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The Quantitative Analysis of the Vascularization Following Two Basic Auditory Canal Skin Incisions

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A B S T R A C T

Three groups of nine patients each were analyzed. The first two groups consisted of those that underwent tympanoplastic due to chronic inflammation of middle ear. Two different standard auditory canal skin incisions were applied, i.e. tympanomeatal flap (TMF) or vascular strip (VS). The third control group consisted of non-operated patients. All the operated patients were subjected to a quantitative analysis of the auditory canal revascularization by means of the Weibel stereological test method, i.e. the B 100 double network system. The density of capillaries, arterioles, venulolymphatic spaces and a total volume density of all vascular elements of the auditory canal skin were measured. The obtained results of vascularization were compared with those of the target control group. It was found out that there were no significant differences in vascularization of auditory canal skin between TMF and VS patients from one side and the control group on the other side.

Key words: *auditory canal, skin vascularization, tympanomeatal flap, vascular strip*

Introduction

Various incisions have been described for the purpose of access to the middle ear when undergoing tympanoplasty[1–3]. Most of them origin from two standard incisions: the one forming tympanomeatal flap (TMF), and the other vascular strip (VS). TMF is formed by means of two longitudinal auditory canal incisions. The upper one runs along the tympanosquamous suture line (approximately in position 12 o'clock), and the lower one at approximately 6 o'clock. The incisions commence close to the annulus and extend about 8 mm towards the auditory canal exit where they are joined with a transversal incision.

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The VS differs from TMF in two features. The VS is exclusively applied in retroauricle approach. Its basis is located laterally from the tympanic membrane, in the level of bony cartilaginous junction. