

Original Article

Evaluation of Urinary Insulin Like Growth Factor-1 as a Maturation Indicator in Females: A Cross-Sectional Study

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ABSTRACT

Aim: the current study was adopted to assess the pubertal growth spurt by evaluating the urinary insulin-like growth factor 1 (UIGF-1) in Egyptian females and estimate the mean chronological age at the peak of urinary IGF-1. **Methodology:** A total sample of 48 subjects was recruited from the Orthodontic Department of the Faculty of Dentistry- Cairo university. They were equally divided (N=16) into three age ranges (8-10), (10 - 12) and (12 - 14). Lateral cephalograms were taken from the subjects for cervical vertebral assessment according to Baccetti et al. and assessed twice by the same rater with a one-week interval and one assessment by another rater. Fresh morning urine samples were collected from subjects to quantify the concentration of IGF-1 by ELIZA technique. **Results:** A compatibility and correlation between the UIGF-1 and CVM and between UIGF-1 and age subgroups was statistically analyzed in females. From the UIGF-1 curve, it was shown that the peak of UIGF-1 at CVM 4, which was significantly different from the mean values of other CVM stages (P <0.05). Females reached the peak UIGF-1 value at 12.42 years. UIGF-1 at CVM 3 preceded the CVM 4 representing the onset of a growth spurt with the mean age of 11.29 years in females. **Conclusion:** UIGF-1 is compatible with the CVM stages. A significant positive moderate correlation was found between UIGF-1 and CVM, and between UIGF-1 and chronological age. Thus, it is a reliable biological indicator employed in orthodontics for growth spurt assessment.

Keywords: IGF-1, UIGF-1, growth factors, biological marker of growth, maturational indicators.

I. INTRODUCTION

Identification of growth spurt is an important diagnostic tool in growing orthodontic patients when dealing with jaws discrepancy. Several indices have been constructed to detect the bone maturation stages, with radiography-based,

hand-wrist analyses, and cervical vertebral maturation (CVM) approaches being the most frequent.^{1,2}

Lateral cephalogram is the common record needed in orthodontics for skeletal, dental, and soft tissue analysis. In addition, it is popularly used to assess CVM stage for assessment of the

level of pre-pubertal/pubertal growth spurt.^{1,3,4,5,6} Other possibilities such as biochemical markers offer a safer procedure for skeletal stage assessment than radiographic exposure, thus representing agents that directly elucidate bone maturity. IGF-1 plays important role in mandibular condyle growth and many sources suggest that modifications of mandibular morphology are caused by changes in function/soft tissue suggesting it may be possible by influencing in mandibular condyle.⁷

Salmon and Daughaday discovered IGF-1 in 1957 as a modulator of growth hormone (GH) activity and have shown that it has a role in systemic and local control of bone growth⁸. It is a growth hormone-stimulated mediator that is synthesized in the liver and supplied to target tissues via the bloodstream in an endocrine manner. However, numerous cell types, including muscle, brain, bone, kidney, hematopoietic cells, nerve cells, and skin, create and respond to IGF-1 via autocrine system by attaching to IGF-1 receptors on the same cell or paracrine activities to the next cell, initiating cell proliferation and differentiation.⁹

The effect of such biochemical indicators on longitudinal bone growth at the pubertal stage has a significant trait. It was reported as a reliable indicator that measured from serum^{10,11,12,13}, and could be evaluated also from other biological fluids such urine and saliva. The IGF-1 is so difficult to obtain from saliva as it needs 30 minutes to collect a pure saliva sample from the parotid duct which makes discomfort to the patient¹⁴. It is unacceptable by the children some extent to obtain it from the blood sample as an injection causing the patient discomfort as well. NAOMI HIZUKA et al (1987) were the first to demonstrate IGF-1 in urine, and they stated that the quantity of IGF-1 in urine was altered in patients with either growth hormone excess or growth hormone deficiency in cases with acromegaly and hypopituitarism patients. It was found in the literature a positive correlation between urinary and serum IGF-1 and has level changes in different ages.^{15,16}

Thus, the present study aimed to evaluate the level of IGF-1 from urine volume as surrogate to serum I at the pubertal and circumpubertal periods with reference to the CVMI as the gold standard and assessment of the mean chronological age at the peak of urinary IGF-1 in female.

