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Relationship of salivary insulin like growth factor 1, serum insulin like growth factor 1 and quantitative cervical vertebral maturational stages. (A longitudinal, Pilot study)

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ABSTRACT: In the field of orthodontic diagnosis IGF-1 has evolved as important tool for identification of skeletal maturation. Circulating concentrations of IGF-1 suggest that serum and salivary concentrations of this peptide reflects bone growth status.

The present study was aimed at assessment of skeletal maturation in monozygotic (MZ) and dizygotic (DZ) twins by using salivary IGF-1 and serum IGF-1 and to correlate these IGF-1 levels with quantitative cervical vertebral maturation (QCVM) given by Li Li Chen et al[2] over a period of 18 months.

The total sample consisted of 20 twin pairs in the age group of 10-16 years (circumpubertal age group); divided into two groups, group A consisted of 10 MZ twins (7 male and 3 female pairs) and group B consisted of 10 DZ twins (4 male and 6 female pairs). Twin pairs in each group was subjected to quantitative cervical vertebral maturation staging given by Li Lin Chen et al[2] and salivary IGF-1 and serum IGF-1 level assessed by IMMUNORADIOMETRICASSAY (IRMA kit).

Mean difference and standard deviation for each group was calculated, student paired t test was applied to find out statistical significance.

Keywords: serum insulin like growth factor 1(serum IGF 1), salivary insulin like growth factor 1(salivary IGF 1), quntitative cervical vertebral method (QCVM), monozygotic twins (MZ), dizygotic twins (DZ). T1 – time of first appointment, T2- time of second appointment, child 1-first child of twin pair, child 2-second child of twin pair.

I. INTRODUCTION:

Skeletal maturity assessment is important and also critical to the modality and timing of treatment of various skeletal abnormalities in field of Orthodontics. Timing of skeletal maturation differs between boys and girls, occurring on average 2 years earlier in girls. Many studies have shown that chronologic age and dental age both is poor predictor of pubertal growth spurt.

In 2008 Li Li Chen et al [1] gave an objective method for assessment of skeletal maturation using a mathematical formula for quantification of bone age using cervical vertebrae.

Masoud et al [2] published an article (2008) on the reliability of serum IGF-1 as a skeletal maturity indicator and concluded that serum IGF-1 correlates well with skeletal ages as determined by cephalometric radiographic technique in his cross sectional study, only limitation is the process of collecting blood sample is an invasive procedure.

Costigan D, Guyda H, Posner B [3] proved that saliva contains free IGF-1 and reflects its level in plasma. **J Ryan** [4] studied the human salivary IGF-1 concentration using Radioimmuno assay from birth through puberty collected from normal population, concluded that salivary IGF-1 concentration were 100-200 fold less than plasma IGF-1 level.

The aim of the study is to assess skeletal maturation in 20 pairs of twins, 10 pairs of monozygotic and 10 pairs of dizygotic twins of same sex, using serum IGF-1 and salivary IGF-1 and to correlate the levels of serum IGF-1 and salivary IGF-1with stages of cervical vertebrae by using Quantitative Cervical Vertebral Method (QCVM) over a period of 18 months.

II. MATERIALS AND METHODS:

The sample of 20 twin pairs in the age group of 10 - 16 years is considered for both monozygous and dizygous twins of same sex. 20 Twin pairs were grouped as monozygotic (Group A-7 male and 3 female pairs) and dizygotic (Group B-4 male and 6 female pairs).

First sample was collected in year 2010, second sample was collected in year 2012 and study conducted at Government Dental College and Hospital, Nagpur.

Zygosity determined by clinical examination, questionnaire method by **S. Ooki** and **A. Asaka [5]** and DNA fingerprinting.

