ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Special issue 1, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISM INSULIN-LIKE GROWTH FACTOR TYPE 1 GENE IN VERTICAL MANDIBULAR ASYMMETRY PATIENTS WITH IDIOPATHIC SCOLIOSIS SYMPTOM

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Received: 07 March 2018, Revised and Accepted:25 March 2018

ABSTRACT

Objective: Previous studies reported insulin-like growth factor type 1 *(IGF-1)* gene expression in mandibular condylar cartilage and idiopathic scoliosis development. This paper aims to correlate the single nucleotide polymorphisms (SNPs) of *IGF-1* gene rs5742632 in vertical mandibular asymmetry patients with idiopathic scoliosis symptom.

Methods: The *IGF-1* gene rs5742632 polymorphism of 49 patients (19.38±3.24 year old) who were treated at the Orthodontics Department Dental Hospital Universitas Sumatera Utara and the Orthopedics Department of Haji Adam Malik Hospital from April to August 2017, were genotyped using polymerase chain reaction-restriction fragment length polymorphism analysis in case control of idiopathic scoliosis symptom. An enzyme-linked immunochemiluminescent assay measured *IGF-1* levels. Analyzing of mandibular asymmetry index based on Kjellberg's technique using the panoramic radiograph.

Results: The scoliosis symptom based on clinical judgment of orthopedics by asymmetry trunk posture and Adam's forward bend test movement analysis. This study showed no statistically significant difference ($p>0.05$) in the genotype distribution (rs5742632) haplotype in vertical mandibular asymmetry based on scoliosis symptom. However, there was a statistically significant difference between early and late adolescent among those subjects in *IGF-1* level measurement ($p=0.033$).

Conclusion: The result was still not conclusive due to variance in mandibular growth and curve lateral spine in adolescent patients. Further study will require subject increment and more specific samples to study the risk factor of vertical mandibular asymmetry.

Keywords: Vertical mandibular asymmetry, Idiopathic scoliosis symptom, Insulin-like growth factor type 1.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11s1.26686

INTRODUCTION

The treatment reason for mandibular asymmetry and scoliosis related to patient's dissatisfaction regarding physical appearance and functional problems. Developing scoliosis in children often invisible until the patient reaches adolescence and enters a rapid growth phase. The most common spinal deformity in adolescent is idiopathic scoliosis and can be classified as congenital, neuromuscular, or idiopathic. There were approximately 2% to 4% of adolescent affected with idiopathic scoliosis, which a polygenic disorder with multiple inheritance pattern [1,2]. Mandibular asymmetry is the asymmetric growth of mandible that due to complicated postnatal growth abnormality of the mandibular condyle [3-5]. In understanding the genetic aspects of congenital and idiopathic scoliosis, Giampietro suggested that craniofacial asymmetry as one of the major clinical features of oculo-auriculo-vertebral spectrum [6]. However, the abnormalities in the skeletal, nerve, endocrine systems, and connective tissue for both asymmetries in the bilateral joint of temporomandibular and spinal still unclear whether these are primary or secondary. The essential of early treatment to prevent scoliosis and mandibular asymmetry development can be a challenge to treat once it has set.

Insulin-like growth factor 1 *(IGF-1)* is similar to insulin in function and structure. It is a member of a protein family that involve in mediating growth and development in adolescent subjects. There was an aberrant expression of *IGF-1* that may influence hormone metabolism which resulted in a gross asymmetry and promotes the progress of adolescent idiopathic scoliosis. In case–control adolescent idiopathic scoliosis, there was a statistically difference in the genotypic frequency at rs6179 [7]. The study of idiopathic scoliosis genetic epidemiology has also reported this gene as the principal mediator of accelerated linear growth and bone dimension development during puberty [8]. It is also one of the growth factors that play an essential role in the proliferation and differentiation of cells in the mandibular condyle. *IGF-1* gene expression depends on to the etiology of mandibular growth in Class III malocclusion [9,10]. *IGF-1* promotes human TMJ cartilage overgrowth in the developing process of condylar hyperplasia by enhancing chondrocytes proliferation through MAPK (Mitogen-activated protein kinase)-eERK pathway [11]. Distribution of *IGF-1* in condylar hyperplasia patients has been a complicated expression of facial asymmetry in postnatal growth abnormality of the mandibular condyle [4]. *IGF-1* has been reported to be involved in growth by regulating endochondral ossification and local unilateral *IGF-1* injection into mandibular condylar cavity successfully induced unilateral endochondral mandibular growth in mice without any systemic adverse effects [5].