II. MATERIAL AND METHODS

The study was conducted at the Orthodontic department clinic, Faculty of Dentistry -Cairo university. The participants were seeking orthodontic or are under orthodontic treatment. The total number of female subjects recruited in this study were forty-eight, equally divided into three age ranges. These patients were selected according to date of birth around circumpubertal age, 8-14 years old. These age ranges were selected according to previous studies results established by Mahmoud et al¹⁷. The whole sample was divided into three age subgroups gained from date of birth; each group included 16 subjects, (8-10 y), (10-12 y), (12-14y).

The study was conducted following the ethical guidelines by the Faculty of Dentistry, Cairo university with approval of research medical committee. Inclusion criteria were healthy patients without systemic or chronic diseases and under recent medication and no history of trauma or surgery in the cervical vertebral area. Exclusion criteria were patients with Bone diseases and syndromic patients.

Personal data chart included the name, age, date of birth, date of examination, address, mobile number, and diagnostic sheet with detailed past and present medical history. The participants and their parents were informed about the research plan, urine morning samples and a copy of the digital lateral cephalogram which was part of records for orthodontic treatment were taken on the same day. participants were asked for a menstruation period to postpone the sample taking to avoid urine contamination with blood, Morimoto et al.¹⁸ All lateral cephalometric radiographs were taken with source image receptors distance (SID) 64-67 inches with magnification 1.01% - 1.13 %. 60-80 kv, with exposure time 0.2 – 23 seconds. Participants who had not lateral cephalogram yet were referred to the radiology center. Lateral cephalograms had been taken with a digital radiographic machine (Planmeca ProLine XC) with high contrast and low effective dose. skeletal assessment of cervical vertebrae examined through later cephalometric radiographs were then staged into six parameters described by Baccetti et al¹ by pencil 0.5 mm with matte acetate paper under optimal clear viewing conditions and the inferior, superior, posterior, and anterior borders of the

second, third, and fourth cervical vertebrae were traced. Assessment of the maturity stages of the CV was done and classified by intra-rater with one week interval and inter-rater by well-trained orthodontic student. All investigators were consistently blinded to patient's name and age. Cervical vertebrae were visible only to eliminate age bias by dental development. Two techniques were used for CVM assessment, visualized and cephalometric analysis.

- **Visual Cephalogram Analysis**

a. Initiation stage

80% to 100% of adolescent growth was expected. The lower borders of all the three vertebrae are flat; the bodies of both C3 and C4 are trapezoid in shape. The peak in mandibular growth will occur on average two years after this stage.

b. Acceleration stage

65% to 85% of adolescent growth is expected. Concavities appear in the inferior borders of C2. The inferior border of C3 and C4 are flat. The bodies of both C3 and C4 are still trapezoid in shape. The peak in mandibular growth will occur on average one year after this stage.

c. Transition stage

25% to 65% of adolescent growth is expected. Concavities at the lower borders of

both C2 and C3 are present. The bodies of C3 and C4 may be either trapezoid or rectangular horizontal in shape. The peak in mandibular growth will occur during the year after this stage.

d. Deceleration stage

10% to 25% of adolescent growth is expected. Concavities at the lower borders of C2, C3, and C4 now are present. The bodies of both C3 and C4 are rectangular horizontal in shape. The peak in mandibular growth has occurred within 1 or 2 years before this stage.

e. Maturation stage

A 5% to 10% of adolescent growth is expected. The concavities at the lower borders of C2, C3, and C4 still are present. At least one of the bodies of C3 and C4 is squared in shape. If not square, the body of the other cervical vertebra still is rectangular horizontal. The peak in mandibular growth ended at least 1 year before this stage.

f. Completion stage

Little or no adolescent growth was expected. The concavities at the lower borders of C2, C3, and C4 still are evident. At least one of the bodies of C3 and C4 is rectangular vertical in shape. If not rectangular vertical, the body of the other cervical vertebra is squared. The peak in mandibular growth ended at least 2 years before this stage.

