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Voice quality assessment in cases of vocal fold gaps after single growth factor injection

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ORIGINAL STUDY

Voice quality assessment in cases of vocal fold gaps after single growth factor injection

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Abstract

Background: **In severe vocal fold (VF) lesions, deposits of disorganized, thick collagen bundles and little hyaluronic acid (HA) are deposited in the superficial lamina propria (SLP). These changes lead to severe dysphonia and aspiration, which could be complicated by fatal pneumonia. Unfortunately, no specific treatment has been recognized due to unpredictable. VF regeneration. Current progress in regenerative medicine has allowed the development of tissue engineering techniques using cells, scaffolds, and growth factors (GFs). Extrinsic GFs application could help in the induction of the regenerative process.**

The aim: **To assess the voice quality after a single GF injection of the VF in cases of VF gaps through pre- and post-injection voice analysis measures. Material and methods: Twenty patients of both genders aged 20, 60 years were selected as candidates for GF injection using "fiberoptic laryngoscopy" under local or general anesthesia. They were subjected to pre- and post-injection (after 3 and 6 months) assessments using objective voice analysis, "computerized speech lab" (CSL), and subjective voice analysis by "auditory perceptual analysis" (APA).**

Results: **There is a statistically significant difference regarding APA with P value less than 0.001, where 85.7 % of cases were improved (no dysphonia) after 3 months postinjection as the growth factor acts for only 3 months.**

Conclusion: **A single GF injection in the VFs in cases of VF gaps revealed improvement in voice quality after 3 months post-injection by APA and CSL.**

Keywords: Growth factor, Vocal folds gap, Immobility, Sulcus vocalis, Injection

1. Introduction

Vocal folds (VFs) normally consist of a pair of mucosa measuring 15−20 mm in length. In terms of mechanics, the 'VFs' structure can be conceptualized as comprising three layers: the outer cover (made up of epithelium and the topmost layer of the lamina propria), the intermediate transition layer (encompassing the intermediate and deep layers of the lamina propria), and the inner body layer (comprising the vocalis muscle). The superficial layer of the superficial lamina propria (SLP) constitutes a stratum containing an undefined substance and microfibrils that facilitate its gliding motion over the underlying deep layer, thereby contributing to its elastic and vibratory capabilities. Under normal circumstances, the SLP is characterized by a lax composition and a high concentration of interstitial proteins [1]. VF closure is responsible for the majority of phonation since the vibratory waves of the VF mucosa are responsible for the quality of voice produced [2].

The SLP accumulates disordered, dense collagen bundles and little hayaluronic acid (HA) in severe VF lesions like VF scars, sulci, and atrophy. These histological alterations lead to VF sclerosis, dampening of vibration, and inadequate closure of the glottis. Consequently, these changes give rise to pronounced symptoms, including voice alterations, phonasthenia, and the risk of aspiration, which can potentially result in fatal pneumonia [1].

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The VFs have a respiratory function: they open during respiration to allow gas exchange protective function, close during swallowing to protect the airway and phonatory function and engage in vibration as air flows through them during speaking or singing. These folds consist of delicate layers with a gentle outer covering. Beneath lies a more rigid ligament that connects deep within the ligament through the thyro-arytenoid muscle. The bulk of the VF is comprised of the thyro-arytenoid muscle. If one or both folds weaken or atrophy, a space emerges between them, leading to a condition known as VF bowing. This phenomenon obstructs full closure and proper vibration, affecting their normal functioning [3].

Management of glottal gaps depends on the type and size of the gap; for all nonorganic and some paralytic gaps from 1 to 1.5 mm, VF augmentation is the treatment of choice; from 1.5 to 3 mm, VF augmentation is done either through permanent or temporary materials; and more than 3 mm are treated by trans-thyroid medialization of the VFs [4]. Temporary VF injection is currently the preferred approach to treating glottic incompetence when the likelihood of recovery remains uncertain, and this is particularly notable in acute unilateral VF paralysis or paresis, a complication observed in 20–60 % of cases following thyroid gland surgery. Within the potential recovery timeframe, typically up to 6 months postonset, using a short-term substance in VF injection has effectively alleviated vocal symptoms and enhanced swallowing capabilities. This strategy serves as a bridge until either function regains normalcy or the patient becomes eligible for a more enduring treatment solution [4].