The location of *IGF-1* gene is on chromosome 12q23.2 that is an intronic *IGF-1* single nucleotide polymorphism (SNP). There were 13 tagging SNPs of *IGF-1* in that showed a minor allele frequency ≥5% in the association of high myopia of Chinese population and reported that potential SNPs of *IGF-1* gene were worthy to be studied related to any phenotype [12,13]. In dentistry, *IGF-1* gene expression affects cell growth regulation and proliferation of the affected organs in skeletal maturity, especially in mandibular condylar cartilage which is related to stimulation of proliferation and promoting myoblastic differentiation or osteoblastic tissues [4,11]. TThe similarity of period in mandibular growth and increasing of spinal curve degree in growing subjects as

multidisiplinary approched nowadays in treatment of growing subjects. In this study, we conducted a systematic case–control study to associate the SNPs of *IGF-1* gene rs5742632 based on idiopathic scoliosis and associated the phenotype of vertical mandibular asymmetry.

METHODS

This study was undertaken in compliance with the Helsinki Declaration and approved by Research Ethics Committee of Universitas Sumatera Utara Medical Faculty no. 100/DATE/KEPK FK USU-RSUP HAM/2017. This case–control study involved 49 subjects that treated in the Orthodontics Department, Universitas Sumatera Utara Dental Hospital and the Orthopaedic Traumatology Department of Haji Adam Malik hospital from April to August 2017. The laboratory analysis was conducted in the Integrated laboratory, Medical Faculty Universitas Sumatera Utara. The inclusion criteria are as followed: The age of patients among 11–25 years old; complete dentition until the second molar; there was no history of previous orthodontic, prosthodontic treatment, or occlusal adjustment; there was also no traumatic facial injury and congenital disease. The scoliosis symptom based on clinical judgment of orthopedics by asymmetry trunk posture and Adam's forward bend test movement analysis. Analyzing of mandibular asymmetry index based on Fig. 1 Analyzing of mandibular vertical asymmetry index based on Kjellberg's technique using the pre-treatment panoramic radiograph [14.15].

The genotype of *IGF-1* rs5742632 SNP by polymerase chain reaction (PCR)-RFLP based on Mak's studies [16]. The following primers were used: Forward 5'-(T)10 GGATGCGACTGCCAGTTAATTGAATTCA-3' and reverse 5'-GCCCACAGACTGTCTAACTTAAGG-3'. PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 37 cycles of denaturation for 1 min at 94°C, annealing for 30 s at 60°C, and an extension for 8.50 s at 72°C. A final extension was carried out for 7 min at 72°C. Restriction enzyme, EcoRI digested PCR product size (223 bp), according to kit instructions (New England Bio Labs, Beverly, MA) at 37°C for 12 h. The PCR product was separated on 3% agarose gel stained with ethidium bromide and visualized under ultraviolet light. The PCR produced bands of 191 and 32 bp for TT genotype, whereas TC genotype generated bands of 191, 116, 75, and 32 bp, and CC genotype produced 116-, 75-, and 32-bp fragments. The 32-bp fragment was not retained on the gel [17]. Measurement of level *IGF-1* concentration was determined using a double antibody sandwich immunochemiluminescent assay (Immulite 1000, Tawada Healthcare). The data were processed by SPSS (version 11; SPSS, Inc., Chicago, IL).

The validity and reliability test for interrater and intrarater digitized panoramic radiograph and cephalometric measurements with a t-test. The relation of condylar height asymmetry based on vertical growth pattern was compared using Pearson's Chi-squared test (SPSS software, version 22.0 for Windows; SPSS, Chicago IL).

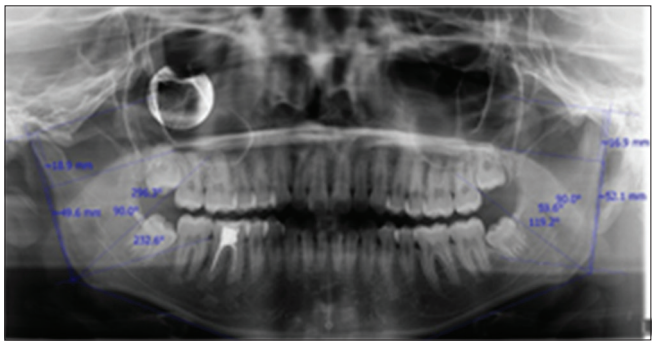

Fig. 1 Analyzing of mandibular vertical asymmetry index based on Kjellberg's technique using the pre-treatment panoramic radiograph