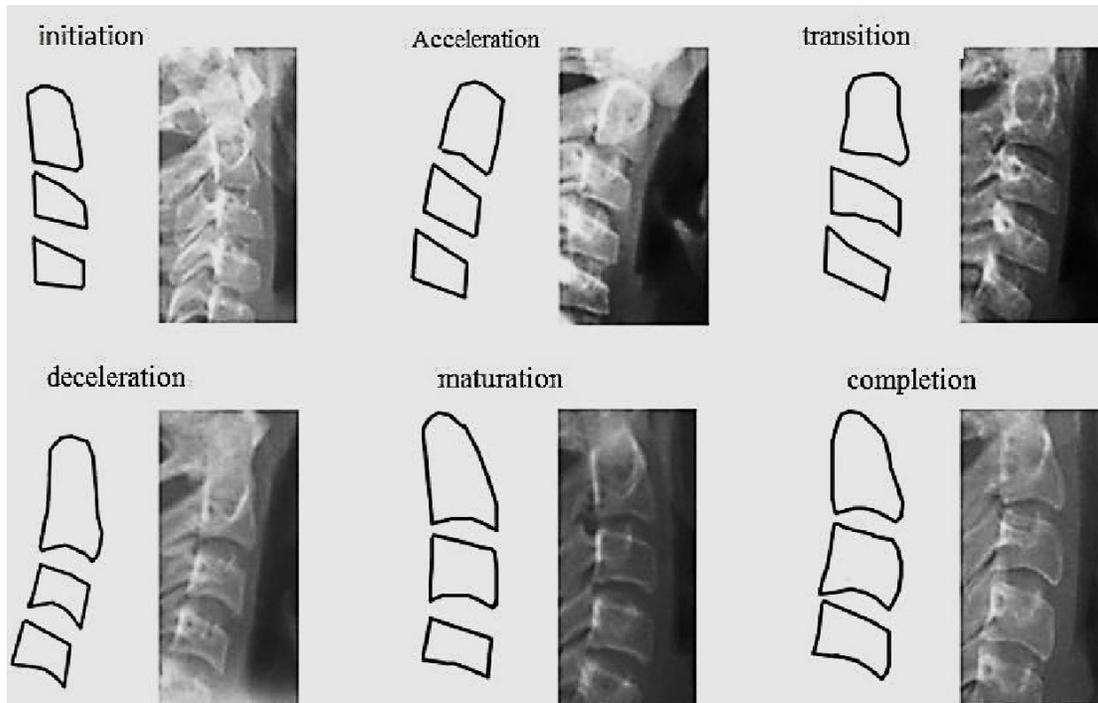


Figure (1): Morphological changes of cervical from second through fourth cervical vertebrae adopted by Bacetti et al.

• **Measurable Cephalometric analysis**

The traced point of C2, C3, and C4 vertebrae describing the height and length were determined according to **Hellsing (1991)**, and the inferior border points described by **Bacetti et al. (2002)** to assess the depth of concavity.

- C2p, C2m, C2a: the most posterior, the deepest, and the most anterior points, respectively, on the lower border of the body of C2.
- C3up, C3ua: the most superior points of the posterior and anterior borders, respectively, of the body of C3.
- C3lp, C3m, C3la: the most posterior, the deepest, and the most anterior points, respectively, on the lower border of the body of C3.
- C4up, C4ua: the most superior points of the posterior and anterior borders, respectively, of the body of C4.
- C4lp, C4m, C4la: the most posterior, the deepest, and the most anterior points, respectively, on the lower border of the body of C4.

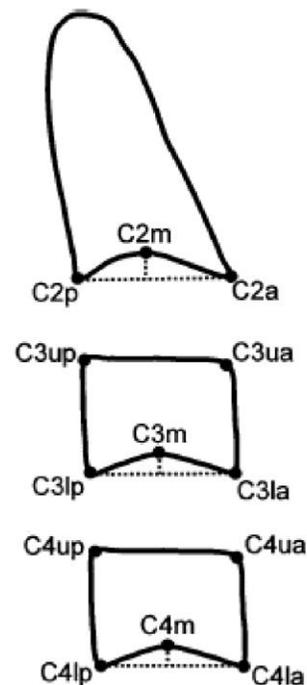


Figure (2): Points indication from second through fourth cervical vertebrae.

