Abstract
Scar formation after palate repair affects maxillary growth in patients with cleft lip and palate. In a previous study on denuded rat palate, basic fibroblast growth factor (bFGF) administration suppressed the postoperative scar formation. We analyzed the correlation between bFGF expression in palatal mucoperiosteal tissue and maxillary growth using the Great Ormond Street, London and Oslo (GOSLON) Yardstick in operated unilateral cleft lip and palate patients.

GOSLON Yardstick from dental casts of 8 operated cleft lip and palate patients was used. Palatal mucoperiosteal tissue samples were collected from the cleft patients during alveolar bone grafting surgery. Microscopic analysis with immunohistochemistry and modified Masson’s trichrome were performed.

GOSLON Yardstick and bFGF expression in palatal mucoperiosteal tissue showed a strong negative correlation ($r = -0.637$, Spearman test). Further, this expression differed significantly between the favorable and unfavorable GOSLON groups ($P < 0.05$, Mann-Whitney test). GOSLON Yardstick and modified Masson’s trichrome staining results showed a strong positive correlation ($r = 0.900$, Spearman test).

bFGF expression was correlated with dental arch relationships in operated cleft lip and palate patients wherein it regulated collagen fiber formation and minimized scar formation. bFGF may promote maxillary growth after cleft lip and palate repair.

Keywords: Basic fibroblast growth factor, palate repair, GOSLON Yardstick, maxillary growth, scar formation

Received date: 09 September 2018  Accept date: 18 November 2018

Introduction
Surgical wound contraction and scar tissue formation after primary palate repair affect maxillary growth of patients with cleft lip and palate. The mucoperiosteal denudation of bone is followed by wound contraction and formation of scar tissue on the denuded bone area. Palatal wound healing starts with the migration of fibroblasts, which generates tension in the matrix, resulting in differentiation of fibroblasts into myofibroblasts. During scar tissue formation, the numbers of endothelial cells and fibroblasts are decreased by apoptosis. Elastin fibers are not reconstituted during wound healing and scar tissue formation, resulting in stiff and rigid scar tissue. As a result, primary palate repair may constrict the maxillary dental arch in both the anteroposterior and transverse dimensions.1-5

The critical part of the assessment of treatment of cleft lip and palate is evaluation of the dental arch relationships. The GOSLON (Great Ormond Street, London and Oslo) Yardstick was introduced by Mars et al. in 1987 to evaluate dental arch relationships in the late
mixed and early permanent dentition stages of patients with cleft lip and palate after primary palate surgery. The GOSLON Yardstick was found to be a sensitive instrument to assess spatial discrepancy between the maxillary and mandibular dental arches. Evaluation of the anteroposterior relationship was considered the most important clinical evaluation, and evaluation of the vertical and transverse relationships helped discriminate borderline cases. Based on the dental arch relationships, patients are placed into GOSLON Yardstick categories ranging from group 5, indicating a very poor outcome and requiring combined orthodontic and orthognathic surgical treatment.8-9

Fibroblast growth factors (FGFs) function in tissue repair and regeneration. There are 23 known FGFs, and many studies have reported their role in the regeneration of damaged tissues, including skin, blood vessels, muscle, ligament, nerve, cartilage, bone, and teeth.10,11 FGFs have a role in the wound healing process, as shown by studies in which local application of FGF1, FGF2, FGF4, FGF7, and FGF10 stimulated tissue repair.12,13

