IGF-1 AND VEGF IN SALIVA AND ITS RELATION WITH CVMI STAGES IN DETERMINING THE SKELETAL MATURITY

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INTRODUCTION

INTRODUCTION

The determination of the growth status of an individual plays a vital role in deciding the orthodontic treatment plan. The growth modification therapy carried out particularly during peak pubertal growth spurt provides a successful treatment outcome within a reduced period of time.[38] It is believed that girls have an earlier onset of puberty when compared to boys. The accelerating phase of growth may last for 2 years on average so this aspect should be taken into consideration while making clinical decisions for any growth modification procedures.[43]

Each and every individuals matures differently according to his or her own biological clock. So there are different methods that had been reported by different authors to determine the skeletal maturity of an individual such as height, weight, chronological age, sexual maturation, frontal sinus, biological age, hand wrist radiograph, cervical vertebrae, dental eruption, dental calcification stages and recently biomarkers.[44]

Greulich and Pyle (1959) and Fishman (1982) determined skeletal maturation using the hand wrist radiograph. It is a popular and reliable indicator for determining the skeletal maturation but it requires an additional radiograph and an unwanted radiation exposure to the patient.

Lamparski (1972) [19] assessed the skeletal maturation based on the morphology of cervical vertebrae. Hassel B and Farman A (1995) [12] used the lateral cephalogram and studied C2, C3 and C4 to develop a reliable method for identifying adolescent growth potential. One advantage of CVM method is that a lateral cephalometric radiograph is routinely required for orthodontic diagnosis and treatment planning, so no additional radiograph is required.[34]

Gandini et al [28] and Flores-Mir et al [6] compared hand wrist bone radiograph with cervical vertebral radiograph for measuring skeletal maturation and found that vertebral analysis on a lateral cephalogram is as valid as the hand wrist bone analysis. The CVM method is comprised

of six maturational stages (CS1-CS6) with the peak skeletal maturation occurring between CS3 and CS4. The detection of CS2 indicates that the growth spurt is approaching and occurs one year after CS2.[52]

Biochemical markers are also used for determining the skeletal maturity status and they include systemic growth factors like growth hormone (GH), insulin like growth factor-1 (IGF1), thyroid hormones, glucocorticoids, estrogen and testosterone and local factors which are involved in growth regulation are Ihh, PTHrP, FGF, BMPs, VEGF, Wnt5a, Wnt5b, Sox 5, 6 & 9, Runx2, HIF-1 alpha, RANKL, OPG, CSF, c-FOS and IGF1. These biochemical markers of skeletal maturity were initially found to be detected in serum. Recently the presence of these growth factors in saliva has been identified.[25]

Salmon and Daughaday discovered IGF1 in 1957 as a mediator of growth hormone (GH) function, which was termed as sulphation factor. IGF1 is a circulating GH factor so its level in serum can be used to identify sexual maturity. Its level does not fluctuate throughout the day so precise assessment of IGF1 is a useful diagnostic tool for determining skeletal maturity.[33,43] Masoud et al and Ishaq et al assessed skeletal maturity by using blood spot IGF1 level.[25,26] Various methods that are used for analyzing IGF-1 are radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA).[33,38,42] There were a significant correlation between cervical skeletal maturity and the biochemical marker from prepuberty to late pubertal stages.

Costigan et al [7] demonstrated the presence of free IGF1 in human saliva. Antonelli et al [1] investigated the presence of IGF1 in saliva and compared it with plasma free IGF1. Salivary IGF1 levels are extremely low i.e less than 1% of serum levels and salivary IGF1 level reflects its plasma level.[25] IGF1 in women was found to be significantly higher than in men.[8]

Nayak et al [42] had studied the relationship between salivary IGF1 and cervical maturation stages for determining the skeletal maturity. They found that highest levels were at the highest velocity stage and thereafter a gradual drop in salivary IGF1 level was detected. Thus salivary IGF1 level is a reliable method for predicting pubertal growth spurt and it is also a noninvasive method.

Rabie et al [31] studied the expression of growth factors regulating mandibular condylar growth. It was found that inactivation of vascular endothelial growth factor (VEGF) suppressed neovascularization and endochondral bone formation in the epiphyseal growth plate of rats. The reintroduction of VEGF caused capillary invasion and bone growth. Also highest levels of VEGF expression coexisted with the highest level of bone formation. VEGF is able to promote ossification by either inducing neovascularization or by directly affecting the bone cells.[57]

Huang et al [14] found that VEGF not only plays a vital role in angiogenesis but also induces bone remodeling. Shum et al [21] assessed the amount of VEGF expression and bone formation in posterior glenoid fossa during stepwise mandibular advancement. There were greatest increase in VEGF expression preceding new bone formation. Therefore, they concluded that an increase of VEGF is temporarily related to the amount of new bone formation. Brozovic et al [45] and Taichman et al [48] studied the presence of VEGF in saliva using enzyme linked immunoassay (ELISA) method.

Routinely radiographs were used for determining skeletal maturity but recently many studies were done on biomarkers to determine skeletal maturity as they avoid radiation exposure. Many researches had been done to evaluate the Insulin like growth factor-1(IGF-1) and Alkaline phosphatase in serum and gingival crevicular fluid. But no study till date has evaluated the level of vascular endothelial growth factor in determining skeletal maturity.

This present study is intended to find out the activity levels of insulin like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in saliva and to correlate it with the various stages of cervical vertebral maturation indicator to confirm whether any definitive change is occurring during the peak pubertal growth period.

AIM AND OBJECTIVE

AIM AND OBJECTIVES OF THE STUDY

AIM:

The aim of this study is to quantitatively evaluate the insulin like growth factor 1 and vascular endothelial growth factor level during various stages of cervical vertebral maturation and to find its correlation with skeletal maturity.

OBJECTIVE:

1) To evaluate the levels of insulin like growth factor - 1 during various stages of cervical vertebral maturation.
2) To evaluate the levels of vascular endothelial growth factor during various stages of cervical vertebral maturation.
3) To find out whether there is any gender differences in insulin like growth factor levels and vascular endothelial growth factor levels.

Also to find whether these two biomarkers can be used to determine the skeletal maturity of an individual by correlating its value with cervical vertebral maturation stage

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Colm Costigan et al (1988) [7] studied the presence of insulin like growth factor 1 (IGF-1) in human saliva and correlated with plasma GH levels. Mixed saliva samples were taken from 14 normal subjects and after centrifugation, the clear supernatant was collected and used for the assay. All the samples were assayed by RIA (Radioimmunoassay) method. In this study salivary IGF1 concentration did not change with increasing salivary flow rates above normal or with storage of samples at room temperature for up to 24h before freezing. The mean IGF1 concentration in mixed saliva was 2.3 ± 0.3 ng/ml and their mean plasma IGF1 level was 315 ± 27 ng/ml. The study concluded that saliva contains free IGF1 and IGF1 level reflects the GH status of the donor.

Maria T.O' Reilly et al (1988) [22] assessed the relationship of cervical vertebral maturation and mandibular growth changes in annual lateral cephalometric radiographs. The samples were obtained from Bolton-Broadbent growth study. It consisted of yearly lateral cephalometric radiographs of 13 Caucasian females with ages ranging down from 9 to 15 years. The measurement of mandibular length, corpus length and ramus height were compared with the stages of cervical vertebral maturation. The result was found to be significant increase in mandibular growth changes associated with specific maturation stages of cervical vertebrae.

Ryan J et al (1992) [36] studied the insulin like growth factor 1 (IGF-1) concentrations in human saliva from birth through puberty. Mixed saliva samples were collected from 327 normal subjects, age ranging from birth to adolescence. The samples were analyzed by RIA (Radioimmunoassay) method. IGF1 level was found to be low during early childhood, rising with age, peaking in puberty and falling again in late adolescence. Salivary IGF1 concentrations

were 100 to 200 folds less than plasma IGF1 levels and also there is no difference between male and female samples apart from pubertal influences.

S. Halimi et al (1994) [13] studied the concentration of insulin like growth factor-1 (IGF-1) in saliva of acromegalic patients and compared with the serum IGF-1 level and growth hormone. The study included 13 healthy adult individuals and 17 acromegalics . IGF-1 was determined in extracted saliva and serum using RIA (Radio immuno assay) method. When compared with healthy subjects, acromegalics had significantly higher level of IGF-1 concentrations and somatotropin levels. However salivary IGF-1 concentrations of active acromegaly patients were within the normal range. Serum IGF-1 and somatotropin concentration were found to follow more closely towards disease activity. These results suggest that IGF-1 levels in serum and saliva are somatotropin dependent. Also measurement of IGF-1 in saliva is less reliable than the determination of IGF-1 and somatotropin in serum.

Johannes Pammer et al (1998) [16] investigated the expression of vascular endothelial growth factor (VEGF) in normal and pathological salivary gland tissues. In situ hybridization revealed distinct labelling of VEGF mRNA in normal and tumoural salivary gland tissues. Saliva samples collected from 24 healthy volunteers revealed high concentrations of VEGF ranging from 0.10 to 2.50ng/ml were detected. Thus the author concluded that VEGF is constitutively expressed in normal human salivary glands and in saliva of healthy individuals. The presence of considerable quantities of VEGF in normal human saliva suggests its important role in the maintenance of homeostasis and accelerates wound healing by neoangiogenesis within the oral cavity.

Taichman NS et al (1998) [48] studied the presence of vascular endothelial growth factor (VEGF), a potent, multifunctional, angiogenic cytokine in normal human saliva. VEGF was measured by ELISA (enzyme linked immunosorbent assay) in whole saliva (median concentration, 460

pg/ml). They found that VEGF seems to be synthesized endogenously by salivary glands because both VEGF mRNA and protein were localized to serious acinar cells and ductal epithelial cells within the parotid, submandibular and minor salivary glands. All findings point the existence of a "Salivary VEGF System".

Martine M. L. Deckers et al (2000) [24] investigated the expression of vascular endothelial growth factor (VEGF) and their receptors during osteoblast differentiation using the mouse preosteoblast like cell line KS483 during early differentiation of osteoblast. KS483 cells express low levels of VEGF mRNA, whereas during mineralization, KS483 cell express high level of VEGF mRNA. VEGF production during osteoblast differentiation was stimulated by insulin like growth factor 1 which enhances osteoblast differentiation and was inhibited by PTH related peptide which inhibits osteoblast differentiation. Thus the expression pattern of VEGF and their receptors play an important role in bone formation by stimulating osteoblast differentiation.

Shoshana Yakar et al (2000) [40] reviewed about the growth hormone/insulin like growth factor-1 system and its implications on organ growth and development. Growth hormone (GH) and insulin like growth factors (IGFs) are essential for normal growth and development during embryonic and postnatal stages. GH has little effect prenatally whereas the IGFs are essential during these stages. GH-IGF1 axis is important for pubertal growth. So in order to determine whether postnatal growth and development are dependent on circulating or locally processed IGF-1, the IGF-gene in the liver were deleted in mice using cre/LoxP system used for tissue specific gene deletion. It was found that these animals demonstrated 75-80% reduction in circulating IGF-1 and four fold increase in circulating GH. Despite the marked reduction in circulating IGF-1 growth and development was normal. Thus the liver production of IGF-1 is not essential, whereas autocrine/paracrine IGF-1 production is sufficient for normal growth and development.

Rabie et al (2002) [30] studied the VEGF expression and bone formation in the glenoid fossa during natural growth and compared with that during forward mandibular positioning. The samples consisted of 150 female Sprague – Dawley rats, 35 day old were randomly divided into 10 experimental and 10 control groups. The rats were then killed at different times. Sections were cut and stained with anti – VEGF antibodies to evaluate VEGF expression. The results showed that there were significant increase of VEGF and new bone formation in the experimental groups when compared with the controls. The highest amount of VEGF expression was found before the highest amount of bone formation has reached.

Rabie et al (2002) [31] studied the factors regulating condylar growth that has not been identified before such as Sox9 transcription factor and vascular endothelial growth factor (VEGF). Samples of 115 Sprague – Dawley rats, 35 day old were used in this study. They were killed at 38, 42, 49, 59 and 65 days of age. Tissue sections were made and the expressions of these factors were identified on protein level by using immuno staining. It was found that the Condylar growth involves a sequence of stages, defined by molecules that are intrinsically synthesized by cells in the condyles and also the expression of VEGF by chondrocytes reaches its maximum level of preceding bone formation.

Rabie et al (2002) [32] investigated the expression of VEGF (Vascular Endothelial Growth Factor) and new bone formation in the condyle during forward mandibular positioning. One hundred and fifty samples of 35 day old female Sprague-Dawley rats were taken in this study. The rats were divided into 10 groups. Each group consisted of 10 rats in which five were fitted with bite jumping appliances and remaining five untreated. One group was sacrificed on each of experimental day 3, 7, 14, 21, 30, 33, 37, 44, 51 and 60 respectively. Sagittal sections were cut and stained with VEGF antibodies. Each section was quantitatively analyzed using computer software. The result was found to have a significant increase in both vascularization and

mandibular bone growth upon forward mandibular positioning. Highest amount of both the VEGF and mandibular bone growth was seen in the posterior region of the condyle.

