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Experimental Research

Randomised clinical trial: Effect of administering platelet-rich fibrin to autologous fat tissue in injection laryngoplasty for vocal cord paralysis

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ABSTRACT

The vocal cord in humans is essential in producing voice used in communication and interaction between us. Vocal cord paralysis causes dysphonia, which interferes with communication, causing disruptions towards social activity and daily activities. One of the managements for vocal cord paralysis is medialization and augmentation of the vocal cord through injection laryngoplasty. Autologous fat is one of the best fillers used in this procedure, but it is highly absorbable and can be reabsorbed very quickly when injected into body tissues. Platelet Rich Fibrin (PRF) is a biomaterial consisting of growth factors that are thought to improve fat tissue viability by increasing adipogenesis and angiogenesis. Improvement in fat viability will improve clinical outcomes after the laryngoplasty procedure, potentially reducing the number of repeated injections needed to achieve a satisfactory resolution to vocal cord paralysis. The study evaluates a combination of PRF and autologous microlobular fat compared with autologous microlobular fat alone on laryngoplasty. This single-blinded randomised control trial recruit a total of 18 patients, which are then randomised into the treatment and control groups. The evaluation was done via computerized acoustic analysis/Multidimensional Voice Program (MDVP) parameters and maximum phonation time. The MDVP results and maximum phonation time in both groups showed clinical improvement after the operation with no statistically significant differences.

1. Introductions

Voice is a medium for communication and interaction between two individuals. The backbone of voice production in humans is supported by an intricate breathing system. Phonation proses started from energy generation from the lung, followed by movement of the vocal cord and contraction of laryngeal intrinsic muscles. Paralysis in the movement of vocal cord will result in abnormal phonation (dysphonia) [1].

Evaluations of dysphonia caused through disruption in phonation quality, structure, and function of the vocal cord can be conducted objectively. Computerized acoustic analysis such as Multidimensional Voice Program (MDVP) and maximum phonation time can give objective analysis on the state of the vocal cord's structure, functions, and quality [2,3]. These modalities (and combination of it) can help clinician diagnose and promptly treat any disruption in the vocal cord which may cause disruption in phonation.

Injection laryngoplasty is one of the methods aimed at medialization and augmentation of the vocal cord which can help treat dysphonia caused by paralysis of the vocal cord [4]. One of the fillers commonly used in injection laryngoplasty is fat. As an autograft medialization material, it is known to had low incidence of complication and rejection from the body [5]. Injection laryngoplasty using microlobular fat autograft had been known to help with vocal cord paralysis causing dysphonia, however, due to the rapid absorption of fat in the tissue, repeated injections are required to maintain the result of the medialization augmentation [6].

Adipose tissue in fat cells contain large number of adipose stem cells (ASC). ASC in fat graft can secrete growth factors and cytokines that can

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increase the angiogenesis process, local vascularization and blood vessel supply, and can slow down the immune response process. With its unique properties, ASC content can reduce the acceleration of reabsorption by inducing adipogenesis and angiogenesis [7,8]. In theory, replacement substance with similar properties, such as growth factors, can be given to induce similar processes that would then increase the viability of the fat graft.

Platelet Rich Fibrin (PRF) is an advanced form of Platelet Rich Plasma (PRP) which is known to contain a high concentration of platelet, fibrin formation, and slower release of growth factor [9]. PRF can play a role in platelet aggregation by pooling free platelets. The presence of platelets is essential because it would release a high concentration of growth factors that will stimulate the processes of angiogenesis and adipogenesis. Thus, a combination of fat autograft with PRF as an augmentation medialization material for vocal cord paralysis is expected to reduce the possibility of fat reabsorption, reduce the occurrence of repeated augmentation, reduce morbidity, and improve quality of life [10,11].

2. Methods and material

2.1. Study design

This research is a randomised clinical trial with a single-blinding approach on the study's participants. Randomization was achieved by block randomization to get an equal number of participants for the treatment and control group. The treatment group will receive injection laryngoplasty with a combination of PRF and microlobular autograph fat, while the control group receive injection laryngoplasty with microlobular autograph fat. A baseline measurement including routine blood examination, chest x-ray and consultation to the internal medicine and anesthesiology department was conducted before surgery. Measurements will be carried out before surgery, 1-week, 4–6 weeks, and 8–10 weeks after injection laryngoplasty to evaluate phonation quality and the vocal cord's structure (see summary in Fig. 1). The research had passed ethical review from the Faculty's Health Medicine Research Ethics Committee and was carried out for six months.

