sharp increase from CS2 and MP3- FG to the peak levels at CS3 and MP3 - G. Between CS4 and CS5 and MP3 - H to I, IGF-1 level gradually declined. The values were specific for each stage, denoting that they can be used to differentiate one stage from another. **Conclusions**: Statistically significant correlation was observed on comparing all the 3 variables. Hence, IGF-1 can be used as a reliable method in predicting growth potential.
Key words: Insulin Like Growth Factor, Skeletal Maturity.

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INTRODUCTION:

The timing and the amount of remaining facialgrowth are important factors in orthodontics. Growth potential of individuals with skeletal discrepancies is of utmost importance to clinical orthodontics because these malocclusions constitute a significant percentage of cases. Therefore, predicting growth potential during preadolescenceand adolescence is useful for estimating severity of the underlying skeletal discrepancy and deciding treatment plan for the same.[1-3]

Various previous methods have been shown to be unreliable and impractical for estimatingthe pubertal growth spurt.[3-6] Over the past few decades, cervical vertebrae bone maturation stages (CVMI) and third finger middle phalanx (MP3) x-rays have been used to identify growth and determine the intensity of growth spurt and locate the end of growth. The chronological timing of puberty and adolescent growth spurt demonstrate much variation due to various factors. Timing also differs between boys and girls.[2-10]

Insulin-like growth factor I (IGF-I) is a polypeptide hormone synthesized mainly by the liver. It is a memberof a group of hormones termed insulin-like growth factors. Most investigators have studied that insulin like growth factor-1 is mediator for growth hormone that plays an essential role in both local and systemic regulation of bone growth.[11]Itplays a major role in postnatal growth; preciselyin the process of longitudinal bone growth. IGF-1 is measurable in serum as well as urine and saliva. Several studies conductedusing radioimmunoassay testson IGF-I

have reported that its serum levels inchildren and adolescents followed a pattern that wasclosely related to the pubertal growth curve[12-13]

Measuring serum IGF-1 is considered a useful diagnostic tool for determining serum growth hormone status, especially since its levels do not fluctuate throughout the day.Blood spot IGF-1 measurement is relatively new, minimally invasive techniqueand has good correlation with skeletal maturity which involves collection of blood and has excellent correlation with regular serum IGF-1. In addition, the samples are stable at room temperature for upto 2 weeks.

The purpose of the study was to evaluate IGF-1 levels in blood using ELISA technique and to determine its correlation with CVMI and MP3 skeletal maturity indicators to predict growth potential.

MATERIALS AND METHODS:

The study conducted was an in – vivo cross sectional observational study.The samples were selected using randomised sampling technique.150 subjects were divided into 5 groups depending on their CVMI staging given by Bacettiet al.[25]Each group included 30 subjects under it and was subdivided into males and females

Subjects within age group of 9-18 yearsof both sexes were included in the study. Patients who would begin orthodontic treatment in our institution, were already undergoing treatment or in post-treatment follow-up phase were chosen. Those with a history of systemic illness, diagnosed hormonal imbalance or growth abnormality were excluded from the study.

For IGF- 1 testing, 1ml of serum was obtained from 2.5 ml of blood sample collected from each subject using minimally invasive technique. (Figure 1)The collected blood sample was numbered sequentially.The blood was centrifuged at 3000 rpm for 5 minutes. After centrifuging the serum was separated and stored in different vials which had the same numbering as per the previous blood sample. The stored test tubes (vials) were transported to the laboratory on the same day for testing of IGF-1 levels.The serum IGF-1 levels determination was done using IGF-1 600 ELISA kit (96 wells) (EIA 4140, DRG Instruments, Germany) using ELISA method at only one laboratory centre. (Figure 2)

