Original Research Article

Cytokines and Defensins in Tissue Biopsies Obtained by Bronchoscopy from Patients with Post-intubation Tracheal Stenosis

ABSTRACT

Aims: The objective of our study was to perform the routine analysis of bronchoscopically obtained tracheal samples to determine the appearance and relative distribution of cytokines and antimicrobial proteins in patients with post-intubation tracheal stenosis (PITS).
Study design: Retrospective.
Place and Duration of Study: Rīga Stradiņš University, Institute of Anatomy and Anthropology, Pauls Stradiņš Clinical University Hospital, between May 2014 and May 2015.
Methodology: Five patients with PITS were involved in this study. Tissue samples were obtained by bronchoscopy from the upper part of trachea, then proceeded for routine histological staining with hematoxylin and eosin. Interleukine-1 (IL-1), interleukine-10 (IL-10) and tumor necrosis factor alpha (TNFα), as well as beta defensin-2 (β def-2) were detected by use of immunohistochemistry (IMH) method. The number of immunoreactive (positive) structures was graded semi-quantitatively.
Results: Squamous metaplasia, inflammatory cell infiltration and formation of granulation tissue were observed in all cases. Significant expression of IL-10 and β def-2 was seen as various number of immunoreactive structures in tracheal tissue. Only few scattered IL-1 and TNFα positive macrophages were found in part of cases.
Conclusions: The leading role in pathogenesis of post-intubation tracheal stenosis is assumed to be the chronic inflammation, fibrous scarring, as well as the remodeling of tracheal wall due to the ischemia. Compensatory expression of antimicrobial peptide β def-2 and anti-inflammatory cytokine IL-10 indicates the intense local tissue defense reactions. TNFα and IL-1 are not among the most significant factors in pathogenesis of PITS.

Keywords: cytokines, defensins, post-intubation tracheal stenosis.

1. INTRODUCTION

The most common reasons for the non-malignant obstruction of central airways (trachea, as well as primary bronchi) are intubation and tracheostomy [1] or combination of both [2], which are considered as iatrogenic causes. The incidence of post-intubation tracheal stenosis diagnosed in patients with prolonged artificial ventilation varies from 1% to 2% of patients requiring treatment, and 10% to 22% due to the presence of clinical signs and symptoms [3], in total estimating 4.9 cases per million per year [4]. ~~On one hand, the incidence of post-intubation tracheal stenosis increases due to the growing number in the use of mechanical ventilation in intensive care departments and for the management of chronic diseases, mainly with the origin of lungs. On the other hand, the incidence of post-intubation tracheal stenosis has decreased because of the recognition of exact etiological factors in pathogenesis of post-intubation tracheal stenosis and design modifications, as well as the precise application of cuffed intubation tubes.~~

Post-intubation tracheal stenosis is a complication of endotracheal intubation using cuffed intubation tube, which usually occurs at the site of the contiguity of cuff and the tracheal wall

in the upper part of the trachea (in the subglottic region) [5]. The following factors could predispose the development of post-intubation tracheal stenosis: prolonged and/or traumatic intubation, history of intubation, previous tracheotomy, use of high dose corticosteroids, advanced age, female gender, severe respiratory failure, severe gastroesophageal reflux disease (GERD), concomitant autoimmune diseases, sleep apnea–hypopnea syndrome, local radiation therapy [5]. ~~The pathogenesis of post-intubation tracheal stenosis have been described with main pathological changes of blood perfusion, due to which the secondary processes take place in all histological layers of tracheal wall. The very first moment of those pathological events is the cuff pressure higher than appoximately 30 mmHg exerted on the tracheal mucosa, that exceeds the pressure in mucosal capillaries. This predominance of cuff pressure over the perfusion capabillities in tracheal capillaries leads to the mucosal ischemia [6]. Interestingly, the amount of pathological damage found in morphological analysis of tracheal wall increases as the period of the mechanical ventilation and therefore the presence of cuffed intubation tube was prolonged [7]. Due to the unique segmental blood supply of the trachea, of which the blood vessels perforate the tracheal wall at interannular cartilage spaces and arborize through the submucosa, lesions of transmural nature occure at the site of cuffed tube compressing tracheal wall within all its layers [8]. This is the most significant explanation to all pathological events, that could be partialy seen describing both bronchoscopical and morphological findings. Not only the regional ischemia causes the reversible edema, congestion, hemorrhages and traces of fibrinous exudate of the tracheal wall that could heal complately within few days [2][9], but also causes the ulceration of the mucosa initiating the necrosis, therefore typically within 3 to 6 weeks initiating the process of complex healing and scarring [8][9]. Fibrous tissue due to scarring in bronchoscopy were detected as circumferential tight and little tight stenosis of tracheal lumen [10].~~

The morphopathogenesis of post-intubation tracheal stenosis includes the development of fibrous tissue along with the local ischemia mainly [6,7,8,9,10].

The pseudostratified ciliated epithelium, which normally lies the trachea, is often replaced by stratified squamous epithelium. Sometimes keratinization and flattening of the surface cells is present [11]. Similar findings were observed in animal (rabbit) model [12].