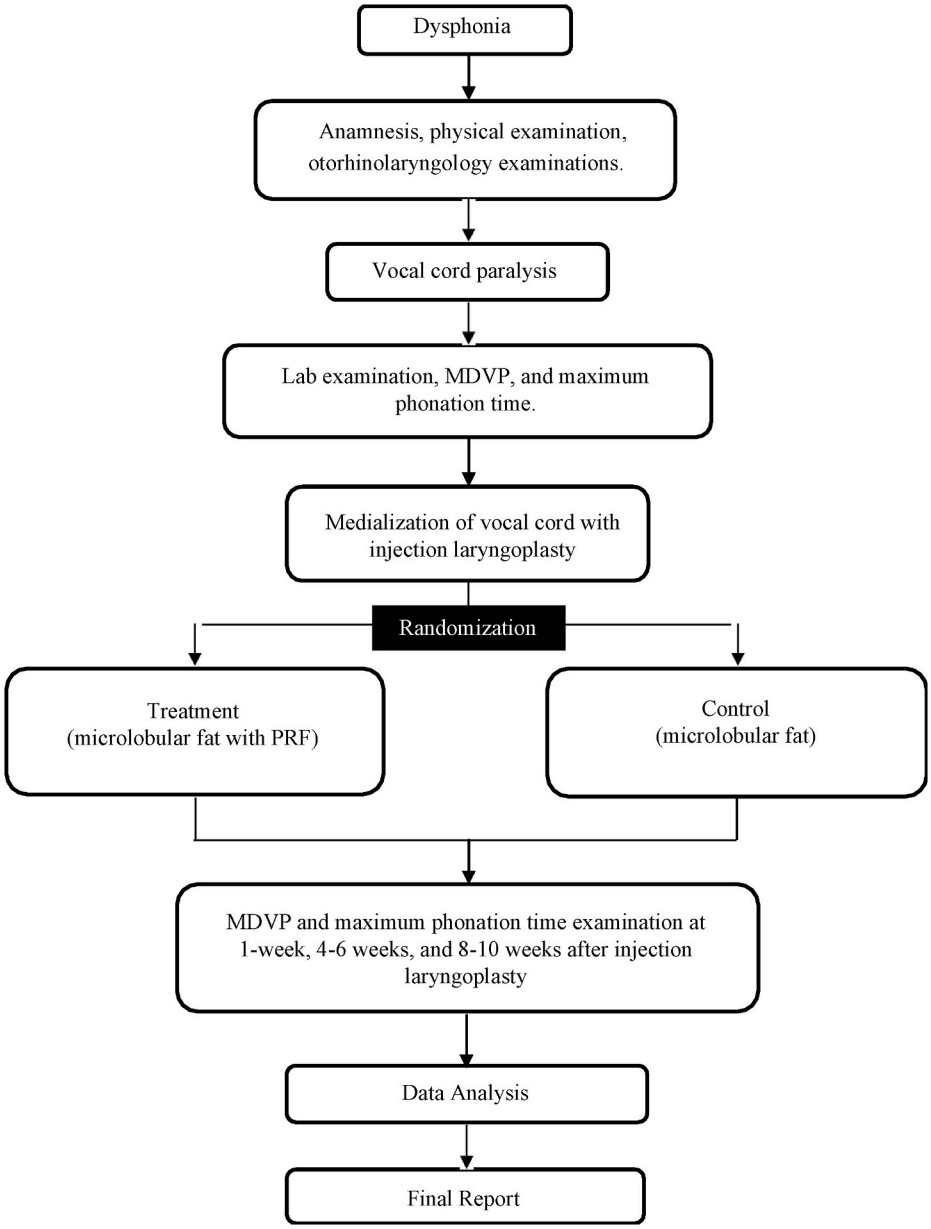

Fig. 1. Research workflow.

2.2. Eligibility criteria

Patient coming to the Otorhinoplaryngology Head and Neck Department who is diagnosed with unilateral vocal cord paralysis are recruited if they fulfill the following inclusion criteria: (1) Participant is diagnosed with unilateral vocal cord paralysis in paramedian position or 3 months lateral onset without movement and mucosal waves of the vocal cords on videostroboscopy. (2) Age between 18 and 70 years old. (3) Willing to give consent. The patient is not eligible to participate if they meet any of the following exclusion criteria: (1) Has a history of malignancy of the larynx or lung. (2) The participant isn't able to undergo an injection laryngoplasty procedure under general anesthesia. (3) Participant with thrombocytopenia.

2.3. Participant enrolment

Patients with dysphonia are assigned to clinical evaluations (Fig. 1). Eligible patients will receive an explanation from the researcher regarding research procedures. Those willing to participate in the study signed informed consent and were assigned as research participants. All available participants were taken by consecutive sampling technique and assigned to groups using block randomization to achieve equal group size. The treatment group was assigned to laryngoplasty injection treatment using PRF added fat filler while the control group was assigned to laryngoplasty injection treatment using only fat filler.

2.4. Preparation of autologous microlobular fat fillers

Autologous microlobular fat were harvested from abdominal fat (area under the umbilical). Lidocaine was infiltrated under the umbilicus and then an incision was made in the area followed by fat removal using scissors. The fat was cleaned with 0.9% NaCl solution and then sheared into microlobular form. Four mL of microlobular fat is mashed by pushing it back and forth 15 times in a container of 2 piston tubes (10 mL) connected with a three-way connector.