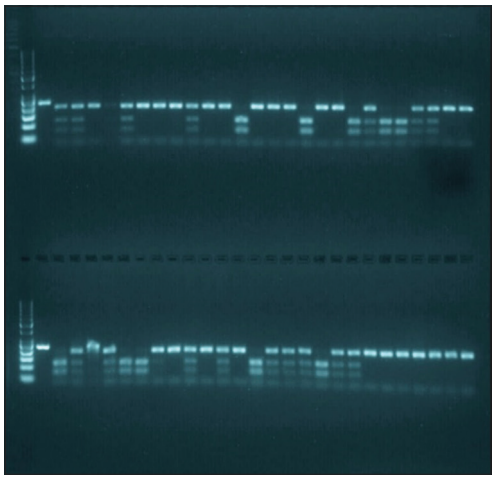

Fig. 2: RLFP Enzim *EcoR1* rs5742632 (Agarose 3%; DNA ladder 25-1000) TT: 191-32 bp; TC: 191, 116, 75, 32 bp; CC: 116-, 75, 32-bp

RESULTS AND DISCUSSION

Results

The studied subjects were 25 adolescent subjects with idiopathic scoliosis symptom and 24 without the symptom in mean age of 19.38±3.24 year old. The PCR produced bands in this study has been shown in Fig. 2. The age (OR 95% =2.674) and vertical mandibular asymmetry showed allele distribution (rs5742632) haplotype with or without scoliosis asymmetry (OR 95% =2.125) as a risk factor for scoliosis symptom (Table 1). The polymorphism of *IGF-1* gene rs5742632 showed risk factor in vertical mandibular asymmetry (OR 95% =1.417) and scoliosis symptom (OR 95% =2.101) in Table 2. The result in Table 3 showed statistically significant differences of level IGF-1 between early and late adolescent in studied subjects [p=0.033]. However, there was no statistically significant difference in scoliosis symptom (p=0.709) and vertical mandibular asymmetry (p=0.798).

Table 1: Distribution of scoliosis symptom characteristics in studied subjects

Clinical Parameters	Scoliosis symptom		
	OR	95% CI	p
Age	2.674	0.666–6.781	0.102
Vertical mandibular asymmetry	2.125	0.668–6.604	0.200

*p<0.05: Significant, CI: Confidence interval

Table 2: Distribution of *IGF-1* gene rs5742632 in vertical mandibular asymmetry with scoliosis symptom

Clinical Parameters	rs5742632		
	OR	95% CI	p
Vertical mandibular asymmetry	1.417	0.45–4.458	0.551
Scoliosis symptom	2.101	0.608–6.604	0.201

*p<0.05: Significant, CI: Confidence interval

Table 3: Distribution of level serum *IGF-1* in studied subjects

Clinical Parameters	*IGF-1* level	
	Mean±SD (ng/µL)	p
Adolescent		
Early	251.55+88.30	0.033*
Late	198.92+64.35	
Scoliosis symptom		
Control	206.71+82.18	0.709
Positive	214.60+64.13	
Vertical mandibular asymmetry		
Control	208.48+82.43	0.798
Symmetry	214.00+58.14	

*p<0.05: Significant, *IGF-1*: Insulin-like growth factor 1 quantitative data presented as mean±SD

Measurement of serum *IGF-1* concentrations by analyzing acid-labile components and binding proteins. Acid treatment is necessary to release *IGF-I* to ensure accurate quantitation. There is no significant difference in *IGF-1* level in scoliosis symptom in vertical mandibular asymmetry regarding *IGF-1* gene (rs5742632) polymorphism.

DISCUSSION

Previous orthodontics and orthopedic findings reported that idiopathic scoliosis might indirectly correlate with facial asymmetry or dental deviations in the transverse dimension within growing subjects. Although the process of facial asymmetry and malocclusion as the prime of pathological states, these deformities can be originated from a faulty posture of the trunk related to a deformity of the spine [18-21]. An extension of interdisciplinary concepts between orthodontic and orthopedic examination had credible evidence in adolescent idiopathic scoliosis. There was a higher incidence of pain in the muscles of the neck, trunk, the upper and lower limbs, and temporomandibular joints of patients with occlusal dysfunction [18,22]. Evaluation of the higher degree of mandibular deviation to scoliosis and trunk imbalance the management of facial asymmetry related to the mandibular difference. A skeletal component in laterality disorder of mandible playing an important role in developing and sustaining facial asymmetry [16,18,23]. Vertical anomalies of occlusion were prevalent for other occlusal defects among 13% of 605 children at 3rd–5th year of Genoa primary schools with pathological gait [24]. In this study, we focus on vertical mandibular asymmetry.