In order to correlate this with skeletal maturity, latercal cephalograms were taken. Lateral cephalograms were taken in centric occlusion with lips in repose in natural head position (NHP).Numbering of each film was done such that it correlated with the IGF-1 numbering for identification.The cervical vertebrae staging technique as described by Baccetti et al was used to stage the cervical vertebrae. Additional MP3 staging was done for each subject. The radiograph of middle phalanx of third finger (MP3) was taken with a standard size (31mm x 41mm Kodak) periapical dental X-ray film by placing the hand with the palm downward on a flat table in such a way that the middle phalanx was located in centre of the film. Numbering was done which was correlated with the IGF-1 and lateral cephalograms numbering.The MP3 were traced and staged according to the technique described by Kansal and Rajagopal's modification. (Figure 3) The staging were correlated with values of IGF-1 and the results were obtained.

One-way ANOVA analysis to evaluate the correlation between and within groups on comparing IGF-1 values with CVMI stages.

RESULTS:

Mean and standard deviation values of IGF-I for each stage of the cervical vertebral maturation index for the whole sample as shown in Table 1 represents that mean IGF-1 serum level increased gradually from its lowest value at stage 1 toward stage 2. A sharper increase was observed between stages 2 and 3 where the peak value was reached. The IGF-1 values then declined toward stage 4 to reach its baseline level at stage 5. In the whole sample, the highest mean IGF-1 value (384.9300 µg/mL) was observed in stage 3. The second highest mean IGF-1 value was observed in stage 2. The lowest mean value (138.2967 µg/mL) was observed in stage1. Mean IGF-I values recorded at each stage of the cervical vertebral maturation index were statistically different from the values recorded at the other stages.One-way ANOVA analysis shows significant correlation between and within groups on comparing IGF-1 values with CVMI stages (Table 2).

Table 3 shows present mean and standard deviation values of IGF-I for males and females subjects correlated with mean age for each stage of the cervical vertebral maturation index of the sample. For female subjects, the mean IGF-I serum level was observed to be highest in stage 3 with a value of 368.0429 µg/mLfollowed closely by stage 2 with a value of 337.8600 µg/mL. For male subjects, the mean IGF-I serum level was observed to be highest in stage 3 with a value of 399.7063 µg/mLfollowed by stage 2 with a value of 286.7800 µg/mL. Mean IGF-I values recorded at each stage of the cervical vertebral maturation index were statistically different from the values recorded at the other stages and had a close correlation with age.Sex differences between male and female subjects were not statistically significant.

Table 1 Descriptive IGF-1 statistics for mean, standard deviation and error for each cervical stage

Cervical stage	n	Mean IGF-1 (µg/L)	Std. Deviation	Std. Error Mean
CVMI 1	30	138.2967	64.80971	11.83258
CVMI 2	30	303.8067	70.45848	12.86390
CVMI 3	30	384.9300	52.61383	9.60593
CVMI 4	30	275.3933	59.08913	10.78815
CVMI 5	30	205.2167	43.96833	8.02748
Total	150	261.5287	102.65971	8.38213

Table 2 p values for 1-way ANOVA showing IGF-1 differences between and within the cervical stages.

	Sum of Squares	Df	Mean Square	F	Significance
Between Groups	1066941.435	4	266735.359	76.835	0.000 HS
Within Groups	503371.852	145	3471.530		
Total	1570313.287	149			

Significance set at p <0.05, Highly Significance set at p <0.001

Table 3 Descriptive IGF-1 statistics for mean, standard deviation and errorfor male, female samples and whole sample correlated with age in each cervical stage

CVMI	Female				Male				Whole Sample			
	n	**Mean Age**	**IGF-1**	**SD**	**n**	**Mean Age**	**IGF-1**	**SD**	**n**	**Mean Age**	**IGF-1**	**SD**
CVMI 1	11	11.50	135.07	59.61	19	11.60	140.16	69.16	30	11.60	138.30	64.81
CVMI 2	10	12.60	337.86	79.61	20	12.70	286.78	60.49	30	12.60	303.81	70.46
CVMI 3	14	13.70	368.04	44.26	16	13.80	399.71	56.17	30	13.80	384.93	52.61
CVMI 4	13	14.60	270.55	56.76	17	15.20	279.10	62.28	30	15.03	275.39	59.09
CVMI 5	13	17.30	217.07	34.97	17	16.70	196.15	48.84	30	17.00	205.22	43.97