From these points the following measurements were performed:

1- The depth of concavity of inferior border

According to the result of Bacetti et al. study, the depth of concavity was divided as follows:

- Less than 0.5mm is flat
 - 0.5-1.0mm initial concavity
 - More than 1.0mm prominent concavity.
- Baccetti et al.** ¹⁹
- C2Conc: a measure of the concavity depth at the lower border of C2 (distance from the line connecting C2p and C2a to the deepest point on the lower border of the vertebra, C2m).
 - C3Conc: a measure of the concavity depth at the lower border of C3 (distance from the line connecting C3lp and C3la to the deepest point on the lower border of the vertebra, C3m).
 - C4Conc: a measure of the concavity depth at the lower border of C4 (distance from the line connecting C4lp and C4la to the deepest point on the lower border of the vertebra, C4m).

2- Morphology of third and fourth cervical vertebrae bodies

Laboratory procedures:

Once the samples were received, the urine was transferred from the container to Eppendorf tubes by pipettes (1.8ml). Samples then were centrifuged 2000-3000 RPM for approximately 20 minutes. All the urine samples were kept at the deep freezer -80° until the time of assay to measure the hormone volume.

The shape of C3 and C4 are trapezoid, square or rectangular could be measured by the following:

- C3BAR: the ratio between the length of the base (distance C3lp-C3la) and the anterior height (distance C3ua-C3la) of the body of C3.
- C3PAR: the ratio between the posterior (distance C3up-C3lp) and anterior (distance C3ua-C3la) heights of the body of C3.

- C4BAR: the ratio between the length of the base (distance C4lp-C4la) and the anterior height (distance C4ua- C4la) of the C4 body.

- C4PAR: the ratio between the posterior (distance C4up-C4lp) and anterior (distance C4ua-C4la) heights of the body of C4.

• **Urine sample collection:**

Urine samples were collected at the Orthodontic department, Faculty of dentistry, Cairo university between 8:00 am and 12 pm inside sterilized plain urine containers. Each sample container was coded by a numerical label indicated for one patient, then transferred to the laboratory of the biochemistry department at the faculty of medicine, Cairo University.

• **Statistical analysis**

Statistical analysis was performed with SPSS 2016®, Graph Pad Prism®1, and Microsoft Excel 20163. All quantitative data were explored for normality by using Shapiro Wilk Normality test and presented as mean ± standard deviation. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that of all groups regarding age and IGF - 1 were originated from normal distribution (parametric data) resembling normal Bell curve. Interrater and inter-rater reliability was also calculated by using Kappa test.

• **Sample size calculation:**

Sample size calculated depending on a previous study (**Carelli et al 2021**) as reference. According to this study, the minimally accepted sample size was 38 when the marginal error was 16 depending on previous study. Sample size was calculated using the following formula as N (Sample size)= $N \times ((N-1)E^2 + x)$.

III. RESULTS

The percentage of CVM stages among whole female subjects were calculated from the lowest to the highest percentage: stage 6, stage 2, stage 4, stage 5, stage 3 and stage 1, respectively.

- **Age and IGF-1 among different age ranges**

From table 1, the UIGF-1 values have a significant difference between different age

ranges as $P=0.01$ (10-12 years was significantly the highest, 8-10 years was significantly the lowest, while 12-14 years revealed insignificant difference with other age ranges.

As shown from table 1, the IGF-1 value start to decrease after the age of 12 years while the peak was at 11.08 years