The lateral cephalogram of each subject was taken in centric occlusion, the second, third and fourth cervical vertebrae were manually traced. For saliva sample collection, patient was made to sit comfortably on the dental chair with arm positioned on the slanting rest, arm extending

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straight from the shoulder. Paraffin block (1cm×1cm) was placed under the tongue to stimulate saliva secretion. After sufficient amount of saliva was accumulated in the floor of mouth, saliva was collected by pipette and transferred into a 25ml sterile container. 4 ml of blood collected from medial cubital vein in the cubital fossa and samples stored at 2-8 ^{0}C for not more than 30 minutes and sent to Diagnostic centre for Immunoradiometricassay analysis. Sample collection done by pathology laboratory technician. After 18 months again lateral cephalogram of same sample taken, blood sample and saliva sample collected by same procedure and analysed in the same laboratory.

III. RESULTS:

Salivary IGF 1, serum IGF 1 and QCVM staging were carried out at 1st

Appointment (T1) and after a period of 1 ½ years (T2) an independent't' test was applied for intragroup comparison. The mean values were calculated for all the variables. Level of significance was judged by p value. For statistical significance $p<0.05$ was considered.

IV. DISCUSSION:

Various methods are available for the detection of pubertal growth spurt in an individual, like height, weight, chronologic age and dental age, and sexual maturation. But in comparison to these methods skeletal age is more reliable parameter in assessing pubertal growth spurt.

Three pairs of monozygotic twins showed QCVM stage I (accelerating stage) with QCVM values in the range of 1.32-1.61, salivary IGF-1 level 2.0 – 2.5 ng/ml and serum IGF-1 level was 160 -236 ng/ml **at T1**, **at T2** the same pairs were in QCVM stage II (high velocity stage) with QCVM value ranging from 1.80 - 2.40, salivary IGF-1 increased to 3.0 – 4.5 ng/ml and serum IGF-1 levels increased to 250-423ng/ml. In seven twin pairs **at T1**, QCVM stage II (high velocity stage) was observed with QCVM value of 1.75 - 2.57 and salivary IGF-1 was 2.0 – 6.8 ng/ml and serum IGF-1 level was 160 - 450ng/ml. **At T2**, same pairs showed QCVM stage III (decelerating stage) with QCVM value of 2.55 – 3.43 and decreased in level of salivary IGF-1 to 2.0 – 3.8 ng/ml and serum IGF-1 level also decreased to 142- 330ng/ml. In all the 10 pairs of MZ twins over a period of 18 months QCVM values increased , salivary IGF-1 and serum IGF-1 levels reached a peak as twins matured from stage I to stage II and the levels declined towards post pubertal stage i,e. stage II to stage III. **Juul et al [6], Jull et al [7], Brabant G. et al [8] and Masoud et al [9] reported in their study the values of serum and salivary IGF 1 decline towards post puberty** In **2008 Masoud et al[2]** reported increased value of serum IGF-1 at cervical vertebral stage CS3-CS5 (Lamparski method) which indicates the stage of puberty and decline in level of serum IGF-1 from CS5 – CS6 (stage of completion). **reported in their study the values of serum and salivary IGF 1 decline towards post puberty** In **2008 Masoud et al[2]** reported increased value of serum IGF-1 at cervical vertebral stage CS3-CS5 (Lamparski method) which indicates the stage of puberty and decline in level of serum IGF-1 from CS5 – CS6 (stage of completion). In **2012 Ramy Abdul [10]** reported increased level of serum IGF-1 at cervical vertebral stage, CS 4 in females and cervical vertebral stage CS5 in males. Values are given in mastersheet. Mean difference in **rate of change** of QCVM values from T1 to T2 for CHILD 1 (rate of change 0.39) and CHILD 2 (rate of change 0.42) of MZ twins showed no statistical significant difference (Table/Fig 1, Table/Fig 2). Mean difference in **rate of change** of salivary IGF-1 from T1 to T2 for both CHILD 1 (rate of change -0.13) and CHILD 2 (rate of change -0.15) of MZ twins showed no statistical significant difference (Table/Fig 1, Table/Fig 2). Mean difference in **rate of change** of serum IGF-1 from T1 to T2 of CHILD 1 (rate of change 0.06) and CHILD 2 (rate of change 0.01) in MZ showed no statistical significant differences (Table/Fig 1, Table/Fig 2). QCVM, salivary IGF-1 and serum IGF-1 showed almost similar rate of skeletal maturation in MZ twins over a period of 18 months showing a high correlation between the three skeletal maturity indicators. MZ twins mature at the same rate as they share the same genetic material. **M. Harella et al** [**11]** showed that a genetic component for IGF-1 is same in MZ twins.