2.5. Preparation of PRF with autologous microlobular fat fillers

The PRF was made by taking 10 mL of peripheral blood from a healthy donor (with no hematological abnormalities and no blood transmitted disease). Blood is then inserted to the tube from the Regen Lab kit. The tube was centrifuged on a RegenLab 642VFD PRP Fixed Angle Centrifuge (Regen Lab, Le Mont-sur-Lausanne, Switzerland) with a force of 1500 g (3000 rpm) for 5 min, after which the tube is turned 3 times over. Four mL of the resulting substance was transferred to a 10 mL test tube followed by adding 1 M CaCl2 with a micropipette. The tube was then turned for 3 times, producing PRF. Autologous fat harvested from the patient's abdomen (as explained in section 2.4) was then mixed with the PRF solution to create the filler for injection laryngoplasty procedures.

2.6. Injection laryngoplasty procedures

The patient is put in a supine position on the operating table under sedation followed by aseptic and antisepsis procedures. The patient is placed in a sniffing position followed by the insertion of intraoral Kleinsasser laryngoscope through the uvula, posterior pharyngeal wall, and epiglottis until the vocal cords are visible. The process of harvesting fat from the abdomen is carried out. The surgical scar from harvesting of fat around the umbilicus area is closed with sutures and a sterile bandage. For the control group, the crushed fat is injected into the affected vocal cord as much as 3 mL using a 12 G laryngoplasty syringe until medialization is achieved. For the treatment group, 4 mL of microlobular fat is added to 4 mL of PRF and is smoothed by pushing it back and forth 15 times between 2 separate 10 mL syringe connected by a three-way connector (Fig. 2). The mixture of fat and PRF is injected into the affected vocal cord as much as 3 mL using a 12 G laryngoplasty syringe until medialization is achieved. After the injection, Kleinsasser's laryngoscope was removed and the procedure was complete. In order to minimize variabilities and confounding factors during the laryngoplasty procedures, all of the operations were done by the same surgeon accompanied by the same two senior surgeons.

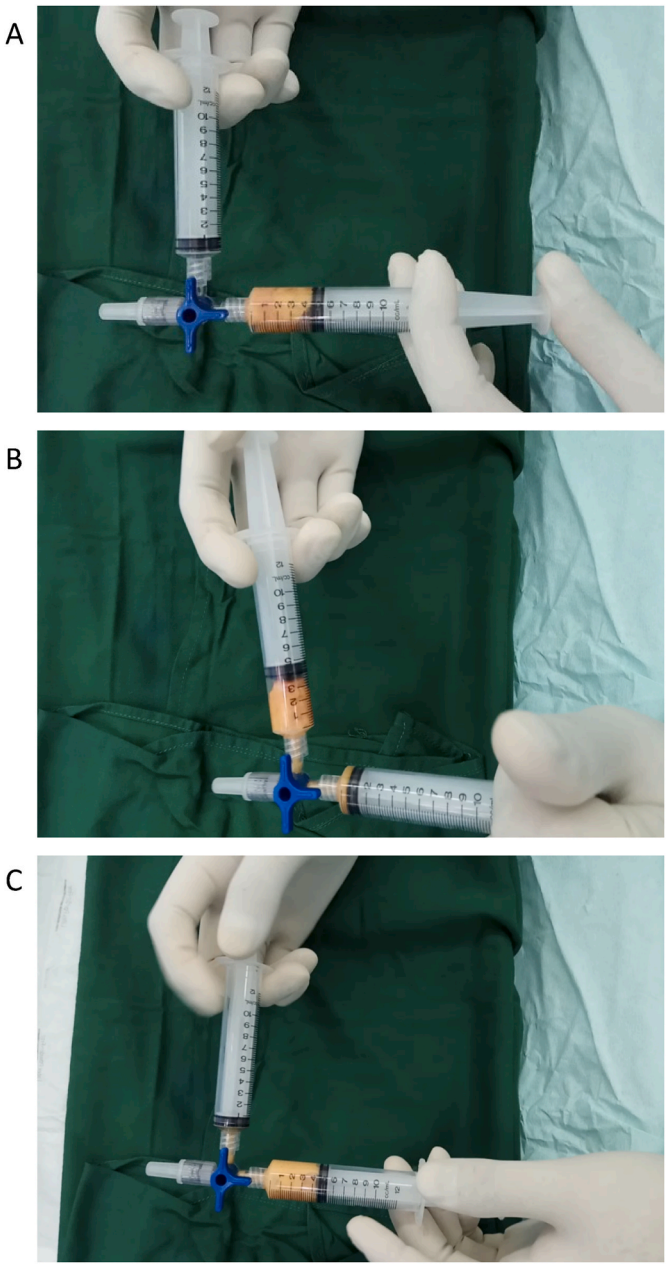

Fig. 2. Smoothing of microlobular fat while mixing it with PRF. (A) A mixture of 4 mL microlobular fat and 4 mL PRF is added to a 10 mL syringe. (B) The mixture is pushed back in forth 15 times in between 2 separate 10 mL syringe connected by a three-way connector. (C) The end product for injection, note that the final mixture is more liquified in consistency.