Shoshana Yakar et al (2002) [41] studied whether the circulating level of IGF-1 directly has a role in regulating bone growth and density. IGF are bound to a family of six structural IGF binding proteins (IGFBP1 through IGFBP6). Majority of IGF (70 – 80%) exists in a 150-kDa complex comprising of one IGF molecule, IGFBP-3 and the acid labile subunit (ALS) in serum. Liver IGF-1 deficient (LID) mice and ALS knockout (ALSKO) mice had relatively normal growth. Whereas double gene disrupted mice generated by crossing LID +ALSKO mice exhibited a reduction in linear growth and serum IGF-1 level. The proximal growth plates of tibia, bone mineral density, periosteal circumference and cortical thickness were reduced in these mice. Treatment with IGF-1 for 4 weeks restored the normal growth and development. Thus the circulating IGF-1 have a role in directly regulates normal bone growth and density.

Suzana Brozovic et al (2002) [45] investigated the association of vascular endothelial growth factor (VEGF) with various stages of recurrent aphthous ulceration (RAU). Samples of saliva were collected from 27 healthy controls age and sex matched and 30 patients with minor and major RAU grouped into three stages. VEGF levels were determined by enzyme linked immuno assay using Human VEGF ELISA Kit. The results were expressed in pg of VEGF/ml. VEGF levels decreased in patients with RAU when compared to healthy controls.

Tiziano Baccetti et al (2002) [51] had done a study to provide an improved version of cervical vertebral maturation (CVM) method for the detection of peak mandibular growth. Samples comprised of 30 orthodontically untreated subjects with not less than six consecutive cephalograms were included in the study from University of Michigan Elementary and Secondary school growth study. The maximum increase in Co-Gn between two consecutive

cephalograms was used to define peak in mandibular growth at puberty. The analysis consisted of both visual and cephalometric appraisals of morphological characteristics of 3 cervical vertebrae. The new CVM method comprises of five maturational stages with the peak in mandibular growth occurring between CVMS II and CVMS III . This method is particularly useful when skeletal maturity has to be appraised on a single cephalogram and second through fourth cervical vertebrae is visible even when a protective radiation collar is worn.

Georg Brabant et al (2003) [10] analyzed the level of serum insulin like growth factor-1 using automated chemiluminescence immunoassay method. Totally 3961 healthy subject (2,201 Males, 1760 Females) aged from 1 month to 88 Years were selected. Samples were collected and processed for the separation of serum and stored frozen at -20° or -70° C until analysis. The result showed a age dependency of serum IGF-1 level. At age less than 20, higher SIGF-1 level were seen in girl's with an estimated peak values of 410 μg/L at age 14 and an estimated peak value of 382 μg/L at age 16 in boys. Thereafter, a rapid decrease was seen to approximately 25 years of age followed by a slow age dependent decrease.

Kouichi Itoh et al (2003) [18] studied the effects of local administration of insulin like growth factor-1 on mandibular condylar growth in rats. Thirty, 3 week old and 20, 12 week old male Sprague Dawley rats were used in the study. They were divided into control and experimental groups. Bacteria derived human recombinant IGF-1 was dissolved in saline to a concentration of 20 μg/ml. The drug was injected into articular capsule on the third, fifth and seventh day. After administration the rats were killed and histomorphometric measurements were made from the prepared sections. The study concluded that local administration of IGF-1 may make it possible for the mandibular condyle to continue grow even after normal growth is complete. However it is difficult to further accelerate mandibular condyle growth during the period of growth.

Lily Shum et al (2004) [21] assessed the amount of vascular endothelial growth factor (VEGF) expression and related new bone formation in posterior glenoid fossa during stepwise mandibular advancement. A total of 250 female Sprague – Dawley rats, 35 day old were randomly divided into 2 groups, each including 5 control and 20 experimental rats. In each group, 10 experimental rats were fitted with functional appliances with single step advancement of 3.5mm. Another 10 were fitted with stepwise advancement. The rats in experimental groups were killed on days 3, 7, 14, 21, 30 , 33, 37, 44, 51 and 60 matched with their controls. Sections were cut through glenoid fossa sagittally and stained with anti - VEGF antibody. The results showed than an increase of VEGF is spatially and temporally related to amount of new bone formation in the posterior glenoid fossa.

Suzuki S et al (2004) [46] studied the effects of local injection of insulin like growth factor -1 (IGF-1) on mandibular condyle growth in mature rats . Samples consisted of sixteen, 15 week old male rats. In experimental group IGF-1 was injected into articular capsules of condyle at a concentration of 50 μg/ml, while rats in the control group were injected with equal volume of saline. Injections were given three times at 7 days intervals and rats were killed on 7th day after last injection. There was found to be significant increase in thickness of cartilaginous layer in IGF-1 treated condyle. The amount of endochondral bone growth was also found to be greater than in the control group. Thus local injection of IGF-1 seemed to reactivate the process of endochondral bone formation.

H.Werner et al (2004) [54] reviewed about the role of insulin like growth factors (IGFs) in physiological and pathological oral processes. It had been postulated that IGF-1 affects growth via endocrine and paracrine/autocrine loops. In addition to its role in normal growth, the IGF is involved in several pathological oral processes. IGFs also regulate various aspects of salivary gland homeostasis.

Agha Hosseini et al (2005) [3] investigated the levels of vascular endothelial growth factor (VEGF) in unstimulated whole saliva of patients with recurrent aphthous stomatitis (RAS). Samples of 31 patients with RAS were selected and their saliva was collected by spitting method. The samples were immediately stored at -20° C until analysis. Salivary levels of VEGF were then determined by Sandwich ELISA using Human VEGF ELISA kit. Results were expressed in pg of VEGF/ml of saliva.

Tiziano Baccetti et al (2005) [52] had done a study using cephalometric files of the University of Michigan Elementary and Secondary School Growth study. The total samples comprised of n=706 subjects. Those subjects with less than six consecutive annual cephalometric observations were excluded from the study. Totally 30 subjects (18 males & 12 Females) were included. The maximum increase in Co-Gn between two consecutive annual cephalograms were used to define peak in mandibular growth at puberty. Two earlier and two later consecutive cephalograms has to be available for each subject. Both visual and cephalometric appraisals of morphological characteristics of cervical vertebrae were analyzed. The study concluded that the use of Cervical Vertebral Maturation method enables the clinician to identify optimal timing for the treatment of dentoskeletal disharmonies occurring in all three planes of space.

Carlos Flores-Mir et al (2006) [6] assessed the correlation between Fishman maturation prediction method (FMP) and cervical vertebral maturation(CVM) method for determining skeletal maturation. Hand wrist and lateral cephalograms were taken from 79 subjects (52 females and 27 males). Hand wrist radiographs were analyzed using FMP method and cervical vertebrae using CVM method for determining skeletal maturation stage. The correlation values was found to be moderately high between FMP and CVM. So either of the methods can be used indistinctively for research purposes.

Paola Gandini et al (2006) [28] compared skeletal maturation using Hand Wrist bone analysis and cervical vertebral analysis. Radiographs of 30 patients (14 males and 16 females, 7-8 years of age) were examined. The hand wrist bone was evaluated by Bjork index and cervical vertebral analysis by cervical vertebral maturation stage (CVMS) method. The results showed correlation of CVMS I with Bjork stages 1&3, CVMS II with Bjork stage 4, CVMS III with Bjork stage 5, CVMS IV with Bjork stages 6&7 and CVMS V with Bjork stages 8&9. Thus vertebral analysis is as valid as hand wrist bone analysis with main advantage of reducing the radiation exposure of growing subjects.

Tancan Uysal et al (2006) [49] investigated the relationship between chronologic age and skeletal maturation using cervical vertebrae and hand wrist radiographs. The samples consisted of lateral cephalometric radiographs and hand wrist radiographs of 503 subjects (213 male, 290 female, ages 5.3 – 24.1 years). Cervical vertebral maturation was evaluated by Hassel and Farman method and hand wrist radiograph by Bjork and Grave method. A high correlation coefficient was found between chronologic age and cervical vertebrae. Also a high correlation coefficient was found between hand wrist skeletal maturation and vertebral maturation. These findings indicate that the cervical vertebrae stages can be used as a skeletal maturity indicator of the pubertal growth spurt with a degree of confidence similar to other indicators.

Antonelli G et al (2007) [2] evaluated the level of free IGF-1 in saliva specimen between young athletes and sedentary females. Saliva samples were collected from well-trained athletes and control group of 14 young sedentary females. Within 10-15 min of collection, samples were centrifuged at 2000 rpm for 10 min to remove particulate material and the clear supernatant was immediately stored in vials at -80 deg C. Samples were assayed by enzyme-linked immunosorbent assay (ELISA) method. There was to found to be a decreased level of free IGF-1

in athletes when compared to sedentary females. This decrease could be related to greater tissue requirement by active muscles.

Silvia Chiappin et al (2007) [39] reviewed about the saliva specimen as a new laboratory tool for diagnostic and various investigation purposes. The various compounds like inorganic, organic non protein, protein/polypeptide, hormones and lipid molecules which are present in saliva. Growth hormone (GH) level in human saliva was found to be 100-1000 times less than the physiological serum levels. Unstimulated whole saliva can be collected by a standard method called passive drooling method. In which saliva is allowed to drain off the lip into a sterile plastic vial. After the collection of saliva samples, store the samples at -20° C if the analysis is carried out days to months later. Thus the use of saliva samples in various assays will continue to expand and provides us a new tool for investigating physiologic as well as pathophysiologic status.

Yan Gu et al (2007) [55] evaluated the mandibular dimensional changes and regional remodeling changes occurring during five intervals of circumpubertal growth. The samples consisted of longitudinal cephalometric records of 20 subjects (13 female, 7 male) included in the Mathews and Ware implant study. In which eleven subjects were orthodontically untreated and nine subjects were without treatment in mandible. Cephalograms which were available at each of six consecutive stages of cervical vertebral maturation were analyzed. A peak in mandibular growth was noted at CS3 to CS4 during puberty. Whereas mandibular remodeling and condylar rotation continue to occurs even after the growth spurt.

Benny M. Soegiharto et al (2008) [5] determined and compared skeletal maturation in Indonesian and white children using hand wrist and cervical vertebrae maturation methods. The study included 2167 patients with hand wrist and lateral cephalometric radiographs. In that 648 were Indonesian boys, 393 white boys (age range of 10-17 years), 774 Indonesian girls and 442 white

girls (age range of 8-15 years). The skeletal maturation index (SMI) was used to evaluate the stages of skeletal maturity from hand wrist radiograph and cervical vertebrae maturation from lateral cephalogram. Results confirmed that variation exists between chronologic age and each stage of skeletal maturity between the two ethnic groups. On average the white children attained each stage of SMI and CVM about 0.5 to 1 year earlier than their Indonesian peers and were more obvious in boys. So there are differences between the timing of skeletal maturity between sexes and the ethnic groups.

Hessa Abdulla Alkhal et al (2008) [15] investigated the correlation between chrological age, cervical vertebral maturation (CVM) and Fishman's hand wrist skeletal maturity indicators. Four hundred subjects with hand wrist and lateral cephalogram were randomly selected and analyzed. The female subjects were between 12 and 17 years of age and male subjects between 12 and 17 years of age, all were within in the circumpubertal period. The hand wrist was assessed using the method developed by Fishman and CVM was assessed by the method given by Bacetti and coworkers. When these two methods were correlated with chronological age. The findings indicated that CVM is a valid indicator of skeletal growth during circumpubertal period and has high correlation with hand wrist maturation (HWM). However, there was found to be a low correlation between chronological and both CVM & HWM showing that chronological age was not a suitable method to identify skeletal maturity.

Juan Dai et al (2008) [17] investigated whether the introduction of specific vascular growth inducting genes would favorably affect the mandibular condylar growth in Sprague – Dawley (SD) rats over an experimental period. Ninety 35 days old female SD rats were randomly divided into 3 groups. Each group received any of the injections of recombinant adeno associated virus mediated vascular endothelial growth factor (rAAV – VEGF), rAAV mediated enhanced green fluorescence protein (rAAV-eGFP) or phosphate buffered saline (PBS) into both mandibular

condyles. Rats of each group were sacrificed on the following experimental days of 7, 14, 21, 30 and 60. Left halves of the mandible was isolated and digital pictures were obtained in a standardized manner. The length of condylar process as well as the mandibular length were significantly increased on 30th day and continued to increase until the end of experiment. Thus gene therapy with VEGF stimulates condylar growth and provides the basis to regulate mandibular condylar growth.

Mohamed Masoud et al (2008) [25] assessed the skeletal maturity by using blood spot insulin like growth factor-1 (IGF-1). The study included 83 patients (44 female, 39 male) between age of 5 and 25 years. Lateral cephalogram and blood sample were collected on the same day for each patient. The samples were assayed by radioimmunoassay and correlated with the stages of cervical vertebrae. The results showed that blood spot IGF-1 level is low in prepubertal CVM stage, then rises sharply to its peak in late puberty and decline in post puberty stages. Thus IGF-1 level can be accurately used to determine the timing and intensity of a patient's growth spurt.

Wai Yip Lei et al (2008) [53] studied the core binding factor alfa1 (cbfa1) and vascular endothelial growth factor (VEGF) expressions in the spheno-occipital synchondrosis in vitro with and without tensile stress. Sixty one day old male BALB/c mice were randomly divided into experimental and control groups. Each group was subdivided into five subgroups of different time frames 6, 24, 48, 72 and 168 hours. Each subgroup consisted of six mice. Animals were sacrificed and cranical base synchondrosis were aseptically removed and mechanical stress was applied using helical springs and incubated as organ culture. Then the tissue sections were subjected to immunohistochemical staining for quantitative analysis of growth factors. Mechanical stress applied to spheno-occipital synchondrosis upregulates alfa1(cbfa1) and VEGF. So increased levels of both the factors could play a role in endochondral bone growth.

Antonelli et al (2009) [1] investigated the effects of physical exercise on salivary free insulin like growth factor-1 and plasma free IGF-1. Eighteen males at an age range of 19±1 year were included in the study. Saliva and blood samples were collected before and at the end of physical exercise. Both the saliva and blood samples were assayed by ELISA method. The results showed that sIGF-1 was significantly increased at the end of physical exercise whereas plasma free IGF-1 concentrations did not demonstrate any difference.

Mohamed I. Masoud et al (2009) [26] predicted the relationship between blood spot insulin like growth factor 1 levels and hand wrist assessment of skeletal maturity. Eighty four subjects (45 female, 39 male) between the ages of 5 and 25 were included in the study. From each subjects hand wrist radiograph and a blood spot sample were collected on the same day. The results showed that IGF-1 levels were significantly higher during pubertal stage than at the prepubertal or postpubertal stage when compared with hand wrist maturation stages.

Ricky W.K Wong et al (2009) [34] evaluated the validity of cervical vertebral maturation (CVM) method as an indicator of skeletal age in the circumpubertal period by correlating with hand wrist method (HWM). Hand wrist and lateral cephalometric radiographs of 400 subjects were randomly selected at age ranging from 10 to 15 years for girls and 12 to 17 years for boys. They were within the circumpubertal period. Skeletal age of each subject was assessed according to CVM method and HWM. All Patients in cervical vertebral stage 3 correspond to stages of MP3-FG or MP3-G in HWM. Thus CVM method is a valid indicator of skeletal growth during circumpubertal period, providing the optimum time period for growth modification procedure.

Tahwinder Upile et al (2009) [47] had done a study to evaluate and compare the salivary VEGF level in healthy population and in oral cancer patients. The samples included twenty one participants (12 male and 9 females) of whom 14 were healthy subjects and 7 oral cancer

patients. Whole saliva samples were taken from each subject and assayed by chemiluminescent VEGF immunoassay is a 5.5 hour solid phase ELISA designed to measure VEGF level in saliva. The result indicated the presence of VEGF in normal healthy population is (mean = 231.609 pg/ml) and found to be increased in the cancer patients. Thus saliva contains biologically active proteins, growth factors and cytokines.

Lili Chen et al (2010) [20] investigated the relative growth rate (RGR) of maxilla and mandible according to quantitative cervical vertebral maturation (QCVM) of adolescents. The samples consisted of 87 adolescents (32 boys and 55 girls) age ranging from 8 to 18 years. Lateral cephalograms and hand wrist films were taken sequentially once a year for 6 consecutive years. The growth magnitude (GM) and relative growth rate (RGR) of maxilla and mandible were measured and analyzed. GM and RGR were not always consistent because each subjects had different periods of growth between various QCVM stages. GM was not as reliable as that of RGR. The greatest RGR of mandibular length and height was in QCVM stage II. So its the best intervention period and can be used as a reference in deciding orthodontic treatment or an orthognathic surgery.

Denise Boechat Leite et al (2011) [8] analyzed insulin like growth factor-1 (IGF-1) in serum using an automated chemiluminescence immunoassay system. Total samples consisted of 484 healthy subjects (251 men, 233 women) age ranging from 18 – 70 years. The blood samples were collected and serum was separated and stored frozen at -20° C until analysis . The samples were analyzed by an automated immuno chemiluminometric assay (ICMA). The results demonstrated that in adulthood, a slow age dependent decrease in IGF-1 was seen and also there exists a significant difference between the genders. IGF-1 in women was found to be significantly higher than in men.

Gina Ball et al (2011) [11] studied the relationship between cervical vertebral maturation and mandibular growth. The samples consisted of 90 boys from the Burlington growth center at age ranging from 9 to 18 years. For determining the incremental growth of mandible the lengths were measured from articulare to gnathion. Cervical vertebral maturation stages were assessed by 6 stage method. Advanced, average and delayed maturation groups were established. Minimum velocity of prepubertal mandibular growth occurred during cervical stage 1 and peak mandibular growth occurred most frequently during stage 4 in all 3 maturation groups with a statistical difference between each group. Progression from cervical stages 1 through 6 does not occur annually, time span in each stage varies depending on maturation group . This cervical vertebral maturation stages cannot accurate identifies the mandibular prepubertal growth. So it better to use with other methods when considering for dentofacial orthopedic treatment or orthognathic surgery.

Mohamed I. Masoud et al (2012) [27] studied the relationship between blood spot insulin like growth factor (IGF-1) and changes in mandibular growth. Twenty five patients (12 female , 13 male) were longitudinally evaluated for annual IGF-1 level, cervical stage and mandibular length. Patients follow up periods ranged between 1 and 5 years. The samples were assayed by radioimmunoassay and the mandibular length was measured from condylion to gnathion. The cervical vertebrae were staged into 6 stages as described by Baccetti T. The group with ascending IGF-1 levels had significantly more mandibular growth than did the group with descending IGF-1 levels . Results suggested that blood spot IGF-1 is a promising tool for predicting the timing and intensity of mandibular growth spurt.

Ramy Abdul Rahman Ishaq (2012) [33] evaluated the applicability of insulin like growth factor-1 (IGF-1) blood level as a maturation indicator by correlating it to cervical vertebral maturation (CVM) method. Samples consisted of 120 subjects, equally divided into 60 males (ages 10-18

years) and 60 females (ages 8-16 years). Lateral cephalogram and blood samples were taken from each subject. Blood samples were analyzed with enzyme linked immunosorbent assay (ELISA) technique. The IGF-1 mean value at each cervical vertebral maturation stage was statistically different. Highest mean values were observed in stages 4 & 5 in males and stage 3 in females. Thus IGF-1 serum level is a reliable skeletal maturation indicator that could be applied in orthodontic diagnosis.

Rodrigo Cesar Santiago et al (2012) [35] reviewed the reliability of cervical vertebrae maturation (CVM) staging to predict the pubertal growth spurt. The selection criteria for considering studies in this systematic review were cross-sectional or longitudinal descriptive studies that evaluated qualitatively or quantitatively on established CVM method on lateral cephalometric radiographs to determine skeletal maturation and those studies that used hand wrist radiographs as the standard method for comparison with the CVM method. Twenty three were finally selected and scored. This systematic review had shown that CVM method can be used as a radiographic assessment for determining skeletal maturation during orthopedic treatment.

Yan Qi Yang et al (2012) [57] reviewed the role of vascular endothelial growth factor (VEGF) in ossification. Studies have shown that VEGF stimulates cell proliferation by upto 70% and directly promotes differentiation of primary human osteoblasts in vitro by increasing nodule formation and alkaline phosphatase activity in a dose dependent manner. Also reported that VEGF is expressed at low level in the beginning of osteoblast differentiation and its expression increased strongly during the period of mineralization. VEGF is able to promote ossification by either inducing neovascularization or by directly affecting the bone cells.

Martin Bidlingmaier et al (2013) [23] had done a study to measure the level of IGF-1 from birth to senescence using a new automated chemiluminescence IGF-1 immunoassay. It is a

multicenter study with samples from 12 cohorts from the United States, Canada and Europe including 15,014 subjects (6697 males and 8317 females) age ranging from 0-94 years. The result of the study concluded that the IGF-1 secretion declined immediately after birth followed by an increase until pubertal peak (at 15 years of age) and later in life, the values decreased continuously.

Sapna Jain et al (2013) [37] studied the association between serum insulin like growth factor – 1 (IGF-1) levels with cervical maturation stages (CS) 3, 4 and 5 on lateral cephalogram. The total samples consisted of 45 males at skeletal maturation stage CS-3, CS-4 and CS-5. Blood samples were taken and serum was separated and stored at 2 to 8 degree Celsius until analysis. The samples were assayed by chemiluminescence immunoassay a fully automated method for determination of IGF-1 level. The results showed that there is a highly significant differences between all cervical stages. Serum IGF-1 levels showed good correlation with skeletal age in males 53.3% at CS-3, 66.7% at CS-4 and 6.7% at CS-5. Thus serum IGF-1 levels can be used as an additional diagnostic tool to optimize orthodontic treatment timing.

Subash Nayak et al (2014) [42] had done a study to find the relationship between salivary insulin like growth factor-1 (IGF-1) and quantitative cervical vertebral maturational stages (QCVM) of skeletal maturity. The study included 24 female and 21 male between the ages of 7 and 23 years. QCVM staging was used to divide the subjects in to four groups. Parotid saliva samples were collected using Lashley cup for about 30 min and the samples were assayed by IRMA (Immunoradiometric assay) method. The results concluded that salivary IGF1 levels and its secretion rate gradually increased to a peak in the high velocity QVCM stage and decreased thereafter. Thus salivary IGF-1 can be used as a marker for predicting residual mandibular growth.

Yangi Yang et al (2014) [56] reviewed about the growth factors involved in mandibular condylar growth. It has been identified that several factors including Sox9, type II collagen, PTHrP, Ihh, FGFR, type X collagen, VEGF, Cbfal/Runx2 and OPG/RANKL play a pivotal role in growth and development of mandibular condyle. The mandibular condylar cartilage is a secondary cartilage influenced by both growth hormones and mechanical forces. Upon forward mandibular positioning by bite jumping appliance, there was found to be a significant uprelation of VEGF. In single step advancement VEGF expression was less when compared with stepwise advancement. Thus VEGF plays a vital role in regulating endochondral ossification of condyle by controlling neovascularization.

Paszynska E et al (2015) [29] had done a study to measure the salivary and serum insulin like growth factor-1 (IGF-1) concentration of patients with anorexia nervosa (AN) in comparison to healthy individuals. Total samples of 121 patients and 77 individuals were selected. Blood and salivary samples were derived and analyzed by enzyme liked immunoassay (ELISA) suitable for measuring free IGF-1. There was found to be a significant reduction in free IGF-1 levels in saliva and serum. Thus salivary and serum IGF-1 analysis also appear to be a reliable biochemical indicator of malnutrition.

Shreya Gupta et al (2015) [38] had done a cross sectional study to assess serum insulin like growth factor-1 (IGF-1) level at various cervical vertebral maturation (CVM) stages. The study included 60 subjects (30 females and 30 males) in age ranging from 8-23 years. All subjects serum IGF-1 levels were estimated by means of chemiluminescence immunoassay (CLIA). CVM was assessed on lateral cephalograms using the method given by Baccetti T. It was found that peak value of serum IGF-1 was observed in cervical stage CS3 in females & CS4 in males. The greatest mean serum IGF-1 for female is 397ng/ml which were slightly higher than in males

394.8 ng/ml. In this study males and females showed a slight difference in IGF-1 level at different cervical stages.

Suchita M T et al (2015) [43] reviewed about the various methods which are currently used as skeletal maturity indicators. The timing of the growth spurt can be assessed by chronological age, skeletal age, physiologic age and dental age. Other methods are radiological and biochemical methods. In radiological method radiographs like hand wrist, lateral cephalogram and orthopantomogram are used. Whereas recently biochemical methods were used for identification of growth factors like IGF-1, alkaline phosphosphate (ALP) and creatinine in saliva and serum IGF-1 is a circulating GH factor, the level of which correlates with sexual maturity. It is measurable in serum, urine and saliva. Various studies have shown a positive correlation between IGF-1 levels with cervical skeletal maturity from the prepubertal to the late pubertal stage.

Sushma Dhiman et al (2015) [44] reviewed about the various methods for the assessment of skeletal maturity in orthodontics. These include body height, body weight, sexual maturation, frontal sinus, chronological age, biological age or physiological age, hand wrist radiograph, cervical vertebrae, dental eruption, dental calcification stages and various biomarkers. Biomarkers are the agents that are involved directly in both growth and remodeling. These biomarker avoids radiation exposure and recently many researchers are being done to explore the role of biomarkers for determination of skeletal maturation.

Hechang Huang et al (2016) [14] studied the effects of vascular endothelial growth factor (VEGF) on osteoblasts and osteoclasts. Preosteoblastic MC3T3-E1 cells were treated with VEGF-A165. Also preosteoclastic RAW264.7 cells were treated with VEGF with or without the receptor activator of nuclear factor kappa–B ligand (RANKL). It was found that VEGF significantly

induced MC373-E1 osteoblast cell proliferation, migration and did not directly induce osteoclastogenesis but by significantly increasing the RANKL/OPG ratio from MC3Y3-E1 cultures.

Taye J Lasisi et al (2016) [50] investigated the effect of acute exercise on salivary flow rate and salivary free insulin like growth Factor-1 (IGF-1). Saliva samples were collected before and immediately after exercise from 22 males with a mean age of 20-46 years. The level of free IGF-1 in saliva was determined by enzyme like immunoassay (ELISA) kits following manufacturer instructions. The result concluded that the salivary flow rate is reduced and there is no change in the level of salivary free IGF-1.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was carried out in 90 patients with no previous history of orthodontic treatment before with their age ranging from 6 to 20 years. The patients were selected from those who reported to the department for seeking orthodontic treatment. Before their entry into the study all the subjects were explained about the study. An informed consent form duly signed by every subject was obtained and ethical clearance for the study had been obtained from the Institutional ethical committee.

