Effect of inhaled fluticasone propionate on laryngotracheal stenosis after balloon dilation: a randomized controlled trial

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Abstract
Purpose Laryngotracheal stenosis describes various airflow compromising conditions leading to laryngeal and tracheal narrowing, including subglottic and tracheal stenosis. Direct laryngobronchoscopy is the diagnostic gold standard for laryngotracheal stenosis. This study aimed to explore the effect of inhaled fluticasone propionate as adjuvant medical therapy in patients with laryngotracheal stenosis after balloon dilation.

Methods This prospective randomized controlled trial was conducted from April 2019 to April 2020. Fourteen adults (≥ 18 years) with laryngotracheal stenosis consented to participate. All patients underwent endoscopic balloon dilation. Seven patients were treated with inhaled fluticasone propionate, and seven acted as controls. Detailed documentation of operative findings and pre- and post-balloon dilation spirometry measurements were recorded. Basic demographic data and operative details, including information about the percentage of laryngotracheal stenosis, distance of laryngotracheal stenosis from the vocal cords, the stenotic segment vertical length, and the largest endotracheal tube used before and after dilation were noted.

Results Spirometry measurements were obtained on 34 occasions (17 before and 17 after balloon dilation). The two groups were similar in spirometry values after treatment. Both groups had significantly improved on most spirometry values after balloon dilation.

Conclusion We found that using inhaled steroids after balloon dilatation in patients with laryngotracheal stenosis had no benefit over non-user patients in spirometry parameters during the short postoperative follow-up. To confirm this outcome, we recommend a large-scale double-blind study with a longer follow-up period.

Keywords Adjuvant medical therapy · Balloon dilation · Fluticasone propionate inhaler · Inhaled corticosteroid · Laryngotracheal stenosis · Pulmonary function test · Subglottic stenosis · Wound healing modifying agent

Introduction
Laryngotracheal stenosis (LTS) describes various airflow compromising laryngeal and tracheal narrowing situations, including subglottic and tracheal stenosis [1–3]. Usually, the patient presents with progressive dyspnea, primarily on exertion, and might present with severe respiratory distress [2, 4–7]. LTS has various etiologies, including trauma, neoplasia, infectious diseases, systemic inflammatory disorders, congenital anomalies, and idiopathic causes [3, 8].

Direct laryngobronchoscopy is the gold standard diagnostic approach for LTS. Multiple adjunct diagnostic methods and surgical planning techniques have been proposed, including pulmonary function tests (PFT) as a non-invasive tool. In particular, flow-volume loops are used to stratify patients with LTS [9, 10]. PFT provides valuable...
information on the nature and intensity of the underlying condition, and can be used during monitoring [9, 11–13]. Expiratory disproportion index (EDI) is the value received by dividing forced expiratory volume during 1 s (FEV1) by the peak expiratory flow (PEF). The index shows a good diagnostic spirometry value with high sensitivity and specificity when comparing stenotic and non-stenotic patients [14]. Post-dilation PFT parameters were reported to show significant improvement in most parameter [12, 13, 15–17].

A great variety of LTS treatments are practiced because, currently, there is no standard reference management approach [2, 3, 18, 19]. Multiple surgical options, including endoscopic, open airway surgery, and tracheotomy, have been described, and the selection between them depends on the patient’s condition. No single optimal option stands out [2–6]. However, the focus has been directed toward endoscopy as a preferred initial treatment, in which different techniques can be employed either alone or in combination. These include cold steel, CO2 laser, and balloon dilation, in addition to adjunct treatment modalities such as corticosteroid injection, and mitomycin C application as a wound healing modifier with controversial outcomes [2, 6, 20–24].

Endoscopic management has gone through multiple phases of improvement, yet it still results in high recurrence rate, especially in idiopathic subglottic stenosis (SGS) patients (40–70%), over months to years [1].

Maldonado et al. conducted a retrospective study of adult patients with idiopathic SGS [22], treated by the standard endoscopic technique, followed by deferent post-operative adjuvant medical treatment regimens. Their primary endpoint was the recurrence rate. Their patients were divided into four groups: no additional medical treatment, only anti-gastroesophageal reflux disease (GERD) regimen, combined anti-GERD regimen and inhaled corticosteroid (ICS), and combined medical therapy that included anti-GERD, ICS, and trimethoprim-sulfamethoxazole. They observed a lower recurrence rate of the disease when using the combined medical treatment regimen. However, due to its retrospective design, the causality could not be determined.