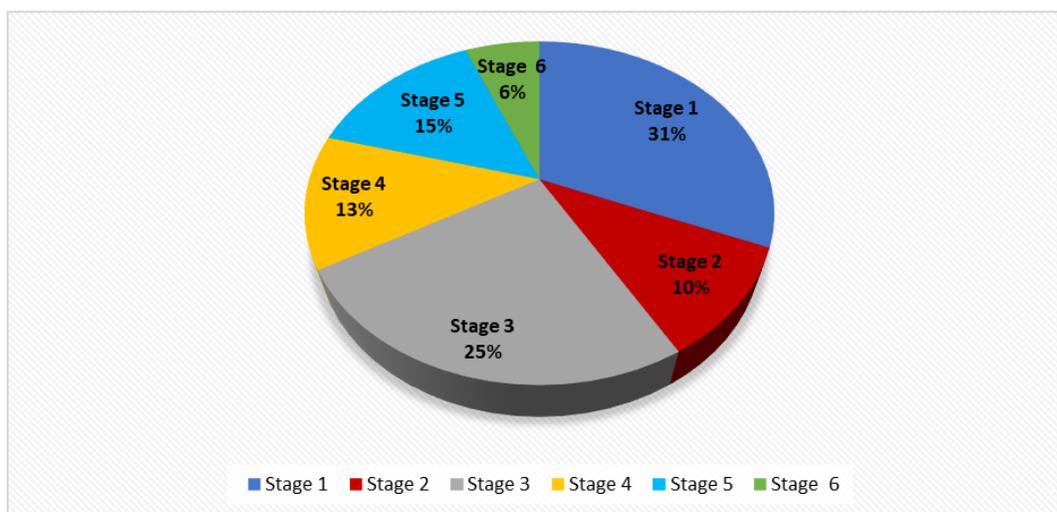


Figure (3): Pie chart representing percentages of different stages among studied sample.

Table (1): Mean & standard deviation of age and IGF – 1 among different age ranges, comparison between them

Age range	N	Age		IGF-1	
		M	SD	M	SD
8-10 years	16	9.06 ^a	0.57	0.61 ^a	0.12
10-12 years	16	11.08 ^b	0.58	0.75 ^b	0.16
12-14 years	16	12.99 ^c	0.60	0.70 ^{ab}	0.12
P value (One Way ANOVA test)		<0.0001*		0.01*	

*M; mean; SD: standard deviation *: significant difference as $P<0.05$
 Means with the same superscript letters were insignificantly different as $P>0.05$.
 Means with different superscript letters were significantly different as $P<0.05$.*

• **Age and IGF-1 among different stage**

Calculation of mean age and IGF-1 values at different stages were calculated, it was shown at table 2 a significant difference between mean age among different stages as $P < 0.0001$ (Age in stage 5 and stage 6 were significantly the highest with insignificant difference between them, while age in stage 1 was significantly the lowest). In UIGF-1, the level was gradually

increased with age until the sharp increase from stage 3 to stage 4 which was significantly the peak of UIGF-1 as $P < 0.0001$, while was significantly the lowest in stage 1, stage 2 and stage 6 with insignificant difference between them). From the data of this study, it was noticed peak of UIGF-1 value was at 12.14 years in females, then the level gradually decreases at stage 5 and stage 6, respectively.

Table (2): Mean & standard deviation of age and IGF – 1 among different stages, comparison between them

Stages	Age		IGF - 1	
	M	SD	M	SD
Stage 1	9.16 ^a	0.79	0.60 ^a	0.13
Stage 2	10.36 ^b	0.76	0.59 ^a	0.09
Stage 3	11.29 ^c	1.04	0.72 ^b	0.11
Stage 4	12.42 ^d	0.78	0.88 ^c	0.13
Stage 5	12.84 ^{de}	0.66	0.74 ^b	0.09
Stage 6	13.60 ^e	0.44	0.60 ^a	0.06
P value (One Way ANOVA test)	$< 0.0001^*$		$< 0.0001^*$	

*M; mean; SD: standard deviation *: significant difference as $P < 0.05$
 Means with the same superscript letters were insignificantly different as $P > 0.05$.
 Means with different superscript letters were significantly different as $P < 0.05$.*