Table 4 Descriptive IGF-1 statistics for mean, standard deviation for male, female samples and whole sample correlated with age in each MP3 stage

MP3	Female				Male				Whole Sample			
	n	**Mean Age**	**IGF-1**	**SD**	**n**	**Mean Age**	**IGF-1**	**SD**	**n**	**Mean Age**	**IGF-1**	**SD**
F	8	11.3	111.65	51.54	14	11.5	133.37	79.09	22	11.5	125.47	69.80
FG	8	12.8	248.66	59.23	15	12.3	217.68	64.12	23	12.5	228.46	62.93
G	12	12.5	391.53	45.89	18	13.1	364.33	65.90	30	12.9	375.21	59.40
H	11	14.18	296.09	63.52	12	14.5	363.26	53.90	23	14.3	328.60	66.81
HI	10	15.2	278.83	67.61	16	15.3	248.97	57.92	26	15.2	260.45	62.27
I	12	17.5	217.21	37.59	14	17	198.35	50.33	26	17.2	207.05	45.06

Table 5 Correlation between MP3 stages and CVMI.

Mp3 stages	F	Fg	G	H	HI	I
Cvmi 1	20	10	-	-	-	-
Cvmi 2	2	11	17	-	-	-
Cvmi 3	-	2	13	14	1	-
Cvmi 4	-	-	-	9	20	1
Cvmi 5	-	-	-	-	5	25

Table 6 Results of Pearson Correlation test showing correlation between CVMI, MP3 stages and IGF-1 levels.

Correlations				
		CVMI	MP3	IGF_1
CVMI	Pearson Correlation	1	.942	.139
	Sig. (2-tailed)		.000**	.090*
	N	150	150	150
MP3	Pearson Correlation	.942	1	.161
	Sig. (2-tailed)	.000**		.049**
	N	150	150	150
IGF_1	Pearson Correlation	.139	.161	1
	Sig. (2-tailed)	.090*	.049**	
	N	150	150	150
**. Correlation is significant at the 0.05 level (2-tailed).				
*. Correlation is significant at the 0.10 level (2-tailed).				

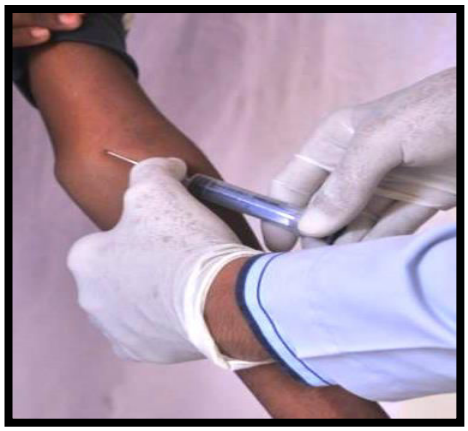

Fig. 1: PROCEDURE OF BLOOD COLLECTION

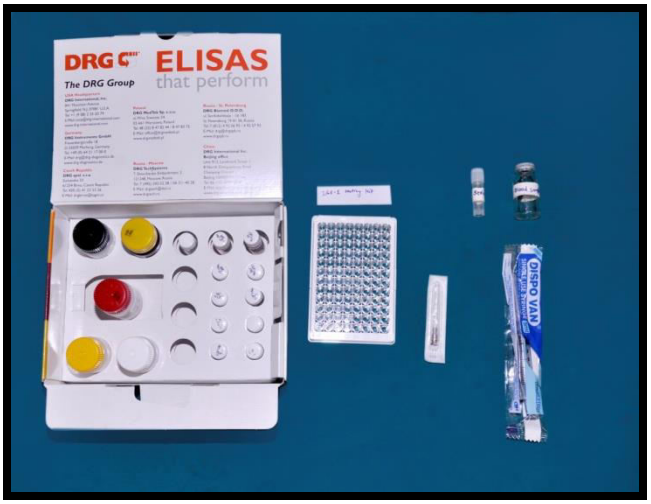

Fig. 2: ELISA KIT AND ARMAMENTARIUM FOR BLOOD COLLECTION (5 cc syringe and needle, vial for collection of blood and serum transportation)