Nine pairs of dizygotic twins were in QCVM stage II (accelerating stage) with QCVM value in the range of 1.76 – 2.01, salivary IGF-1 level was 3.5 – 4.5 ng/ml and serum IGF-1 level was 164-407ng/ml **at T1**. **At T2** same pairs showed QCVM stage III (decelerating stage) with QCVM value 2.62 – 3.11 , salivary IGF-1 decreased to 2.0 – 3.0 ng/ml and decreased serum IGF-1 level to 142 – 336 ng/ml (Master sheet). In one pair QCVM stage III (decelerating stage) was observed with QCVM value 2.67 & 2.90, salivary IGF-1 level was 3.5 ng/ml and serum IGF-1 level was 164-423ng/ml at **T1**. At **T2** same pair showed

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QCVM stage IV (completion stage) with QCVM salivary IGF-1 to 2.0 & 2.5 ng/ml and decreased serum IGF-1 level to 123 ng/ml & 160 ng/ml.

Salivary IGF-1 and serum IGF-1 levels were at peak in the accelerating stage and decreased 0.46) of DZ twins showed statistical significant difference (Table/Fig 3, Table/Fig 4). Mean difference of **rate of change** of salivary IGF-1 from T1 to T2 for both CHILD 1 (rate of change of **rate of change of** serum IGF-1 from T1 to T2 for both CHILD 1(rate of change -0.26) and CHILD 2 (rate of change -0.21) of DZ showed statistically significant difference (Table/Fig 3, Table/Fig 4). QCVM, salivary IGF-1 and serum IGF-1 showed a statistically significant difference in the rate of maturation in DZ indicating that DZ twins differ in skeletal maturation over a period of 18 months, DZ twins show different genetic pattern. QCVM, salivary IGF-1 and serum IGF-1 showed good correlation for assessment of rate of maturation in DZ twins. The lowest detection limit of IRMA kit used is 2 ng/ml. In **MZ twins** peak value of salivary IGF-1 found was 3.0 – 6.8 ng/ml in QCVM stage II and decreased level of salivary IGF-1 in QCVM stage I and stage III to 2.0 – 3.8ng/ml (Table/Fig 5) **In DZ twins** peak value of salivary IGF-1 was 3.5-4.5 ng/ml in QCVM stage II and decreased level of salivary IGF-1 in QCVM stage I and III with values of 2.0 – 3.5ng/ml (Table/Fig 5).**Costigan et al [3] reported the value of IGF 1 .**with mean concentration of 2.3 ±0.3 ng/ml.**J Ryan**[4] reported low levels of salivary IGF-1 in early childhood, rising level with age, peak in puberty and falling again in late adolescence, salivary IGF-1 levels were 100-200 fold less than plasma IGF-1 levels. Since the test is a radioimmunoassay and is usually not undertaken routinely, limits the sample size, short duration study only of $1^{1/2}$ year because long follow up is not possible is the limitation for this study.

value 2.72 & 3.61, decreased towards the deceleration and completion stage of maturity. Mean difference of **rate of change** of QCVM from T1 to T2 for both CHILD 1 (rate of change 0.54) and CHILD 2 (rate of change -0.33) and CHILD 2 (rate of change -0.42) of DZ twins showed statistical significant differences (Table/Fig 3, Table/Fig 4). Mean difference

V. CONCLUSION:

A good correlation exist between the three skeletal maturity indicators i.e. QCVM, salivary IGF-1 and serum IGF-1.Increased value of IGF-1 obsereved at prepuberty, peak at puberty and decline in post puberty, salivary IGF-1 has a positive correlation with serum IGF-1, salivary IGF-1.