Our study aimed to explore the effect of inhaled fluticasone propionate as adjuvant medical therapy in post-balloon dilation LTS patients using spirometry values to measure its effect in comparison to non-user group. The time to recurrence requiring further surgical intervention was also compared between the groups.

Methods

Study design and setting

This prospective, randomized controlled trial was conducted from April 2019 to April 2020. The Institutional Review Board (IRB) approved the study, and it was carried out in accordance with the Declaration of Helsinki.

Study participants

Due to the rarity of the disease, the study enrolled all adult patients (≥18 years) referred to the Laryngology and Airway Clinic with suspected or confirmed diagnosis of laryngotracheal (subglottic, tracheal, or both) stenosis. Only patients undergoing endoscopic evaluation and balloon dilation who agreed to participate and signed the informed consent forms were included. The patients’ privacy and confidentiality were strictly secured.

We excluded patients regularly using medication for uncontrolled asthma, pregnant women, patients who were not fit for endoscopic management (e.g., grade 4 stenosis), having other airway narrowing conditions (e.g., bilateral vocal cord immobility or supraglottic stenosis), tracheostomized patients, and mentally disabled patients.

Study protocol and data collection

We have enrolled a total of 16 patients. However, unfortunately, two patients could not complete the post-operative spirometry assessment due to the COVID-19 pandemic. These patients were thus excluded from the analysis (Fig. 1). The patients were assigned to one of two groups postoperatively, using computer-generated randomization. Demographic data, including patient sex, age, height, weight, smoking status, etiology of stenosis, and co-morbidities, were gathered. All patients underwent a standard spirometry test in a clinical physiology laboratory within the month before the operation. The patients’ signs and symptoms dictated the decision on surgical intervention. All patients underwent endoscopic evaluation of stenosis grade, using the Cotton–Myer stenosis grading scale (grade 1, up to 50%; grade 2, 51–70%; grade 3, 71–99%, and grade 4, no detected lumen) [25]. The percentage of stenosis was assessed both subjectively by the most senior surgeon in the operating room and by passing the largest possible endotracheal tube (ETT) while testing for an audible leak at a pressure of 10–25 cm H2O, and then documenting the stenosis grade and percentage. The stenosis vertical length and site (subglottic, tracheal, or both) were also documented. Radial cold steel incisions were then done in three different areas. After that, a 15-mm balloon dilation catheter (CRE®, Pulmonary Balloon Dilation Catheter, Boston Scientific Ltd., Cork, Ireland) was used three times, performing dilation for 40 s in each. After dilation, the largest suitable ETT was passed while testing for an audible leak at a pressure of 10–25 cm H2O (Fig. 2). At the end of the procedure, a cottonoid patty soaked with mitomycin-C (4 mg/mL in 2.5 mL of saline)
Fig. 1  The study design and patient distribution

Enrollment

- 16 Patients
- All patients underwent:
  - Preoperative spirometry test
  - Surgical intervention

Allocation

- 8 Patients in control group
- 8 Patients in treatment group

Follow-Up

- Both groups after 4 weeks underwent:
  - Postoperative spirometry test clinical assessment
  - One patient in each group did not complete postoperative spirometry test (total 2 patients)

Re-Allocation

- 2 recurrences
- 1 recurrence
- Total 8 Observations in control group
- Total 9 Observations in treatment group

Fig. 2  Endoscopic view of laryngotracheal stenosis

- a pre-dilation of acquired traumatic stenosis
- b post-dilation of acquired traumatic stenosis
- c pre-dilation of idiopathic stenosis
- d post-dilation of idiopathic stenosis
Continuous variables were compared using the independent samples \( t \) test when the normality assumption was met and the Mann–Whitney \( U \) test with exact \( p \) values when it was not. For comparison of spirometry measurements before and after the surgery, we used the paired-samples \( t \) test. The level of significance used was \( \alpha = 0.05 \).

### Results

In this prospective study, we split the 14 patients equally between the control and the treatment (inhaled steroids) groups. Recurrence happened twice in one patient and once in another patient. Thus, we had eight observations in the control group and nine in the treatment group (Fig. 1). The demographic variables, including sex, age, height, weight, smoking status, etiology of stenosis, and co-morbidities, are presented, along with the summary statistics for the 14 unique patients, in Table 1. However, we used all 17 observations for the clinical measurements since they were assessed before every surgery.