A routine diagnostic lateral cephalogram (ORTHOPHOS XG 3D, Sirona) taken prior to orthodontic treatment in 90 individuals were used to assess the skeletal maturation stage.

Grouping of samples

The 90 subjects were divided into 3 groups namely group I, II and III comprising of 30 individuals in each group based upon the pubertal growth status determined by Hassel and Farman CVMI staging.

Group I – Individuals in the pre-pubertal growth stage (CS1 & CS2)

Group II – Individuals in the pubertal growth stage (CS3 & CS4)

Group III – Individuals in the post pubertal stage (CS5 & CS6)

Materials used in the study

1) Tarsons 15 ml centrifuge tubes for collecting samples
2) Eppendorf tubes for storage of samples
3) Micropipette and disposable micropipette tips
4) Laboratory centrifuge

5) Laboratory deep freezer
6) IGF1 and VEGF kits
7) 96 well micro-titer plates
8) Microplate reader

Inclusion criteria

1) Age ranging from 6 to 20 years
2) No previous history of orthodontic treatment
3) No history of any systemic disease, hormonal imbalance and drug intake
4) Good oral hygiene

Exclusion criteria

1) Craniofacial anomalies
2) Growth retardation
3) Any oral ulcers
4) Xerostomia

Determination of CVMI

Hassel and Farman's method of CVM index were used for determining the cervical vertebral maturation stages. It consists of 6 stages of skeletal maturation from CS1 to CS6. The inferior vertebral borders were flat when immature and they were concave when mature. The concavities occurs sequentially from CS2 to CS4 as skeleton matures. Also the shapes of the vertebral bodies change from wedge shaped, to rectangular, followed by square shape. In addition they increase in height as skeleton matures.

Stage 1 (Initiation) : Inferior borders of C2, C3 and C4 were flat and wedge shaped. The superior border tapers from posterior to anterior.

Stage 2 (Acceleration) : Concavities were developing in the inferior borders of C2 and C3. The inferior border of C4 was flat and vertebral bodies of C3 & C4 were rectangular in shape.

Stage 3 (Transition) : Distinct concavities were seen in the inferior borders of C2 and C3. Whereas concavity is started developing in inferior border of C4. Vertebral bodies of C3 and C4 were rectangular in shape.

Stage 4 (Deceleration) : Distinct concavities were seen in the inferior borders of C2, C3 and C4. Vertebral bodies of C3 and C4 were becoming squarer in shape.

Stage 5 (Maturation) : More accentuated concavities were seen in the inferior borders of C2, C3 and C4. Vertebral bodies of C3 and C4 were nearly square in shape.

Stage 6 (Completion) : Deep concavities were seen in the inferior borders of C2, C3 and C4. Vertebral bodies of C3 and C4 were square and were more greater in vertical dimension.

Patient preparation for saliva collection

Thorough oral prophylaxis was carried out before sample collection in all the participants who were included in the study. Mouth washes were not used by the subjects prior to sample collection. If so the subjects were asked to rinse with water for more than two times. Also subjects were asked not to drink or eat two hours prior to sample collection.

Collection of the saliva sample

Unstimulated whole saliva sample was collected in this study. Saliva sample collection was performed according to Brozovic et al in which all the subjects were asked to retain mixed saliva in their mouth for 5 min without swallowing and then to expectorate into a sterile 15ml Tarzons centrifuge tube. Then samples were centrifuged for 10 min at 1500g at room temperature and stored at -20 deg C until analysis.

Estimation of IGF-1 and VEGF

Before the assay thawed saliva was again centrifuged and the clear supernatant sample were used for the assay. Quantitative determination of IGF-1 was done by sandwich chemiluminescence immunoassay. A monoclonal antibody is used for coating magnetic particles (solid-phase) and another monoclonal antibody is linked to an isoluminol derivative (isoluminol – antibody conjugate). During the incubation, IGF-1 present in calibrators, samples or controls binds to the solid phase as well as to the conjugate. After the incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IGF-1 concentration present in calibrators, samples and controls.

Remaining samples were used for determination of VEGF by sandwich Enzyme Linked Immunosorbent Assay (ELISA) method using human VEGF ELISA Kit. Add 50 µL of the incubation buffer to all 96 wells expect chromogen blanks. Then add 100 µL of standards to the appropriate microtiter wells. For samples and controls, add 50 µL of Standard Diluent Buffer to each appropriate well followed by 50 µL of sample or controls. Cover the plate with plate cover and incubate for 2 hours at room temperature. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer. Add 100 µL Hu VEGF Biotin Conjugate solution into each well except chromogen blanks. Cover the plate with plate cover and incubate for 1 hour at room temperature. Thoroughly aspirate the solution and wash wells 4 times with diluted Wash Buffer. Add 100 µL Streptavidin – HRP into each well except the chromogen blanks. Cover the plate with plate cover and incubate for 30 minutes at room temperature. Then thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue. Cover the plate with plate cover and incubate for 30 minutes at room temperature in the dark. Add 100 µL Stop Solution to each well and tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the absorbance at 450 nm within 2 hours after adding the Stop Solution. Use curve fitting software to generate the standard curve. Concentrations for unknown samples and control were read from the standard curve.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

The statistical analysis was performed with SPSS windows software (version 21, IBM USA). The significance level was set at $P \leq 0.05$. The Kruskal – Wallis test and ANOVA was used to compare the mean IGF-1 and VEGF values in different cervical vertebral maturation stages. The Mann – Whitney U test was used to compare the mean IGF-1 and VEGF values between the genders in each cervical vertebral maturation stages. For multiple comparisons of samples among groups Post Hoc test – Bonferroni were used.

Kruskal – Wallis test

$$H = 12\frac{12}{n(n+1)}\sum_{i-1}^{k}\frac{Ri^2}{} - 3(n+1)$$

Where

H = Kruskal-Wallis Test statistic

N = total number of observations in all samples

T_i = Sum of the ranks assigned

ANOVA analysis

$$\text{ANOVA} = \frac{\text{BMS} - \text{WMS}}{\text{BMS} + (\text{n} - 1)\text{WMS}}$$

Where

BMS = between subjects mean sum of squares

WMS = within subjects mean sum of squares

n = Number of measurements

P value of less than 0.05 was considered to be statistically significant.

Mann – Whitney U test

$$U = n_1 n_2 + \frac{n_2\ (n_2\ + 1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Where

U=Mann-Whitney U test

N1 = sample size one

N2= Sample size two

Ri = Rank of the sample size.

RESULTS

RESULTS

The results of the study are based on the levels of insulin like growth factor (IGF-1) and vascular endothelial growth factor (VEGF) at various stages of cervical vertebral maturation (CVM).

Table 1 shows the total number of samples in pre-pubertal stage (CS1 and CS2) with a mean age of 9.3 yrs. Also it expresses the mean level of VEGF (189.73±200.63 pg/ml) and IGF-1 (4.40±0.17 ng/ml). Table 2 shows the total number of samples in peak-pubertal stage (CS3 and CS4) with a mean age of 12.9 yrs. The VEGF level decreases to 97.31±141.99 pg/ml but the IGF-1 level was found to be increased to 6.15±1.04 ng/ml. Table 3 shows the total number of samples in post-pubertal stage (CS5 and CS6) with a mean age of 17.4 yrs. The level of VEGF decreases steadily to 58.05±87.85 pg/ml and the IGF-1 level also decreases to 4.43±0.29 ng/ml during this stage.

Table 4 shows the total number of female samples in all the 3 groups. The mean level of VEGF (97.25±151.65 pg/ml) and IGF-1 (4.9±1.03 ng/ml) in females were measured. Table 5 shows the total number of male samples taken into the study with mean VEGF level of 127.56±163.32 pg/ml and IGF-1 level of 5.11±1.05 ng/ml.

Table 6 shows the total number of female samples in pre-pubertal stage (CS1 & CS2) with a mean age of 9.1 yrs. The mean level of VEGF in female was measured to be 199.29±242 pg/ml and IGF-1 was found to be 4.42±0.15 ng/ml.

Table 7 shows the total number of female samples in peak-pubertal stage (CS3 & CS4) with a mean age of 12.11 yrs. The measured level of VEGF in female during this stage decreases to 87.49±74.84 pg/ml and the IGF-1 level increases to 6.47±1.07 ng/ml.

Table 8 shows the total number of female samples in post-pubertal stage (CS5 & CS6) with a mean age of 17 yrs. In this stage the VEGF level decreases steadily to 50.62±89.90 pg/ml and the IGF-1 level also decreases to 4.42±0.33 ng/ml.

Table 9 shows the total number of male samples in pre-pubertal stage (CS1 & CS2) with a mean age of 9.5 yrs. The mean VEGF level measured in males was (184.43±181.24 pg/ml) and the IGF-1 level was (4.39±0.18 ng/ml).

Table 10 shows the total number of male samples in peak-pubertal stage (CS3 & CS4) with a mean age of 13.2 yrs. Similarly to females the mean level of VEGF decreases to 101.53±164.108 pg/ml and IGF-1 level increases to 6.02±1.02 ng/ml during this stage.

Table 11 shows the total number of male samples in post-pubertal stage (CS5 & CS6) with a mean age of 18.4 yrs. The mean level of VEGF decreases steadily to 74.57±85.85 pg/ml and IGF-1 level also decreases to 4.44±0.19 ng/ml.

Table 12 shows the comparison of VEGF and IGF-1 across the CVM stages. There is a significant difference in VEGF (P=.004) and IGF-1 level (P=.000).

Table 13 shows the multiple comparisons and mean difference of VEGF and IGF-1 across the CVM stages. The data demonstrated a significant difference in VEGF level when group 1 and group 3 were compared (P=.004). In IGF-1 there is a highly significant difference when group 2 is compared with group 1 and group 3 (P=.000).

Table 14 shows the comparison of females across the CVM stages. There is a significant difference in VEGF (P=.035) and IGF-1 level(P=.000)

Table 15 shows the multiple comparisons and mean difference of females across the CVM stages. There is a significant difference in VEGF level when group 1 and group 3 were compared (*P*=.031). In IGF-1 level also there is a highly significant difference when group 2 is compared with group 1 and group 3 (*P*=.000).

Table 16 shows the comparison of males across the CVM stages. There is no significant difference in VEGF level (*P*=.161) but in IGF-1 level there is a highly significant difference (*P*=.000).

Table 17 shows the multiple comparisons and mean difference of males across the CVM stages. There is no significant difference in VEGF level was found when the three groups were compared. In IGF-1 level there is a highly significant difference when group 2 was compared with group 1 and group 3 (*P*=.000).

Table 18 shows the comparison of CVM stages across genders. There is no significant difference in VEGF and IGF-1 level when females and males were compared.

Table 19 and Graph 1 shows the mean value of IGF-1 level in both females and males across the CVM stages. In both the genders the IGF-1 level was found to be increased in group 2 (F - 6.47±1.07 ng/ml, M - 6.02±0.19 ng/ml) than in group 1 (F - 4.42±0.15 ng/ml, M - 4.39±0.18 ng/ml) and group 3 (F - 4.42±0.33 ng/ml, M - 4.44±0.19 ng/ml).

Table 20 and Graph 2 shows the mean value of VEGF level in both females and males across the CVM stages. In both the genders the VEGF level decreases steadily from group 1 (F - 199.29±242 pg/ml, M - 184.43±181.24 pg/ml) to group 2 (F- 87.49±74.84 pg/ml, M - 101.53±164.108 pg/ml) and group 3 (F - 50.62±89.90, M – 74.57±85.85).

Table 21 and Graph 3 shows the mean value of VEGF and IGF-1 across the CVM stages irrespective of genders. The VEGF level decreases steadily from group 1 (189.73±200.63 pg/ml) to group 2 (97.3±141.99 pg/ml) and group 3 (6.15±1.04 pg/ml). whereas IGF-1 level was found to be greater in group 2 (6.15±1.04 ng/ml) than in group 1 (4.40±0.17 ng/ml) and group 3 (4.43±0.29 ng/ml).