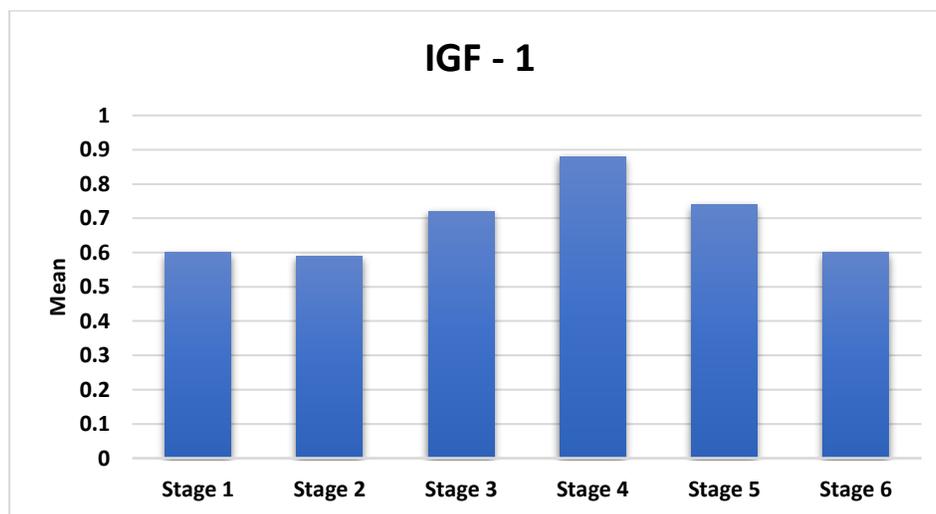


Figure (4): Bar chart representing mean IGF-1 among different stages.

• **IGF – 1 at different age ranges in all stages**

Comparison between different stages within each age range revealed significant difference ($P < 0.0001$) at all age ranges as:

- **8-10 years:** stage 1, 2, 3 were significantly the highest.
- **10-12 years:** stage 4 was significantly the highest.
- **12-14 years:** stage 4 was significantly the highest.

Comparison between different age ranges within each stage revealed significant difference ($P < 0.0001$) at all age ranges as:

- **Stage 1 and 2:** 8-10 years and 10-12 years were significantly the highest.
- **Stage 3 and 4:** 10-12 years was significantly the highest.
- **Stage 5 and 6:** 12-14 years was significantly the highest.

Table (3): Mean and standard deviation of IGF – 1 at different age ranges among different stages, comparison between them

Stages	IGF - 1 at different age stages						P value (One Way ANOVA)
	8-10 years		10-12 years		12-14 years		
	M	SD	M	SD	M	SD	
Stage 1	0.60 ^{aA}	0.13	0.63 ^{aA}	0.16	0.00 ^{aB}	0.00	<0.0001*
Stage 2	0.64 ^{aA}	0.04	0.56 ^{aB}	0.11	0.00 ^{aC}	0.00	<0.0001*
Stage 3	0.66 ^{aA}	0.00	0.77 ^{bB}	0.11	0.61 ^{bA}	0.04	<0.0001*
Stage 4	0.00 ^{bA}	0.00	0.96 ^{cB}	0.03	0.80 ^{cC}	0.16	<0.0001*
Stage 5	0.00 ^{bA}	0.00	0.00 ^{dA}	0.00	0.74 ^{cB}	0.09	<0.0001*
Stage 6	0.00 ^{bA}	0.00	0.00 ^{dA}	0.00	0.60 ^{bB}	0.06	<0.0001*
P value (ONE Way ANOVA)	<0.0001*		<0.0001		<0.0001*		

M: mean; SD: standard deviation

*Significant difference as $P < 0.05$.

Means with different superscript letters (small per column / capital per row) were significantly different as $P < 0.05$