2.7. Participant follow-up and outcome measures

Follow-up was conducted on the 1st week, 4th - 6th weeks, and 8th - 10th weeks after injection laryngoplasty. A time range for follow-up was given because the participant needed to go and visit the hospital clinic. Examination of the patient's clinical condition was done followed by MDVP and maximum phonation time.

Multidimensional Voice Program (MDVP) 4500 produced by Kay Elemetric Corp was used for voice evaluation. The parameters measured are (1) F0 (Average Fundamental Frequency) which represents the number of vocal cord vibration cycles in 1 s (2) Jitter and shimmer which are variations that occur in the fundamental frequency. (3) NHR (Noise to Harmonic Ratio) represents the ratio of non-harmonic and harmonic waves in a certain sound wave period. (4) VTI (Voice Turbulence Index) represents the ratio between non-harmonic waves at high frequencies of 2800–5800 Hz and harmonic waves at frequencies of 70–4500 Hz. (5) ATRI (Amplitude Tremor Intensity Index) represents the mean ratio of the low-frequency amplitude to the total amplitude of the sound being examined.

Maximum phonation time was done by evaluating the longest phonation time after maximum inspiration. Research participants emitted a vowel sound/"a"/as long as possible after deep inspiration and which were then measured using a counter. The examination was carried out 3 times to gain average phonation time. The abnormal value for men is under 8 s and women is under 6.4 s. Changes in all the aforementioned parameters was noted and compared throughout the follow-up periods.

2.8. Sample size considerations

Primarily, sample size was calculated using the formula bellow:

$$n = \left(\frac{(2\alpha + 2\beta)SD}{X_1 - X_2}\right)2$$

Where $2\alpha = 1.96$ and $2\beta = 0.842$, but no data is available for SD (standard deviation of mean difference in value of participants who were given the addition of PRF and without being given PRF) and X_1-X_2 (change in value between PRF and fat and no PRF addition). Due to the absence of data, sample for the study was assigned to a minimal of 10, so $n = 10$ samples. This study has 2 group, so the total required $n = 2 \times 10 = 20$. Anticipating drop out cases in this study, additional 10% of were added, leading to a final number of 22 samples.

2.9. Statistical analysis

Data that had been collected is edited and coded, then entered in a worksheet, processed on a computer using SPSS 20 (Statistical Package for Social Science) software. Parameter with numerical scale will be tested by the Mann Whitney, Friedman and Wilcoxon tests. The Mann Whitney test is used to assess the proportion between the treatment group and the control. Friedman test is carried out to assess the proportion before and after the procedures on each group. The Wilcoxon test is used to assess the compared proportion from each group based on the time of the follow up. Nominal scale research parameters will be tested with the Fischer Exact and McNemar tests. The Fischer Exact test is a non-parametric test used to assess the proportion between the treatment group and the control group, while the McNemar test is a test used to assess the proportion before and after the treatment of each study group based on the time of the examination.

3. Results

3.1. Demography

Eighteen research participants were recruited and divided into treatment and control groups. The characteristics of the treatment group towards the control were analyzed using the Fischer test, the Kolmogorov Smirnov test, and the Student's t-test. The overall assessment of the proportion shows $p > 0.05$, which means that there is no significant difference in characteristics between the two groups.

In the treatment group, there were 4 (44.4%) male participants and 5 (55.6%) female participants. Five (55.6%) participants were in the 22–50 years age group and 4 (44.4%) participants in the 51–64 years age group. Paralysis of the paramedian position was present in 4 (44.4%) participants while the lateral position paralysis was found in 5 (55.6%) participants. In the control group, there were 2 (22.2%) male participants and 7 (77.8%) female participants. There were 3 (33.3%) participants in the 22–50 years age group and 7 (66.7%) participants in the 51–64 years age group. Paralysis of the paramedian position was present in 2 (22.2%) participants while the lateral position paralysis was present in 7 (77.8%) participants.

The etiology of vocal cord paralysis varied, idiopathic and post-thyroidectomy had the highest number among other etiologies in the two study groups. Idiopathic causes in the treatment group account for 4 (44.4%) of the participants and 5 (55.6%) participants in the control group. Post-thyroidectomy accounts for the cause of paralysis in 4 (44.4%) participants in the treatment group and 2 (22.2%) participants in the control group. Post-sternotomy was present in 1 (11.1%) person for each group. Post-resection Cerebellopontine Angle (CPA) tumor was found in 1 (11.1%) participant in the control group.