Basic fibroblast growth factor (bFGF or FGF2) is found in all tissues of the human body.11,14,15 bFGF influences the proliferation of fibroblasts and other cells involved in the healing process, including endothelial cells, keratinocytes, and smooth muscle cells. It can increase the rate of wound healing in many models, including full-thickness and incisional wounds.15 bFGF has a role in the regulation of connective tissue cell migration and in the synthesis of intracellular protein and extracellular matrix.16-18 Administration of bFGF during early wound healing results in less mature scar tissue formation.19 bFGF is utilized for accelerating wound healing, improving scar quality, and regeneration with administered somatic stem cells. bFGF inhibits terminal differentiation of fibroblasts to myofibroblasts, cells that are associated with keloid and hypertrophic scar. This action is confirmed by a significant decrease of α-smooth muscle actin (α-SMA)-positive cells in dermal cell culture supplemented with bFGF. Decrease in the number of myofibroblasts may also inhibit the transition from endothelial or epithelial cells to mesenchymal cells, because the activation of dermal fibroblasts to α-SMA-positive myofibroblasts is the most important mechanism in scar formation.20,21 In a previous study, bFGF administration regulated collagen type I generation and postoperative scar tissue formation after mucoperiosteal denudation in the rat palate. These results suggest that administration of bFGF after primary palate repair may improve maxillary growth.18

Studies of the relationship of bFGF to maxillary growth in patients with cleft lip and palate after primary palate repair are very limited. Therefore, this study aimed to investigate the correlation between bFGF expression in palatal mucoperiosteal tissue and maxillary dental arch constriction in 8 operated nonsyndromic patients with unilateral complete cleft lip and palate. The bFGF expression in the palatal mucoperiosteal tissue from each patient was examined with GOSLON Yardstick using immunohistochemistry examination. Microscope sections stained with modified Masson’s trichrome were also analyzes to characterize the collagen fibers configuration in the samples.

Materials and methods

The Ethical Committee of Dental Research, Faculty of Dentistry Universitas Indonesia, approved this study, with regards of the protection of human rights and welfare in medical and dental research (No.16/Ethical Approval/FKGUI/IV/2017). The informed consent for the patients and their parents consist of information about the study objectives and procedure. The information provided included the collection of a small portion of palatal soft tissue will not interfere the results of alveolar bone grafting treatment. In addition, the procedure will also not increase the pain and will not increase the inconvenience that will be felt by the patients after the bone graft surgery. Furthermore, the wound will also heal quickly.22 The analgesic that will be administered after the alveolar bone graft surgery is adequate to overcome postoperative pain in alveolar bone graft and also in the small wound of the palate. Every parent of the patients have agreed to participate in the study and have signed a detailed informed consent form.

Research Subjects

The subjects in this research are eight female patients with non-syndromic unilateral
complete cleft lip and palate. All subjects had undergone primary lip repair with Cronin technique at the age of 3-6 months and primary palate repair with modified pushback technique at the age of 18-24 months at Harapan Kita Mother and Children Hospital, Jakarta by an expert oral and maxillofacial surgeon (MSH). The palatal mucoperiosteal tissue was obtained during alveolar bone grafting surgery, and the subjects were 11-16 years old at the time when the alveolar bone grafting surgery was performed.

The Dental Arch Relationships Evaluation

The dental casts in this study were obtained from the eight operated unilateral cleft lip and palate patients at the time they came back to Harapan Kita Hospital Cleft Lip and Palate Center, Jakarta to have orthodontic treatment. The dental casts obtained before any orthodontic appliances treatment. The age of the patients at the time of dental cast obtained was ranged from 5 years old to 15 years old. The dental arch relationships evaluation of the dental casts using GOSLON Yardstick was performed by 2 Orthodontists (AM and SN) from Kagoshima University, Japan. All of the Orthodontist examiners were completely independent from the Harapan Kita Hospital Cleft Lip and Palate team, and they have calibrated before the evaluation. The dental casts were given in random numbers to blind their origin and evaluation was performed separately on the same occasion by the examiners. After a week, the dental casts were randomized again and evaluated again. The categories of the GOSLON Yardstick range from group 1, indicating an excellent treatment outcome, group 2, indicating a good treatment outcome, group 3, indicating a fair treatment outcome, group 4, indicating a poor treatment outcome, and group 5, indicating a very poor outcome.6 The strength of agreement was assessed by calculation of the weighted kappa statistic.