Besides the scarring fibrosis and squamous metaplasia, the mucosa demonstrates the ulceration sites ~~lacking epithelium and layered with granulation tissue containing a moderate number of small capillaries and various infiltrating cells. These ulceration sites are characterised as decubitus sores with extensive proliferation of connective tissue cells, as well as vast production of connective tissue fibers. Also~~ and the wrinkling of mucosal layer and cartilage, as well as atrophic glands ~~becoming atrophic could be present~~ [9,13].

Inflammatory cell infiltrations containing lymphocytes and plasma cells, but mostly neutrophils could be found both in mucosa and submucosa [14,15,16]. ~~although the specific initial processes – the signalling, migration and infiltrating – of these cells have not been only partially described [14]. Animal studies indicate early inflammatory cell infiltration in murine model of subglottic stenosis [16].~~

~~Previously listed pathological cell and tissue processes within tracheal wall validated by morphological, clinical and bronchoscopical findings play a significant role in the complex formation of post-intubation tracheal stenosis, however,~~ The cruicial player in the pathogenesis of post-intubation tracheal stenosis appears to be also the local inflammatory responses to the injury. Initial inflammatory phase of wound healing includes the release of the inflammatory signals from different cell types, not always derived from the immune system, that further enchances the accumulation of immunological cells (macrophages, granulocytes, lymphocytes) in tissue of exact organ. Inflammatory signals enchances the

ingrowth of fibroblasts, depositions of connective tissue components, angiogenesis and the formation of granulation tissue [14,17].

The presence of immune cells accumulated in the tissue occur due to the different cytokine expression, so the cytokine interleukine-1 (IL-1) family have autoinflammatory and autoimmune properties, but IL-1 itself is a proinflammatory cytokine. ~~One of the most important functions is the stimulation of thermoregulation centers in brain, thus the IL-1 works as endogenous pyrogen, also~~ IL-1 possesses also the regulatory function on innate immunity and inflammation by amplifying the humoral innate immunity and the resistance to infections, regulating tissue damage, recruiting leukocytes, prolonging the lifespan and stimulating the functions of neutrophils and macrophages [18,19]. ~~Also several changes in immunological responses could be detected due to the release of IL-1 – increased antibody production (adjuvant effect), increased lymphokine synthesis (IL-1β, IL-2, -3, -4, -5, -6, -7, -10, -12), enchanced development of T cell clones and other, also increased expression of various genes by IL-1 have been described: several cytokines, cytokine receptors, proinflammatory mediators, hepatic acute phase reactants, growth factors, clotting factors, tissue remodeling factors, components of extracellular matrix and other [20].~~

~~The main functions of cytokine interleukine-10 (IL-10) are immunoregulatory properties with multiple biologic effects on different cell types.~~ Other interesting cytokine is IL-10. IL-10 inhibits cytokines associated with cellular imunity and allergic inflammation while stimulating humoral responses [21] and promotes the growth of the B lymphocytes [18,20]. Primary sources of IL-10 are regulatory T cells, however, the most important IL-10 sources in human organism are monocytes and B lymphocytes. The dual nature of IL-10 have been described, firstly, IL-10 inhibits the synthesis of proinflammatory cytokines, therefore this cytokine has anti-inflammatory potential. Secondly, due to the activated immunogenic cells, IL-10 could promote the inflammation processes. However, IL-10 works as anti-inflammatory cytokine blocking pro-inflammatory and inflammatory signaling [18,20,21].

Tumor necrosis factor alpha (TNFα) is a potent cytokine with wide activity of pro-inflammatory properties [18,22]. TNFα have a wide spectrum of activities. It promotes the degranulation of neutrophils, previously of what the chemotaxis of neutrophils could be observed. TNFα also activates macrophages to excrete matrix metalloproteinases (MMPs), as well as promotes the transcription of pro-inflammatory genes. TNFα induces several cytokines, for example, IL-1 and IL-6 [23].

Beta defensin-2 (β def-2), an antimicrobic protein, prevents the skin and mucosa in respiratory, genitourinary, gastrointestinal systems from bacterial infections, therefore works as effectors of innate imunity and enchances the antigen specific humoral and cellular imunity [24]. β def-2 is a great example of defensins being activated by bacterial products and pro-inflammatory cytokines, which is released during the inflammatory response in normal tissue [25]. β def-2 is active against several Gram-negative bacteria and works synergically with antibacterial proteins, for example, lysozyme and lactoferrin. In vertebrates, β def-2 works not only as microbicid agent of innate immunity, but also promotes adaptive immunity, promotes the chemotactic recruitment of monocytes, macrophages, neutrophils and immature dendritic cells. β def-2 also stimulates the migration, proliferation of endothelial cells [26].

Previously described pathological findings – fibrous scarring, development of granulation tissue, squamous metaplasia, immune cell infiltration due to the ischemia at the site of cuffed tube – can not be seen exclusively, therefore exact local mechanisms, intercellular interactions between different cell types must be analyzed for the understanding of complex pathological events found in post-intubation tracheal stenosis. Intercellular signaling in form

of cytokine expression must be investigated to find specific pathogenetic stepways or how exactly the cuff pressure could lead to tracheal stenosis.

