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TANAFFOS

Histopathological Changes in the Stenotic Part of the Airway in Post-Intubation Tracheal Stenosis

Hossein Kalantari Dehaghi [1], Fariba Ghorbani[2] , Mohammad Bayat [3], Amirbahador Abbasi [1], Maryam Kalantari Dehaghi [1], Makan Sadr [4], Nafiseh Mohammadizadeh [2], Bardya Mohseni [3], Mohammad Behgam Shadmehr [2]

[1] Lung Transplantation Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti. University of Medical Sciences (NRITLD), Tehran, Iran, [2] Tracheal Diseases Research Center, National Research. Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3] Department of Anatomy, Arak University of Medical Sciences, Arak, Iran. [4] Virology Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Correspondence to: Shadmehr MB
Address: Tracheal Diseases Research Center (TDRC), NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email address: mbshadmehr@yahoo.com

Background: Post-intubation tracheal stenosis (PITS) is one of the most important early complications of intubation. In this study, a detailed examination of the histopathological changes of the trachea is presented.

Materials and Methods: In this cross-sectional study, specimens of the stenotic region of the trachea in 25 patients with type III, type IV, and subglottic airway stenosis were evaluated using hematoxylin/eosin and Masson's trichrome staining. Also, the prevalence of microscopic changes in the different structures of the tracheal wall was assessed.

Results: The mean age of the patients was 37.12±13.96 years, and the intubation duration was 12.6 ± 4.8 days. The length of stenosis part was 30.12± 6.4 mm. The location of stenosis in 19 patients was in the trachea, in five patients both the trachea and the subglottic area were affected, and in one patient only the subglottic area was restricted. In grade III, the epithelial layer was disorganized, and widespread cell destruction occurred. Also, the mucosal and submucosal layers were destroyed, and the accumulation of extracellular matrix and collagen fibers was clear, but the hyaline cartilage of the trachea was still intact and non-pathological. In Grade IV lesions, the tracheal tissue was extensively damaged, with complete destruction of the epithelial layers, lamina propria, mucosa, submucosa, seromucous glands, and tracheal cartilage. The extracellular matrix and collagen fibers were also extensively disrupted and replaced by fibrotic tissue.

Conclusion: Tissue destruction in tracheal stenosis sometimes progresses to the depth of the cartilage layer and is irreversible. Thus, in many cases, anastomotic resection surgery is the only treatment.

Keywords: Post-Intubation; Tracheal Stenosis; Prolong Intubation; Histological Findings

INTRODUCTION

Tracheal intubation can cause changes in the structure of the tracheal wall, including mucosal damage, inflammation, tissue destruction, granulation formation, tracheomalacia, and cartilage injury. Post-intubation tracheal stenosis (PITS) is one of the most important early complications of intubation, which can occur at any age. This acquired, non-malignant, and rare condition can be life-threatening if mismanaged or if there is a delay in diagnosis (1). The development of this complication can be due to insufficient care of the tracheal tube, selection of an inappropriate endotracheal tube, prolonged intubation, cuff pressure, and other factors (2).

PITS occurs in 6–22% of adults following prolonged intubation or tracheostomy (3) and should be managed

properly to prevent other complications. Symptomatic patients may undergo bronchoscopy and dilatation or airway resection and anastomosis based on their clinical condition. However, these approaches may not always be feasible, and in cases of prolonged stenosis with anatomical and clinical restrictions, tracheostomy may be necessary to save the patient's life. More specifically, when the lesion extends over more than half of the tracheal length in adults or one-third of the tracheal length in children, complete surgical resection may not be feasible. These limitations are influenced by several factors, including patient age, regional anatomy, underlying pathology, and previous treatment (4, 5).