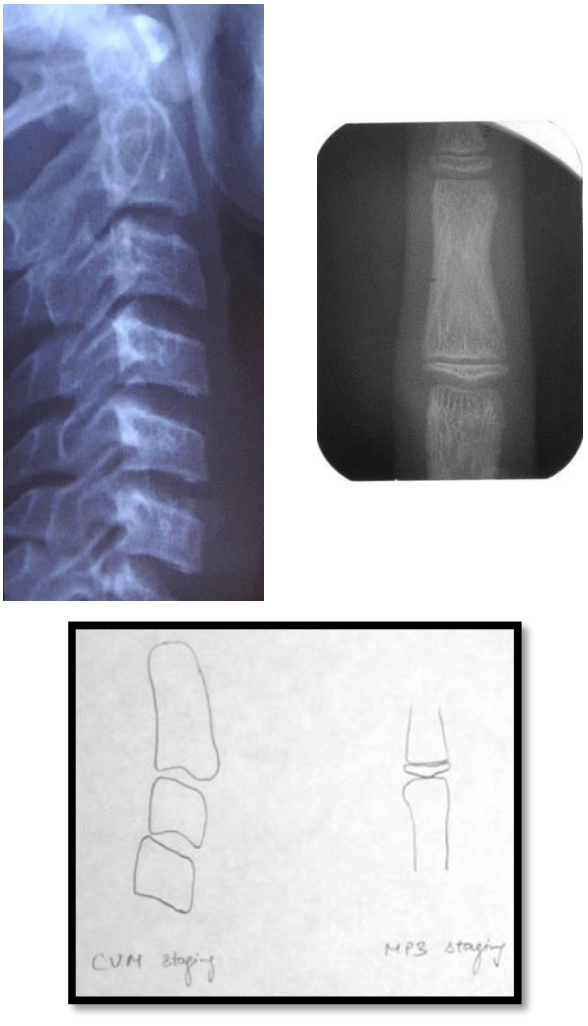

Fig. 3: TRACING OF CVMI STAGE 3 AND MP3 STAGE G

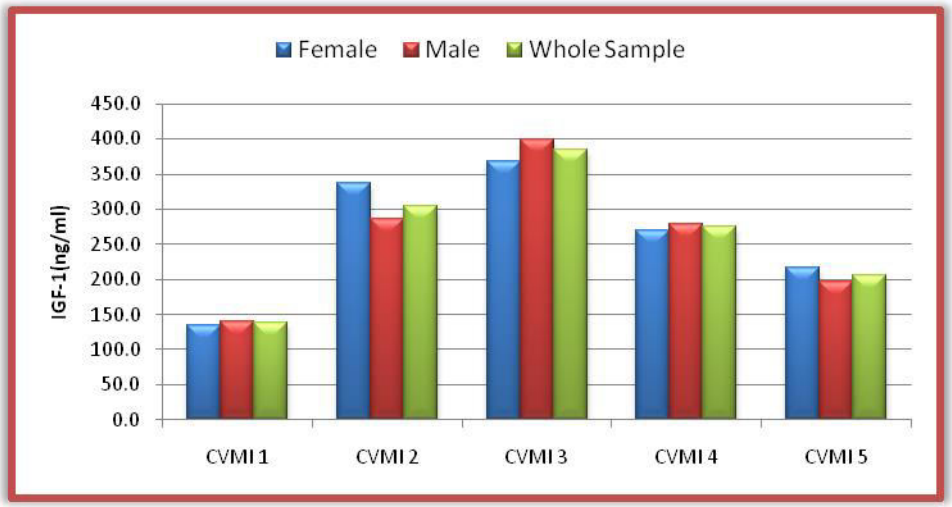

Fig 4. Bar chart representing mean values of IGF-I with different cervical vertebral maturation stages

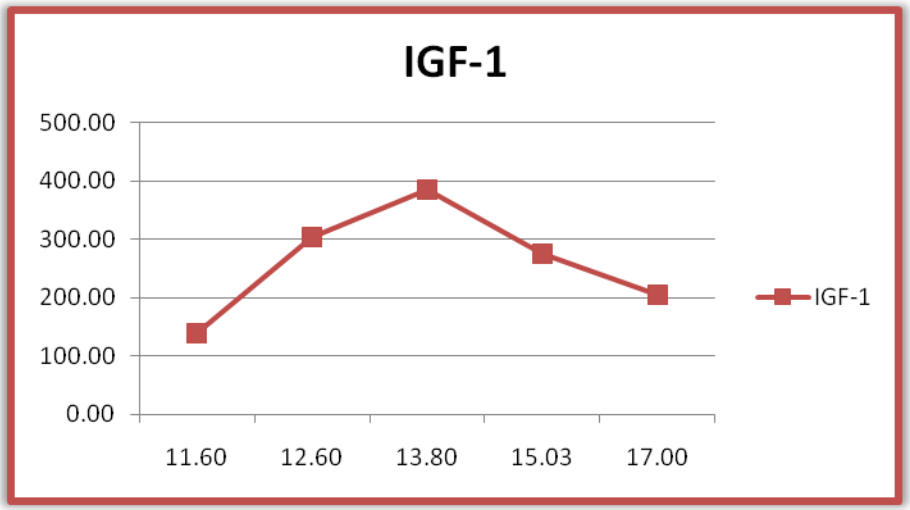

Fig 5: Pattern of IGF-I in relation to the age in whole sample

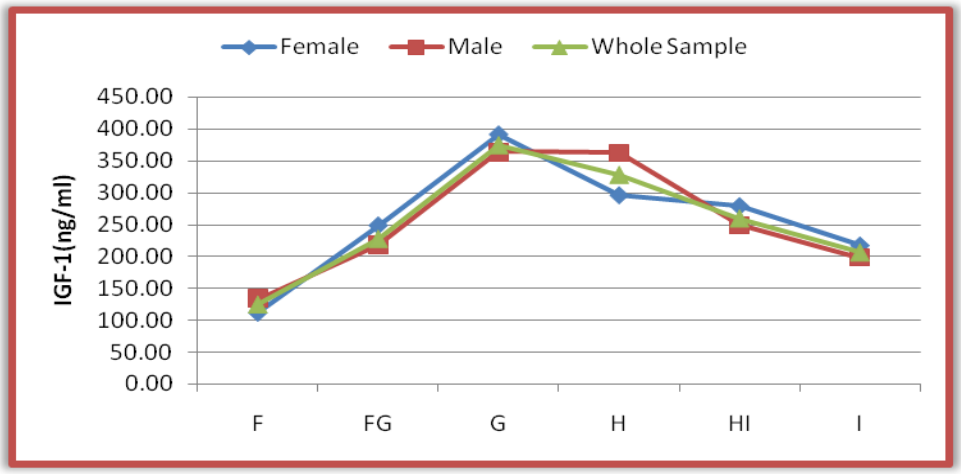

Fig 6Pattern of IGF-I in relation to the stages of the MP3 index.

DISCUSSION:

Growth is a series of anatomic and physiologic changes taking place with increasing age from the beginning of prenatal life to infancy, childhood and adulthood. All the structures have a uniform pattern of changes which are consistent for that person but the timing for these changes are different for each person according to his or her own biologic clock. In girls, pubertal growth spurts usually start between the ages of 10 to 12 years, in boys between 12 to 14 years with variations of 3 to 6 years on either side.