2. MATERIAL AND METHODS

2.1 Patients

The tissue was obtained from five patients (age 28 to 70) with post-intubation tracheal stenosis at Pauls Stradiņš Clinical University Hospital within a period of time in year 2013. Diagnosis of post-intubation tracheal stenosis was confirmed during the bronchoscopy, which was indicated for patients due to the clinical status. In all cases material was taken at the upper part of trachea from patients with prolonged intubation. The extension of tracheal stenosis evaluated through bronchoscope varies from 50% to 70% of the tracheal lumen; the length of fibrous tissue varies from 2 to 3 cm. All clinical information about the patients is summarized in Table 1. Patients with severe respiratory failure, severe GERD, concomitant autoimmune diseases, sleep apnea–hypopnea syndrome, local radiation therapy and administration of high-dose corticosteroids were not included in this study.

Table 1. Clinical data about the patients with post-intubation tracheal stenosis involved in study.

Patient / Data	Age	Gender	Cause of tracheal stenosis	Length of intubation (days)	Obesity (BMI of 30-39.9 kg/m^2)	Smoking	Cardio-vascular disease	Diabetes
1	66	Female	Prolonged intubation	7	Yes	No	Yes	Yes
2	69	Male	Prolonged intubation	5	No	15 pack years	Yes	Yes
3	70	Male	Prolonged intubation	7	No	20 pack years	Yes	Yes
4	28	Male	Prolonged intubation	6	No	6 pack years	No	No
5	29	Male	Prolonged intubation	5	No	3 pack years	No	No

2.2 Methodology

1) Soft tissue specimens of 1-10 mm^3 were taken under control of bronchoscope within local anaesthesia (submucosal administration on 1-2 mL of 1% lidocaine solution).
2) The mixture of 2% formaldehyde and 0,2% picric acid in 0,1 M phosphate buffer (ph 7,2) was used for tissue fixation. Afterwards tissue samples were rinsed in Thyroid solution, containing 10% sucrose for 12 hours, then were embedded into paraffin. Six to seven micrometers (μm) thin tissue sections were cut.
3) Routine histological staining with hematoxylin and eosin was used for each case to get review picture of the slide.
4) Sections were proceed for detection of cytokines IL-1α, IL-10, TNFα and defensin β def-2 by use of biotin-streptavidin immunohistochemistry (IMH) method [27]. The characteristics of primary antibodies were following:
    - IL-10 (code: P22301, rabbit, work dilution 1:400, BioSite),
    - TNFα (code: sc-52250, mouse, work dilution 1:100, Santa Cruz Biotechnology, INC),
    - IL-1α (code: sc-9983, mouse, work dilution 1:50, Santa Cruz Biotechnology, INC),

- β defensin-2 (code: 015263, goat, work dilution 1:100, R&D Systems).

5) The samples were examined under *Leica DC 300F camera* microscope for conventional histological picture. The relative number of positive immunohystochemical structures was graded semi-quantitatively [28,29]. The following scale of semi-quantitative method was used, counting the immunoreactive (positive) structures seen in visual field: 0 – no positive structures, 0/+ – occasional positive structures, + – few positive structures, +/++ – few to moderate number of positive structures, ++ – moderate number of positive structures, ++/+++ – moderate number to numerous positive structures, +++ – numerous positive structures, +++/++++ – numerous to abundance of positive structures, ++++ – abundance of positive structures seen in visual field.

3. RESULTS

3.1 Routine morphology

The squamous metaplasia of tracheal epithelium (stratified squamous epithelium instead of pseudostratified ciliated epithelium) was found in all tissue specimens (Figure 1). Moreover, the irregular thickening of basal membrane was seen in one case. Also numerous infrequent infiltration regions of inflammatory cells (macrophages, neutrophils, lymphocytes) were found in mucosa and submucosa of all tissue specimens (Figure 2), however, intraepithelial infiltrations were not present. The granulation tissue with various amounts has been found in submucosa of all cases, showing well-formed connective tissue with the presence of numerous blood vessels. Prominent connective tissue fiber bundles with fibroblasts and morphologically modified fibroblasts, as well as sclerotized blood vessels located diffusely were found in part of patients.