By the close of the 20th century, advancements in tissue engineering and regenerative medicine had emerged to revitalize lost organs and restore their capabilities. Research revealed that the basic fibroblast growth factor (bFGF) spurred the generation of HA by VF fibroblasts and curtailed collagen production in animal trials. The practical application of bFGF was extended to human patients afflicted with VF scars, atrophy, paralysis, and sulcus. This approach exhibited noteworthy enhancements in VF voice functions, signifying a substantial step forward in this field [5].

According to Bradshaw et al. [6] and Andreopoulos and Persaud [7], fibroblast growth factors (FGFs), operating via FGF receptors, play a pivotal role in overseeing a broad spectrum of biological processes. Organ transplantation and synthetic implants are the prevailing and extensively employed techniques for addressing human tissue and organ loss. Nonetheless, there persists a need for novel solutions and methodologies to combat tissue failure, as a definitive remedy remains elusive; this has led to a heightened focus on regenerative medicine and tissue engineering, emerging as promising alternatives for repairing or regenerating compromised tissues.

The concept of this study depends on the promising results of Kanazawa et al. [8], which employed bone marrow-derived mesenchymal stem cells to regenerate damaged VFs in a canine model. In addition, another animal study [9] revealed that hepatocyte growth factor and bFGF induced the production of HA by VF fibroblasts while concurrently diminishing collagen production.

This study aims to assess the voice quality after single growth factor injection of the VFs in cases of VF gaps through preinjection and postinjection voice analysis measures.

2. Patients and methods

The institutional committee's ethical criteria were followed during all proceedings. The Ethics Committee approved the study.

This prospective study involved a cohort of 20 adult patients spanning both sexes, aged between 20 and 60 years. Of the participants, 14 (70 %) were male, whereas six (30 %) were female, with VF gaps (atrophy, sulcus vocalis, and VF paralysis) who were candidates for GF injection (LC Revive) into the VF using a fiber-optic laryngoscope under local or general anesthesia and presented at ENT and phoniatrics' outpatient clinic, Kasr El Ainy Hospital, in the period from February 2022 till August 2022.

2.1. Inclusion criteria

(1) A VF gap was diagnosed using a laryngoscope (phonatory gap >2 mm from the midline posteriorly).
(2) Male and female patients over 20 years.
(3) No response for voice therapy for 6 months or more.

2.2. Exclusion criteria

(1) Other co-existing laryngeal pathologies include MAP lesions, functional VF disorders, and premalignancy (e.g. leukoplakia).
(2) Presence of thrombocytopenia, platelet dysfunction, or coagulopathy.
(3) Bilateral VF paralysis near the midline.
(4) Underlying liver disease, cancer, autoimmune disease, or compromised respiratory function.

All patients underwent the following assessments:

2.2.1. Preliminary diagnostic procedures

(1) Conducted patient interviews involving history-taking and clinical examinations. Written consent from the guardians of all patients was obtained for their participation in the study and potential publication.
(2) Two experts performed an auditory perceptual assessment (APA) of voice. This assessment covered overall severity, roughness, breathiness, strain, pitch, and loudness, graded on a 0–3 GRBAS scale [2].

2.2.2. Clinical diagnostic aids

Flexible laryngoscopy was employed for laryngoscopic assessment.