3.2. MDVP

Some of the acoustic parameters used in assessing vocal cord paralysis include F0, Jitter, Shimmer, NHR, VTI, SPI and ATRI. Table 1 shows an initial description of MDVP parameters before intervention in treatment and control groups with no significant difference in all the parameters.

Changes in the parameters during follow-up were recorded and statistically analyzed. Median between treatment group and control group on each follow-up were compared with Mann Whitney test, none of the comparison yield statistical significance. Friedman tests were then used to compare median values before operation and in 8–10 weeks. Parameters in each group with a significant difference in median value were further analyzed using the Wilcoxon test (Table 2).

Observation in F0 reveals that there is no significant difference in median between the two group during each follow-up. However, it can be observed that the median value for F0 in the treatment group show a trend of decrease, while the control group remained the same. There's a decrease in median value for jitter and shimmer in both study group, but lower overall value can be seen in treatment group.

3.3. Maximum phonation time (MPT)

MPT is a parameter for evaluating sound output after maximum inspiration. Examination is repeated for 3 times before the result is recorded. The MPT value is considered abnormal in men if it is under 8 s and in women if it is under 6.4 s. The median WFM value of the two groups before surgery were assessed, the treatment group (3.6 s) had a higher WFM value than the control group (2.6 s), but there was no significant difference between the two groups. Table 3 shows the change in median WFM based on time of follow-up; the treatment group shows a continuous increase in the median value from before surgery to the end of the evaluation while the median value for control group tends to stay the same after 1 week follow-up. There was no significant difference in median value between the two groups during all the follow-up points.

Table 1
Comparison of median value for MDVP parameters before interventions.

Parameters	Median		p-value
	Treatment Group (n = 9)	Control Group (n = 9)	
F0 (Hz)	293 (167–436)	215 (124–431)	0,113
Jitter (%)	2,0 (0,9–24,8)	5,4 (1,4–7,8)	0,356
Shimmer (%)	9,0 (2,0–20,5)	13,3 (6,5–25,7)	0,133
NHR	0,16 (0,11–1,01)	0,29 (0,18–0,79)	0,113
VTI	0,05 (0,03–0,75)	0,06 (0,05–0,41)	0,549
ATRI (%)	8,3 (2,1–17,6)	9,6 (3,4–19,5)	0,842

Table 2
Median value for MDVP parameters during follow-up for both treatment and control group. There is no significant difference between treatment group and control group for all parameters.

Parameters	Median					
	Treatment Group			Control Group		
	1 Week	4–6 Weeks	8–10 Weeks	1 Week	4–6 Weeks	8–10 Weeks
F0 (Hz)	207[a,b]	165	168	208	224	214
Jitter (%)	0.8[a,b]	0.6	0.5	2.8	1.1	1.3
Shimmer (%)	4[a,b]	3.6	3.4	8.9[a]	6.4	7.5
NHR	0.13[a,b]	0.13	0.12	0.18[a]	0.15	0.16
VTI	0.05	0.04	0.05	0.11	0.06	0.06
ATRI (%)	4.3[a,b]	4.3	3.2	5[a,b]	2.1[c]	4.3[d]

[a] Friedman's p-value < 0.05 for median before intervention and 8–10 weeks.
[b] Wilcoxon's p-value < 0.05 for median before intervention and 1 week.
[c] Wilcoxon's p-value < 0.05 for median in 1 week and 4–6 weeks.
[d] Wilcoxon's p-value < 0.05 for median in 4–6 weeks and 8–10 weeks.

Table 3
Median value for MPT during follow-ups for both treatment and control group.

Parameters	Median					
	Treatment Group			Control Group		
	1 Week	4–6 Weeks	8–10 Weeks	1 Week	4–6 Weeks	8–10 Weeks
MPT (seconds)	7.6[a,b]	8.3	10.9	5.2[a,b]	5.4	5.5

[a] Friedman's p-value < 0.05 for median before intervention and 8–10 weeks.
[b] Wilcoxon's p-value < 0.05 for median before intervention and 1 week.

Nevertheless, a statistically significant changes between median value for each follow-up points inside the groups were observed.

4. Discussions

This study involved 18 participants with unilateral vocal cord paralysis. Based on the history and physical examination, it was found that idiopathic and post-thyroidectomy were the most common etiologies of unilateral vocal cord paralysis in this study. This finding is consistent with the study conducted by Toutounchi et al. where etiological observation of vocal cord paralysis found idiopathic as the cause in 31.11% of patients while postoperative was found in 28.89% of patients. The study also noted that malignancies are sometimes forgotten and should be ruled out before idiopathic is assigned as an etiology [12]. This study also observed that post-thyroidectomy caused 3.5% unilateral vocal cord paralysis while 2.3% caused bilateral paralysis, which suggests that post-thyroidectomy causes more unilateral rather than bilateral vocal cord paralysis as observed by other studies [13,14].