ACKNOWLEDGEMENT:

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MASTER SHEET 1

QCVM and salivary IGF-1 in Monozygotic Twins at T1 and T2

SR NO	PAIR NO	AGE	QCVM stages				QCVM values by formula				Salivary IGF-I (ng/ml)				Serum IGF-I ng/ml			
			T1		T2		T1		T2		T1		T2		T1		T2	
			CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD2	CHILD 1	CHILD 2	CHILD 1	CHILD 2				
1.	1	14	II	II	III	III	1.75	1.8	3.43	3.02	3.5	3.0	2.0	2.0	250	242	142	136
2.	2	13	II	II	III	III	1.78	1.82	2.65	2.71	3.5	3.5	2.5	2.5	210	200	155	160
3.	3	11	II	II	III	III	2.01	2.11	2.88	2.7	3.	3.5	2.5	2.0	190	200	178	171

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											5							
4.	4	12	II	II	III	III	1.87	1.78	2.55	2.71	3.5	4.5	2.0	2.0	173	160	160	150
5.	5	14	II	II	III	III	2.57	2.40	3.10	3.01	6.1	6.8	3.8	3.0	435	450	235	250
6.	6	11	II	II	III	III	2.50	2.34	2.62	2.66	2.5	4.0	2.0	2.0	200	223	153	148
7.	7	10	I	I	II	II	1.50	1.32	1.88	1.80	3.5	3.0	4.0	4.5	178	199	320	300
8.	8	11	I	I	II	II	1.61	1.49	2.11	1.89	2.5	2.0	4.0	3.0	210	236	423	420
9.	9	12	II	II	III	III	1.81	1.88	2.66	2.80	3.5	3.5	2.0	2.5	440	436	340	330
10.	10	11	I	I	II	II	1.55	1.43	2.05	2.40	2.5	2.5	3.0	3.5	160	178	253	250

T1- saliva sample collected at baseline/first appointment.
T2-saliva sample collected after 18 months

MASTER SHEET 2

QCVM and salivary IGF-1 in Dizygotic Twins at T1 and T2

SRNO	PAIR NO	AGE	QCVM				QCVM				Salivary IGF-I (ng/ml)				Serum IGF-I (ng/ml)			
			T1		T2		T1		T2		T1		T2		T1		T2	
			CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD2	CHILD1	CHILD 2	CHILD1	CHILD2	CHILD1	CHILD2
1.	1	13	II	II	III	II	1.80	1.87	2.82	2.72	3.5	4.5	2.5	2.0	250	170	176	136
2.	2	11	II	II	III	II	1.82	1.93	2.63	2.67	3.5	4.5	2.5	2.5	260	164	223	142
3.	3	12	II	II	III	II	1.76	1.84	2.65	2.65	3.5	3.5	3.0	2.5	320	264	167	178
4.	4	13	II	II	III	II	1.84	2.01	2.70	2.81	4.0	3.5	2.5	2.0	295	270	160	153
5.	5	13	II	II	III	II	1.78	1.87	3.11	3.02	3.5	4.0	2.5	3.0	275	210	210	200
6.	6	12	II	II	III	II	1.76	1.80	3.09	3.22	4.5	4.5	2.5	2.5	407	336	336	278

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7.	7	13	II	II	III	III	1.81	1.92	2.89	2.70	4.0	4.0	2.5	2.0	372	310	199	224
8.	8	12	II	II	III	III	1.87	1.76	2.71	2.64	4.5	4.5	3.0	2.5	395	350	254	219
9.	9	12	II	II	III	III	1.79	1.87	2.77	2.67	4.5	3.5	2.0	2.0	360	423	278	334
10.	10	14	III	III	IV	IV	2.67	2.90	3.61	2.72	3.5	3.5	2.5	2.0	182	174	160	123