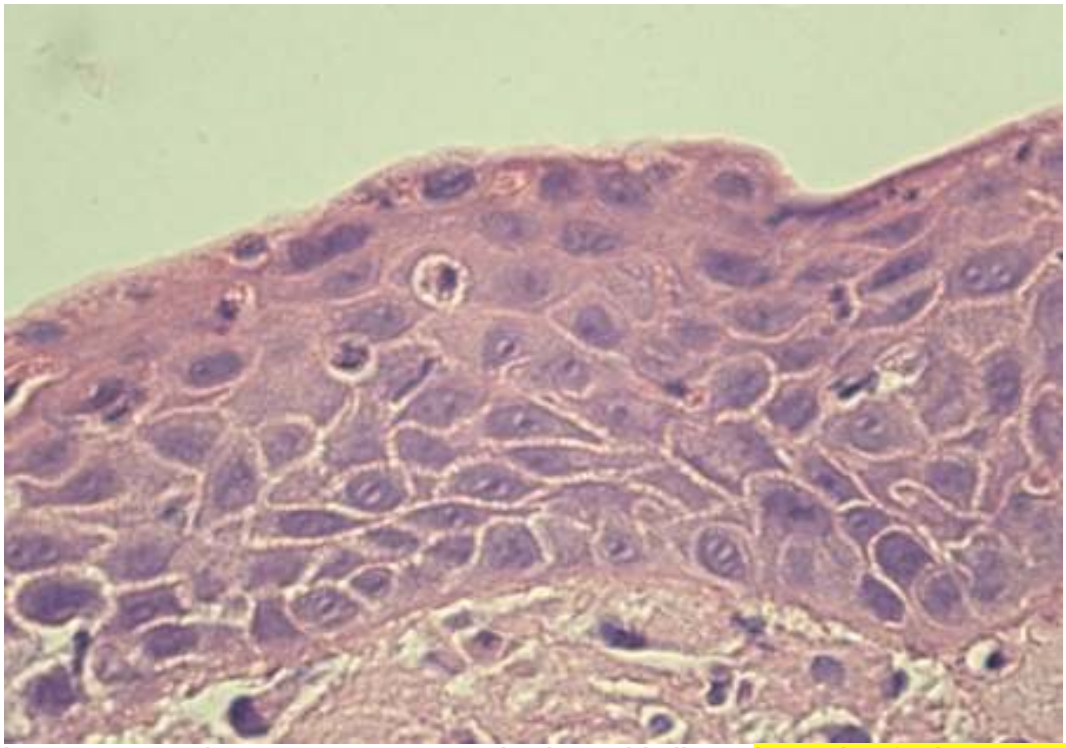

Fig. 1. Note the squamous metaplastic epithelium ~~instead of the normal pseudostratified ciliated epithelium~~ in tracheal mucosa of 28 years old male. Hematoxylin and eosin, X 400.

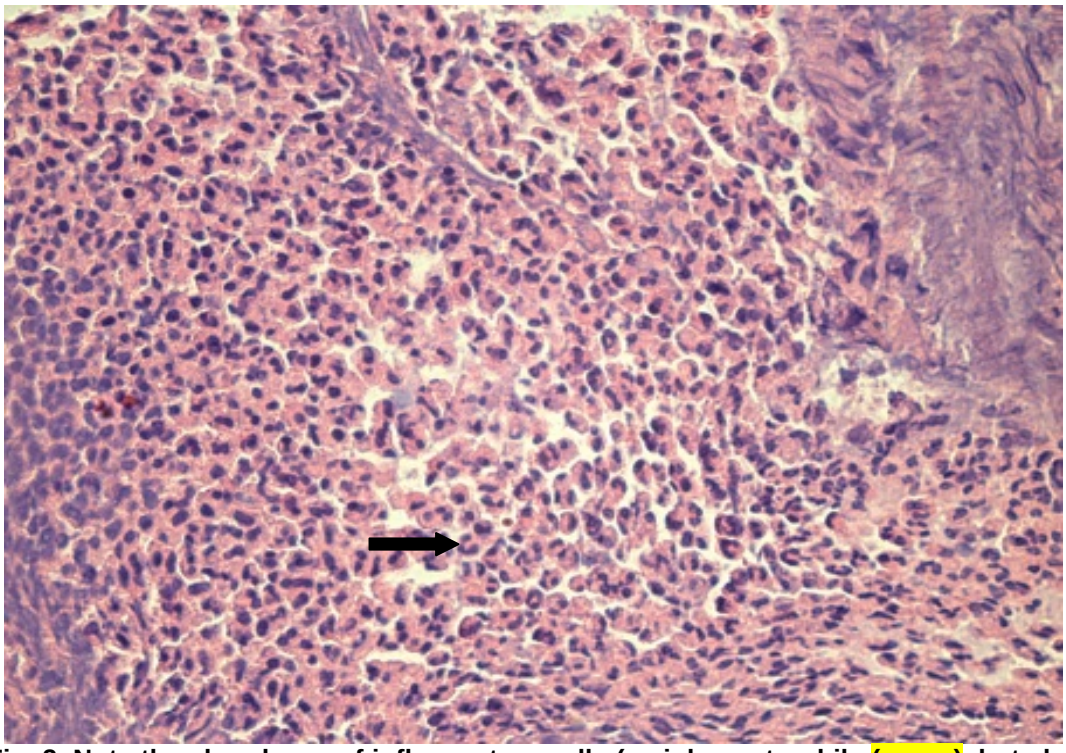

Fig. 2. Note the abundance of inflammatory cells (mainly neutrophils (arrow), but also lymphocytes) in the tracheal submucosa of 69 years old male. Hematoxylin and eosin, X400.

3.2 Immunohistochemistry

The tissue demonstrated a moderate number („++") to abundance („++++") of IL-10 positive inflammatory cells (macrophages, neutrophils, lymphocytes) both in mucosa and submucosa. Also few to abundance of fibroblasts (including modified fibroblasts), epithelial and endothelial cells for this cytokine were observed in tissue. In summary, the prominent expression of IL-10 was seen as numerous immunoreactive structures in both mucosal and submucosal layers of trachea (Table 2).

Table 2. Semiquantitative distribution of immunoreactive (positive) structures in the tissue of patients with post-intubation tracheal stenosis.