There were more women (5/7, 71.4%) in the control group and more men (5/7, 71.4%) in the treatment group, although the difference was not significant. The main etiology was acquired traumatic stenosis, being 57.1% (4/7) in both groups. The sample consisted of predominantly non-smokers (6/7, 85.7% in both groups). Most patients had no co-morbidities. However, some patients were treated with bronchial asthma (BA) on/off medication in both groups, with no difference between them. The groups were also similar when compared for the three grades of LTS (Table 1).

Characteristics of the patients and their stenosis in the two groups are presented in Table 2. The patients in the treatment group were found to be taller than those in the control (167.14 ± 7.56 cm vs. 156.57 ± 9.95 cm; \( p = 0.045 \)). The groups were similar in all other characteristics, and from these assessments, we observe that none of the physical and clinical variables, except for height, differed between the groups.

Tables 3, 4 summarize the comparison outcome in lung functions before and after the balloon dilation procedure. An independent samples \( t \) test was used. No difference was found between the groups in any of the spirometry values.

Finally, we present in Table 5 a comparison between pre- and post-surgery measurements within each group, using the paired-samples \( t \) test. Improvement was noted in both groups in FEV1, PEF, PEF 25%, and PEF 50%. In the treatment group, mid maximum expiratory flow (MMEF) and EDI values have also improved.

Subgroup analysis of five idiopathic stenosis patients was done, three in the control group and two in the treatment group, without statistically significant difference on pre and post-surgery spirometry values except for PEF 75% (\( p = 0.042 \)) in pre-surgery values (Tables 6, 7).

All nine observations in the treatment group submitted the compliance chart of medication usage with a minimum of 90% adherence to medication regimen per patient. None of them reported any adverse effects.
Table 1  Summary statistics of demographic and clinical categorical variables

| Variable                                | Control | Inhaled steroid | Total | p value |
|------------------------------------------|---------|-----------------|-------|---------|
| N                                        | 7       | 7               | 14    |         |
| Sex                                      |         |                 |       |         |
| Female                                   | 5 (71.4%) | 2 (28.6%)     | 7 (50%) | 0.286  |
| Male                                     | 2 (28.6%) | 5 (71.4%)     | 7 (50%) |         |
| Etiology of stenosis                     |         |                 |       |         |
| Acquired traumatic stenosis              | 4 (57.1%) | 4 (57.1%)     | 8 (57.1%) | 1.000  |
| Idiopathic stenosis                      | 3 (42.9%) | 2 (28.6%)     | 5 (35.7%) |         |
| Systematic inflammatory-associated stenosis | 0 (0.0%) | 1 (14.3%)     | 1 (7.1%) |         |
| Smoking                                  |         |                 |       |         |
| No                                       | 6 (85.7%) | 6 (85.7%)     | 12 (85.7%) | 1.000  |
| Yes                                      | 1 (14.3%) | 1 (14.3%)     | 2 (14.3%) |         |
| Comorbidity                              |         |                 |       |         |
| BA on/off medication                     | 1 (14.3%) | 1 (14.3%)     | 2 (14.3%) | 1.000  |
| DM                                       | 1 (14.3%) | 0 (0.0%)      | 1 (7.1%) |         |
| Hypothyroidism                           | 0 (0.0%) | 1 (14.3%)     | 1 (7.1%) |         |
| IHD, HTN                                 | 0 (0.0%) | 1 (11.1%)     | 1 (5.9%) |         |
| None                                     | 5 (71.4%) | 4 (57.1%)     | 9 (64.3%) |         |
| Grade of SGS/tracheal stenosis           |         |                 |       |         |
| Grade 1                                  | 4 (50.0%) | 3 (33.3%)     | 7 (41.2%) | 0.650  |
| Grade 2                                  | 2 (25.0%) | 5 (55.6%)     | 7 (41.2%) |         |
| Grade 3                                  | 2 (25.0%) | 1 (11.1%)     | 2 (17.6%) |         |

The table reports the counts and percentages along with p values from Fisher’s exact test.

BA bronchial asthma, DM diabetes mellitus, IHD ischemic heart disease, HTN hypertension, SGS subglottic stenosis.

