Correlation Between Serum IGF-1 Levels and CVM Stages for the Assessment of Skeletal Maturity

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OBJECTIVE: The major disadvantage in cervical vertebral maturation stages (CVMS) is the involvement of radiographic exposure. This study was undertaken to assess the applicability of insulin-like growth factor I (IGF-I) blood level as a maturation indicator by correlating it to the CVMS.

METHODOLOGY: This cross-sectional study was conducted at orthodontic department of our institute. With 80% power of study, 5% desired level of significance and using 0.67 correlation value, a sample size of 75 was calculated. There are five stages of CVMS and in each CVM stage 15 subjects were allocated, therefore collective sample size was 75. The technique of sampling was purposive (non-probability) sampling. Out of 75 patients, 47 (62.7%) were males and 28 (37.3%) were females. The mean ages of the patients were 12.5 ± 2.6 years. Analysis of variance (ANOVA) test was performed to evaluate the blood serum IGF-1 levels among five stages of CVMS.

RESULTS: There was a statistically considerable difference in mean IGF-1 among five stages of CVM. The mean IGF-1 (ng/ml) of CVMS I was 204.9 ± 21.1, mean IGF-1 of CVMS II was 272.5 ± 39.5, mean IGF-1 of CVMS III was 343.1 ± 38.6, mean IGF-1 of CVMS IV was 287.7 ± 22.3 and mean IGF-1 of CVMS V was 171.5 ± 24.5. The highest mean values were observed in stage III followed by stage IV, II, I and V. IGF-1 levels were maximum in females at CVM stage 3 and were maximum in male at CVM 4.

CONCLUSION: IGF-1 levels at the pubertal stage were significantly higher than the pre-pubertal and post-pubertal stages. IGF-1 levels might prove to be a valuable skeletal maturity indicator.

KEY WORDS: Insulin Like Growth Factor-1; IGF-1; Cervical Vertebral Maturation Stages; CVMS.

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INTRODUCTION

The correct treatment planning in orthodontics is significantly dependent on identification of peak growth spurt as growth modification is more effective during rapid pubertal growth spurt period. Profit and Fields suggested that timing difference between boys and girls as girls mature two years earlier as compared to boys. However, Fishman has revealed that dental age and chronologic age are pitiable interpreters of the pubescent growth spurt. Various radiographic techniques are in use for skeletal maturation assessment. The most common among these include hand-wrist radiography and cervical vertebral maturation stages (CVMS) through lateral cephalometric radiographs (LCR). The assessment of CVMS is more appealing to orthodontists since LCR are normally in use for orthodontic patients, this method comprises of five distinct stages of anatomic modifications in 2nd (C2), 3rd (C3), and 4th (C4) cervical vertebrae.
In recent times, many researchers have studied growth factors like, estrogens, androgens such as testosterone, thyroid hormones, growth hormone, and insulin-like growth factor-1 (IGF-1). Both local and systemic bone growth regulation is mediated by IGF-1 through growth hormone.

The CVMS method indicates completion of growth at stage V, however, Goto et al., and Mitani et al., showed that some growth still remains. For this reason, there is a need for more reliable biomarker for the skeletal maturity assessment. Chronologic age and sexual maturity stages co-relation with serum IGF-1 is investigated in the past already. Masoud et al., and Juul A et al., used the blood-spot technique and correlated the IGF-1 levels with CVMS and hand-wrist growth stages.

Following this rationale, the objective of this research was to examine and assess different stages of CVMS and then determine serum levels of IGF-1 along with its correlation with different stages of CVMS. Hypothesis was that there is a correlation between serum IGF-1 levels and different stages of CVM.

METHODOLOGY

Present cross-sectional study was conducted at orthodontic department. Duration of study was one year. With 80% power of study, 5% desired level of significance and using 0.67 correlation value, a sample size of 75 was calculated. There are five stages of CVMS, and in each CVM stage 15 subjects were allocated, therefore collective sample size was 75. The technique of sampling was purposive (non-probability) sampling.

Following patients were included: patients who were about to begin orthodontic treatment, both genders and age range of 8 to 16 years. Patients with history of systemic illness or bleeding disorder, having growth abnormalities, vertebral issues and syndromic conditions, were excluded.