Microlobular fat was used as a filler material for the injection laryngoplasty in this study. Fat cells contain adipose stem cells (ASC), which have mesenchymal stem cells (MSC)-like characteristics – multipotential, capable of regenerating and increasing adipogenesis and angiogenesis. Fat tissue is a good augmentation material, but it can be reabsorbed quickly and has low viability; therefore, re-augmentation is required to maintain good results [15]. Angiogenesis and adipogenesis from ASC helped in preventing the acceleration of adipocyte tissue reabsorption. ASC can integrate with host tissue and release cytokines and tissue growth factors which include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), platelet-derived growth factor (PGDF), and transforming growth factor-beta (TGFβ). Cytokines and growth factors will be stimulated after harvesting the autologous fat which will start the adipogenesis and angiogenesis processes [7,8].

Based on in vitro studies, ASC was reported to demonstrate myogenic potential. ASC-derived myogenic progenitors can be implanted into muscle, stimulating myofibers synthesis and restoring dystrophin [16]. The use of ASC in patients with Duchenne muscular dystrophy (who had paralysis and death at around 20 years of age) found that 81% of participants were able to maintain muscle strength and prevention to loss of muscle mass. This is consistent with what is written by Maclean et al. and Dunn et al. that injection of fat into the vocal cords through the side of the thyroarytenoid muscle has the potential to increase muscle strength and mass [17,18]. This property of ASC in fat is suspected to be the main reason for a similar increase in phonation quality between the treatment and control groups during evaluations.

Platelet-rich plasma (PRP) was first introduced by Marx in 1998 when it was used for mandibular reconstruction. It was found that PRP accelerated bone growth by accommodating the transfer of growth factors to the reconstructed tissue. The PRP is made via blood centrifugation with an anticoagulant. The centrifuged plasma will produce three layers: platelet-poor plasma (PPP), platelet-rich plasma (PRP), and red blood cells. PRP can stimulate angiogenesis and adipogenesis [19]. The presence of PRP will maintain the viability of fat tissue. PRF is an advanced form of PRP. The original manufacturing method introduced by Marx was enhanced by Reksodiputro [20]. PRF is made by centrifugation of PRP at 3000 rpm and then adding 1 M Calcium Chloride (CaCl2) to obtain active thrombin from prothrombin. Thrombin degranulation will cause the release of cytokines which can stimulate cell migration and proliferation of the fibrin matrix to form fibrin from fibrinogen. In comparison to PRP, PRF is superior because it has the ability to slowly release growth factors which in turn will increase and lengthen the fat enhancement viability.

The thought of using microlobular fat in combination with PRF as a good soft tissue graft material is based on Koento's study, that observed a higher number of adipocytes and fibroblasts when using a combination of PRF and microlobular fat compared to using fat graft alone. Koento also found that increase in adipocytes would be in direct relation with the increase in fibroblasts [11]. The increase in fibroblasts will be associated with an increase in fibrinogen and fibrin, where these molecules will play a role in the platelet aggregation process during hemostasis by bringing together free platelets which will protect blood vessel branches during the coagulation process. The presence of platelets is essential because it contains a high concentration of growth factors that will stimulate the processes of angiogenesis and adipogenesis [10].

The hypothesis of this study relies on the premise that a combination of ASC in fat autograft and PRF as medialization material for vocal cord paralysis will reduce the possibility of fat reabsorption, reduce the need for repeated augmentation, reduce morbidity, and improve quality of life. Although no statistically significant differences were observed when comparing the treatment group to the control group, real clinical improvement can still be observed in the MDVP parameters (Fig. 3) and changes in MPT. A larger sample size might be needed to improve statistical accuracy.

On another note, the study also resulted in an improvement in the preparation procedure for microlobular fat or a combination of microlobular fat and PRF for fillers in injection laryngoplasty. The key update is in the filler refining method using 2 of 10 mL syringes connected using a three-way connector. This improvement eases the process of injecting fat into the vocal cord using the 12 G laryngoplasty needle, while at the same time reducing the amount of leaked fat residue during the injection. The refining method can also be used to mix PRF with microlobular fat into a liquid that can be easily injected into the vocal cord. Observation throughout the study revealed that a combination of PRF with microlobular fat will coagulate into viscous, jelly-like 10–20 min after the mixing procedures. The aforementioned property helps the filler follow the vocal cord's contours resulting in a more flexible movement and thought to be the main reason behind clinical improvement seen in the treatment group.