Despite numerous studies aimed at clarifying the management of tracheal stenosis, our knowledge of the histologic processes underlying its development and stricture remains insufficient. Some studies have reported that fibrosis and scar formation are the prominent pathological features of stenosis (6). To be more precise, in some cases, high cuff pressure can lead to mucosal ischemia within the first few hours after intubation (7). Prolonged ischemia can cause mucosal necrosis, resulting in epithelial ulcers. Long-term ischemia can lead to mucosal necrosis, resulting in superficial and deeper ulcers that may expose the cartilage. This can then lead to partial or complete destruction of the tracheal rings with a loss of structural integrity. The healing process can result in sequelae such as fibrosis within 3-6 weeks after intubation, ultimately leading to progressive stenosis (8). In a study conducted by Badr El Din et al., it was reported that during tracheal intubation, the mucosa, submucosa, and the area around the mucus glands are the most commonly affected areas, with ulcerations, erosion of the epithelium, submucosal gland atrophy, and cartilage necrosis being observed (9). Evidence of squamous metaplasia was also reported in their study. The causes and risk factors for post-intubation airway stenosis are still unclear, but further research can aid in prevention and treatment. In this study, we aimed to identify pathological changes in patients undergoing resection-anastomosis surgery by evaluating different tissue layers observed in tracheal stenosis.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted at Masih Daneshvari Hospital. Data were obtained from the Alborz database, a registry of patients with tracheal stenosis. The Ethics Committee of the Research Institute of Tuberculosis and Lung Diseases registered and approved this study (IR.SBMU.NRITLD.REC.1394.147).

Participants

Patients were categorized into three clusters, including type III, type IV, and laryngotracheal stenosis, according to Cotton-Myer classification regarding location and severity of the stricture (10). Tracheal samples of 25 patients with tracheal stenosis were gathered to evaluate histological changes. A part of the affected trachea after resection and anastomosis surgery was sent to an expert histologist for examination in a Falcon tube containing buffered formalin for tissue fixation, and it was transferred to the relevant laboratory for Hematoxylin-Eosin (HE) and Masson's Trichrome (MT) staining (11)

Inclusion and Exclusion criteria for both study groups

- Patient or family consent
- Absence of underlying diseases, including diabetes mellitus, malignancy, and autoimmune diseases.
- Age of 18 to 40 years
- In case of a history of multiple bronchoscopies (more than 3 times), the sample was excluded.
- No history of laser therapy as a treatment method for airway stenosis.
- No history of direct trauma or tracheal damage.

RESULTS

The mean age of the patients was 37.12±13.96 years, and the duration of intubation was 12.6±4.8 days. The mean length of tracheal stenosis was 30.12±6.4 mm. The

stenosis was located in the trachea in 19 patients, involved both the trachea and subglottic region in five patients, and was confined to the subglottic region in one patient.

Table 1. Description of destruction of the tracheal structure

Histological Layer	
Epithelium	25 patients (the superficial epithelium layer was destroyed in all patients of the case group)
Mucous and submucous layer	Tracheal destruction in 16 patients had progressed to the depth of the mucosal and submucosal layers
Perichondrium	Tracheal destruction in 12 patients had progressed to the depth of the inner layer of the perichondrium
Hyaline cartilage	9 patients had different degrees of tracheal cartilaginous layer destruction

In Figure 1, normal histology of the trachea is presented with both Masson's trichrome (MT) and hematoxylin-eosin (HE) staining (13).

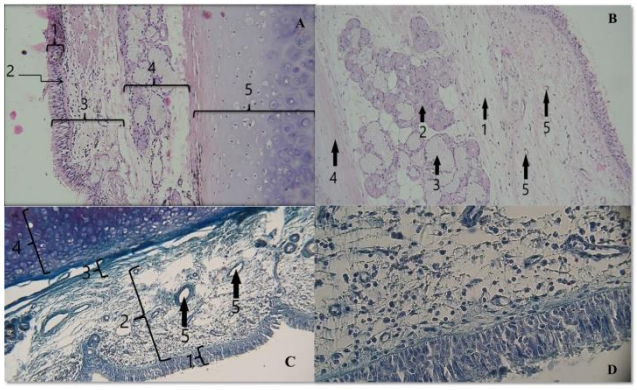

Figure 1. Histology of normal trachea from control group: (A) HE, 100X: 1: Respiratory epithelium. 2: lamina propria. 3: mucous layer. 4: Submucosal layer (including serosal and mucosal glands). 5: cartilaginous layer. (B) HE, 400X: 1: Collagen fibers (frequently seen in the mucosal tissue). 2: mucosal glands. 3: Serous glands. 4: The perichondrium layer around the tracheal cartilage. 5: Vessels of the mucous layer. (C) MT, 100X: 1: Respiratory epithelium and lamina propria. 2: Mucosal and submucosal layers. 3: Cartilage perichondrium (It should be noted that the detachments in the perichondrium layer are related to cutting the samples and are not pathological.). 4: Cartilaginous layer. 5: Blood vessels of the mucosal and submucosal layer. (D) MT: Collagen fibers can be seen significantly in the mucosa and submucosa of the trachea