The GOSLON Yardstick results were divided into two groups, namely the favorable GOSLON group (consisting of group 1, 2 and 3 GOSLON Yardstick), and the unfavorable GOSLON group (consisting of group 4 and 5 GOSLON Yardstick). The division of the results into favorable GOSLON group and unfavorable Goslons group is based on the predicted need for orthognathic surgery. The favorable GOSLON group is a group that requires only orthodontic treatment alone, while the unfavorable GOSLON group in addition to orthodontic treatment also requires orthognathic surgery.23,24

Palatal Mucoperiosteal Tissue Sample

The palatal mucoperiosteal tissue samples were collected from the palatal mucosa of the first molar region at the minor segment and located 5 mm from the gingival margin (Figure 1). This region is considered to be one of the sites where the scarring occurred after primary palate repair at the age of 18-24 months.25 The tissue samples were collected during the alveolar bone grafting surgery with a size of 8 mm x 5 mm and covers the entire full thickness of mucoperiosteum (Figure 2).

Immunohistochemistry Analysis

Immediately after collected from the patients, the tissue samples were fixated in 10% formalin and afterward, embedded in paraffin. The paraffin blocks were sectioned for 3 µm slices and mounted onto glass sections. The sections were deparaffinized in xylene and then
 Modified Masson's Trichrome Analysis

The paraffin blocks were sectioned for 4 μm slices and mounted onto glass sections. After the sections were dried (20 minutes in a 40°C warmer followed by 30 minutes in a 60°C oven), they were deparaffinized in xylene and graded alcohols and then hydrated in flowing water. The sections were treated in 10% potassium dichromate with 10% trichloroacetic acid for 40 minutes and rinsed in flowing water. After that, the sections were rinsed in 70% ethanol and immersed in resorcin fuchsin for 10 minutes to stain the elastic fibers. Next, the sections were submerged in 100% alcohol and rinsed in flowing water. Then, the sections stained in hematoxylin for 10 minutes and rinsed again in flowing water. Subsequently, the sections were immersed in hydrochloric acid, followed by immersed in ammoniac. The sections then were checked under the microscope for blue staining of the nucleus and purple staining of the elastic fiber. After that, the sections were immersed in Orange G solution for 20 minutes. Next, the sections submerged in 1% acetic acid and immediately submerged in Masson solution for 1 minute to stain the collagen fibers. Subsequently, the sections were immersed in 1% acetic acid again and followed by immersed in 1% phosphotungstic acid for 2 minutes. After that, the sections were immersed again in 1% acetic acid. Next, the sections were treated in light green solution for 5 minutes. Then, the sections were rinsed in 100% alcohol and followed by immersed in xylene and finally mounted with glass coverslip. The modified Masson's Trichrome staining technique (a combination of Elastic Verhoeff-Van Gieson staining and Masson's Trichrome staining) was used to differentiate investigation of collagen fiber (green), muscle fiber (red), elastic fiber (purple), red blood cell (orange), and nuclei (dark blue). The collagen configuration in the sections was observed microscopically and evaluated blindly by 2 examiners (HAG and AT) from Universitas Indonesia. Every section was evaluated twice, and the strength of agreement was assessed by calculation of the weighted kappa statistic. The collagen fiber configuration categories are Score 1 (normal collagen fiber configuration), in which the collagen fiber spread in parallel direction longitudinally; Score 2 (mild irregular collagen fiber configuration), which there are only a few collagen fibers spread in unparallel direction; Score 3 (less dense collagen fiber configuration with irregular orientation), which there are some collagen fibers spread perpendicularly; Score 4 (dense collagen fiber configuration with irregular orientation), which dense collagen fibers spread irregularly in circular direction; and Score 5 (disorientation and irregular collagen fiber configuration), which is the worst collagen fibers configuration observed in the sections.

Statistical Analysis

Correlation analysis between dental arch relationships evaluation using GOSLON Yardstick results and bFGF expression using real-time PCR was performed using Pearson
correlation test. On the other hand, the correlation between dental arch relationships evaluation using GOSLON Yardstick results and analysis of collagen fiber configuration using modified Masson’s Trichrome was performed using Spearman correlation test. The GOSLON Yardstick results were divided into two groups, namely the favorable GOSLON group (consisting of group 1, 2 and 3 GOSLON Yardstick), and the unfavorable GOSLON group (consisting of group 4 and 5 GOSLON Yardstick). The significance level for all tests was set at $P < 0.05$.