No side effect or adverse event was observed in participants of this

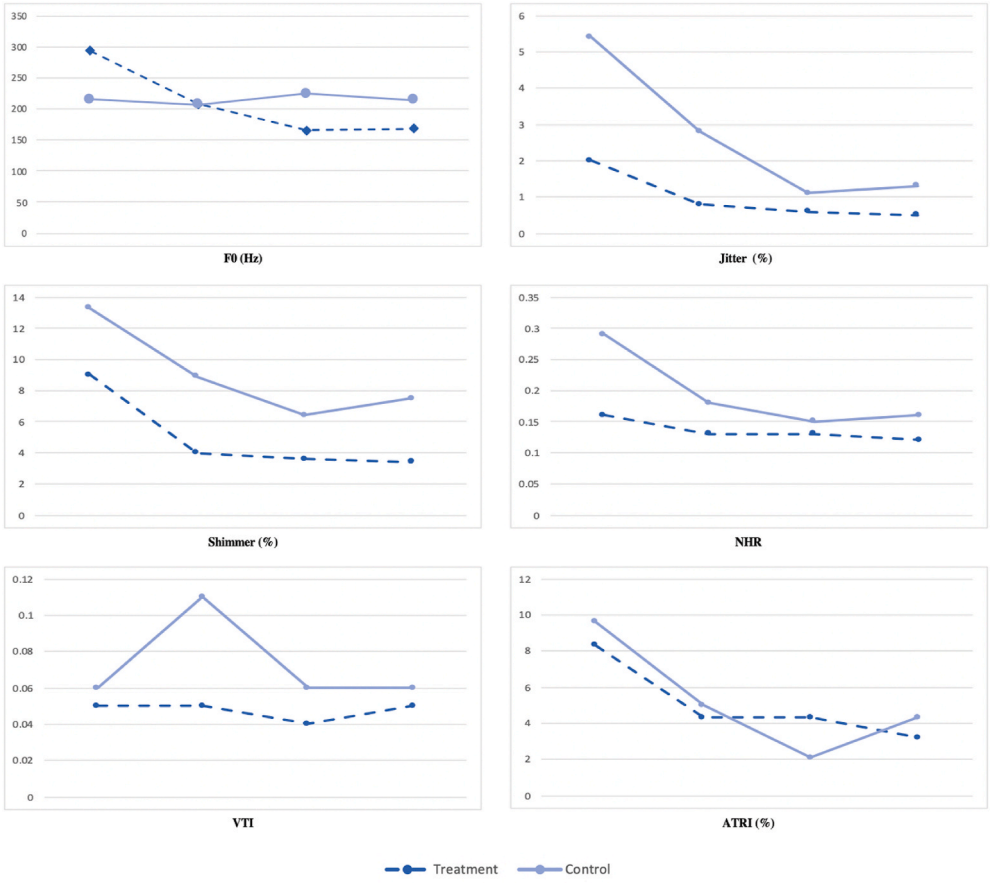

Fig. 3. The changes in MDVP parameters in treatment and control group. The four points in the graph represents median value before intervention, 1 week, 4–6 weeks, and 8–10 weeks.

study. However, it should be noted that the long-term side effect or return of dysphonia is not explicitly observed in this study. Researchers of this study continue to pay close attention to all the participants for any complaints. The utilization of PRFM as a filler for laryngoplasty is still uncommon; thus, studies regarding its side effect are limited at best. The researcher of this study suggests that further study should be done to see any short term or long term side effects in the utilization of PRFM as filler for laryngoplasty.

5. Conclusion

A combination of PRF and autologous microlobular fat for injection laryngoplasty may have the potential in enhancing fat tissue viability, reduce the need for repeated augmentation, reduce morbidity, and improve the quality of life. The MDVP results and maximum phonation time in treatment and control groups showed clinical improvement after the injection laryngoplasty procedure with no statistically significant differences between the median value of parameters between the two groups.

Ethical approval

The study had received ethical approval from Universitas Indonesia Faculty of Medicine's Health Medicine Research Ethics Committee (Approval Number: 1351/UN2·F1/ETIK/2018).

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Author contribution

Mirta Hediyati Reksodiputro, M.D., PhD., ORL-HNS. – Conceptualization, Methodology, Formal Analysis, Supervision.

Syahrial Marsintha Hutauruk, M.D., ORL-HNS. – Conceptualization, Methodology, Formal Analysis, Supervision.

Trimartani Koento, M.D., PhD., ORL-HNS., MARS – Validation, Supervision.

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Razki Yorivan Rustam Hakim, M.D., ORL-HNS. – Conceptualization, Formal Analysis. Investigation, Writing – Original Draft, Project Administration

Sacha Audindra, M.D., BMedSci. – Writing – Review & Editing

Mikhael Yosia, M.D., BMedSci, PGCert, DTM&H. – Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

Conflicts of interest

The author states no conflict of interest.

Registration of research studies

1.Name of the registry: ClinicalTrial.gov
2.Unique Identifying number or registration ID: NCT04839276
3.Hyperlink to your specific registration (must be publicly accessible and will be checked): https://clinicaltrials.gov/show/NCT04839276

Guarantor

Mirta Hediyati Reksodiputro and Syahrial Marsintha Hutauruk.