Factors/ Patients	IL-10			β def-2		
	e	*f*	*i c*	*e*	*f*	*i c*
1	++	+	+++	+++	++	+++
2	0	+	++	0	++	+++/++++
3	++++	+++	+++	+++	0	+++
4	++	+/++	+++	+++/++++	+++	+++
5	+++	+++	++	++	0	+++

"0" – no positive structures seen in the visual field, "0/+" – occasional positive structures seen in the visual field, "+" – few positive structures seen in the visual field, "+/++" – few to moderate number of positive structures seen in the visual field, "++" – moderate number of positive structures seen in the visual field, "++/+++" – moderate number to numerous positive structures seen in the visual field, "+++" – numerous positive structures seen in the visual field, "+++/++++" – numerous to abundance of positive structures seen in visual field, "++++" – abundance of positive

structures seen in visual field, "e" – epithelium, "f" – fibroblasts, "i c" – inflammatory cells.

Moderate number („++”) to abundance („++++”) of β def-2 positive structures were found in all cases: inflammatory cells (mostly neutrophils), fibroblasts, epithelial cells (Figures 3 and 4). Moderate („++”) number of β def-2 positive glandulocytes in submucosal tracheal glands were found in one tissue specimen.

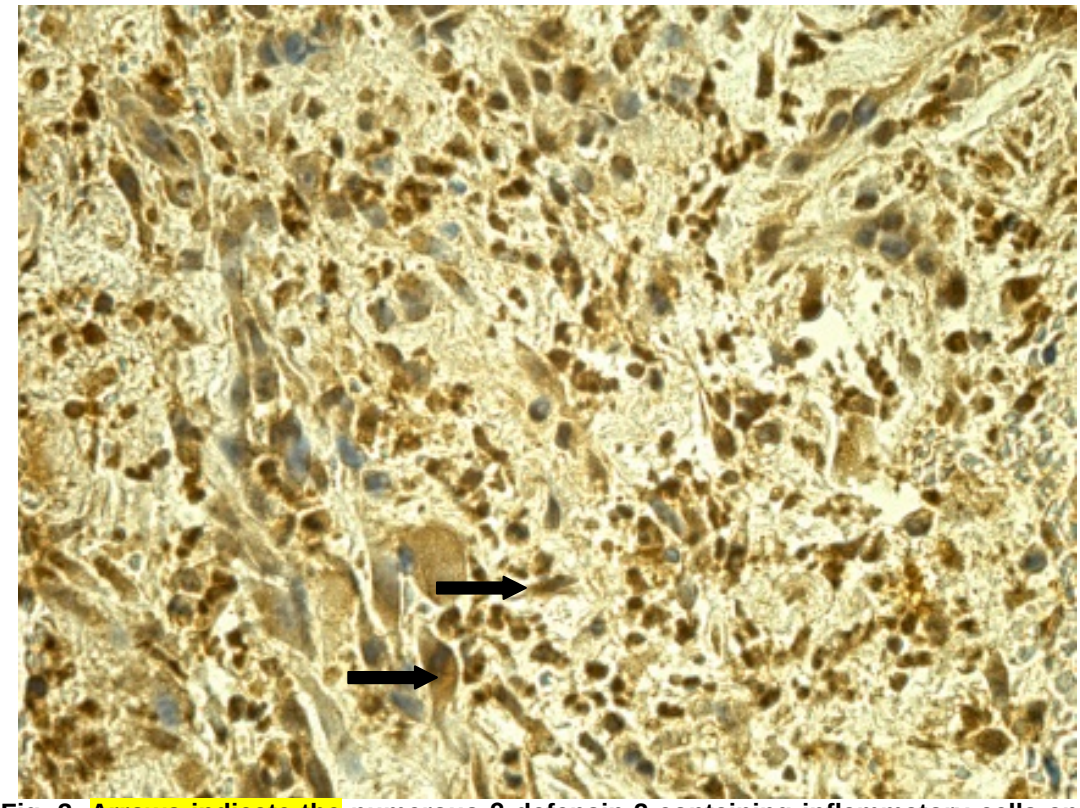

Fig. 3. Arrows indicate the numerous β defensin-2-containing inflammatory cells and moderate number of β def-2-positive fibroblasts in tracheal submucosa of 28 years old male. β defensin-2 IMH, X400.

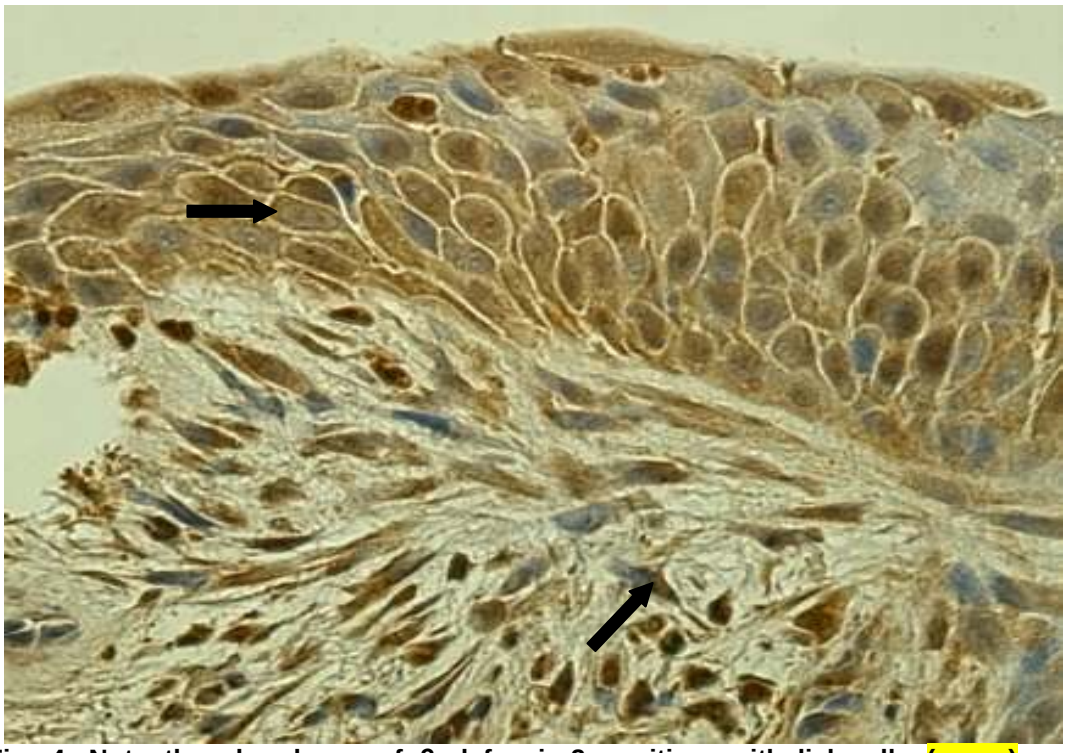

Fig. 4. Note the abundance of β defensin-2 positive epithelial cells (arrow) and numerous β defensin-2 positive fibroblasts (arrow) in tracheal mucosa of 28 years old male. β defensin-2 IMH, X400.

Only occasional („0/+") TNFα and IL-1 positive scattered macrophages were found in two cases (Fig.5). In three cases tracheal tissue samples were observed as TNFα and IL-1 negative due to the none of positive structures observed in the visual field.

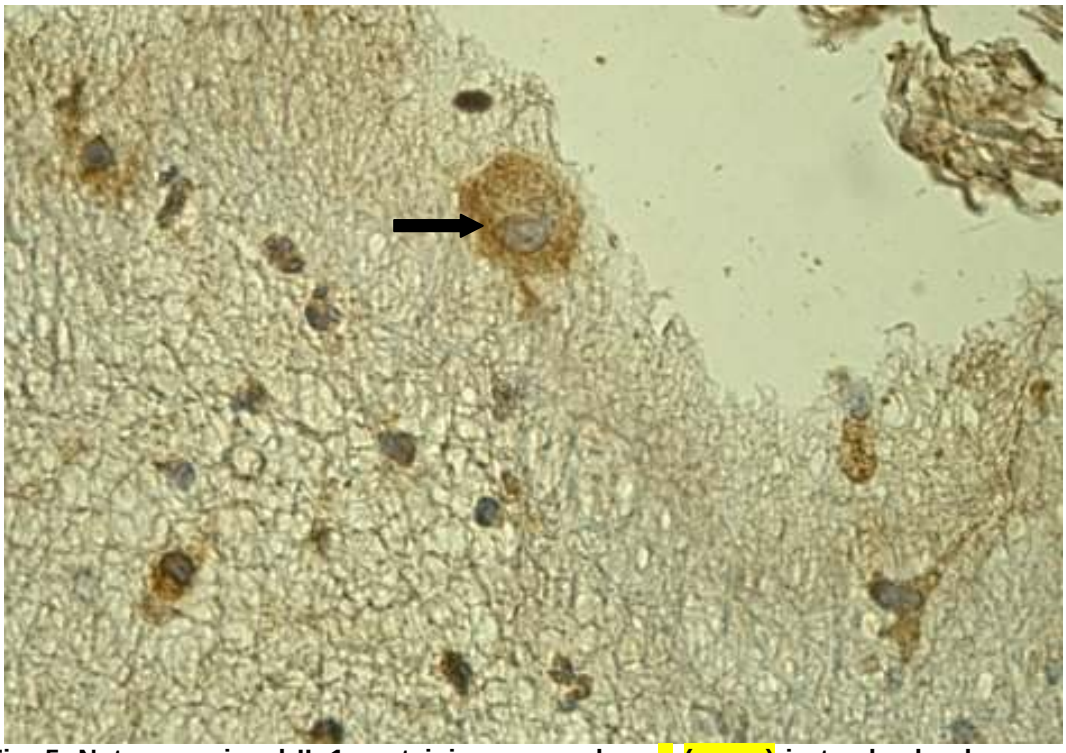

Fig. 5. Note ocassional IL-1-containing macrophages (arrow) in tracheal submucosa of 70 years old male. IL-1 IMH, X 400.

4. DISCUSSION

From all the factors predisposing the occlusion of tracheal wall capillaries and consecutive ischemia caused by the prolonged intubation with cuffed tube [5], we found smoking, cardiovascular disease and diabetes to be possible coexisting contributory factors in the development of post-intubation tracheal stenosis.