Skeletal maturation is an integral part of an individual's pattern of growth and development. The efficacy of dental procedures including dentofacial orthopaedic therapy and orthognathic surgery is highly dependent on the amount of active skeletal growth remaining in an individual. Dentofacial orthopaedic therapy is best carried out during active growth stage as it takes the advantage of growth to correct skeletal discrepancy. If the active growth phase for an individual has ceased, correction of skeletal discrepancyhas to be carried out by camouflage/ orthognathic surgery to achieve facial harmony.The pubertal growth spurt is considered to be an advantageous period for orthopaedic (skeletal) corrections of the facial complex.

Various other methods for predicting pubertal growth spurt have been known [1-4,15-16,27,32-43] but most of the methods either involve radiographic exposure, or are expensive or not constantly reproducible.[19-20,44]Previous studies have shown a large amount of intra operator variations.[19,20]Quantitative assessment of growth potential is not possibleand the final stage of development does not necessarily indicate the completion of growth, especially mandibular growth,also they are highly subjective techniques and lack the ability to determine the intensity of the growth spurt and the end of growth. Hence, the need for better methods of determining skeletal maturity was felt.

Insulin like growth factor-I (IGF-I) is a circulating growth hormone dependent factor which reflects growth hormone status.[45-47] Many physiological effects of growth hormone are mediated by IGF-1, hence a potent growth and differentiation factor. Serum IGF-1 increases in puberty and declines with age. No previous studies have been documented in literature which has correlated IGF-1 with CVMI and MP3.

The IGF-I assay was performed with an ELISA IGF-I technique that was advocated by Byme et al (2000),[48]Zumbado et al (2010)[46]andIshaq et al (2012).[11]Previously different techniques were adopted such as radioimmunoassay, immunoradiometric and chemiluminesce assays for determination of IGF-1 and compared for accuracy.[1,15-16,49,50]It was concluded that the different assays were comparably accurate, especially in healthy subjects. The ELISA technique was used in this research because of the applicability and accuracy of the technique.

Results of this study shows that the mean IGF-1 levels were significantly higher in pubertal stage (CVMI 3 andMP3 stage G) compared to the prepubertal (CVMI 1 &MP3 stage F) and post pubertal stage (CVMI 5 and MP3 stage I). Linear correlation showed a significant positive correlation with skeletal maturity from the prepubertal stages to the post pubertal stage&a negative linear correlation with increasing time from the onset of puberty, as well as chronological age. A statistically significant correlation between the mean IGF-1 levels of individuals in their pubertal growth spurt according to CVMI and MP3 stagingwas noticed.The values were specific for each stage, denoting that they can be used to differentiate one stage from another.

For female subjects, mean IGF-I serum level was observed to be highest in CVMI stage 3 and MP3 stage G. The values in CS2 and CS3 in female subjects are almost similar suggestive of shorter peak height velocity or sudden attainment of peak height velocity. The mean age difference between CS2 and CS3 is 1.1yrs which shows that there is shorter working window in female patients for growth modulation and accurate assessment with IGF-1 will be helpful for effective treatment during growth.

For male subjects, Mean IGF-I serum level was observed to be highest in CVMI stage 3and MP3 stage G followed by stage H. The values in stage G and H in male subjects are almost similar suggestive of longer peak height velocity in male subjects compared to female subjects. A sharp increase in mean IGF-1 value was noted between stage 2 and 3. The difference of 112.9263 µg/mL between CS2 and CS3 is not statistically significant but this difference is greater compared to the difference among the female subjects which could be attributed to the longer peak height velocity in male subjects compared to female subjects. Thus accurate assessment with IGF-1 will be helpful for effective treatment during growth so as not to tax the compliance of the patient by starting the treatment very early. The mean age difference between CS2 and CS3 is 1.1 yrs.