Consent

Yes, informed consent form and patient information sheet are made available in Indonesian for all the participants.

References

[1] S. Ahmad, A. Muzamil, M. Lateef, A study of incidence and etiopathology of vocal cord paralysis, Indian J. Otolaryngol. Head Neck Surg. 54 (4) (2002) 294–296.

[2] A. Mattei, G. Desuter, M. Roux, B.J. Lee, M.A. Louges, E. Osipenko, et al., International consensus (ICON) on basic voice assessment for unilateral vocal fold paralysis, Eur Ann Otorhinolaryngol Head Neck Dis 135 (1) (2018) S11–S15.

[3] M. Brockmann-Bauser, B. Balandat, J.E. Bohlender, Immediate lip trill effects on the standard diagnostic measures voice range profile, jitter, maximum phonation time, and dysphonia severity Index, J. Voice 34 (6) (2020) 874–883.

[4] C.Q. Phua, Y. Mahalingappa, J. Homer, Y. Karagama, Injection laryngoplasty, Otorhinolaryngologist 6 (2) (2013) 111–118.

[5] J.B. Salinas, D.K. Chhetri, Injection laryngoplasty: techniques and choices of fillers, Curr Otorhinolaryngol Rep 2 (2) (2014) 131–136.

[6] T.J. Fang, H.Y. Li, R.E. Gliklich, Y.H. Chen, P.C. Wang, H.F. Chuang, Outcomes of fat injection laryngoplasty in unilateral vocal cord paralysis, Arch. Otolaryngol. Head Neck Surg. 136 (5) (2010) 457–462.

[7] Y. Ren, Fat grafting with adipose stem cells: the successes and challenges, Cell, Stem cells Regen Med. 1 (2) (2015).

[8] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, et al., Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Engineering, 2001, pp. 211–228.

[9] E. Kobayashi, L. Flückiger, M. Fujioka-Kobayashi, K. Sawada, A. Sculean, B. Schaller, et al., Comparative release of growth factors from PRP, PRF, and advanced-PRF, Clin. Oral Invest. 20 (9) (2016) 2353–2360.

[10] A. Modarressi, Platlet rich plasma (PRP) improves fat grafting outcomes, World J. Plast. Surg. 2 (1) (2013) 6–13 [Internet], http://www.ncbi.nlm.nih.gov/pubmed/25489498%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4238337.

[11] T. Koento, M.H. Reksodiputro, O.N. Marimpa, Role of platelet-rich fibrin in enhancing the viability of autologous microlobular fat graft: an animal-based experimental study, Med J Indones 28 (3) (2019) 246–251.

[12] S.J. Seyed Toutounchi, M. Eydi, S.E. Golzari, M.R. Ghaffari, N. Parvizian, Vocal cord paralysis and its etiologies: a prospective study, J. Cardiovasc. Thorac. Res. 6 (1) (2014) 47–50.

[13] D.O. Francis, E.C. Pearce, S. Ni, C.G. Garrett, D.F. Penson, Epidemiology of vocal fold paralyses after total thyroidectomy for well-differentiated thyroid cancer in a medicare population, Otolaryngol. Head Neck Surg. 150 (4) (2014) 548–557.

[14] L.H.S. Rosenthal, M.S. Benninger, R.H. Deeb, Vocal fold immobility: a longitudinal analysis of etiology over 20 years, Laryngoscope 117 (10) (2007) 1864–1870.

[15] T.E. Havas, K.J. Priestley, Autologous fat injection laryngoplasty for unilateral vocal fold paralysis, ANZ J. Surg. 73 (11) (2003) 938–943.

[16] N.M. Vieira, V. Brandalise, E. Zucconi, T. Jazedje, M. Secco, V.A. Nunes, et al., Human multipotent adipose-derived stem cells restore dystrophin expression of Duchenne skeletal-muscle cells in vitro, Biol. Cell. 100 (4) (2008) 231–241.

[17] S. MacLean, W.S. Khan, A.A. Malik, S. Anand, M. Snow, The potential of stem cells in the treatment of skeletal muscle injury and disease, Stem Cell. Int. 1 (2012) 1–9.

[18] A. Dunn, M. Marcinczyk, M. Talovic, K. Patel, G. Haas, K. Garg, Role of stem cells and extracellular matrix in the regeneration of skeletal muscle, in: Muscle Cell and Tissue - Current Status of Research Field, 2018.

[19] R.E. Marx, Platelet-rich plasma: evidence to support its use, J. Oral Maxillofac. Surg. 62 (4) (2004) 489–496.

[20] M.H. Reksodiputro, Peran Faktor Pertumbuhan Pada Platelet Rich Fibrin Matrix Dan Platelet Rich Plasma Autologus Terhadap Percepatan Proses Penyembuhan Luka Tandur Kulit, Univ Indones., 2014.