Our results show fibrotic tissue seen as adhesions located mostly in mucosa and submucosa, containing large amount of fibroblasts and components of extracellular matrix, as well as modified fibroblasts in two specimens, assuming this is the most important cause of bronchoscopically detected tracheal stenosis due to the web-like scarring. Secondary pathological events – scarring or cicatrization, web like fibrosis leading to local stricture – take place at previous necrotic site not only within mucosa, but also deeper in submucosa of tracheal wall [8]. Histological research of fibrosis reveal significant changes in in all layers of the tracheal wall [14]. Immunohistochemistry (IMH) investigation also reveals a strong martix associated subepithelial expression of transforming growth factor beta (TGF-β), which is one of the strongest inducers of myofibroblast differentiation and maturing, and is a mitogen to immature fibroblasts [30], as well as α-smooth muscle actin (α-SMA) for myofibroblast detection and collagen Type I was used to investigate the tissue of tracheal stenosis. Significant increase of collagen Type I deposits and intense web-like network of spindle shaped α-SMA positive cells were found in the subepithelial layer, suggesting that tracheal wall thickening found in post-intubation tracheal stenosis is related to myofibroblast activation which afterwards arranges collagen remodeling processes [6,31]. α-SMA, a marker of myofibroblasts, determines the activity of fibroblasts and probably their number in different phases [32]. Fibroblasts overexpressing the extracellular matrix (ECM) components (such as collgen Type I, collagen Type III, fibronectin) play an important role in the formation

of granulations within the tracheal wall, wound contraction and scar formation [7,32]. We can assume the obtained biopsy materials matches proliferation and mature phase, regarding the results of Cai et al. (2013), where authors observed the highest transforming growth factor (TGF)-β, α-smooth muscle actin (α-SMA), type I and III collagen expression in the proliferation phase and mature phase [32].

In our study, biopsy specimens obtained from patients using bronchoscopy can not be analyzed within all layers of tracheal wall, as the tissue specimens using bronchoscopy could be taken only from upper surface of lining mucosa and submucosa. Previous researches confirm the fibrotic changes or cicatrization through all tracheal layers, however, analysis of whole tracheal wall is not relevant to diagnostic observations in everyday clinical practice. We could speculate if fibrosis found only in mucosa and submucosa indicates fibrotic processes deeper in tracheal wall as well.

In the analysis of tissue specimens stained with hematoxylin and eosin, we found squamous metaplasia in all study subjects. Proliferating Ki-67 (nuclear protein, necessary for cellular proliferation) positive cells were investigated and found mainly localized in the basal epithelial layer also with squamous epithelial metaplasia alltogether in most of the specimens [30], proposing the squamous metaplasia appears as a result of actively proliferating basal (stem) cells in epithelium, but specific inductor has not been found yet. Since the epithelium is exposed to the cuffed tube first, also the squamous metaplasia could appear first at the site of cuffed tube compressing tracheal wall. We could not prove the sequence of squamos metaplasia occuring before any other pathological events due to the findings of our study, where squamous metaplasia was found together with immune cell infiltration in all cases. Similar findings were found in the morphological analysis of iatrogenic subglottic tracheal stenosis mainly due to the long-term intubation [14]. In rabbit model, significant narrowing of the lumen occured due to the increase in submocasal thickness of tracheal stenosis induced by intubation [12,33]. In murine model, chemically and mechanically injured tracheal specimens showed either attenuated or regenerated epithelium within few pathological findings regarding fibrosis and granulations deeper in mucosa and submucosa [16].

In our results, granulation tissue was present in tissue specimens of all patients involved in this study. Granulation tissue occured within various presentation – from few to moderate number of structural components found in both mucosal and submucosal layers. Besides the fibrosis found in part of tissue examples taken from patients with post-intubation tracheal stenosis, also the granulation tissue plays an important role in pathogenesis of tracheal stenosis and thus is related to the clinical and bronchoscopic findings. Granulation tissue and fibrosis shows complex and yet interacting wound healing seen in tracheal wall. Hypoxic conditions due to the ischemia caused by cuffed tube pressure on mucosa and submucosa in trachea is an important pathogenetic member. The expression of hypoxia-inducible factor (HIF)-1α (a nuclear transcription factor that facilitates the adaption of cells under hypoxic conditions) is seen highest in the granulation phase of the tracheal healing process, therefore suggesting HIF-1α may be a potential key regulator in both the initiation and facilitation of post-intubation tracheal stenosis pathogenesis [32]. Newly formed connective tissue, as well as rapid angiogenesis in granulation tissue may show the capabilities of healing and tissue remodelation instead of fibrous scarring (that could be seen as tissue replacement stage in damaged areas without further remodeling possibilities), and the at least partial renewal of previous structures at the wound site. Modified fibroblasts within the ingrowth of newly formated connective tissue are a characteristics of successful tracheal wall remodeling process.

We found numerous immune cell (neutrofils, lymphocytes, macrophages) infiltration sites within mucosal and submucosal layers of tracheal wall. The expression of pro-inflammatory cytokines IL-1α and TNFα in our study of post-intubation tracheal stenosis affected patients briefly is characterised as weak, therefore we can assume the early inflammation part of wound healing ir replaced by further changes.