2.2.3. Supplementary instrumental measurements

Acoustic analysis was conducted using the computerized computerized speech lap model 4500 (Kay Elemetrics, Bridgewater Lane, Lincoln Park, NJ 07035, USA). The patients were asked to produce a sustained vowel "\a" at their comfortable frequency and amplitude levels for the analysis. The signal was captured in an acoustically treated room using a dynamic microphone positioned 20 cm anterior to the patient's mouth. Each of the following parameters was recorded: mean fundamental frequency (F0) in Hz, shimmer%, jitter%, maximum phonation time (MPT), and noise to harmonic ratio (N\H).

2.2.4. Injection set up

The procedure was done under local or general anesthesia. GF was injected in the SLP of the anterior two-thirds of the VFs. The material used is GF (LC Revive), a rejuvenating formula with human adipose stem cells that enhances collagen production and accelerates cellular regeneration. So, it is widely used by physicians worldwide with injection techniques. It is a synthetic peptide containing 13 amino acids and an elastin-derived peptide that upgrades elastin and hyaluronic acid expression and increases fibroblastic activity [10]. In this study, we consider a single bFGF injection of 1–2 ml in the VFs, whether office-based (after injection of local anesthesia lidocaine 2 % through the cricothyroid membrane using a 22-G syringe) or under general anesthesia while visualization of the larynx is done by flexible nasal endoscopy. However [9], the clinical trial involved the administration of bFGF through a local four-time injection method. Each injection consisted of 10 mg of bFGF dissolved in 0.5 ml saline solution. These injections were applied to each VF while the patient was under topical anesthesia. The injections were performed repeatedly during the trial (Fig. 1).

2.3. Statistical analysis

Descriptive analysis was conducted on the collected data, ensuring its completeness and logical coherence. Data was precoded and input into Microsoft Office Excel Software Program 2019. Subsequently, it was transferred to the Statistical Package of Social Science Software program, version 26 (SPSS), for statistical analysis. Data was presented as mean, SD, median, and interquartile range for quantitative variables. Group comparisons were performed using the Mann–Whitney *U* test and Friedman's test. Group comparisons were conducted using the χ^2 test and Fisher's exact test.

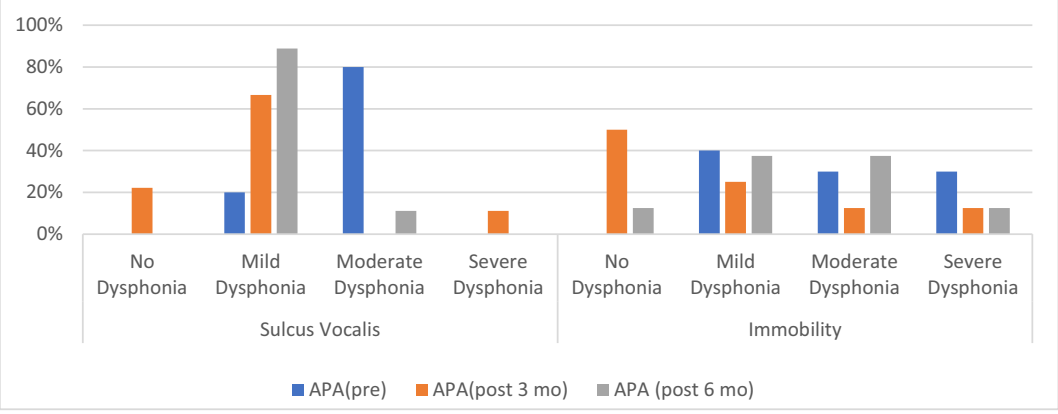

Fig. 1. APA preinjection and postinjection between sulcus vocalis group and immobility groups over time.

3. Results

3.1. Demographic data

Twenty patients were recruited from the ENT and phoniatric outpatient clinics in Kasr El Ainy. Their ages range from 20 to 60 years. The mean age was 33 ± 11 years, and the median was 32 years. Fourteen (70 %) patients were males, while six (30 %) were females.