**Results**

All of the subjects were operated female patients with left unilateral complete cleft lip and palate. The clinical profiles of the subjects are summarized in Table 1.

**Dental arch relationships**

The kappa values of the intraexaminer agreement of the two examiners were 0.8261 (AM) and 1.00 (SN), indicating a very good agreement. Similarly, the kappa values of the interexaminer agreement between the two examiners were 0.8261 (first evaluation) and 1.00 (second evaluation), also indicating a very good agreement. The results of the evaluation showed in Table 1. The examples of GOSLON Yardstick classification of the dental casts showed in Figure 3.

**Immunohistochemical analysis of bFGF expression.**

Immunohistochemical evaluation results using BZ-X700 KEYENCE light microscope were shown in Table 2 and Figure 4. The kappa value of the intraexaminer agreement from the examiner (HAG) were 1.00, indicating a very good agreement. There are 2 samples showed very weak positive of bFGF expression or score 1 (Figure 4A). Three samples showed weak positive of bFGF expression (Figure 4B), while only one sample showed moderate positive of bFGF expression (Figure 4C). Two samples were evaluated as score 4 with moderate strong positive of bFGF expression (Figure 4D).

Spearman test analysis showed a strong negative correlation between GOSLON Yardstick and bFGF expression in the palatal mucoperiosteal tissue of operated cleft palate subjects ($r = -0.637$) (Figure 5). Comparative analysis using Mann-Whitney test between favorable GOSLON group and unfavorable GOSLON group of bFGF expression in palatal mucoperiosteal tissue samples showed a significant difference ($P < 0.05$).

**Figure 3.** Dental casts of GOSLON Yardstick classification in this study. One dental cast from favorable GOSLON group (A, B, C) and unfavorable GOSLON group (C, D, E) respectively.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>*GOSLON Classification of the dental casts</th>
<th>**bFGF expression</th>
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**Table 2.** bFGF expression in palatal mucoperiosteal tissue samples of operated patients with unilateral cleft lip and palate.

**Collagen fiber configuration analysis**

Collagen fiber configuration analysis was performed using modified Masson’s trichrome staining. Evaluation results were shown in Table 3. The kappa values of the interexaminer agreement between the two examiners (HAG and AT) were 0.8974, indicating a very good agreement. There is only 1 sample out of eight palatal mucoperiosteal tissue samples showed normal collagen fiber configuration or score 1 (Figure 6A).
One sample showed mild irregular collagen fiber configuration, while three samples showed less dense collagen fiber configuration with irregular orientation. Two samples were evaluated as score 4 with dense collagen fiber configuration with irregular orientation. One sample showed disorientation and irregular collagen fiber configuration evaluated as score 5 (Figure 6E).

The correlation analysis using Spearman test between GOSLON Yardstick result and modified Masson's trichrome staining evaluation of collagen fiber configuration showed strong positive correlation ($r=0.900$) (Figure 7). Comparative analysis using Mann-Whitney test between favorable GOSLON group and unfavorable GOSLON group of collagen fiber configuration in palatal mucoperiosteal tissue samples showed a significant difference ($P < 0.05$).

**Correlation between collagen fiber configuration and bFGF expression**

Spearman test analysis showed a strong negative correlation between collagen fiber configuration using modified Masson's trichrome staining evaluation and bFGF expression in the palatal mucoperiosteal tissue of operated cleft palate subjects ($r = -0.761$) (Figure 8).

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**Table 3.** Collagen fiber configuration evaluation with modified Masson's trichrome staining. *Collagen fiber configuration evaluation with modified Masson's trichrome staining. 1 = normal collagen fiber configuration; 2 = irregular collagen fiber configuration; 3 = less dense collagen fiber with irregular orientation; 4 = dense collagen fiber with irregular orientation; 5 = disorientation and irregular collagen fiber configuration.