Regarding wound healing cascade described by Hirshoren and Eliashar (2009), we can assume our study findings show mostly the proliferation and maturation phase of wound-healing modulation processes in trachea, where massive cell proliferation within several cell types (fibroblasts, macrophages, keratinocytes and endothelial cells)~~, as well as granulation, after which the maturation stage of wound healing cascade is followed by remodeling and scarring processes~~. Therefore, previous early release of inflammatory mediators (the most important, IL-1, TNFα) at the inflammation phase of wound healing cascade, cell migration of polymorphonuclear granulocytes (mostly neutrophils), monocytes, macrophages [15] was not observed in this study due to negative findings of active inflammation factors (none of IL-1 and TNF-α positive cells).

The weak expression of pro-inflammatory cytokines within our study suggest that the initiatory part of occuring inflammation occured some time before the clinical signs (due to which the endoscopy was performed) appeared. TNFα and IL-1α was detected only in few scattered macrophages within mucosal and submucosal layers of tracheal wall, also fibrotic tissue was present. It is known, that IL-1 could induce the mitosis for smooth muscle cells and fibroblasts, thus it has has pro-fibrotic effects in many chronic inflammatory diseases. Also several changes in immunological responses could be detected due to the release of IL-1 – increased antibody production (adjuvant effect), increased lymphokine synthesis (IL-1β, IL-2, -3, -4, -5, -6, -7, -10, -12), enchanced development of T cell clones and other, also increased expression of various genes by IL-1 have been described: several cytokines, cytokine receptors, proinflammatory mediators, hepatic acute phase reactants, growth factors, clotting factors, tissue remodeling factors, components of extracellular matrix and other [20]. The IL-1α, one of the IL-1 family cytokines, within the form of its precursor (pro IL-1α) is present in all epithelial cell layers of the entire lung, endothelial cells. Upon cell death by necrosis, as occurs in ischemic diseases, the IL-1α precursor is released. Hypoxia, ischemia along with the reperfusion is know as inducers of IL-1. Furthermore, the IL-1α precursor rapidly initiates a cascade of inflammatory cytokines and chemokines. Another type – a membrane form of IL-1α – is seen on activated monocytes and B lymphocytes [19,20]. IL-1α also induces the release of IL-1β, which strongly amplifies the inflammation processes by recruiting macrophages [34], as well as induce the proliferation stage of wound healing cascade [15]. In the study by Haft et al., some cytokines were elevated – including IL-1β, IL-10, TNFα –, suggesting that symptomatic tracheal granulation tissue is mostly seen as the early inflammatory phase of wound healing, also the early fibrotic and angiogenesis remodeling processes could be described within detecting different cytokines [34].

IL-10 was widely expressed in many cell types – starting from inflammatory cells (neutrophils, macrophages, lymphocytes) and continuing with epithelial cells, fibroblasts and modified fibroblasts (myofibroblasts), endothelial cells. The anti-inflammatory properties of IL-10 persisted as normal response to the inflammation, suggesting the all cell types expressing IL-10 has sufficient sources of controling the immune response and maintaining the proliferation and maturation phases of wound healing. We hypothese, the early initiation of inflammatory processes, where IL-1 and TNFα work towards the intensifying the inflammation process, has been replaced by next stage of wound healing with, firstly, the modulating action, and afterwards an inhibiting abilities of anti-inflammatory cytokines, particularly, IL-10. The cytokine IL-10 have several functions regarding inhibition of pro-inflammatory processes – it affects antigen presenting cells, supressing their ability to

activate Th cells, as well as decreases the synthesis of IL-1β, IL-2, -4, -5, -6, -8, -12, particulary important, also TNF-α, thus working as anti-inflammatory cytokine [18,20,21].

The expression of β def-2 was found as moderate to numerous positive structures located both in mucosa and submucosa – inflammatory cells, fibroblasts, epithelial cells – , also modified fibroblasts and glandulocytes of submucosal glands were found in one tissue specimens. We can hypothese the wide expression of β def-2 in outer structures, as well as in fibroblasts, inflammatory cells and endothelium of tracheal wall, suggest the tissue itself has activated its defense systems against potential inhalated antigenes, also show readiness to initiate immune responses to protect wounded tracheal wall. Due to the antimicrobic properties of β def-2 in the outermost respiratory tract parts (epithelium, glands with the secrete) that serve as natural barriers towards the environment full of different antigenes [35], we could suggest our β def-2 expression results show the antibacterial abilities of wounded tracheal wall that has been firstly induced by proinflammatory cytokines at first (mostly IL-1) [36], and afterall joined by wide expression throughout the tracheal wall.

The exact interactions between native cells from different tissue found in tracheal wall and migrating inflammatory cells would be revealed performing the analysis of whole tracheal wall, also the analysis of broader spectrum of pro- and anti-inflammatory cytokines and other proteins.

5. CONCLUSIONS

The chronic inflammation with the formation of scarring tissue as a result of the previous formation of granulation tissue, as well as the remodeling of tracheal wall with the presence of fibroblasts and modified fibroblasts, also the ingrowth of connective tissue, assumedly, has the leading role in complex pathogenesis of post-intubation tracheal stenosis.

The intense local tissue defense reactions are presented as compensatory expression of antimicrobial peptide β defensin-2 and anti-inflammatory cytokine IL-10 found in different cell types within all tracheal wall layers.

TNFα and IL-1 are not among the most significant factors in pathogenesis of PITS despite of the presence of numerous inflammatory cells.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

This study was approved by the Ethical Committee of Pauls Stradiņš Clinical University Hospital dated of 23th January, 2013.

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