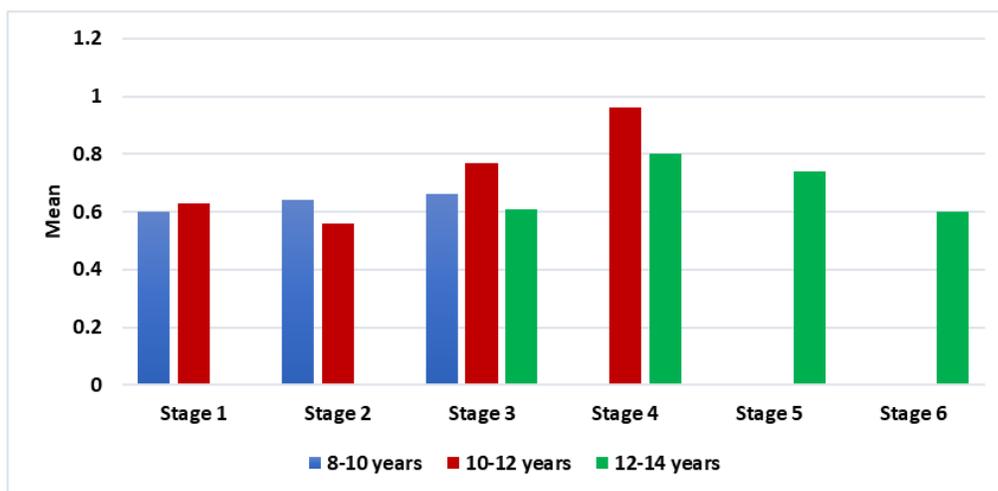


Figure (5): Bar chart representing IGF – 1 at different age ranges in all stages

- **Correlation between age, IGF-a and CVM**

Pearson's correlation coefficient was performed and revealed positive significant correlation between age and IGF-1 and CVM and IGF-1.

IV. DISCUSSION

By ALARA principle of radiation safety, it was crucial to search for a non-hazard method providing an effective diagnostic tool for growth assessment to meet the criteria of ideal biological indicator, as stated by Frachi et al.²⁰ Thus, the current study was conducted to investigate the validity of urinary insulin-like growth factor-1 (UIGF-1) as a diagnostic tool for the human growth assessment, instead of the radiographic method as long as safer, simple and low cost effective.

The current study used IGF-1 in the urine, it was proved to be found in the urine and changes according to the GH excess or deficiency.²¹ IGF-1 is found in other body fluids such as saliva and serum. Several investigators declared that IGF-1 in serum is accurate and reliable. Its value during puberty is more than in the average adult in both genders and declined after puberty with females reaching the peak value 1-2 years earlier than males^{10, 22, 12, 23}.

As blood is more disturbing for the children and there is a positive correlation between urinary and serum IGF-1 levels during childhood and adolescence²⁴, the IGF-1 in urine was selected in the present study as a surrogate to IGF-1 in serum.

The selection of the age range of female subjects from 8 to 14 years and further subdivision to subgroups according to the result documented by several investigators who have conducted their study on the Egyptian population to include all subjects in the circumpubertal period. They were categorized into all six CVM stages within which the pubertal growth spurt might occur. females were subdivided chronologically into three age subgroups (8-10 y), (10-12 y) and (12-14) years to ensure all prepubertal, pubertal, and postpubertal stages were included.^{15, 17, 25}

The current study included adolescent females who met the inclusion criteria. Exclusion of subjects was ruled out by direct questioning of participants and/or their parents, in which the existence of systemic disease might impair the process of growth and maturation as well as renal function. Furthermore, trauma or surgery in the cervical region may alter the integrity and morphology of the structure to be radiographed.

The concentration of GH and IGF-1 increases during sleep which ensures its availability in a fresh morning urine sample to be detectable and measurable by laboratory investigation²⁶. Hence, sample collection from 48 patients was unexpectedly challenging. Few patients did not adhere to the instructions that they had to avoid urination until the time of sample collection, so they were excluded from the study.

Measuring the quantified level of IGF-1 using the ELIZA technique was advocated by many researchers, it could be measured by the blood, saliva, and urine as reported by Ishaq et al.¹³ Sinha et al.¹⁶ and Tripathi et al.²⁸ respectively. Different laboratory techniques are used to detect and quantify the IGF-1 or other hormones such as radioimmunoassay, immunoradiometric, and ELIZA. We used an ELIZA IGF-1 kit because it is simpler, more accurate, and its availability compared to other techniques that need more laboratories equipped with specialized protective measures.

Data distribution was normal (parametric data) as P value was insignificant, indicating that alternative hypothesis was rejected, so a one-way ANOVA test was used to perform a comparison between the variables. In the current study, results by ANOVA analysis revealed that the comparisons among the mean UIGF-1 values at all CVM stages were significantly different. The sample showed that the lowest level of IGF-1 was at CVM stage 1 (initiation) then gradually rose to peak at CVM stage 4 (deceleration) in females. Tuckey's Post Hoc test was used for multiple comparisons between each mean of UIGF-1 value at the different CVM stages. It was shown that the peak of UIGF-1 at CVM 4 had a significant difference compared to other values at the other CVM five stages. The sudden and significant rising of the urinary IGF-1 curve starting from CVM 3, it was indicated that CVM

3 is the onset of a growth spurt and the optimal time of functional orthodontic intervention.