The cases under study underwent a single injection of GF in the VFs. This study focused on two types of pathological lesions [sulcus vocalis in 10 (50 %) patients and unilateral VF immobility in 10 (50 %) patients]. Regarding the presence and absence of reflux, 15 (75 %) patients had reflux. Regarding reflux disease, 50 % of patients with sulcus vocalis had reflux. In comparison, 100 % of patients with unilateral VF immobility had reflux, so the treatment protocol included treatment for reflux. We assume that reflux could negatively affect the patients' voice outcome, so it should be included in the treatment protocol in further studies .

Table 2. Comparison between mean fundamental frequencies (F0), jitter, shimmer, N\H ratio, and maximum phonation time (preoperatively, postoperatively 3 months and post 6 months.

	P value
Mean F0 (pre) vs. mean F0 (post 3 months)	0.088
Mean F0 (pre) vs. mean F0 (post 6 months)	0.060
Mean F0 (post 3 months.) vs. mean F0 (post 6 months)	0.650
Jitter% (pre) vs. Jitter% (post 3 months)	0.496
Jitter% (pre) vs. Jitter% (post 6 months)	0.248
Jitter% (post 3 months) vs. Jitter% (post 6 months)	0.433
Shimmer% (pre) vs. Shimmer% (post 3 months)	0.820
Shimmer% (pre) vs. Shimmer% (post 3 months)	0.859
Shimmer% (post 3 months) vs. Shimmer% (post 6 months)	0.530
N\H ratio (pre) vs. N\H ratio (post 3 months)	0.496
N\H ratio (pre) vs. N\H ratio (post 6 months)	0.505
N\H ratio (post 3 months) vs. N\H ratio (post 6 months)	0.972
MPT (pre) vs. MPT (post 3 months)	0.071
MPT (pre) vs. MPT (post 6 months)	0.969
MPT (post 3 months) vs. MPT (post 6 months)	0.022

MPT, maximum phonation time.

There is a statistically significant difference regarding the (maximum phonation time) in favor of 3 months postoperatively with a *P* value of 0.022.

3.2. Comparison data

Normally, MPT ranges from 15 to 30 s for normal people. When the MPT is under 10 s, many individuals experience breathlessness during conversation, indicating an underlying pathology (Table 2).

Regarding subjective voice evaluation, the extent of dysphonia was appraised using APA scores, spanning from no to severe. No statistically significant difference was observed between the severity of dysphonia in the two distinct pathological lesions before and at 3 and 6 months postsurgery (Table 1).

However, before the surgical intervention, most cases in the sulcus vocalis group exhibited moderate dysphonia (80 %). Top of form eight patients out of 10, while most unilateral VF immobility cases were mild dysphonia (four out of eight). Three months postoperatively, 60 % of patients in the sulcus vocalis group experienced mild dysphonia, and 20 % of patients had no dysphonia, versus 50 % of patients (four out of eight) in the unilateral VF immobility group who experienced total improvement.

Six-month follow-up showed 80 % of patients with mild dysphonia in the sulcus vocalis group versus three patients who experienced mild dysphonia and 30 % with moderate dysphonia in the unilateral VF immobility group (Table 3).

4. Discussion

Over the past year, glottal insufficiency has been a challenge for the patient's quality of life. Voice therapy, office-based or operative injections, and medialization laryngoplasty procedures improve glottal insufficiency [11].

This study aims to stimulate the regeneration of VF structures and their augmentation for better glottal closure. Recent technologies use regenerative medicine and tissue engineering to regenerate lost organs and restore their functions.

Concerning demographic data, this study encompassed 20 patients: 10 were diagnosed with

Table 1. Comparing the APA preoperatively, after 3-month and 6-month postoperatively.