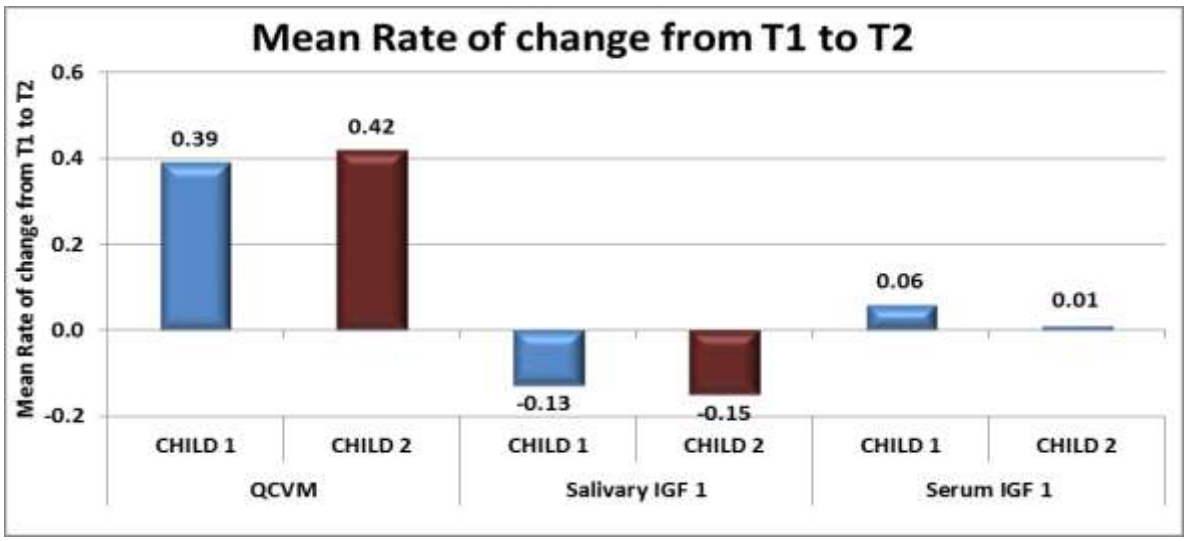
T1- sample collected at baseline/first appointment.
T2- sample collected after 18 months.

TABLES/FIGURES:

Parameters		Mean diff. of rate of change (SD)	p value	Sinificance
QCVM	CHILD 1	0.39 (0.24)	0.59	NS
	CHILD 2	0.42 (0.18)		
Salivary IGF-1	CHILD 1	-0.13 (0.33)	0.77	NS
	CHILD 2	-0.15 (0.44)		
Serum IGF-1	CHILD 1	0.06 (0.53)	0.06	NS
	CHILD 2	0.01 (0.42)		

P>0.05; NS , not significant

Table/Fig 1: Rate of change of parameters from T1 to T2 for both CHILD 1 and CHILD 2 in MZ twins (Mean values of differences between T1 and T2)



Table/Fig 2: Rate of change of QCVM, salivary IGF-1 and serum IGF-1 from T1 to T2 for both CHILD 1 and CHILD 2 in MZ twins (Mean value of difference between T1 and T2).