WORKING & RESULT TABLES

TABLE 1: TOTAL NUMBER OF SAMPLES IN PRE PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	11/F	Pre pubertal	1	32.568	4.41
2	10/F	Pre pubertal	1	128.965	4.25
3	11/F	Pre pubertal	1	26.631	4.39
4	10/M	Pre pubertal	1	250.798	4.14
5	10/M	Pre pubertal	1	11.278	4.19
6	11/M	Pre pubertal	1	46.035	4.44
7	11/M	Pre pubertal	1	15.594	4.48
8	7/F	Pre pubertal	1	36.533	4.67
9	12/M	Pre pubertal	1	41.413	4.6
10	10/M	Pre pubertal	1	93.718	4.69
11	9/F	Pre pubertal	1	32.769	4.36
12	10/M	Pre pubertal	1	25.798	4.46
13	9/M	Pre pubertal	1	261.766	4.53
14	11/M	Pre pubertal	1	250.301	4.43
15	10/M	Pre pubertal	1	244.165	4.24
16	10/F	Pre pubertal	1	371.423	4.39
17	8/F	Pre pubertal	1	648.55	4.49
18	9/M	Pre pubertal	1	629.675	4.54
19	10/M	Pre pubertal	1	90.484	4.2
20	8/F	Pre pubertal	1	573.799	4.32
21	9/M	Pre pubertal	1	475.73	4.63
22	9/F	Pre pubertal	1	123.7	4.69
23	8/M	Pre pubertal	1	133.349	4.28
24	6/M	Pre pubertal	1	379.047	4.17
25	9/M	Pre pubertal	1	32.595	4.43
26	6/M	Pre pubertal	1	6.709	4.56
27	8/F	Pre pubertal	1	17.971	4.27
28	11/M	Pre pubertal	1	331.348	4.11

Total no: 28, Mean age: 9.3 years

Mean±SD of VEGF = 189.73±200.63, Mean±SD of IGF-1 = 4.40±0.17

TABLE 2: TOTAL NUMBER OF SAMPLES IN PEAK PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	14/M	Pubertal	2	734.42	5.14
2	14/M	Pubertal	2	21.425	5.1
3	12/F	Pubertal	2	122.413	6.34
4	13/M	Pubertal	2	7.799	4.94
5	13/M	Pubertal	2	36.112	7.12
6	13/F	Pubertal	2	188.192	5.87
7	13/M	Pubertal	2	45.226	5.32
8	12/F	Pubertal	2	194.173	7.55
9	13/M	Pubertal	2	145.641	5.78
10	11/M	Pubertal	2	30.993	7.13
11	13/M	Pubertal	2	251.571	4.89
12	14/M	Pubertal	2	101.07	7.01
13	12/M	Pubertal	2	-11.43	4.87
14	12/M	Pubertal	2	67.01	7.45
15	14/F	Pubertal	2	30.965	5.67
16	11/F	Pubertal	2	53.076	7.56
17	13/M	Pubertal	2	22.059	7.31
18	11/F	Pubertal	2	-1.833	4.78
19	12/F	Pubertal	2	-3.903	5.56
20	15/M	Pubertal	2	-17.566	5.78
21	13/M	Pubertal	2	33.833	5.45
22	15/M	Pubertal	2	223.533	7.21
23	14/M	Pubertal	2	-4.836	7.28
24	13/M	Pubertal	2	146.149	7.51
25	13/M	Pubertal	2	71.96	5.13
26	13/M	Pubertal	2	175.561	5.29
27	14/M	Pubertal	2	4.005	5.77
28	13/M	Pubertal	2	47.614	4.95
29	11/F	Pubertal	2	80.655	7.25
30	13/F	Pubertal	2	123.706	7.73

Total no: 30, Mean age: 12.9 years

Mean±SD of VEGF: 97.31±141.99, Mean±SD of IGF-1: 6.15±1.04

TABLE 3: TOTAL NUMBER OF SAMPLES IN POST PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	19/F	Post pubertal	3	-23.25	4.47
2	15/F	Post pubertal	3	12.911	4.18
3	16/F	Post pubertal	3	178.87	4.23
4	20/M	Post pubertal	3	7.096	4.32
5	17/F	Post pubertal	3	-9.971	4.01
6	15/F	Post pubertal	3	336.324	5.01
7	19/F	Post pubertal	3	192.34	4.54
8	17/F	Post pubertal	3	7.443	4.34
9	15/F	Post pubertal	3	4.08	4.78
10	20/F	Post pubertal	3	-2.675	4.14
11	16/F	Post pubertal	3	48.377	4.76
12	14/F	Post pubertal	3	8.806	4.71
13	18/M	Post pubertal	3	118.368	4.61
14	18/F	Post pubertal	3	67.216	4.36
15	18/F	Post pubertal	3	-18.536	4.1
16	15/F	Post pubertal	3	104.051	5.21
17	20/F	Post pubertal	3	38.6	4.18
18	19/M	Post pubertal	3	-6.907	4.22
19	20/M	Post pubertal	3	2.138	4.31
20	17/M	Post pubertal	3	92.6	4.44
21	16/M	Post pubertal	3	67.656	4.56
22	17/M	Post pubertal	3	270.356	4.82
23	20/M	Post pubertal	3	87.143	4.51
24	16/F	Post pubertal	3	-3.479	4.75
25	19/F	Post pubertal	3	-7.799	4.34
26	19/F	Post pubertal	3	22.62	4.13
27	15/F	Post pubertal	3	22.717	4.24
28	17/F	Post pubertal	3	33.755	4.07
29	19/M	Post pubertal	3	32.703	4.23

Total no: 29, Mean age: 17.4 years

Mean±SD of VEGF: 58.05±87.85, Mean±SD: 4.43±0.29

TABLE 4: TOTAL NUMBER OF FEMALE SAMPLES

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	11/F	Pre pubertal	1	32.568	4.41
2	10/F	Pre pubertal	1	128.97	4.25
3	11/F	Pre pubertal	1	26.631	4.39
4	7/F	Pre pubertal	1	36.533	4.67
5	9/F	Pre pubertal	1	32.769	4.36
6	10/F	Pre pubertal	1	371.42	4.39
7	8/F	Pre pubertal	1	648.55	4.49
8	8/F	Pre pubertal	1	573.8	4.32
9	9/F	Pre pubertal	1	123.7	4.69
10	8/F	Pre pubertal	1	17.971	4.27
11	12/F	Pubertal	2	122.41	6.34
12	13/F	Pubertal	2	188.19	5.87
13	12/F	Pubertal	2	194.17	7.55
14	14/F	Pubertal	2	30.965	5.67
15	11/F	Pubertal	2	53.076	7.56
16	11/F	Pubertal	2	-1.833	4.78
17	12/F	Pubertal	2	-3.903	5.56
18	11/F	Pubertal	2	80.655	7.25
19	13/F	Pubertal	2	123.71	7.73
20	19/F	Post pubertal	3	-23.25	4.47
21	15/F	Post pubertal	3	12.911	4.18
22	16/F	Post pubertal	3	178.87	4.23
23	17/F	Post pubertal	3	-9.971	4.01
24	15/F	Post pubertal	3	336.32	5.01
25	19/F	Post pubertal	3	192.34	4.54
26	17/F	Post pubertal	3	7.443	4.34
27	15/F	Post pubertal	3	4.08	4.78
28	20/F	Post pubertal	3	-2.675	4.14
29	16/F	Post pubertal	3	48.377	4.76
30	14/F	Post pubertal	3	8.806	4.71
31	18/F	Post pubertal	3	67.216	4.36
32	18/F	Post pubertal	3	-18.536	4.1
33	15/F	Post pubertal	3	104.05	5.21
34	20/F	Post pubertal	3	38.6	4.18
35	16/F	Post pubertal	3	-3.479	4.75
36	19/F	Post pubertal	3	-7.799	4.34

37	19/F	Post pubertal	3	22.62	4.13
38	15/F	Post pubertal	3	22.717	4.24
39	17/F	Post pubertal	3	33.755	4.07

Total no: 39,

Mean±SD of VEGF: 97.25±151.65, Mean±SD of IGF-1: 4.9±1.03

TABLE 5: TOTAL NUMBER OF MALE SAMPLES

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	10/M	Pre pubertal	1	250.798	4.14
2	10/M	Pre pubertal	1	11.278	4.19
3	11/M	Pre pubertal	1	46.035	4.44
4	11/M	Pre pubertal	1	15.594	4.48
5	12/M	Pre pubertal	1	41.413	4.6
6	10/M	Pre pubertal	1	93.718	4.69
7	10/M	Pre pubertal	1	25.798	4.46
8	9/M	Pre pubertal	1	261.766	4.53
9	11/M	Pre pubertal	1	250.301	4.43
10	10/M	Pre pubertal	1	244.165	4.24
11	9/M	Pre pubertal	1	629.675	4.54
12	10/M	Pre pubertal	1	90.484	4.2
13	9/M	Pre pubertal	1	475.73	4.63
14	8/M	Pre pubertal	1	133.349	4.28
15	6/M	Pre pubertal	1	379.047	4.17
16	9/M	Pre pubertal	1	32.595	4.43
17	6/M	Pre pubertal	1	6.709	4.56
18	11/M	Pre pubertal	1	331.348	4.11
19	14/M	Pubertal	2	734.42	5.14
20	14/M	Pubertal	2	21.425	5.1
21	13/M	Pubertal	2	7.799	4.94
22	13/M	Pubertal	2	36.112	7.12
23	13/M	Pubertal	2	45.226	5.32
24	13/M	Pubertal	2	145.641	5.78
25	11/M	Pubertal	2	30.993	7.13
26	13/M	Pubertal	2	251.571	4.89
27	14/M	Pubertal	2	101.07	7.01
28	12/M	Pubertal	2	-11.43	4.87
29	12/M	Pubertal	2	67.01	7.45
30	13/M	Pubertal	2	22.059	7.31
31	15/M	Pubertal	2	-17.566	5.78
32	13/M	Pubertal	2	33.833	5.45

33	15/M	Pubertal	2	223.533	7.21
34	14/M	Pubertal	2	-4.836	7.28
35	13/M	Pubertal	2	146.149	7.51
36	13/M	Pubertal	2	71.96	5.13
37	13/M	Pubertal	2	175.561	5.29
38	14/M	Pubertal	2	4.005	5.77
39	13/M	Pubertal	2	47.614	4.95
40	20/M	Post pubertal	3	7.096	4.32
41	18/M	Post pubertal	3	118.368	4.61
42	19/M	Post pubertal	3	-6.907	4.22
43	20/M	Post pubertal	3	2.138	4.31
44	17/M	Post pubertal	3	92.6	4.44
45	16/M	Post pubertal	3	67.656	4.56
46	17/M	Post pubertal	3	270.356	4.82
47	20/M	Post pubertal	3	87.143	4.51
48	19/M	Post pubertal	3	32.703	4.23

Total no: 48,

Mean±SD of VEGF: 127.56±163.32, Mean±SD of IGF-1: 5.11±1.05

TABLE 6: FEMALE SAMPLES IN PRE PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	11/F	Pre pubertal	1	32.568	4.41
2	10/F	Pre pubertal	1	128.97	4.25
3	11/F	Pre pubertal	1	26.631	4.39
4	7/F	Pre pubertal	1	36.533	4.67
5	9/F	Pre pubertal	1	32.769	4.36
6	10/F	Pre pubertal	1	371.42	4.39
7	8/F	Pre pubertal	1	648.55	4.49
8	8/F	Pre pubertal	1	573.8	4.32
9	9/F	Pre pubertal	1	123.7	4.69
10	8/F	Pre pubertal	1	17.971	4.27

Total no: 10, Mean age: 9.1 years

Mean±SD of VEGF: 199.29±242, Mean±SD of IGF-1: 4.42±0.15

TABLE 7: FEMALE SAMPLES IN PEAK PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	12/F	Pubertal	2	122.41	6.34
2	13/F	Pubertal	2	188.19	5.87
3	12/F	Pubertal	2	194.17	7.55
4	14/F	Pubertal	2	30.965	5.67
5	11/F	Pubertal	2	53.076	7.56
6	11/F	Pubertal	2	-1.833	4.78
7	12/F	Pubertal	2	-3.903	5.56
8	11/F	Pubertal	2	80.655	7.25
9	13/F	Pubertal	2	123.71	7.73

Total no: 9, Mean age: 12.11 years

Mean±SD of VEGF: 87.49±74.84, Mean±SD of IGF-1: 6.47±1.07

TABLE 8: FEMALE SAMPLES IN POST PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	19/F	Post pubertal	3	-23.25	4.47
2	15/F	Post pubertal	3	12.911	4.18
3	16/F	Post pubertal	3	178.87	4.23
4	17/F	Post pubertal	3	-9.971	4.01
5	15/F	Post pubertal	3	336.32	5.01
6	19/F	Post pubertal	3	192.34	4.54
7	17/F	Post pubertal	3	7.443	4.34
8	15/F	Post pubertal	3	4.08	4.78
9	20/F	Post pubertal	3	-2.675	4.14
10	16/F	Post pubertal	3	48.377	4.76
11	14/F	Post pubertal	3	8.806	4.71
12	18/F	Post pubertal	3	67.216	4.36
13	18/F	Post pubertal	3	-18.536	4.1
14	15/F	Post pubertal	3	104.05	5.21
15	20/F	Post pubertal	3	38.6	4.18
16	16/F	Post pubertal	3	-3.479	4.75
17	19/F	Post pubertal	3	-7.799	4.34
18	19/F	Post pubertal	3	22.62	4.13
19	15/F	Post pubertal	3	22.717	4.24
20	17/F	Post pubertal	3	33.755	4.07