It was shown that the level of IGF-1 with no significant difference between CVM 3 and CVM 5, gives a confusing decision in whether the patient is a candidate for orthodontic treatment or not. In this situation we suggest further IGF-1 investigation within a few months to distinguish if the hormone directed toward up level indicating the bone age tends to CVM 4 or down level toward CVM 6.

Our findings were similar to the serum IGF-1 curve of Ishaq et al.¹³ except for the difference in the hormone values in all skeletal stages; the serum IGF-1 were higher than urinary IGF-1 of the present study suggesting that IGF-1 in urine is remanent and an excess of the body's demand, which subsequently is excreted through the urine in minor amounts.

From the literature and the current study, it is clear that whether IGF-1 is measured from urine or serum, the course of this hormone varies throughout life with chronological age and significantly with the staging of skeletal development, it characterizes by the highest level at puberty. From the current result, it was demonstrated a significant positive moderate correlation between the UIGF-1 and CVM indicators, and between UIGF-1 and chronological age. Depending on the studied population, the chronological age at the time of pubertal growth has a wide variety. Our findings showed the female subjects reached the peak urinary IGF-1 value at 12.42 years, while a study adopted by Sinha et al. on the Indian population reached the peak at 13.67 years.²⁷

In contrast, as the age ranges of the current study sample were selected according to the previous studies that had been adopted on the Egyptian population, there were similarities in the findings. For example, Mahmoud et al. found that the onset of pubertal growth in females started at the mp3-G stage - which is closely related to CVM 3 - with the mean age of 11.17 years¹⁷, and in current findings, the growth spurt onset was at CVM 3 with 11.29 years. As a consequence, our findings were almost compatible with previous studies as they were conducted on the same population.

The current results show that the UIGF-1 could be considered as a reliable indicator for the

skeletal growth assessment despite the orthodontists admitting that pre-treatment radiographs cannot be neglected as it is an essential record for skeletal, dental, soft tissue analysis, pathologies investigation, and skeletal growth assessment.

According to Hassal and Farman, the radiographic method should not be relied on due to the difficulty in tracing all references anatomical changes of one stage as it integrated with the next stage.⁶ Thus, we can state that the UIGF-1 should be used as an indicator that assists in growth assessment in cases of doubtful radiographic readings. Accordingly, to eliminate tracing error in the current study, the cephalograms of participants were highly precise selected with good contrast and well defined cervical vertebral images displaying the apparent criteria for each stage that is not ensured in orthodontic practice which is frequently attained with vague radiographs then urge the patient for further radiographic exposure is inadvisable for ethical reason. Furthermore, this biochemical indicator has a possible benefit to diagnose the growth spurt especially in rural areas which do not provide or lack high qualified radiologic services.

The IGF-1 is the primary mediator of the growth hormone (GH) that induces IGF-1 production and secretion from many tissues, most of it is by the liver and then is transported to the target tissue by the blood in an endocrine manner. In addition, IGF-1 is also released from neighboring cells, e.g., chondroblast, and acts locally as paracrine or from the same cell as an autocrine hormone. This mechanism reveals that GH could be an indicator of body growth. However, IGF-1 is more reliable than GH to discover a body growth status as reported in the literature review that in some short stature patients with a normal serum GH level they had sign of a declined IGF-1 due to GH-cell resistance preventing its entrance to the target cell to produce IGF-1.³⁰ Another reason that supports the reliability of IGF-1 than GH, is that it was found at puberty IGF-1 is stimulated not only by GH it is also by androgens.³¹

V. CONCLUSION

From the findings of this study, it was concluded that:

- 1- There is a significant relation and positive correlation between UIGF-1 and CVM stages; therefore, it can be a promising futuristic tool for growth assessment in orthodontics.
- 2- UIGF-1 levels in urine steadily rise from stage 1 (initiation) to peak at stage 4 (deceleration), then decline to baseline levels at stage 6 (completion).
- 3- The peak of UIGF-1 value is 0.88 ng/mg in females and related to the CVM stage 4 and the mean chronological age of 12.42 years

More studies are recommended in larger sample sizes to validate these results to facilitate the use of UIGF-1 as simple growth indicator with specific norms in Egyptian population.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry - Cairo university, approval number (19-4-9).

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