Table 2  Summary statistics of demographic and clinical continuous variables

|                  | Group                  | MD       | 95% CI                  | p value |
|------------------|------------------------|----------|-------------------------|---------|
|                  | Control                | Mean     | SD                      |         |
|                  | Inhaled steroid        | Mean     | SD                      |         |
| Age (years)      | 43.57 ± 15.32          | 32.29 ± 12.75 | 11.29 ± (−5.13, 27.70) | 0.160   |
| Height (cm)      | 156.57 ± 9.95          | 167.14 ± 7.56 | −10.57 ± (−20.86, −0.28) | 0.045   |
| Weight (kg)      | 71.54 ± 10.32          | 86.36 ± 18.40 | −14.81 ± (−32.19, 2.56) | 0.088   |
| Body mass index (kg/m²) | 29.44 ± 5.17       | 30.05 ± 4.75 | −0.61 ± (−5.74, 4.52)  | 0.804   |
| Follow-up (months) | 7.75 ± 3.73          | 8.78 ± 3.31 | −1.29 ± (−5.14, 2.56)  | 0.556   |
| Vocal cord to top of stenosis distance (mm) | 20.38 ± 10.04        | 19.11 ± 6.03 | −0.43 ± (−10.02, 9.16) | 0.754   |
| Vertical stenosis length (mm) | 14.3 ± 5.24         | 18.0 ± 7.79 | −3.43 ± (−9.20, 2.34)  | 0.285   |
| Pre-dilation ETT diameter (mm) | 6.73 ± 1.77          | 6.52 ± 1.11 | 0.13 ± (−1.71, 1.97)   | 0.778   |
| Post-dilation ETT diameter (mm) | 10.26 ± .60          | 9.48 ± 0.96 | 0.59 ± (−0.10, 1.27)   | 0.066   |
| Cotton Myer grade (%)  | 54.25 ± 23.82         | 56.44 ± 13.34 | −1.43 ± (−24.86, 22.00) | 0.815   |

The mean, standard deviation, and p values from the independent samples t test are reported.

SD standard deviation, MD mean difference, CI confidence interval, ETT endotracheal tube.
We also performed a similar analysis with one measurement per patient ($n = 14$), taking the first occurrences in each, but that did not yield a different result.

**Discussion**

The present study did not find any difference between the groups in the means before or after the balloon dilatation treatment. In both groups, there was a significant improvement in the spirometry parameters after the balloon dilatation surgery. Steroids are a well-known wound healing modifying agents, with multiple mechanisms of action [27–29]. The role of the steroid inhaler in subglottic and tracheal stenosis cases after balloon dilation as an adjuvant medication has not been studied well, and there is still no solid evidence to support its use. Our study aimed to compare the outcome in a control group and a group treated with inhaled steroids, using spirometry values and the time to recurrence as measures of effect.

Fluticasone propionate is a powder inhaler, usually used for bronchial asthma patients. Particle size in the pharmacologic component is 2.4 µm. At a particle size of 6–8 µm, the steroid inhalers are used as an anti-inflammatory medication to target the vocal cord in patients with vocal cord granuloma [30]. Yet, there is no evidence supporting a specific particle size to target the laryngotracheal mucosa below the vocal cord.

The two groups were similar in the initial characteristics, as shown in Tables 1, 2, and the spirometry values before and after the balloon dilation procedure, as shown in Tables 3, 4. This similarity indicates no advantage for steroid.
inhaler users after balloon dilation, when measured by the spirometry values. Our findings support previous studies that no superiority for ICS with anti-GERD medication over anti-GERD alone among iSGS patients [22]. This similarity might be because the best steroid powder characteristics targeting laryngotracheal mucosa below the vocal cord is unknown. Even so, some reports support the use of ICS to treat tracheal stenosis after intubation [31–33].