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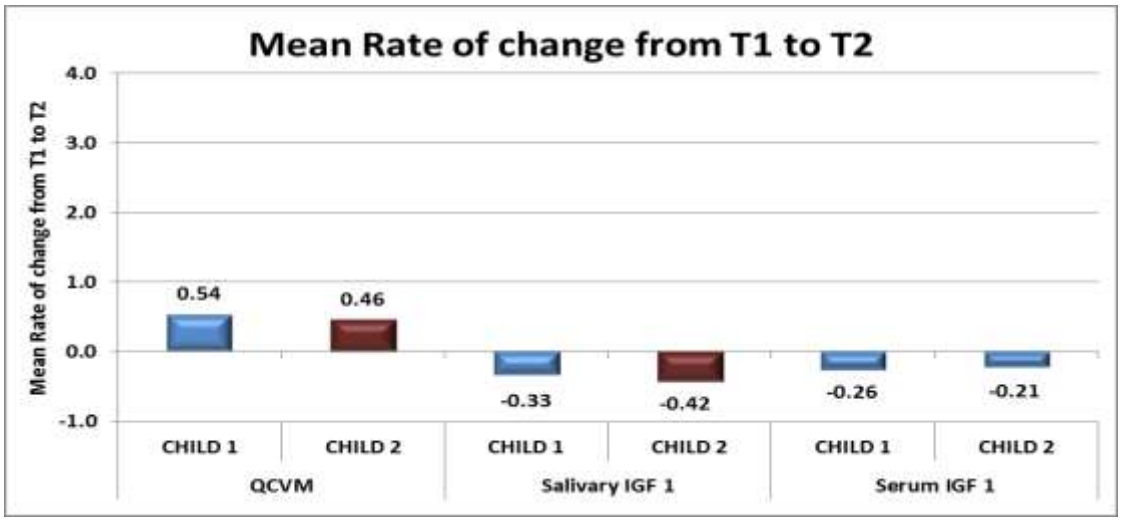
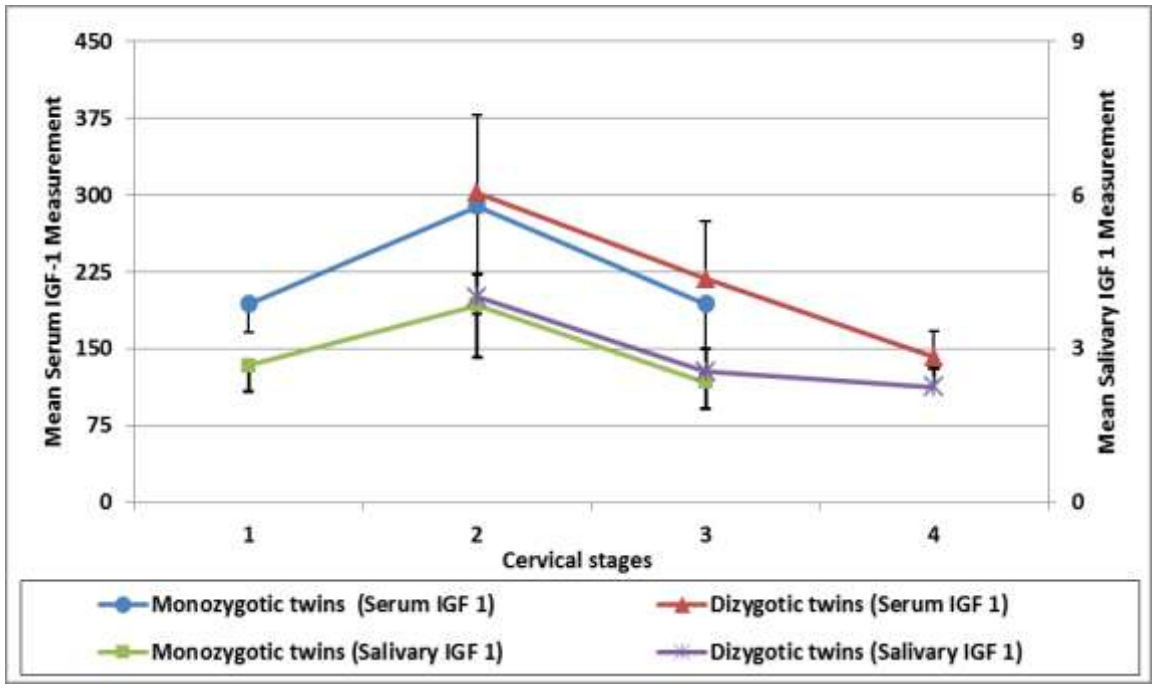
Parameters		Mean difference (SD)	p value	Significance
QCVM	CHILD 1	0.54 (0.13)	0.0093	S
	CHILD 2	0.46 (0.15)		
Salivary IGF-1	CHILD 1	-0.33 (0.11)	0.04	S
	CHILD 2	-0.42 (0.09)		
Serum IGF-1	CHILD 1	-0.26 (0.12)	0.02	S
	CHILD 2	-0.21 (0.17)		

a P< 0.05; S, Significant

Table/Fig 3: Rate of change of parameters from T1 to T2 for CHILD 1 and CHILD 2 in DZ twins (Mean values of differences between T1 and T2)



