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Comparative study of neovascularization density in tracheal defect reconstruction with external oblique muscle aponeurosis patch and dry amniotic membrane (A study with New Zealand rabbits)

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Abstract: The high incidence of complications makes tracheal defect reconstruction challenging to found the ideal technique to repair tracheal defect. The use of dry amniotic membrane to promoted wound healing processed and aponeurosis as a surgical patch and flap in reconstruction is not uncommon, but no studies had been conducted to analyze its combination uses in tracheal defect reconstruction, though its effectivity in wound healing was significant in reconstruction of other organs. This study aims to compare neovascularization in tracheal defect reconstruction using external oblique muscle aponeurosis patch only and using external oblique muscle aponeurosis patch combine with dry amniotic membrane patch. This randomized control trial is a laboratory experimental study using male New Zealand white rabbits. Randomization was carried out with permuted block randomization into a control group (reconstruction with external oblique muscle aponeurosis patch only) and a treatment group (reconstruction with external oblique muscle aponeurosis patch combine with dry amniotic membrane). Histopathological assessments were carried out 14 days after surgery. We got homogeneous samples in both groups (16 samples each) in terms of age (p=0.712), weight before procedure (p=0.247), and weight after procedure(p=0.534). Histopathological analysis showed that there were significant differences in neovascularization (p=0.007) between the two groups. It can be concluded that there are significant differences in neovascularization in New Zealand rabbits with tracheal defect using external oblique muscle aponeurosis patch only and external oblique muscle aponeurosis patch combine with dry amniotic membrane patch.

Keywords: *Aponeurosis patch, Dry amniotic membrane, External oblique muscle aponeurosis, Neovascularization, Tracheal reconstruction.*

1. Introduction

The trachea, besides carrying out the function of air conduction between the larynx and the lungs, plays a vital role in protecting sensitive lung tissue from injury and invasion by microorganisms. Defects in the trachea will increase airway resistance and increase the patient's respiratory workload. Although rare, tracheal injury whether due to trauma, disease, or iatrogenic is a medical condition that requires attention due to its potential to be life-threatening.

In large tracheal defect, tracheal reconstruction is often necessary. However, tracheal reconstruction is a challenge in itself due to the lack of ideal materials and the high incidence of post-reconstruction complications. The use of aponeurosis as a surgical patch and flap for reconstruction is not uncommon, however, no studies had been conducted to analyze its use in tracheal defect reconstruction. In fact, previous studies have shown increased effectiveness of wound healing with the use of aponeurosis patches in the reconstruction of other organs.

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Neovascularization is pivot components in the process of wound healing. Neovascularization is important for wound healing because neovascularization modulated granulation tissue growth and stimulated wound healing.

2. Method

This study used a randomized control trial, a laboratory experimental study using 32 male New Zealand white rabbits with age between 6 until 9 month, weight between 1900 until 2500 gram, and no anatomies abnormality. Randomization was carried out with permuted block randomization into a control group (reconstruction with external oblique muscle aponeurosis patch only) and a treatment group (reconstruction with external oblique muscle aponeurosis patch combine with dry amniotic membrane), each group had 16 rabbits. Exclusion criteria were rabbits that had abnormality behavior (aggressive) during acclimatization period and rabbits that had death during acclimatization period. Drop out criteria were rabbits that dead and had ill during experimental period. Experiment had placed in laboratory of medical animal faculty of Airlangga university. Histopathological assessments were carried out 14 days after surgery. Trachea were harvested 14 days after surgery. Neovascularization was observed with hematoxylin and eosin under microscope with 400x zoom. The obtained data were processed based on the research variables using SPSS 23.0 for windows, presented in the form of tables and analyzed with t-test non parametric.

Table 1.
Sample distribution.

Characteristics	Group		P-Value
	Control (n=16)	Treatment (n=16)	
Age (Month)			
Mean	7.04	7.32	0.712
Standard deviation	0.88	1.04	
Median	7 (6-8.5)	7.5 (6-9)	
Weight before experiment (g)			
Mean	2213.2	2212.7	0.247
Standard deviation	103.53	93.23	
Median	2190 (2055-2330)	2135 (2000-2310)	
Weight after experiment (g)			
Mean	2074.4	2043.3	0.534
Standard deviation	77.54	82.12	
Median	2035 (2010-2030)	2023 (1900-2180)	

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Table 2.
Neovascularization distribution degree in control and treatment groups.

No.	Control group		Treatment group	
	Sample code	Neovascularization degree	Sample code	Neovascularization degree
1	K1	20	PK1	40
2	K2	10	PK2	30
3	K3	10	PK3	20
4	K4	20	PK4	20
5	K5	10	PK5	40
6	K6	20	PK6	50
7	K7	20	PK7	40
8	K8	20	PK8	30
9	K9	20	PK9	20
10	K10	10	PK10	60
11	K11	20	PK11	20
12	K12	30	PK12	20
13	K13	30	PK13	40
14	K14	30	PK14	70
15	K15	10	PK15	20
16	K16	10	PK16	20
Amount		290		540
Average		18,12		33,75

Table 3.
Comparison of neovascularization density between control and treatment group.