In Figure 2, tracheal stenosis grade III is presented with both MT and HE staining in different magnifications. As shown in Figure 2, the epithelial layer exhibits marked architectural disorganization accompanied by extensive cellular destruction. Also, the mucosal and submucosal layers are destroyed, and the accumulation of extracellular matrix and collagen fibers is evident, but the hyaline cartilage of the trachea is still intact and non-pathological.

Indeed, tissue and cellular destruction are evident in the superficial epithelium along with the lamina propria of the trachea, and the cellular structure is destroyed. Mucous and serous glands and blood vessels have disappeared and have been replaced by collagen fibers.

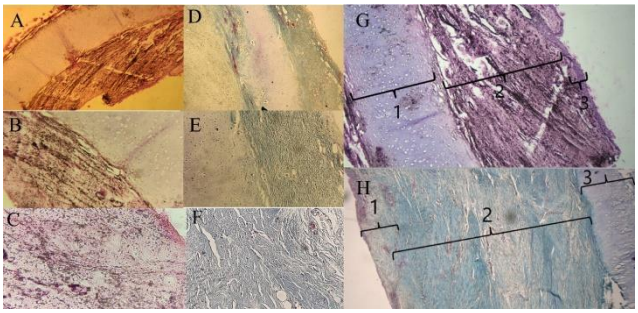

Figure 2. Tracheal stenosis grade III: (A) HE, 4X: Accumulation of collagen fibers in the ciliated subepithelium, but perichondrium and hyaline cartilage are normal. (B) HE 10X: Collagen fibers are seen in the epithelium, but perichondrium and hyaline cartilage are normal. (C) HE 40X: Accumulation of collagen fibers in the epithelium and extensive destruction of serous-mucosal glands. (D) MT 4X: Destruction of serous-mucosal glands in the epithelium and the accumulation of fibrotic tissue. (E) MT 10X: Destruction of serous-mucous glands and accumulation of fibrous tissue in the epithelium are seen, but perichondrium and hyaline cartilage are normal. (F) MT 40X: The destruction of serous-mucous glands in the epithelium and the accumulation of fibrotic tissue are seen. (G): HE 100X: 1: Cartilaginous layer. 2: Mucosal and submucosal layers. 3: Epithelium and lamina propria. (H): MT 100X: 1: Epithelium and lamina propria. 2: Mucosal and submucosal layers. 3: Cartilaginous layer

In Figure 3, tracheal stenosis grade 4 is presented with both MT and HE staining with 4X, 10X, and 40X magnification. In Figure 3, the tracheal tissue shows extensive structural damage, characterized by destruction of the epithelial layers, lamina propria, mucosa, submucosa, serosa, seromucous glands, and tracheal cartilage, with extensive disruption of the extracellular matrix and collagen fibers and their replacement by fibrotic tissue. Indeed, extensive tissue destruction is evident, and all histological structures of the trachea have been destroyed and replaced by collagen fibers and fibrous tissue.

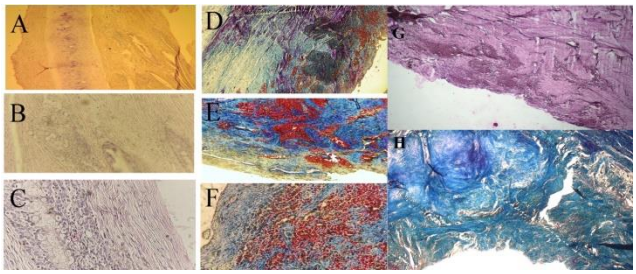

Figure 3. Tracheal stenosis grade IV: (A) 4X: Massive destruction of the ciliated epithelium, serous glands, vasculature, blood clots, and collagen fibers are also seen. (B) HE, 10X: Pathologic findings similar to 4X magnification. (C) HE, 40X: Pathologic findings similar to 4X and 10X magnification. (D) MT, 4X: Massive accumulation of fibrotic tissue in the epithelium and destruction of serous-mucous glands. (E) MT, 10X: Massive fibrosis in the epithelium and destruction of serous-mucous glands are seen. (F) MT, 40X: Findings similar to 4X and 10X magnification. (G) HE, 100 X: Cartilaginous layer is destroyed. (H) MT, 100 X: Destruction of cartilage

In Figure 4, subglottic stenosis is presented with both Mt and HE staining with 4X, 10X, and 40X magnification.

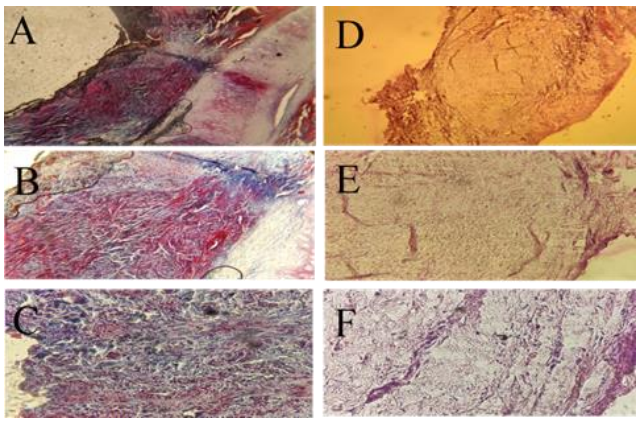

Figure 4. Subglottic stenosis: (A) HE, 4x: Destruction of the ciliated epithelium and accumulation of collagen fibers are evident, but the hyaline cartilage is free of pathologic changes. (B) HE,10X: Destruction of the ciliated epithelium is evident, serous-mucous glands are totally destroyed, and the accumulation of collagen fibers in the epithelium can be seen. Perichondrium and hyaline cartilage have no pathological lesions. (C) HE 40X: Similar findings to 4X and 10X are seen. (D) MT, 4X: Destruction of serous-mucosal glands and the accumulation of fibrotic tissue. (E) MT, 10X: Similar findings to 4X are seen. (F) MT, 40X: Similar findings to 4X and 10X are seen

DISCUSSION

In this study, we investigated the histology of the stenotic section of the trachea using specific dyes. We aimed to determine the pathological changes to clarify the exact process of stenosis and identify the causes of this complication.

Tracheal stenosis is a serious complication of prolonged endotracheal intubation. Patients with tracheal stenosis may present with symptoms such as dyspnea, stridor, respiratory distress, and, in severe cases, airway obstruction (13).

Numerous studies have been conducted to investigate the pathogenesis of tracheal stenosis, but the exact mechanism remains unclear. Prolonged intubation and tracheostomy have been associated with tracheal stenosis, but the reasons why most patients with a history of prolonged intubation do not develop this condition are unknown (14).

Tracheal stenosis after long-term intubation is due to traumatic injuries to the trachea, including tracheal wall ischemia induced by the cuff and stromal injury, and also granulation proliferation after decannulation (15). Several studies have reported laryngopharyngeal reflux as an etiologic factor in tracheal stenosis (16). Genetic susceptibility is considered a predisposing factor to airway stenosis following intubation (17).

The main process in the pathogenesis of airway stenosis seems to be an individual's inflammatory response to initial traumatic injury (18). Insertion of an endotracheal tube can cause pressure necrosis and ulceration of the mucosa. During the healing process, granulation tissue and fibrosis may result from the ulceration. In most cases, the granulation tissue resolves completely without any subsequent issues. However, in some cases, the lesion may become a fibrotic scar. The accumulation of collagen and fibrotic tissue in the submucosal layer can then lead to a reduced luminal diameter (6). Involvement of the subglottic area, anastomotic site infection, and tension on the anastomosis after repair have been linked to restenosis following anastomosis and resection surgery (19)