Table/Fig 4: Rate of change of QCVM, salivary IGF-1 from and serum IGF-1 T1 to T2 for both CHILD 1 and CHILD 2 in DZ twins (Mean value of difference between T1 and T2).



Table/Fig 5: Correlation between QCVM, salivary IGF-1 and serum IGF-1 in MZ and DZ twins at T1 and at T2

REFERENCES:

[1]. **Li-Li Chen, Tian-Min Xu, Jiu-Hui Jiang, Xing-Zhong Zhang, and Jiu-Xiang Lind** Quantitative cervical vertebral maturation assessment in adolescents with normal occlusion: A mixed longitudinal study. Am J Orthod Dentofacial Orthop 2008;134:720.e1-720.e7

[2]. **Mohamed Masoud, Ibrahim Masoud, Ralph L. Kent Jr, Nour Gowharji.** Assessment skeletal maturity by using blood spot insulin like growth factor 1 (IGF-1) testing, Am J Orthod Dentofacial Orthop 2008;134:209-16.

[3]. **Costigan D, Guyda H, Posner B.** Free insulin like growth factor 1 (IGF-1) in human saliva. J Clin Endocrinol Metab 1988;66:1014-8.

[4]. **Ryan J, Mantle T, Costigen D**. A normal study of human salivary insulin like growth factor-1 concentrations from birth through puberty. J Clin Endocrinol Metab 1992;72:774-778.

[5]. **Ooki S, Asaka A.** Zygosity Diagnosis in Young Twins by Questionnaire for Twins' Mothers and Twins' Self-reports. Twin Research. 2004;7(1):5–12.

[6]. **Juul A, Bang P, Hartel N, Main K, Dalgaard P, Jorgensen K, et al.** Serum insulin – like growth factor-1 in 1030 healthy children, adolescents and adults relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 1994;78:744-52.

[7]. **Jull A,Holm K, W. Knud Kastrup, A Søren,et al** Free Insulin-Like Growth Factor 1 Serum Levels in 1430 Healthy Children and Adults, and Its Diagnostic Value in Patients Suspected of Growth Hormone Deficiency. Journal of Clinical Endocrinology & Metabolism 1997;82(8): 2497-2502.

[8]. **Barant G. Muhlen A. Wuster C**. Serum Insulin Like Growth Factor J. values for IGF-1 an Automated Chemiluminescence Immunoassay System. Results from a multicenter Study. Horm Res 2003;60:53-60.

[9]. **Mohamed I. Masoud, Ibrahim Masoud, Ralph L. Kent, Jr, Nour Gowharji, Ali H. Hassan, Laurie E. Cohen**. Relationship between blood-spot insulin-like growth factor 1 levels and hand-wrist assessment of skeletal maturity. Am J Orthod Dentofacial Orthop 2009;136:59-64.

[10]. **Ramy Abdul Rahman Ishaq, Sanaa Abou Zeid Soliman, Manal Yehya Foda, Mona Mohamed Salah Fayed.** Insulin-like growth factor 1: A biologic maturation indicator. Am J Orthod Dentofacial Orthop 2012;142:654-61.

[11]. **M Harrela, H. Koistinen, J. Kaprio, M. Lehtovirta,J. Tuomilehto, J.Eriksson, L.Toivanen, M.Koskenvuo, P.Leinonen, R.Koistinen, M.Seppala.** Genetic and environmental component of individual variation in circulating levels of IGF-1, IGF-2, IGFBP-1 and IGFBP-3. J.Clin.Invest.1996.98: 2612-2615.