CVM stages were determined by two blinded expert examiners as per conventional 5 stages method. Five cephalograms were randomly taken from each group, 2 weeks later, to reassess CVM stage to find out interexaminer and intra-examiner reliabilities. For determination of IGF-1 levels, blood samples were taken from the median cubital vein, between 9am to 10am on the same day of taking lateral cephalograms, samples were collected in anticoagulant free BD vacutainer serum tube, were stored in thermocool box with ice cubes (kept between 2°C and 8°C), and were centrifuged at 3000rpm. The resulting supernatant was designated serum, which was shifted into 0.5 ml aliquots using Pasteur pipette and stored at -20 C. IGF-1 600 Enzyme Immunoassay Kit (Labor Diagnostika Nord GmbH& Co. KG) was used for calculation of IGF-1 levels (ng/ml) in serum samples.

DATA ANALYSIS

The data were analyzed using PASW 18 (Predictive Analytic Software 18). IGF-1 levels (ng/ml), and age variables were presented in form of Mean ± SD. Data of gender, and CVMS were presented in form of percentages and frequencies. The data were tested for normality by Shapiro-Wilk test. Comparison between both the genders was also performed. Spearman’s correlation test was used to determine the correlation of IGF-1 values at different CVMS. Kappa statistic was used to measure interexaminer and intra-examiner reliabilities in CVM stages determination.

RESULTS

The mean age of the patients were 12.5 ± 2.6 years, out of which 47 (62.7%) were male and 28 (37.3%) were female. Kappa statistics showed no significant difference in inter-examiner and intra-examiner readings (0.88 and 0.89, respectively).

The mean IGF-1 (ng/ml) of CVMS I was 204.9 ± 21.1, mean IGF-1 of CVMS II was 272.5 ± 39.5, mean IGF-1 of CVMS III was 343.1 ± 38.6, mean IGF-1 of CVMS IV was 287.7 ± 22.3 and mean IGF-1 of CVMS V was 171.5 ± 24.5. The highest mean values were observed in stage III followed by stage IV, II, I and V.

Shapiro Wilk test was used to check the normality of the data. As data was normally distributed, Analysis of variance (ANOVA) test was performed to compare the IGF-1 among five stages of CVM, which revealed that there was a statistically significant difference in mean IGF-1 among 5 CVM stages (Table 1 & 2).

For multiple comparisons, post hoc Tukey test was used which showed that mean IGF-1 levels at each CVM stage was statistically different from the mean values at the other stages whereas there was no significant difference was found between stage II and IV (Table 3).

<table>
<thead>
<tr>
<th>CVM Stages</th>
<th>N</th>
<th>Mean ± SD</th>
<th>SE</th>
<th>95% CI for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>CVMS I</td>
<td>15</td>
<td>204.9 ± 21.1</td>
<td>5.4</td>
<td>193.2</td>
</tr>
<tr>
<td>CVMS II</td>
<td>15</td>
<td>272.5 ± 39.5</td>
<td>10.2</td>
<td>250.9</td>
</tr>
<tr>
<td>CVMS III</td>
<td>15</td>
<td>343.1 ± 38.6</td>
<td>10.0</td>
<td>321.7</td>
</tr>
<tr>
<td>CVMS IV</td>
<td>15</td>
<td>287.7 ± 22.3</td>
<td>5.8</td>
<td>275.2</td>
</tr>
<tr>
<td>CVMS V</td>
<td>15</td>
<td>171.5 ± 24.5</td>
<td>6.3</td>
<td>158.0</td>
</tr>
</tbody>
</table>
IGF-1 levels were maximum in females at CVM stage 3 and were maximum in male at CVM 4. Significant difference between the IGF-1 levels in males and females was observed at CVMS 2 (P < 0.01), and 3 (P < 0.01), while the difference was not significant in other stages. Spearman’s Rho correlation test revealed that there was strong positive correlation between IGF-1 level and CVMS I-III and strong negative correlation was observed between IGF-1 and CVMS III-V (Table 4).