Total no: 20, Mean age: 17 years

Mean±SD of VEGF: 50.62±89.90, Mean±SD of IGF-1: 4.42±0.33

TABLE 9: MALE SAMPLES IN PRE PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	10/M	Pre pubertal	1	250.8	4.14
2	10/M	Pre pubertal	1	11.278	4.19
3	11/M	Pre pubertal	1	46.035	4.44
4	11/M	Pre pubertal	1	15.594	4.48
5	12/M	Pre pubertal	1	41.413	4.6
6	10/M	Pre pubertal	1	93.718	4.69
7	10/M	Pre pubertal	1	25.798	4.46
8	9/M	Pre pubertal	1	261.77	4.53
9	11/M	Pre pubertal	1	250.3	4.43
10	10/M	Pre pubertal	1	244.17	4.24
11	9/M	Pre pubertal	1	629.68	4.54
12	10/M	Pre pubertal	1	90.484	4.2
13	9/M	Pre pubertal	1	475.73	4.63
14	8/M	Pre pubertal	1	133.35	4.28
15	6/M	Pre pubertal	1	379.05	4.17
16	9/M	Pre pubertal	1	32.595	4.43
17	6/M	Pre pubertal	1	6.709	4.56
18	11/M	Pre pubertal	1	331.35	4.11

Total no: 18, Mean age: 9.5 years

Mean±SD of VEGF: 184.43±181.24, Mean±SD of IGF-1: 4.39±0.18

TABLE 10: MALE SAMPLES IN PEAK PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	14/M	Pubertal	2	734.42	5.14
2	14/M	Pubertal	2	21.425	5.1
3	13/M	Pubertal	2	7.799	4.94
4	13/M	Pubertal	2	36.112	7.12
5	13/M	Pubertal	2	45.226	5.32
6	13/M	Pubertal	2	145.641	5.78
7	11/M	Pubertal	2	30.993	7.13
8	13/M	Pubertal	2	251.571	4.89
9	14/M	Pubertal	2	101.07	7.01
10	12/M	Pubertal	2	-11.43	4.87
11	12/M	Pubertal	2	67.01	7.45
12	13/M	Pubertal	2	22.059	7.31
13	15/M	Pubertal	2	-17.566	5.78

14	13/M	Pubertal	2	33.833	5.45
15	15/M	Pubertal	2	223.533	7.21
16	14/M	Pubertal	2	-4.836	7.28
17	13/M	Pubertal	2	146.149	7.51
18	13/M	Pubertal	2	71.96	5.13
19	13/M	Pubertal	2	175.561	5.29
20	14/M	Pubertal	2	4.005	5.77
21	13/M	Pubertal	2	47.614	4.95

Total no: 21, Mean age: 13.2 years

Mean±SD of VEGF: 101.53±164.108, Mean±SD of IGF-1: 6.02±1.02

TABLE 11: MALE SAMPLES IN POST PUBERTAL STAGE

SAMPLE NO	AGE/SEX	CVMI STAGE	GROUP	VEGF pg/ml	IGF-1 ng/ml
1	20/M	Post pubertal	3	7.096	4.32
2	18/M	Post pubertal	3	118.368	4.61
3	19/M	Post pubertal	3	-6.907	4.22
4	20/M	Post pubertal	3	2.138	4.31
5	17/M	Post pubertal	3	92.6	4.44
6	16/M	Post pubertal	3	67.656	4.56
7	17/M	Post pubertal	3	270.356	4.82
8	20/M	Post pubertal	3	87.143	4.51
9	19/M	Post pubertal	3	32.703	4.23

Total no: 9, Mean age: 18.4 years

Mean±SD of VEGF: 74.57±85.85, Mean±SD of IGF-1: 4.44±0.19

TABLE 12: COMPARISON OF VEGF AND IGF-1 ACROSS CVMI STAGES

		Sum of Squares	df	Mean Square	F	Sig.
VEGPgml	Between Groups	259739.121	2	129869.560	5.779	.004
	Within Groups	1887710.008	84	22472.738		
	Total	2147449.129	86			
IGF1ngml	Between Groups	59.394	2	29.697	71.127	.000
	Within Groups	35.072	84	.418		
	Total	94.466	86			

TABLE 13: MULTIPLE COMPARISONS OF VEGF AND IGF1 - POST HOC TEST

Dependent Variable	(I) NEW GRP	(J) NEW GRP	Mean D (I-J)	Std.Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VEGFPgml	1	2	92.419948	39.391485	.064	-3.80823	188.64812
		3	131.686163*	39.718003	.004	34.66035	228.71198
	2	1	-92.419948	39.391485	.064	-188.64812	3.80823
		3	39.266215	39.038613	.952	-56.09994	134.63237
	3	1	-131.686163*	39.718003	.004	-228.71198	-34.66035
		2	-39.266215	39.038613	.952	-134.63237	56.09994
IGF1ngml	1	2	-1.75229*	.16979	.000	-2.1671	-1.3375
		3	-.02773	.17120	1.000	-.4459	.3905
	2	1	-1.75229*	.16979	.000	1.3375	2.1671
		3	1.72455*	.16827	.000	1.3135	2.1356
	3	1	.02773	.17120	1.000	-.3905	.4459
		2	-1.72455*	.16827	.000	-2.1356	-1.3135

TABLE 14: COMPARISON OF FEMALES ACROSS CVMI STAGES

		Sum of Squares	df	Mean Square	F	Sig.
VEGPgml	Between Groups	148467.246	2	74233.623	3.684	.035
	Within Groups	725471.800	36	20151.994		
	Total	873939.046	38			
IGF1ngml	Between Groups	29.167	2	14.583	45.105	.000
	Within Groups	11.640	36	.323		
	Total	40.807	38			

TABLE 15: MULTIPLE COMPARISONS OF FEMALES - POST HOC TEST

Dependent Variable	(I) NEW GRP	(J) NEW GRP	Mean D (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VEGFPgml	1.0	2.0	111.797122	65.225071	.285	-51.98566	275.57991
		3.0	148.670900*	54.979989	.031	10.61393	286.72787
	2.0	1.0	-111.797122	65.225071	.285	-275.57991	51.98566
		3.0	36.873778	56.979911	1.000	-106.20508	179.95263
	3.0	1.0	-148.670900*	54.979989	.031	-286.72787	-10.61393
		2.0	-36.873778	56.979911	1.000	-179.95263	106.20508
IGF1ngml	1.0	2.0	-2.05489*	.26126	.000	-2.7109	-13989
		3.0	-.00350	.22022	1.000	.5565	.5495
	2.0	1.0	2.05489*	.26126	.000	1.3989	2.7109
		3.0	2.05139*	.22824	.000	1.4783	2.6245
	3.0	1.0	.00350	.22022	1.000	-.5495	.5565
		2.0	-2.05139*	.22824	.000	-2.6245	-1.4783

TABLE 16: COMPARISON OF MALES ACROSS CVMI STAGES

		Sum of Squares	df	Mean Square	F	Sig.
VEGPgml	Between Groups	97719.522	2	48859.761	1.902	.161
	Within Groups	1156016.740	45	25689.261		
	Total	1253736.263	47			
IGF1ngml	Between Groups	30.554	2	15.277	31.106	.000
	Within Groups	22.101	45	.491		
	Total	52.655	47			

TABLE 17: MULTIPLE COMPARISONS OF MALES - POST HOC TEST

Dependent Variable	(I) NEW GRP	(J) NEW GRP	Mean D (I-J)	Std.Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VEGFPgml	1.0	2.0	82.902595	51.482806	.343	-45.12384	210.9290
		3.0	109.860944	65.433504	.300	-52.85782	272.57971
	2.0	1.0	-82.902595	51.482806	.343	-210.92903	45.12384
		3.0	26.958349	63.856562	1.000	-131.83890	185.75560
	3.0	1.0	-109.860944	65.433504	.300	-272.57971	52.85782
		2.0	-26.958349	63.856562	1.000	-185.75560	131.83890
IGF1ngml	1.0	2.0	-1.62492*	.22511	.000	-2.1847	-1.0651
		3.0	-.05111	.28610	1.000	.7626	.6604
	2.0	1.0	1.62492*	.22511	.000	1.0651	2.1847
		3.0	1.57381*	.27921	.000	.8795	2.2681
	3.0	1.0	.5111	.28610	1.000	-.6604	.7626
		2.0	-1.57381*	.27921	.000	-2.2681	-.8795

TABLE 18: COMPARISON OF GENDERS ACROSS CVMI STAGES

		Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig
VEGF	Pre pubertal	89.000	144.000	-.048	.962
	Pubertal	84.000	315.000	-.475	.635
	Post pubertal	67.000	277.000	-1.084	.278
IGF-1	Pre pubertal	86.500	257.500	-.168	.867
	Pubertal	63.000	294.000	-1.426	.154
	Post pubertal	76.500	286.500	-.637	.524

TABLE 19: MEAN IGF-1 LEVEL IN THE THREE STUDY GROUPS

CVM Stage	Group	Mean Age(yrs)	Female Mean ± SD (ng/ml)	Male Mean ± SD (ng/ml)
Pre pubertal	1	9.1	4.42 ± 0.15	4.39 ± 0.18
Pubertal	2	12.11	6.47 ± 1.07	6.02 ± 1.02
Post pubertal	3	17	4.42 ± 0.33	4.44 ± 0.19

GRAPH 1: IGF-1 LEVEL IN BOTH THE GENDERS ACCORDING TO CVMI STAGES

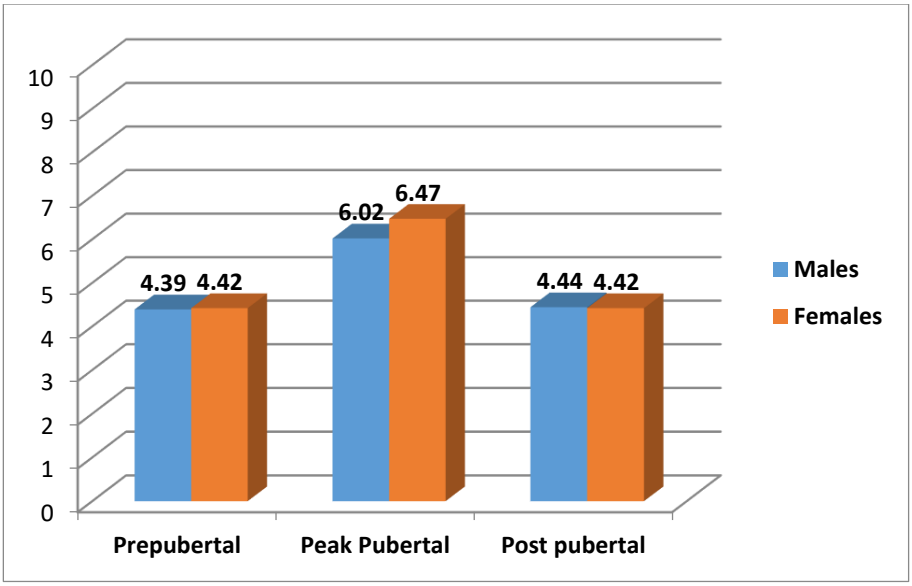


TABLE 20 : MEAN VEGF LEVEL IN THE THREE STUDY GROUPS

CVM Stage	Group	Mean Age(yrs)	Female Mean ± SD (pg/ml)	Male Mean ± SD (pg/ml)
Pre pubertal	1	9.1	199.29 ± 242	184.43±181.24
Pubertal	2	12.11	87.49 ± 74.84	101.53 ± 164.108
Post pubertal	3	17	50.62 ± 89.90	74.57 ± 85.85

GRAPH 2: VEGF LEVEL IN BOTH THE GENDERS ACCORDING TO CVMI STAGES

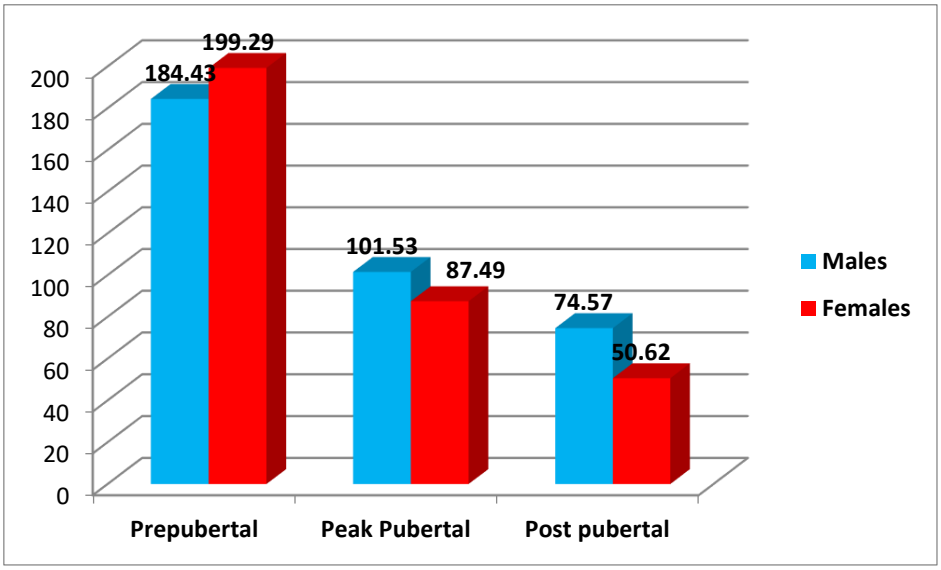
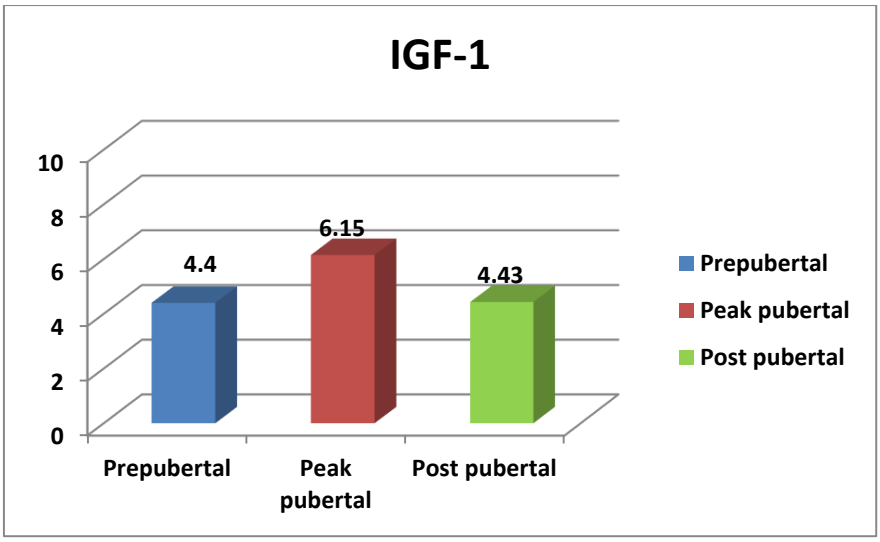