Number of patients	10 patients	17 patients	17 patients	*P* value
APA [*n* (%)]	Pre	3 months post	6 months post	
No dysphonia	0	6 (85.7)	1 (14.3)	<0.001
Mild dysphonia	6 (24.0)	8 (32.0)	11 (44.0)	
Moderate dysphonia	11 (68.8)	1 (6.3)	4 (25.0)	
Severe dysphonia	3 (50.0)	2 (33.3)	1 (16.7)	

There is a statistically significant difference regarding APA with *P* value less than 0.001, where 85.7 % of cases were improved (no dysphonia) after 3 months postinjection as the growth factor acts for only 3 months.

sulcus vocalis and another 10 with unilateral VF immobility. Their ages ranged between 20 and 60 years. The average age of the patients was 33 ± 11 years, with a median age of 32 years. Fourteen (70 %) participants were male, while the remaining six (30 %) were female, with a 6-month follow-up. In our study, a smaller sample size was taken to assess the GF efficiency in cases of sulcus vocalis and immobility as a first step for a series of studies on a larger sample size with a longer follow-up period. No cases of atrophy or scarring were presented in the Kasr Al-Ainy outpatient clinic during the study period.

A history of reflux was observed in 75 % of the cases in this study, including five patients with sulcus vocalis and 10 patients with unilateral VF immobility, which may affect voice outcome as the patient has congestion and increased salivation with a gag reflex that affects the view for injection. As a result of the small sample size, there was no statistical difference. Control of reflux is critical, so medical treatment is not curative. Lifestyle changes and diet modifications are extremely effective at managing acid reflux [12].

This study improved the MPT and the subjective voice quality assessment by APA. There was a statistically significant difference in favor of post-3-month follow-up versus preinjection with a *P* value less than 0.022 regarding the maximum phonation time; however, there is no statistical difference in 6-month follow-up versus preinjection. Regarding the subjective voice quality assessment in this study using the APA score, there was a statistically significant difference with significant improvement in the degree of dysphonia. According to Hirano et al. [9], the group that received injections exhibited improvement in both VHI-10 values and subjective assessments of voice quality. In line with a previous study [8], significant differences were noted between preinjected and postinjected values of MPT, jitter, and VHI. These findings suggest that the single injection method yields comparable results to the four-time repeated injection method or the regenerative surgery explored by the study [9]. MPT offers a rough gauge of VF closure. The degree of closure directly affects air conservation and the duration of sound maintenance. Generally, individuals with an MPT under 10 s often experience breathlessness while speaking. Individuals with healthy vocal function can typically exceed 20–30 s.

Numerous variables influence this test, including lung capacity and the vocal techniques employed to produce sound. However, maintaining constant pitch and volume enhances the assessment's focus on VF approximation [13].

There is no statistically significant difference between the two pathological lesions regarding the APA scores, although a small sample size caused this finding and did not indicate the effectiveness of the bFGF injection, as there was an improvement in the majority of cases regarding the APA score, including the two pathological lesions. After the 3-month follow-up, there were eight (80 %) patients out of 10 with mild dysphonia (60 % in sulcus vocalis and 20 % in unilateral VF immobility). However, six patients improved regarding dysphonia (two out of nine), 20 % in the sulcus vocalis, and four out of eight (50 %) in unilateral VF immobility. In a previous study by Kanazawa et al. [8], there was no significant difference in any parameters of computerized speech lap or subjective assessment in the sulcus subgroup. This difference may be because our study identified and

Table 3. Statistical analysis of mean fundamental frequency (F0), jitter %, shimmer%, noise to harmonic ratio (N\H), and maximum phonation time preoperatively, 3-month and 6-month postoperatively.