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<th>Subjects</th>
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**Figure 4.** Micrograph of bFGF expression in palatal mucoperiosteal tissue samples obtained from operated cleft palate subjects (immuno histochemistry examination). A, Sample with a very weak positive of bFGF expression (score 1). B, Sample with a weak positive of bFGF expression (score 2). C, Sample with a moderate positive of bFGF expression (score 3). D, Sample with a moderate strong positive of bFGF expression (score 4). Scale bar indicates 100 μm.

**Figure 5.** Spearman correlation between GOSLON Yardstick classification and bFGF expression in the mucoperiosteal tissue of operated cleft palate subjects ($r = -0.637$, $P < 0.05$).
Figure 6. Micrograph of collagen fiber configuration in palatal mucoperiosteal tissue samples obtained from operated cleft palate subjects (modified Masson's trichrome staining). A, Sample with normal collagen fiber configuration (score 1). B, Sample with mild irregular collagen fiber configuration (score 2). C, Sample with less dense collagen fiber configuration with irregular orientation (score 3). D, Sample with dense collagen fiber configuration with irregular orientation (score 4). E, Sample with disoriented collagen fiber configuration (score 5). Scale bar indicates 100 µm.

Figure 7. Spearman correlation between dental arch relationships using GOSLON Yardstick classification and collagen fiber configuration using modified Masson's trichrome staining evaluation of the mucoperiosteal tissue from operated cleft palate subjects ($r=0.900$, $P < 0.05$).

Figure 8. Spearman correlation between collagen fiber configuration using modified Masson's trichrome staining evaluation and bFGF expression of the mucoperiosteal tissue from operated cleft palate subjects ($r=0.761$, $P < 0.05$).

Discussion

The scar tissue formation after primary palate repair in cleft palate patients has been known to affect the maxillary growth. During primary palate repair, mucoperiosteal flaps were elevated to close the cleft palate, leaving raw surface of the palatal bone. The mucoperiosteal flap elevation is followed by formation of scar tissue on the raw surface. Collagen fibers between scar tissue and raw surface of palatal bone were contributed to the rigidity of the scar tissue. The excess of collagen fibers in palatal mucoperiosteal tissue is thought to be caused by an imbalance between collagen synthesis and degradation during palatal wound healing.

bFGF is a multipotential glycoprotein that can inhibit terminal differentiation to myofibroblast, which is a crucial mediator in scar tissue. The scar tissue is known as hypertrophy of fibrous tissue with reduced numbers of blood vessels following the formation of granulation tissue in the wound healing process. In a previous study conducted by Ono et al. (2007), bFGF local administration in humans showed that there were no hypertrophic scars, and on the contrary, there were hypertrophic or extensive scars in the control group. In another study, Choi et al. (2008) reported that bFGF could suppress collagen type I generation and scar tissue formation, reducing connective strength with the adjacent teeth and palatal bone in denuded rat palate.
The study of bFGF expression in palatal mucoperiosteal tissue from operated unilateral cleft palate patients associated with maxillary growth has not been reported yet. In this study, real-time PCR analysis was used to evaluate bFGF expression in palatal mucoperiosteal tissue from 8 operated cleft palate patients associated with GOSLON Yardstick classification. Comparative study between favorable GOSLON Yardstick group (consist of GOSLON class 1, 2 and 3) and unfavorable GOSLON group (consist of GOSLON class 4 and 5) showed a significant difference. This result means that bFGF played an important role in preventing maxillary dental arch constriction.

Correlation analysis between GOSLON Yardstick classification and bFGF expression and also with collagen fiber configuration showed a strong correlation. The increase of bFGF expression level will follow by the decrease of GOSLON Yardstick classification. On the other hand, the better collagen fiber configuration analyzed using modified Masson's trichrome, will result in the better GOSLON Yardstick classification. These result also showed the important role of bFGF in preventing maxillary dental arch constriction after primary palate repair.

Collagen fiber plays a dominant role in preserving the anatomic integrity of wound healing process. The characterization of collagen fiber regarding geometrical features such as bundle orientation is required for the histopathological evaluation of wound healing. However, the collagen fiber production can be a double-edged sword. On the one hand, collagen fiber is needed for wound healing, and on the other hand, the excess deposition of collagen fiber can result in scarring. In this study, modified Masson's trichrome was used to analyze the collagen fiber configuration and also to observe the presence of elastic fiber.