Groups	Neovascularization (Mean ± SD)	P-value
Control group	18.12 ± 7.50	0.07
Treatment group	33.75 ± 15.86	

3. Result

Based on Table 1, Ages of control group sample between 6-8,5 month, mean 7,04 month, median 7 month, and standard deviation 0,88. Meanwhile, ages of treatment group sample between 6-9 month, mean 7,32 and median value 7,5 , with standard deviation 1,04. The result of age analyzed showed no differentiation between control group and treatment group (p-value=0.712). The weight before experiment of control and experiment group sample were mean 2.213,2 gr and 2.212,1 (p-value=0,247). And weight after experiment of control and experiment group sample were mean 2.074,4 gr and 2.043,3 gr (p-value=0,534).

3.1. Histopathological Analysis of Neovascularization Levels

Neovascularization levels will be observed by recording the distribution of neovascularization in 5 different microscopic fields of view. It was found that of the 16 control samples, 6 samples had the lowest neovascularization distribution of 10 neovascularizations, 3 samples with the highest degree of neovascularization distribution of 30 neovascularizations. In the treatment group, 7 samples had the lowest neovascularization distribution of 20 neovascularization and 1 sample with the highest neovascularization distribution of 70 neovascularization. The histopathological features of neovascularization for each distribution is depicted in Figure 1. The mean neovascularization in the control group was 18.12 + 7.50, while the mean neovascularization in the treatment group was 33.75 + 15.86. While the results of the comparative analysis of the mean neovascularization in both groups showed significant results with a value of p = 0.007.

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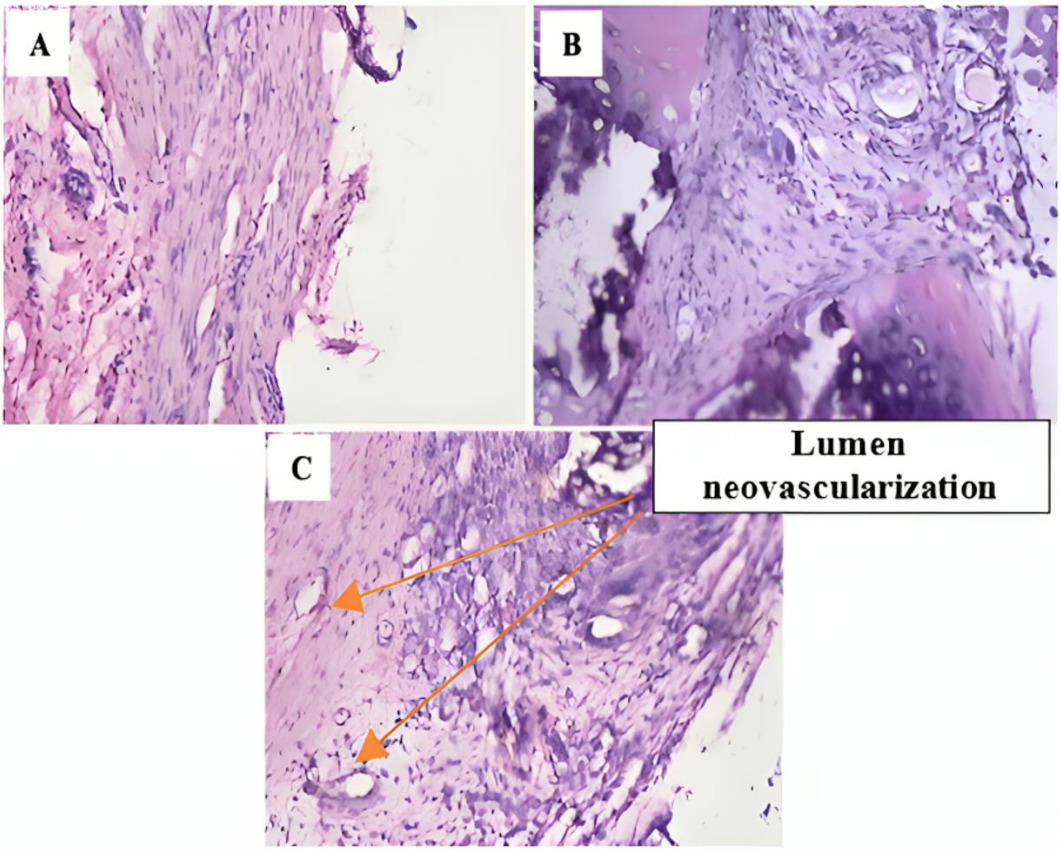

Figure 1.
Histopathological features of neovascularization distribution: (A) Low density, (B) Medium density, and (C) High density.

4. Discussion

This study is an experimental study with a single-blinded randomized control trial study design that aims to analyze the comparison of the results of tracheal defect reconstruction in experimental animals using the external musculus oblique aponeurosis patch and using the external musculus oblique aponeurosis patch and dry amniotic membrane. The test animals used were rabbits, with all rabbits used being the same breed of New Zealand rabbits with male sex. Based on the characteristics, there was no significant difference between the control group and the treatment group both in terms of age and body weight before and after the action ($p>0.05$). This indicates that the sample in this study is homogeneous, so it has low potential bias.