After the balloon dilation, most spirometry values have dramatically and significantly improved in both groups. In the control group, these included FEV1, PEF, PEF25%, and PEF50%. Similarly, in the treatment group, these included FEV1, PEF, PEF25%, PEF50%, MMEF, and EDI. Such

Table 5 Comparison of pre- and post-surgery spirometry measurements, separately for the control and treatment groups

| Group          | MD     | Std. Error | 95% CI | t statistic | df | p value |
|----------------|--------|------------|--------|-------------|----|---------|
| Control        |        |            |        |             |    |         |
| FVC (L)        | 0.111  | 0.084      | − 0.088| 0.31        | 1.323| 7 | 0.227 |
| FEV1 (L)       | 0.52   | 0.156      | 0.152  | 0.888       | 3.344| 7 | 0.012 |
| PEF (L/s)      | 1.334  | 0.545      | 0.045  | 2.622       | 2.448| 7 | 0.044 |
| PEF 25%        | 1.353  | 0.545      | 0.063  | 2.642       | 2.481| 7 | 0.042 |
| PEF 50%        | 1.123  | 0.35       | 0.295  | 1.95        | 3.209| 7 | 0.015 |
| PEF 75%        | 0.209  | 0.207      | − 0.281| 0.699       | 1.007| 7 | 0.347 |
| MMEF (L/s)     | 0.715  | 0.321      | − 0.043| 1.473       | 2.23 | 7 | 0.061 |
| EDI (s)        | 0.183  | 0.097      | − 0.047| 0.413       | 1.883| 7 | 0.102 |
| Inhaled steroid|        |            |        |             |    |         |
| FVC (L)        | 0.336  | 0.187      | − 0.097| 0.768       | 1.79 | 8 | 0.111 |
| FEV1 (L)       | 0.554  | 0.177      | 0.145  | 0.964       | 3.124| 8 | 0.014 |
| PEF (L/s)      | 1.602  | 0.521      | 0.401  | 2.804       | 3.075| 8 | 0.015 |
| PEF 25%        | 1.57   | 0.526      | 0.357  | 2.783       | 2.984| 8 | 0.017 |
| PEF 50%        | 1.01   | 0.337      | 0.233  | 1.787       | 2.997| 8 | 0.017 |
| PEF 75%        | 0.197  | 0.267      | − 0.420| 0.813       | 0.736| 8 | 0.483 |
| MMEF (L/s)     | 0.711  | 0.287      | 0.049  | 1.373       | 2.477| 8 | 0.038 |
| EDI (s)        | 0.211  | 0.076      | 0.035  | 0.386       | 2.769| 8 | 0.024 |

The mean difference, the standard error of the mean difference, 95% confidence intervals, and paired-samples t statistics, with corresponding p values are reported

MD mean difference, Std. standard deviation, CI confidence interval, FVC forced vital capacity, FEV1 forced expiratory volume in 1 s, PEF peak expiratory flow, MMEF mid maximum expiratory flow, EDI expiratory disproportion index

Table 6 Summary statistics of spirometry measurements before surgery for the sub-group of idiopathic stenosis patients only

| Group          | Mean deviation | 95% CI | t statistic | p value |
|----------------|----------------|--------|-------------|---------|
| Control        |                |        |             |         |
| FVC            | 2.257 0.808    |        |             | 0.784   |
| FEV1           | 0.970 0.331    | − 0.070| (− 0.856, 0.716) | 0.086 | 0.950 |
| PEF            | 1.027 0.328    | 0.017  | (− 0.763, 0.796) | 0.068 | 0.950 |
| PEF 25%        | 0.970 0.334    | − 0.025| (− 0.822, 0.772) | 0.010 | 0.927 |
| PEF 50%        | 0.843 0.326    | − 0.142| (− 0.916, 0.633) | 0.058 | 0.601 |
| PEF 75%        | 0.650 0.115    | − 0.295| (− 0.571, − 0.019) | 3.403 | 0.042 |
| MMEF           | 0.757 0.259    | − 0.728| (− 2.053, 0.597) | 1.749 | 0.179 |
| FEV1/PEF       | 0.943 0.063    | − 0.087| (− 0.238, 0.063) | 1.843 | 0.163 |

The mean, standard deviation, t-statistic, and the corresponding p values are reported