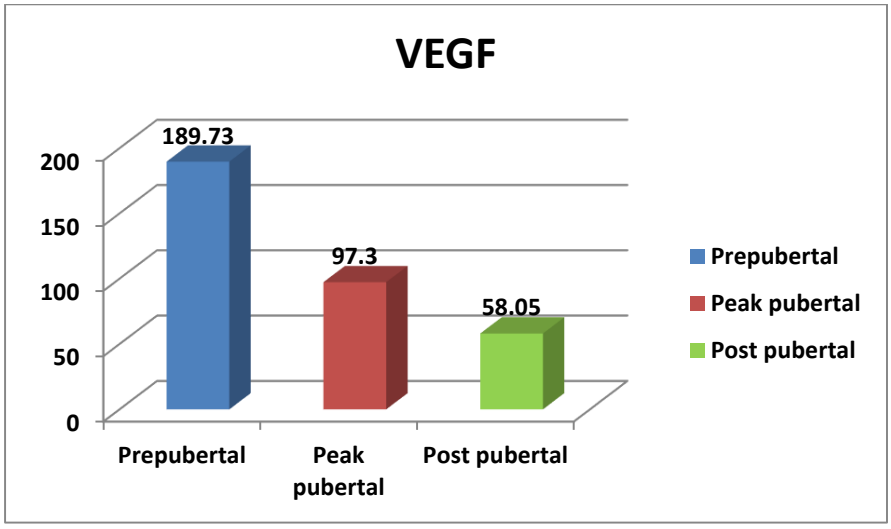
TABLE 21: MEAN VEGF AND IGF-1 LEVEL IN THE THREE STUDY GROUPS

CVM Stage	Group	VEGF(pg/ml) Mean ± SD	IGF-1(ng/ml) Mean ± SD
Pre pubertal	1	189.73 ± 200.63	4.40 ± 0.17
Pubertal	2	97.3 ± 141.99	6.15 ± 1.04
Post pubertal	3	58.05 ± 87.85	4.43 ± 0.29

GRAPH 3: IGF-1 LEVEL IN THREE STUDY GROUPS IRRESPECTIVE OF GENDERS



GRAPH 4: VEGF LEVEL IN THREE STUDY GROUPS IRRESPECTIVE OF GENDERS



DISCUSSION

DISCUSSION

Assessment of growth is of prime importance in the practice of orthodontics as it influences the diagnosis, treatment planning and eventual outcome of the orthodontic treatment. Decisions regarding the use of functional appliances, extraoral traction forces, extraction vs non extraction treatment or orthognathic surgeries are based on growth considerations. The growth modulation procedure carried out during the active growth period is more effective and produces a more successful treatment outcome. There is a wide variation between chronological age and skeletal age. So it is unreliable in prediction of the actual growth status.[49] There are different methods reported by different authors to determine the skeletal maturity. The skeletal maturation staging by the routine lateral cephalometric radiographic method is a widely used approach to predict the precise timing, growth velocity and the amount of remaining growth.

The standard old and reliable method of evaluating skeletal growth maturation is by using hand wrist radiograph. The British Orthodontic Society guideline has stated that the use of handwrist radiograph is not indicated to predict the pubertal growth due to unwanted additional radiation exposure.[15,20,34] Lamparski (1972) developed a method by using cervical vertebral maturation (CVM) index to assess the skeletal maturation. It was found to be effective and clinically reliable method for assessing skeletal maturity.[5,11,28,35,44,51]

Hassel and Farman (1995) developed an index for assessing the skeletal maturation based on the second, third and fourth cervical vertebrae even with the use of a lead collar to protect the thyroid which may have hindered the image of the cervical spine.[11,28,35,44] Many studies had compared the correlation between cervical vertebrae and hand wrist bone analysis and found a significant correlation between both the analysis. Suggesting that either of the methods could be used indistinctively for research purposes. [6,15,28,34,39,4]

Baccetti et al [51] gave an improved version of CVM method for assessment of mandibular growth. The new method consists of CVMS I through CVMS V instead of Cvs 1 through Cvs 6 in the former method and the peak in mandibular growth occurs between CVM II & III. Yan Gu et al [55] evaluated the mandibular dimensional changes and regional remodeling changes occurring during five intervals of circumpubertal growth. They found a peak increase in mandibular length and greatest bone apposition at condylion was seen at CS3-CS4. This is in accordance with the study conducted by Ball et al.[11] It has been stated that the CVM method is valid regardless of individual variations and between various ethnic groups.[11]

The new emerging method for determining skeletal growth is evaluation of biomarkers which avoids radiation exposure. Biomarkers are those agents which are directly involved in bone growth and remodeling. These biochemical markers of skeletal maturity were initially detected in serum, but now it has also been detected in saliva and its an noninvasive method.[43,44] There are many researches that are going on in this field to establish these as a new method of growth estimation. Recent studies evaluated the salivary IGF-1 with CVM method and found a significant correlation during growth period. Also presence of VEGF in normal human saliva has been proved by many studies.[3,16,45,47,48] This study evaluates both the salivary biomarkers as a parameter for precise growth estimation.

Vascular endothelial growth factor (VEGF) was first identified as an endothelial - specific growth factor from bovine pituitary follicular cells by Ferrara and Davis Symth. VEGF is a multifunctional angiogenic cytokine involved in angiogenesis. Many studies found that VEGF is able to promote ossification by either inducing neovascularization or directly affecting the bone cells.[57] Rabie et al [31] and Lei et al [53] studied the factors regulating endochondral bone ossification in rats and the expression of these factors were identified by immunohistochemical

staining. The result found an increased VEGF expression by chondrocytes preceding bone formation. So increased level of VEGF would have a role in bone growth. Rabie et al and Shum et al identified the relationship between VEGF expression and bone formation during forward mandibular positioning in Sprague Dawley (SD) rats. Tissue sections were made and stained with anti-VEGF antibodies to evaluate VEGF expression. A highest amount of VEGF expression was found before bone formation.[21,30,32] This is in accordance with the result of prior study. It was found that in vitro introduction of VEGF to cell culture stimulates osteoblast differentiation, proliferation and migration.[14,24]

Jaun Dai et al [17] introduced a specific vascular growth inducting genes into SD rats to find its effect on mandibular condylar growth. The length of the condylar process as well as the mandibular length were significantly increased. Thus gene therapy with VEGF have a role in stimulating condylar and mandibular growth.[17]

VEGF is a multifunctional cytokine and many studies had found that VEGF is constitutively expressed in normal human salivary glands and is secreted in the saliva of healthy individuals.[3,16,45,47,48] For the determination VEGF whole saliva sample was taken and assayed either by ELISA or chemiluminescent immunoassay method. The median concentration of VEGF determined by ELISA method in whole saliva is (460 pg/ml) and in ductal secretions obtained from the parotid is (277 pg/ml) and submandibular & sublingual salivary gland is (80 pg/ml). VEGF seems to be synthesized endogenously by salivary glands because both VEGF mRNA and protein are revealed in reverse transcriptase-PCR and by immunohistochemistry.[48]

None of the studies in literature correlated the levels of salivary VEGF with various stages of cervical vertebral maturation for predicting the skeletal growth. In our study the mean salivary VEGF level in group 1 was found to be (189.73±200.63 pg/ml) and it decreases in group

2 (97.3±141.99 pg/ml) & group 3 (58.05±87.85 pg/ml). When multiple comparisons were made across CVM stages there is a highly statistically significant difference between group 1 and group 3 (*P*=.004). When females were compared there is a significant difference between group 1 and group 3 (*P*=.031) and in males there is no significant difference. Also there is no significant difference in VEGF level when genders are compared. The mean VEGF level decreases from pre pubertal to post pubertal stages. This decrease in salivary VEGF level may be due to decreased need of tissue angiogenesis.

Insulin like growth factor-1 (IGF-1) was initially identified as liver derived "sulfaction factors" that mediates the effects of growth hormone by Salmon and Daughaday.[54] IGF-1 is a circulating GH factor, the level of which correlates with the sexual maturity.[43] Masoud et al evaluated the blood spot IGF-1 level with hand wrist radiographic analysis and cervical vertebral analysis. The results showed a significant raise in IGF-1 level during peak pubertal growth stage than at pre pubertal or post pubertal growth stages. So the blood spot IGF-1 can be taken as a promising tool for predicting the timing and intensity of mandibular growth spurt.[25,26,27]

Gupta et al [38] and Jain et al [37] correlated the serum IGF-1 level with various stages of CVM and found that there is a significant increase in IGF-1 level during peak pubertal growth period. Between males and females also there was a significant difference. The greatest mean serum IGF-1 level in female is 397 ng/ml which were slightly higher than in males 394.8 ng/ml.[38] Similarly Brabant et al [10] and Leite et al [8] determined the IGF-1 factor using chemiluminescence immunoassay and found a age dependency of IGF-1 level. In female IGF-1 level was found to be significantly greater than in men. This is in accordance with the study conducted by Gupta et al.[38]

Local administration of IGF-1 into articular capsule of rats at different time intervals produced a significant increase in thickness of condyle at cartilaginous layer. The amount of endochondral bone growth was also found to be greater in IGF-1 treated group. Local administration of IGF-1 makes it possible to extend the growth of mandibular condyle even after normal growth is complete but its difficult to further accelerate the mandibular growth in its growth period.[18,46]

The determination of IGF-1 in blood is an invasive method and many studies had been done in the saliva to evaluate the level of IGF-1. The whole saliva samples were taken for the assay.[1,2,7,13,29,36] Antonelli et al [1,2] evaluated the level of salivary IGF-1 in young athletes and sedentary females. There was a decreased level of free IGF-1 in athelets when compared to sedentary females and this decrease could be attributed to greater tissue requirements. Halimi et al [13] and Costigan et al [7] compared the salivary IGF-1 and serum IGF-1 in acromegalic patients to evaluate the disease activity. There was a significant increase in serum IGF-1 level than salivary IGF-1 which reflects the growth hormone status of an individual. The salivary IGF-1 level was found to be less when compared to serum IGF-1. Ryan et al [36] found a significant raise in salivary IGF-1 level during puberty and then decreases in late adolescence. Salivary IGF-1 concentrations were 100 to 200 folds less than the plasma IGF-1 level. Nayak et al [42] studied the relationship between salivary IGF-1 level and CVM stages. There was a significant correlation between salivary IGF-1 and CVM stages suggesting that it can be used as a biochemical marker for predicting growth.

In our study whole saliva sample was taken for the determination of IGF-1 level and compared with the CVM stages. There is a highly statistically significant difference between peak pubertal growth stage and the other two stages (P=.000). IGF-1 level was found to be at

baseline during pre-pubertal stage (4.40±0.17 ng/ml), then increases to its maximum level at peak pubertal growth (6.15±1.04 ng/ml) and decreases again in post pubertal stage (4.43±0.29 ng/ml).

When females and males were compared separately across the CVM stages the result showed a significant difference when group 2 is compared with group 1 and group 3 (*P*=.000). The result is in accordance with the study conducted by Nayak et al [42] and Ryan et al.[36] If females and males were compared across the CVM stages there is no significant difference between genders. This was also in accordance with the study conducted by Ryan et al. [36]

As VEGF level is highly variable it cannot be used as a biomarker for studying growth. But salivary IGF-1 level correlates well with the CVM stages. So IGF-1 is a valid indicator for predicting growth status of an individual with the advantage of avoiding the radiation exposure, misleading nature of the two dimensional radiograph and also it's a non-invasive method.

SUMMARY AND CONCLUSION

SUMMARY

The present study was undertaken

1. To find out the level of salivary insulin like growth factor - 1 during the various stages of cervical vertebral maturation.
2. To find out the level of vascular endothelial growth factor during the various stages of cervical vertebral maturation.
3. To find out whether there is any gender differences in vascular endothelial growth factor level and insulin like growth factor - 1 level.
4. To find out whether the salivary insulin like growth factor - 1 and vascular endothelial growth factor can be used as a biomarker to determine the overall skeletal maturity of an individual by correlating its value with the CVMI stages.