Modified Masson's Trichrome staining technique (a combination of Elastic Verhoeff-Van Gieson staining and Masson's Trichrome staining) was used to differentiate investigation of collagen fiber (green), muscle fiber (red), elastic fiber (purple), red blood cell (orange), and nuclei (blue). This study showed a strong correlation between collagen fiber configuration in modified Masson's trichrome staining of palatal mucoperiosteal tissue samples and GOSLON Yardstick classification of the operated cleft palate subjects. The lower the score of collagen fiber configuration in palatal mucoperiosteal tissue, the lower also the GOSLON classification of the cleft palate subjects. On the contrary, the higher the score of collagen fiber configuration in palatal mucoperiosteal tissue, the higher also the GOSLON classification of the cleft palate subjects. This correlation result means that the more irregularity of the collagen fiber in palatal mucoperiosteal tissue, the poorer also the intermaxillary relation.

In a previous study, the injection of bFGF into palatal tissue after mucoperiosteal denudation showed the suppression of excessive collagen fibers. Thus, reducing connective strength with the adjacent teeth and palatal bone, and minimize scar tissue formation. Other study examined the effect of bFGF administration in the formation of hypertrophic scar of dorsal skin tissue and got limitation of hypertrophic scar results. These studies result strengthen the vital role of bFGF in preventing scar tissue formation.

One of the limitations of this study is the small number of research subjects that can be included. This number of research subjects seems to be a limitation for statistical analysis. The other limitation of this study is the age difference of the study subjects. The age of the subjects varied from 11 to 16 years when palatal mucoperiosteal tissue was obtained. It was difficult to find patients who were ready to undergo an alveolar bone graft surgery. Furthermore, many patients did not return for orthodontic treatment after palate repair. Therefore, operated unilateral cleft lip and palate patients who come to the cleft lip and palate clinic for alveolar bone grafting were included in the study despite their varying age.

Another limitation was the presence of dental caries that make it difficult in GOSLON Yardstick assessment. Dental caries probably because many research subjects did not return for dental treatment and orthodontic treatment after completion of palate repair and speech therapy.

Conclusions

bFGF expression correlated with dental arch relationships in operated cleft palate patients through its ability to regulate the level of collagen fiber formation and minimized the scar tissue formation. This result leads opportunity for
bFGF application in cleft palate patient to provide more favorable maxillary growth after primary palate repair. Nevertheless, the use of bFGF needs clinical trials to determine the efficacy and safety of its administration in human.

**Acknowledgements**

DA would like to thank Orie Iwaya for the supervision in Pathology Laboratory, Kagoshima University Hospital. This study was supported by The Directorate of Research and Community Engagement Universitas Indonesia (DRPM UI) through the Doctoral Student Final Task Grant 2018 (Hibah TADOK DRPM UI 2018). All authors contributed to manuscript preparation and approved the full version.

**Table 1.** Clinical Profiles of the Operated Complete Unilateral Cleft Lip and Palate Subjects.

<table>
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<tr>
<th>Number</th>
<th>Gender</th>
<th>Side affected by the unilateral cleft lip and palate</th>
<th>Age at the time mucoperiosteal palatal tissue collected during alveolar bone grafting surgery (y)</th>
<th>Age at the time study model was obtained before orthodontic treatment (y)</th>
<th>GOSLON Classification of the dental casts*</th>
<th>Favorable/unfavorable GOSLON Classification**</th>
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Table 1. Clinical Profiles of the Operated Complete Unilateral Cleft Lip and Palate Subjects.

*GOSLON Yardstick classification; 1=excellent; 2=good; 3=fair; 4=poor; 5=very poor; **Favorable/unfavorable GOSLON Classification; 1=favorable GOSLON group consists of group 1,2,3,4,5; 2=unfavorable GOSLON group consists of group 4 and 5 GOSLON.

**References**