	Mean	SD	Median	Percentile 25	Percentile 75	*P* value
Mean F0 (pre)	199.8	71.1	213.7	145.0	238.4	0.104
Mean F0 (post 3 months)	185.4	63.7	198.2	141.1	242.8	
Mean F0 (post 6 months)	187.5	74.5	180.6	148.1	230.3	
Jitter% (pre)	3.0	2.7	1.8	1.5	3.7	0.404
Jitter% (post 3 months)	2.9	2.3	1.7	1.3	3.8	
Jitter % (post 6 months)	2.4	2.4	1.6	1.3	2.6	
Shimmer% (pre)	7.1	6.1	4.7	3.6	8.8	0.786
Shimmer% (post 3 months)	9.0	8.8	4.7	4.0	10.1	
Shimmer% (post 6 months)	6.6	3.6	4.7	3.5	9.6	
N\H ratio (pre)	0.2	0.1	0.1	0.1	0.2	0.601
N\H ratio (post 3 months)	0.2	0.2	0.1	0.1	0.3	
N\H ratio (post 6 months)	0.2	0.2	0.1	0.1	0.3	
MPT (pre)	8.3	2.6	8.0	6.5	11.0	0.092
MPT (post 3 months)	10.1	4.5	9.0	7.0	12.0	
MPT (post 6 months)	8.3	3.7	8.0	6.0	10.0	

MPT, maximum phonation time.

According to the objective analysis of voice by measures of voice parameters using the computerized speech lap, there is no significant difference preoperatively, 3 and 6 months postoperatively.

treated patients with reflux in the postinjection treatment protocol. As we assumed, reflux could worsen voice outcomes.

As regards [9], whose study incorporated voice therapy as a preliminary measure before administering bFGF injection, specifically for patients with mild to moderate VF atrophy, after the injection, voice therapy was provided to all patients, except for those who did not necessitate or wish for this intervention due to satisfactory vocal improvement resultant solely from the injection. The objective of voice therapy was to guide patients inappropriately utilizing their regenerated VFs, considering that these folds were now more voluminous than their condition before the injection, which could be the same assumption as our study. In the current study, we assume that voice therapy could positively affect the quality of voice postinjection because the patients were trained to use their voice properly since they used to strain to overcome the bad quality of voice resulting from the VF gap, as in sulcus patients.

In this study, we found that four patients underwent VF injection under general anesthesia, including two female patients who had difficulty identifying the cricothyroid membrane due to a scar from a previous thyroidectomy and a short, wide neck, and two older males (>50 years) with an ossified larynx. Otherwise, past studies only underwent office-based injections [8,9].

VF injection presents itself as a surgical alternative to laryngeal framework surgery. While both approaches have merits and limitations, no definitive algorithm exists for determining when one method might be preferable. Generally, VF augmentation is employed to temporarily address incompetence arising from unilateral VF immobility and permanently correct mild-to-moderate glottic insufficiency [14].

Temporary VF injection is the preferred treatment for cases of 'glottic incompetence' when the prognosis for recovery remains uncertain; this applies particularly to situations like acute unilateral VF paralysis or paresis. Within the window of potential functional recovery, typically spanning up to 6 months post-onset, the administration of a short-term substance through VF injection has demonstrated efficacy in alleviating voice-related symptoms and enhancing swallowing until normal function is restored or the patient becomes eligible for a more permanent treatment alternative [4].

As mentioned in Macri and Clark [15], the most suitable materials for such procedures should be biodegradable, biocompatible, and capable of serving as a supportive artificial extracellular matrix until neighboring cells generate natural tissue as the biomaterials gradually degrade. Utilizing GFs has gained significant appeal in achieving these objectives, given their capacity to influence and regulate various cellular processes integral to tissue healing.

According to Hirano et al. [9], video stroboscopic examination exhibited improved 'mucosal wave' and 'complete glottic closure,' with these positive effects persisting for up to 3 months. 'Mucosal wave amplitude' (normalized) and 'glottis closure' (normalized glottal gap) also demonstrated enhancements following the administration of bFGF. Patients experienced stronger voices with reduced dysphonia. MPT showed improvement, and acoustic parameters similarly indicated enhancements from 1 week to 3 months after treatment.

Finally, we assume that GF injection of the VFs has a promising effect on patients' voice outcomes and quality of life since it improves dysphonia and participates in tissue regeneration with HA production. So, GF may be used alone or combined with other injection methods for better results as a temporary treatment option.

Institutional review board (IRB) approval number

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Conflicts of interest

Authors declare no conflict of interest. The authors hereby have no funding agency and the research was self-funded.

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