**DISCUSSION**

The exact knowledge regarding the mandibular developmental stage and remaining total MG is important for orthodontic diagnosis, treatment planning and treatment. This study was designed to check the hypothesis that the blood serum IGF-I level could be used as a skeletal maturity indicator in orthodontics. Physiologically balanced subjects were included in our study because diabetes mellitus and liver diseases have a direct influence on the metabolism of IGF-I and blood serum IGF-1 levels.\(^{18,19}\) During the collection of blood samples, we ruled out the patients with bleeding disorders which is according to the study by Masoud et al.\(^{15}\)

Cervical vertebral maturation techniques were established as the most reliable diagnostic tool.\(^{20}\) It was useful in orthodontics as a marker for the status of skeletal maturity.\(^{21}\) The CVMS method is proved more advantageous and beneficial as compared to hand-wrist method because of no additional radiation exposure. But the major disadvantage regarding CVMS or hand-wrist method is that radiographically growth is completed, yet mandibular growth remains.\(^{12,13}\) For this reason other methods of biologic maturity assessment are needed along with CVMS stages, when considering any procedure regarding orthognathic surgery or dentofacial orthopedic treatment.\(^{22}\) There are lot of methods are available for cervical vertebral maturation, in our research, CVMS method as described by Baccetti et al., was used.\(^{6}\)

In the present study blood samples were taken to find out the serum IGF-1 levels. In different studies saliva, gingival crevicular fluid, and urine were used as non invasive sources for the estimation of IGF-1.\(^{23-25}\) In case of urinary IGF-1, patient cooperation is a major factor, because sample collection without contamination is very difficult especially for patient.\(^{25}\)

In the present study the IGF-I evaluation was perform with ELISA Kit because of simplicity of this procedure and it was supported by other investigators.\(^{26,27}\) Other investigators adopted different techniques such as immunoradiometric assays and radioimmunoassays.\(^{15,28}\) Studies showed that all assays techniques were comparatively précised and accurate when performed in physically fit persons.\(^{29}\)

Results showed that the highest mean values were observed in stage III followed by stage IV, I and V. At CVMS III blood serum IGF-I levels were at peak with a signify value of 343.05ng/mL, and these peak levels can be linked with upcoming pubertal growth spurt, and residual mandibular growth.\(^{15,28,20}\) Differences in results from other studies can be attributed to difference of chronological age for peak IGF-1 levels. Differences might also be due to the disparity in inclusion criteria, racial backgrounds, genetic factors, environmental factors, and the technique adopted for this study.

In the present study blood serum IGF-1 levels were maximum in females at CVM stage 3 and were maximum in male at CVM 4. This is in agreement with the various previous studies where such similar rise was seen for both.
males and females.\textsuperscript{8,10,31-33} But findings are in contrast with findings of Masoud et al. who showed that peak IGF-1 values were at CVM stage 5 for both the genders.\textsuperscript{15}

Results showed that in girls blood serum IGF-1 levels rise from CVMS I to rapid increase seen from CVMS I to peak at CVMS III, followed by a rapid reduction from CVMS III to CVMS IV continuing to CVMS V; whereas in boys there was a stable rise in IGF-1 levels from CVMS I to CVMS IV, which steadily spiky at CVMS IV follow by a sluggish decline to CVMS V. These results reconfirm prior studies signifying that female subjects have a shorter and earlier growth burst denote by sharp rise and sharp fall in IGF-1 levels, while in boys, there is a later and longer growth spurt.\textsuperscript{34}

In the present study a marked positive relationship was observed between IGF-1 levels and CVMS stages from prepubertal phase (CVMS I) to pubertal phase(CVMS III), whereas there was a negative correlation between IGF-1 levels and CVMS IV and CVMS V stages that is from pubertal phase to postpubertal phase. Our study’s results matched with previous studies that show blood serum IGF-1 peak in early puberty followed by a decrease in late puberty.\textsuperscript{35,36}

CONCLUSION

IGF-1 levels at the pubertal stage were significantly higher than the pre-pubertal and post-pubertal stages. IGF-1 levels might prove to be a valuable skeletal maturity indicator.

CONFLICT OF INTEREST

None declared

REFERENCES