Previous studies suggested that the assessment of growth and development by measuring serum *IGF-1* level might anticipate the incorrect neck position while undertaking radiography in visualizing cervical vertebrae stage. This biomarker has provided an edge over radiographic skeletal maturity assessment method recently. *IGF-1* serum levels have been an additional tool to optimize the timing of orthodontic treatment. There was a significant difference in trends and levels of *IGF-1* at different cervical stages for both sexes [25-27]. The combination of level *IGF-1* and the presence of mandibular length due to abnormal growth in condylar hyperplasia can affect dentocraniofacial development in adolescents [26,27]. Our result showed a significant difference of *IGF-1* level between early and late adolescent (Table 3). The presence of *IGF-1* was found mainly in the proliferative and hypertrophic chondrocyte layer, vice versa only a few in the calcified chondrocyte layer. There was the correlation of *IGF-1* gene with age and cartilaginous thickness [4,11,18]. The study of SNPs can help to discover the multifactorial etiologies which interaction among of genetic factors with hormonal, neurological, biochemical, and biomechanical [1,8,10].

Commonly, chest and trunk asymmetry can be used as the guidance to detect scoliosis based on physical examination screening in healthy children and adolescent according to Bunnel 1994 [1,28]. Previous studies reported that difference in ramus length and anterior nasal spine-menton angles showed a statistically significant correlation ($p<0.05$) to the difference of coracoid height, clavicular angle, radiographic shoulder height, and clavicle-rib intersection [19,20,29]. Class II division 1 malocclusion was a common malocclusion in idiopathic scoliosis subjects that had a significant effect on the condylar asymmetry index compared to other malocclusions [30].

In this study, we used *IGF-1* gene rs5742632 which reported that *IGF-1* gene was not a single marker in myopia of Chinese population [12,13]. The result of this study showed the positive correlation of *IGF-1* rs5742632 in vertical mandibular asymmetry and scoliosis symptom and might be related to the *IGF-1* role as mediating growth and development of any organ (Table 3). Development of jaw posture may influence muscles and cause postural adaption, such as asymmetric jaw growth and unbalanced muscle activity as risk factors for occlusal disease and posture in neuromuscular dentistry [31]. This condition might also be related to the variance of phenotype and multifactorial of vertical mandibular asymmetry with the variation of scoliosis symptom. In orthodontics, different clinical studies have shown different treatment results on the skeletal response of functional appliances in animal studies or growing patients. The assessment of growth potential is essential because of different maturational statuses as well as the type of maturation and mandibular growth influence the diagnosis and prognosis of orthodontic treatment. Unraveling the genetic contributions for both conditions can help to provide improved genetic counseling, prevention, and treatment strategies for this phenotype [6]. Some studies reported that the benefits of early detection of IGF-1 gene might be as one of the principal mediators in promoting muscular and skeletal growth to optimize the timing of orthodontic treatment, indeed in orthopaedic treatment [25-27]. Even though this study showed a non-significant association between *IGF-1* level and any of the SNP (rs5742632), the chronological age might be a reliable indicator for skeletal maturity concurrently with *IGF-1* level assessment in vertical mandibular asymmetry and scoliosis symptom (Table 3). Thus, we recommend limiting the degree of vertical mandibular asymmetry and scoliosis severity to assess the relationship between *IGF-1* gene polymorphisms for future studies. In understanding this gene as one of the molecular markers that are essential in genetic epidemiology studies for growing patients, we should consider the variance of *IGF-1* gene SNPs and multidisciplinary approach.

CONCLUSIONS

The result was still not conclusive due to variance in mandibular growth and curve lateral spine in adolescent patients. The clinicians should be alert to inscrutable biologic phenomenon during the active phase of scoliosis and malocclusion as well as retention phase because mandibular growth and degree of the spinal curve are unpredictable show the random variation in timing and amount. Further study will require subject increment and more specific samples to study the risk factor of vertical mandibular asymmetry.

ACKNOWLEDGMENTS

We express our gratitude to all the subject participants in this study. We would like to thank Derek and Tommy for the technical assistance. This research is supported by Ministry of Research and Technology and the Higher Education Republic of Indonesia. The support is under the research grant DRPM of the Year 2017, contract Number 003/sp2H.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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