Sex differences between male and female subjects were not statistically significant in this study but mean serum IGF-1 level infemale subjects at CS-2 was greater compared male subjects at CS-2. In contrast, mean serum IGF-1 level inmale subjects at CS-3 was greater compared to female subjects at CS-3.Apossible explanation for the difference observed in stageswhere peak in IGF-1 levels between the groups isobserved could be related to gender difference since thetiming of puberty differs in male and female subjects.This is in accordance with the statement by Proffit:"Girls mature earlier, and finish their growth muchsooner. The differences arise because in males slow but steady growth occurs before the growth spurt."

Previous studies by Abdel-Kader,[55] Hegde[56] have shown a significant correlation between CVMI and MP3. In our study, a significant correlation between CVMI and MP3 was observed.Massoudet al[1] reported that serum levels of

IGF-1 peaked at stage 5 of cervical vertebral maturation whereas in our study peak IGF-1 value is observed in stage 3. This isbecausewe used the modified CVM 5 stage method of staging as compared to the 6 stage method used in the study by Massoud et al. Ishaq et al[11] reported that serum levels of IGF-1 peaked at stage 4 of cervical vertebral maturation, and the highest mean IGF-I value was 835.6 mg/L. The differences in the values compared to our study can be attributed to the fact that the procedure in their study involved an pretreatment step to enhance the clinical performance of the assay using acid ethanol procedure and also the ELISA kit used was different. Jain S et al[50] showed that the range of serum IGF-1levels was 171 to 433 ng/ml for CS-3, 252 to 525 ng/mlfor CS-4, and 206 to 372 ng/ml for CS-5. The clinicalusefulness of the Jain S et al study was limited because of overlappinglevels of serum IGF-1 in all three cervical stages was found, possiblydue to the relatively small sample size, cross-sectional designof the study, different body types, and different maturational groups (advanced/average/delayed) and determining sampling using their chronological age.In our study, CVMI was used along MP3 to correlate with IGF-1. In our study also, the values of IGF-1 were higher in post pubertal period compared to pre pubertal period which could be attributed to late mandibular growth.

Various skeletal indicators are poor predictors of end of growth as growth continues for a prolonged period of time. Residual facial growth can be of utmost importance for relapse after orthodontic or orthognathic procedures. Hence, a longitudinal study for measuring growth increments and end of growth can be carried out. The results of our study showed a highly significant correlation between mean values of IGF-1 in between and within groups of CVMI on ANOVA analysis. The correlation between all the 3 groups, CVMI, MP3 and IGF-1 showed significant correlation between. The differences from the previous studies can be attributed to (1) the difference in the population studied; (2) laboratory techniques implemented in the studies to measure the IGF-I levels; and (3) sample size studied of our study is larger. Many methods are available for measuring the skeletal maturation (radiographic and non radiographical) but hardly any of these match the ideal needs of biologic indicator of skeletal maturity.

Our results indicate that assessing IGF-1 levels is an accurate means of determining skeletal maturity. This would be of clinical significance while determining the peak height velocity or assessing the remaining growth for other subjects. IGF-1 levels not only shows the amount of growth remaining in an individual but also indicates the intensity of the remaining growth.The sample size used in this study was greater compared to other previous studies but further studies can be done for validating our results.The combined use of CVM, MP3 and IGF-1 for selection of orthopedic and orthodontic treatment would be beneficial to the patient.

CONCLUSIONS:

1. IGF-I mean values can be used in orthodontic diagnosis as a reliable maturation indicator that is compatible with the cervical vertebral maturation indicator and MP3.
2. IGF-I levels are low in the prepubertal cervical skeletal stages i.e. CVMI 1, rise sharply to their peak during puberty, i.e., CVMI 3 and decline to approach prepubertal levels after puberty, i.e. CVMI 5
3. IGF-I levels are low in the prepubertal MP3 stages i.e. stage F, rise sharply to their peak during puberty, i.e., stage G and decreases from stage H to approach prepubertal levels after puberty, i.e. stage I
4. IGF-1, CVMI and MP3 showed statistically significant correlation on comparing all the 3 variables.
5. There was no statistical difference found between males and females.

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