Microscopic examination of tracheal specimens from patients with stenosis secondary to prolonged intubation typically reveals a range of histopathological changes. These changes can include inflammation, fibrosis, scarring,

and metaplasia. Inflammation is often seen in the submucosal layer of the trachea and may be characterized by the presence of inflammatory cells such as neutrophils, lymphocytes, and macrophages. Fibrosis, or the excessive deposition of collagen and extracellular matrix, can lead to significant narrowing of the tracheal lumen. Scarring and granulation tissue can also be present and may contribute to the narrowing of the airway (9). On the other hand, metaplasia, or the transformation of one type of tissue into another, may occur in the epithelial layer of the trachea. This can lead to the formation of goblet cells, which produce mucus, or squamous cells, which are not typically present in the respiratory tract (20). However, in our study, this finding was not reported. Overall, microscopic examination and special staining of tracheal samples can provide valuable information on the histopathological changes that occur in tracheal stenosis caused by prolonged intubation. This information can help guide diagnosis and treatment decisions for patients with this condition.

The severity of histological changes in tracheal stenosis can vary depending on the duration of intubation, as well as other factors such as the size of the endotracheal tube and the presence of infection or other complications (21).

To the best of our knowledge, relatively few experimental studies have specifically investigated the histopathological changes associated with tracheal and subglottic stenosis. Mayer et al. demonstrated dense submucosal fibrosis in experimentally induced subglottic stenosis (22), while Charous et al. reported prolonged inflammation, delayed epithelialization, and increased collagen deposition in a canine model (23). Similarly, Roh et al. observed mucosal ulceration, inflammation, and granulation tissue during the early phase of subglottic injury, followed by cartilage collapse, submucosal thickening, and fibrosis (24). Nevertheless, detailed histopathological characterization of severe tracheal and subglottic stenosis, particularly high-grade lesions, remains limited.

As mentioned earlier, in tracheal stenosis grade III and subglottic stenosis, the epithelial layer was found to be completely atrophic. Mucosal and submucosal glands were destroyed, and interstitial fibrosis was evident, but the perichondrium was still intact without any pathological changes.

The histopathological changes seen in grade IV stenosis include destruction of all histological layers of the trachea, as well as perichondrium and fibrosis replacement in all layers. In fact, in some cases of grade IV stenosis, the destruction is so extensive that it reaches the cartilage.

In agreement with the findings of Dohar et al. on a canine model, our study suggests that deeper involvement of the trachea (lamina propria and perichondrium) is associated with more extensive fibrotic and stenotic changes in the tracheal wall. However, Dohar et al. reported that superficial injuries (epithelium only) regenerated completely without any sequelae (25).

The mean duration of intubation in our patients was 12.6±4.8 days. We assume that a longer duration of intubation is an important variable in the development of tracheal stenosis. In this regard, El Din et al. reported that tracheal fibrosis developed in many patients by the seventh day of intubation, leading to tracheal stenosis (9). This is consistent with the findings of the study by Stauffer et al. (26), which demonstrated that 19% of patients with intubation developed tracheal stenosis.

Tracheal lesions that developed within 24-48 h after intubation only showed ischemic mucosa, edema, congestion, and evidence of collagen fibers in extracellular matrix. These lesions usually recover within 12–48 h without any fibrotic tissue and evidence of stenosis (9)

This exploratory study had some limitations. Firstly, we only included patients with tracheal stenosis following long-term intubation. Therefore, no statements can be made regarding the morphological characteristics of subglottic stenosis caused by other factors such as Wegener's granulomatosis. It is important to note, however, that the histologic characteristics of tracheal stenosis in this study population were uniform regardless of the cause of the stenosis. Nevertheless, stratification by cause was not possible due to the limited sample size.

CONCLUSION

Our findings demonstrate distinct histopathological changes across different grades of tracheal and subglottic stenosis. Higher-grade stenosis was associated with more extensive tissue involvement, including epithelial and glandular destruction, increased fibrosis, and, in severe cases, involvement of the perichondrium and cartilage. These findings suggest that the severity of stenosis is accompanied by progressively bigger structural changes in the tracheal wall. Histopathological evaluation may provide further insight into the tissue remodeling associated with post-intubation airway stenosis and contribute to a better understanding of its pathological progression. Further studies with larger sample sizes are warranted to confirm these findings and clarify the mechanisms underlying the progression of tracheal stenosis.

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