SD standard deviation, MD mean difference, CI confidence interval, FVC forced vital capacity, FEV1 forced expiratory volume in 1 s, PEF peak expiratory flow, MMEF mid maximum expiratory flow, EDI expiratory disproportion index
Table 7 Summary statistics of spirometry measurements after surgery for the sub-group of idiopathic stenosis patients only

| Group                | Control | Inhaled steroid | Mean difference | 95% CI       | t statistic | p value |
|----------------------|---------|-----------------|-----------------|--------------|-------------|---------|
|                      | Mean    | Std. deviation  | Mean            | Std. deviation |             |         |
| FVC                  | 2.447   | 0.666           | 2.645           | 0.841        | −0.198      | (−2.317, 1.920) | −0.298  | 0.785   |
| FEV1                 | 1.877   | 0.560           | 2.155           | 0.233        | −0.278      | (−1.664, 1.107) | −0.639  | 0.568   |
| PEF                  | 2.073   | 0.811           | 2.840           | 0.042        | −0.767      | (−2.692, 1.159) | −1.267  | 0.295   |
| PEF 25%              | 2.037   | 0.767           | 2.630           | 0.255        | −0.593      | (−2.463, 1.276) | −1.010  | 0.387   |
| PEF 50%              | 1.893   | 0.648           | 2.205           | 0.856        | −0.312      | (−2.414, 1.791) | −0.472  | 0.669   |
| PEF 75%              | 1.243   | 0.335           | 2.145           | 0.530        | −0.902      | (−2.094, 0.291) | −2.406  | 0.095   |
| MMEF                 | 2.047   | 0.926           | 1.745           | 0.191        | 0.302       | (−1.918, 2.522) | 0.432   | 0.695   |
| FEV1/PEF             | 0.936   | 0.130           | 0.758           | 0.071        | 0.178       | (−0.152, 0.507) | 1.715   | 0.185   |

The mean, standard deviation, t statistic, and the corresponding p values are reported

SD standard deviation, MD mean difference, CI confidence interval, FVC forced vital capacity, FEV1 forced expiratory volume in 1 s, PEF peak expiratory flow, MMEF mid maximum expiratory flow, EDI expiratory disproportion index

Improvements in spirometry values after balloon dilation have been reported in the scientific literature before [12, 13, 15, 16].

Two patients developed recurrence during the study period; in both of them, the etiology of stenosis was acquired traumatic stenosis. In one patient, the stenosis recurred twice. The first time occurred 4 months after the balloon dilatation to a 58%, 13-mm vertical length of stenosis noticed 30 mm from below vocal cord, while he was treated with inhaled steroids. The patient was treated with balloon dilatation again to a 38%, 5 mm vertical length of stenosis noticed 33 mm from below vocal cord, and then shifted to the control group. Three months later, he developed stenosis again, and thus treated by balloon dilatation for the third time, which shows a 42%, 25 mm vertical length of stenosis noticed 19 mm from below vocal cord. For the 5 months’ follow-up since the last treatment, the stenosis did not recur. The second patient was in the control group, stenosis characteristics showing a 17%, 14-mm vertical length of stenosis noticed 6 mm from below vocal cord. The symptoms developed again in this patient after 7 months. Balloon dilatation was performed again with stenosis of 60%, 3-mm total vertical length 20 mm apart from the vocal cord, and the patient was allocated to the treatment group. Through the 6 months of follow-up since the treatment, the patient remains without symptoms.

Our study was subject to limitations. Only two of the patients in this study were treated in both groups after recurrence of stenosis. A short-term follow-up due to the limited study period could have diminished the accuracy of the evaluation of recurrence in both groups. Furthermore, spirometry is a patient-dependent test with well-documented test–retest variability. Moreover, thus far, no study has suggested the best steroid inhaler based on particle size to target the subglottic or tracheal segment, as was done in vocal cord granuloma [30]. A large-scale double-blind randomized controlled study should be performed to prove or disprove our findings.

In conclusion, we found that using inhaled steroids in LTS patients after the intervention had no benefit over non-user patients when assessed by spirometry parameters shortly after balloon dilatation. Also the recurrence rate did not differ between the groups, possibly due to a small sample and a short duration of follow-up. To confirm this outcome, we recommend a large-scale double-blind study with a longer follow-up.

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Compliance with ethical standards

Conflict of interest  The authors declare that there is no conflict of interest.

Ethical approval  The Institutional Review Board (IRB) approved the study (Project Reference No. E-19-3754), and it was conducted in accordance with the Declaration of Helsinki. The IRB is registered with the Office for Human Research Protection (OHRP) (Institution Registration No. IORG0006829, OHRP IRB Registration No. IRB000008189, and IRB from King Abdulaziz City for Science and Technology/National Committee for Biological Ethics Registration No. H-01-R-002).

Consent to participate  Informed consent taken from all participant.

Consent for publication  All research team agreed to approve the final manuscript submission.

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