The study shows a significant increase in insulin like growth factor - 1 during peak pubertal growth when compared with the cervical vertebral maturation method. Vascular endothelial growth factor level was found to be highly variable between the three study groups. When females and males were compared there is no significant difference in both of these biomarkers. The mean salivary IGF-1 level found in our study during the three growth stages are pre pubertal = 4.40±0.17 ng/ml, peak pubertal = 6.15±1.04 ng/ml and at post pubertal = 4.43±0.29 ng/ml. The statistical difference between the study groups could only be established for VEGF level and no normal or standard VEGF values could be established for the three groups which requires a longitudinal study with great deal of scientific basis and a large sample size.

CONCLUSION

There is a significant increase in salivary insulin like growth factor - 1 level during the peak pubertal growth when compared with pre-pubertal and post-pubertal growth stages.

This increased salivary insulin like growth factor - 1 level correlates with the cervical vertebral radiograph.

The salivary vascular endothelial growth factor level is highly variable among the three groups.

There is no significant difference in salivary insulin like growth - 1 level and salivary vascular endothelial growth factor level between the genders.

The mean salivary IGF-1 level found in our study during the three growth stages are pre pubertal = 4.40±0.17 ng/ml, peak pubertal = 6.15±1.04 ng/ml and at post pubertal = 4.43±0.29 ng/ml.

No normal and standard values for salivary vascular endothelial growth factor could be established for each cervical vertebral maturation stages because of the varying velocity of growth between the individuals.

Hence salivary insulin like growth factor - 1 level can be used to predict the growth status of a healthy individual.

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ANNEXURE

KSR INSTITUTE OF DENTAL SCIENCE AND RESEARCH

DEPARTMENT OF ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS

INFORMED CONSENT FORM

For questions about the study contact:

PRINCIPAL INVESTIGATOR : Dr.S.SHARMILAA,
Post graduate student,
Department of Orthodontics and Dentofacial Orthopaedics,
KSR Institute of Dental Science and Research,
Tiruchengode.
Contact no : 9442493644

DESCRIPTION :

You are invited to participate in the research conducted in the Department of Orthodontics and Dentofacial Orthopaedics, KSR Institute of Dental Science and Research.

The study is to evaluate the IGF-1 and VEGF levels in saliva and its relation with the CVMI stages in determining the skeletal maturity

RISK AND BENEFITS :

We do not expect any adverse effects to the subject during this study.

TIME INVOLVEMENT :

Your participation time in this study will involve approximately 30 minutes during the procedure.

We kindly inform you that you will not receive any financial benefits from this study. Your decision whether or not to participate in this study will not affect your employment/medical care.

PARTICIPANTS' RIGHTS :

If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled.

If you agree, your identity will be made known in all written data resulting from the study. Otherwise, your individual privacy will be maintained in all published and written data resulting from the study.

An extra signed copy of this consent form will be provided to you.

CONSENT :

I Mr/Ms/Mrs._____, read the consent form completely and have explained about the study well to my knowledge and also about the risks and benefits involved in the study by the principle investigator. I, without any compulsion, voluntarily is willing to participate in this study.

Signature of the participant : _____

Signature of the investigator : _____

Date : _____

KSR பல் மருத்துவக் கல்லூரி மற்றும் ஆராய்ச்சி மையம்

ஒப்புதல் படிவம்

விளக்கம்

எங்கள் பல் சீரமைப்புத் துறையில் நடைபெறும் இந்த ஆராய்ச்சியில் கலந்து கொள்ள உங்களை அழைக்கிறோம்.

இந்த ஆராய்ச்சி, உமிழ் நீரைக் கொண்டு, உங்களின் வளர்ச்சியைக் கணக்கிடுவதற்காக அமைக்கப்பட்டதாகும்.

இந்த ஆராய்ச்சியின் போது, பங்கேற்பவரின் வாய் முழுதாக சுத்தம் செய்யப்படும். பின்னர் உமிழ்நீர் சேகரிக்கப்படும்.

பக்க விளைவுகள் மற்றும் நன்மைகள்

இந்த ஆராய்ச்சியின் போது எந்த ஒரு பக்க விளைவும் ஏற்படாது.

இந்த ஆராய்ச்சியில் பங்கேற்பதன் மூலம், பங்கேற்பவரின் வளர்ச்சி நிலையும் மற்றும் அவரது வளர்ச்சி நிலையில் சாத்தியமான சிகிச்சை முறைகளும் விளக்கப்படும்.

இந்த ஆராய்ச்சியில் பங்கேற்க, பங்கேற்பவர் மருத்துவமனையில் ஏறத்தாழ 30 நிமிடங்கள் இருக்க வேண்டும்.

இந்த ஆராய்ச்சியில் பங்கேற்பதன் மூலம், தங்களுக்கு எந்த ஒரு நிதி உதவியும் வழங்கப்பட மாட்டாது.

இந்த ஆராய்ச்சியில் பங்கேற்பதும், பங்கேற்காததும், தங்களின் மருத்துவ சிகிச்சையை எந்த விதத்திலும் பாதிக்காது.

பங்கேற்பவரின் உரிமைகள்

இந்தப் படிவத்தைப் படித்த பின்பு, தங்களுக்கு இந்த ஆராய்ச்சியில் பங்கேற்க விருப்பம் இருந்தால், அது உங்களின் முழு விருப்பமே தவிர கட்டாயம் இல்லை என்பதை உணர வேண்டும்.

தங்களின் சம்மதத்தின் பேரில், தங்களின் அடையாளம் இந்த ஆராய்ச்சியின் முடிவில் வெளியிடப்படும். இல்லையெனில், இந்த ஆராய்ச்சியின் முடிவு வெளியிடப்படும் போது, தங்களின் அடையாளம் வெளியிடப்படமாட்டாது.

இந்தப் படிவத்தின் நகல் உங்களுக்கு வழங்கப்படும்

ஒப்புதல்

திரு / திருமதி ..... ஆகிய நான், மேற்கண்ட ஒப்புதல் படிவத்தை தெளிவாகப் படித்தும், மருத்துவரின் முழு விளக்கங்களை கேட்டும், இந்த ஆராய்ச்சியின் முழு விவரங்களை அறிந்து கொண்டு யாருடைய தூண்டுதலும், கட்டாயமும் இன்றி என் மகன் / மகளை இந்த ஆராய்ச்சியில் பங்கேற்க முழு மனதுடன் சம்மதிக்கிறேன்.

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பெற்றோர் / பாதுகாவலர் கையொப்பம் **தேதி**

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ஒப்புதல் வாங்குபவரின் கையொப்பம் **தேதி**

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சாட்சிக் கையொப்பம் **தேதி**

S.NO	PATIENTS NAME	AGE	SEX	OP.NO	CVMI STAGE	GROUP
1.	N.Nagalakshmi	11	F	432328	Stage 2	1
2.	G.Haswanth	14	M	322213	Stage 3	2
3.	M.Dhanujasree	10	F	10534C	Stage 2	1
4.	R.Tamil Selvi	19	F	433536	Stage 6	3
5.	S.Sureka	15	F	355034	Stage 5	3
6.	M.Ajay Kumar	14	M	436015	Stage 4	2
7.	B.Divya Lakshmi	16	F	384101	Stage 5	3
8.	A.Srinath	20	M	11903C	Stage 6	3
9.	R.Deepika	12	F	431434	Stage 3	2
10.	M.Mumtaj Begam	17	F	437808	Stage 5	3
11.	A.Kiruthiga	11	F	454702	Stage 2	1
12.	R.Naveen	13	M	450692	Stage 4	2
13.	A.Kavin	13	M	432035	Stage 3	2
14.	R.Abirami	15	F	439888	Stage 5	3
15.	S.Sanmuga Priya	13	F	432043	Stage 4	2
16.	R.Sujan	13	M	430793	Stage 3	2
17.	B.Madhumalathi	12	F	437805	Stage 3	2
18.	V.Kabiyashwanth	13	M	435992	Stage 3	2
19.	M.Rama Prabha	19	F	416872	Stage 6	3
20.	A.Naren Karthick	11	M	434464	Stage 3	2
21.	S.Gokuladharani	17	F	435988	Stage 6	3
22.	S.Chandru	9	M	251071	Stage 2	1
23.	P.Tharun	10	M	447220	Stage 2	1
24.	S.Asvitha	15	F	274179	Stage 5	3
25.	A.Makilesh	13	M	351649	Stage 3	2
26.	A.Guhan Karthik	10	M	434465	Stage 2	1
27.	S.Monish	11	M	353048	Stage 2	1
28.	R.Ramya	20	F	353376	Stage 5	3
29.	R.Aravindhan	11	M	402628	Stage 2	1
30.	S.Preethi	16	F	376667	Stage 5	3
31.	B.Dineshwaran	14	M	353344	Stage 4	2
32.	V.Dhanusri	7	F	369532	Stage 2	1
33.	J.Nithya Shree	14	F	432654	Stage 5	3
34.	N.Sabareeswaran	12	M	314970	Stage 2	1
35.	S.Swamidharanidharan	10	M	351125	Stage 2	1
36.	K.Dharneesh	12	M	285128	Stage 3	2
37.	R.Lokeshwaran	12	M	362721	Stage 3	2
38.	S.Harini	9	F	10463C	Stage 2	1
39.	S.Gokula Kannan	18	M	431413	Stage 6	3
40.	R.Aarthi	18	F	448156	Stage 5	3
41.	M.Devi Priyadharshini	18	F	463539	Stage 5	3
42.	S.Perarasu	10	M	267537	Stage 2	1
43.	M.Surya	9	M	353372	Stage 2	1

44.	K.Manoj Kumar	11	M	427146	Stage 2	1
45.	R.Shivani Sree	15	F	437478	Stage 5	3
46.	J.Gopika	14	F	440176	Stage 4	2
47.	P.Roobalakshmi	11	F	456250	Stage 3	2
48.	S.Thiyaneshwaran	10	M	343491	Stage 2	1
49.	C.Thamizhazhagan	13	M	436284	Stage 3	2
50.	S.Vasundhara Devi	10	F	12663C	Stage 2	1
51.	S.Bavika	8	F	344926	Stage 2	1
52.	G.Priyanka	20	F	359917	Stage 5	3
53.	S.Siva Ranjani	11	F	452881	Stage 3	2
54.	S.Vidhya Sri	12	F	462347	Stage 3	2
55.	T.Sathish	10	M	417471	Stage 2	1
56.	R.Shashagan	19	M	435676	Stage 6	3
57.	R.Jashwanth	10	M	436887	Stage 2	1
58.	V.Prakash	20	M	448424	Stage 6	3
59.	A.Manoj	17	M	436581	Stage 5	3
60.	M.Mukilan	16	M	434171	Stage 5	3
61.	G.Praveen	15	M	439902	Stage 4	2
62.	R.Vishnu Priya	8	F	379792	Stage 2	1
63.	S.Yogeshwaran	9	M	364455	Stage 2	1
64.	N.Subashree	9	F	337399	Stage 2	1
65.	K.Nandha Kumar	17	M	433232	Stage 5	3
66.	A.Saravana Surya	20	M	411908	Stage 6	3
67.	S.Mahanandhin	13	M	353892	Stage 4	2
68.	M.Dhevadharshini	16	F	440483	Stage 5	3
69.	S.Tuhin	8	M	386118	Stage 2	1
70.	B.Bharathi	19	F	442557	Stage 6	3
71.	J.Faijur Rahman	15	M	411930	Stage 4	2
72.	S.Pranesh	6	M	402300	Stage 2	1
73.	S.Aparna	19	F	423542	Stage 5	3
74.	S.T.Ragunath	9	M	426837	Stage 2	1
75.	R.Dharshan	6	M	339415	Stage 2	1
76.	S.Srinidhi	8	F	451138	Stage 2	1
77.	A.S.Kiruthik	14	M	402628	Stage 4	2
78.	S.Bhuvaneshwari	15	F	428662	Stage 5	3
79.	S.Kavin	13	M	431419	Stage 3	2
80.	S.Hariprasath	13	M	432332	Stage 3	2
81.	T.Nadhiya	17	F	426244	Stage 5	3
82.	S.Mohanraj	12	M	448755	Stage 2	1
83.	K.Sownther	13	M	347745	Stage 3	2
84.	K.Sridhar	11	M	10537/C	Stage 2	1
85.	V.Yukesh	14	M	445548	Stage 4	2
86.	P.Sabari	13	M	246646	Stage 3	2
87.	A.Harshini	11	F	432027	Stage 3	2
88.	J.Appas	19	M	432360	Stage 6	3

89.	R.Janani Priya	13	F	431800	Stage 3	2
90.	S.Arsath	17	M	432949	Stage 5	3

INSTITUTIONAL ETHICAL COMMITTEE

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KSR Kalvi Nagar, Tiruchengode.

Ref.: 101 /KSRIDSR/EC/2015 **Date : 18.04.2015**

To

Dr.S.Sharmilaa,
Postgraduate Student,
Dept. of Orthodontics,
KSR Institute of Dental Science & Research,

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Your dissertational study titled "IGF-1 AND VEGF IN SALIVA AND ITS RELATION WITH CVMI STAGES IN DETERMINING THE SKELETAL MATURITY" presented before the ethical committee on 15th Apr. 2015 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)