The results of this study were found in the analysis of neovascularization distribution. The general mean value of neovascularization levels in the treatment group was higher than the control group, the analysis also showed a significant difference in both groups ($p<0.05$). These results prove our hypothesis that the treatment group with external oblique musculus aponeurosis patch and dry amniotic membrane has better neovascularization than the control group which only uses external oblique muscular aponeurosis patch.

Right now, there are no studies assessing tracheal defect reconstruction using aponeurosis patches and dry amniotic membrane to compare the results of this study. In a previous study conducted by Kamal, et al. [1] reported cases of tracheal reconstruction with primary sutures and with the external oblique musculus aponeurosis flap, there were no significant results in both control and treatment

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groups in increasing neovascularization in the wound healing process of tracheal defect reconstruction in New Zealand rabbits .

Several other studies have used pericardium patches [2-4] which are considered the best material for tracheal defect repair due to their smooth surface, strength, and sufficient air tightness. Cheng, et al. [5] reported two cases in which histopathological changes were analyzed after the use of pericardial patches for tracheoplasty.

The analysis in the first case was a post-mortem analysis two years postoperatively, where observation by light microscopy with HE staining showed the surgical defect area in the tracheal cartilage was filled with dense collagen tissue accompanied by mature vascular canals, which are commonly found in mature scar tissue. The second case was histopathologically assessed 20 months postoperatively, with mature scarring found in the surgical area, with the grafted area having undergone complete re-epithelialization with pseudostratified columnar epithelium [5].

The use of fascia as a patch includes reconstruction of abdominal wall defects after tumor resection and infected incisional abdominal hernias [6, 7]. Abdominal wall reconstruction with autologous fascia transplantation can use the anterior layer of the rectus sheath, the aponeurosis of the external oblique musculus as in this study, and the fascia lata patch, but the fascia lata patch is most commonly used because it is larger and stronger, microscopically found to have a thick and wavy collagen layer accompanied by a number of nourishing blood vessels. Another study in 2012 assessed the use of the anterior aponeurosis of the rectus sheath as a graft to close the abdominal wall in rabbits. Histologic analysis seven days postoperatively in this study showed an inflammatory process with maturing collagen and myofibroblasts. There were no signs of acute rejection or dehiscence, herniation, cellulitis, abscess, or hematoma in the wound [8].

The use of dried amnion as a patch for tracheal defects has been done before by comparing the control group with primary suture and the treatment group using MAK patches. However, this study cannot be used as a comparison because the measurement is a measurement of fibroblast levels in wound healing. The research conducted by Corputty, et al. [9] proved that the use of MAK in tracheal defects can improve wound healing by measuring fibroblast levels in the wound area. This is in accordance with the theory that amniotic membrane expresses many neurotrophic and angiogenic factors, such as endothelin-2 and endothelin-3, vascular endothelial growth factor, vascular endothelial growth factor-B, angiopoietin Tie-2 receptor, ephrin-A2, ephrin-A2, B1, B3, B4, B5, neuropilin-2, nerve growth factor receptor, and semaphorin-F19 as well as erythropoietin and its receptors that contribute to wound healing. Several studies have shown the effectiveness of amniotic membrane grafts for wound healing [10].

Some other autologous tissues that have been tested for tracheal replacement include fascia, cartilage, dermis graft, momentum, periosteum, perichondrium, buccal mucosa and auricular cartilage, dura mater, urinary mucosa, periosteum, and jejunum patch as free grafts, as well as intercostal latissimus dorsi, trapezius muscle, periosteum, buccal mucosa, and concha cartilage as vascularized flaps [11]. This is a distinct advantage of this study, as it is new in tracheal reconstruction and has never been studied before. In addition, there is also no study of tracheal reconstruction with the use of any patch that performs specific histopathological analysis to assess the components that play a role in wound healing, one of which is neovascularization as in this study. Considering the gross specimen analysis of the external musculus oblique aponeurosis patch that fused and firmly closed the tracheal defect, the use of other markers of wound healing can be used in the future such as immunohistochemistry or electron microscopy for tissue tensile strength.

However, this study does not escape some limitations. One of the basic ones is the small number of samples with limited time, so the inference from these results can be said to be not optimal. Another limitation of this study is that the histopathological assessment was only performed by one anatomical pathologist. Histopathological data were analyzed semi-quantitatively, which may cause bias that should be minimized by the assessment of two or more anatomical pathologists.

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5. Conclusion

There is a clinically and statistically significant difference in neovascularization in New Zealand rabbits with tracheal defects reconstructed using external oblique musculus aponeurosis patch alone and with external oblique musculus aponeurosis patch and dry amniotic membrane.

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Transparency:
The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Competing Interest:
The authors declare that they have no competing interests.

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Author's Contributors:
All major contributions, including conceptualization, data analysis, and manuscript preparation, were undertaken by Muhammad Afifudin, under the supervision of Marjono Dwi Wibowo and Dwi Hari Susilo.

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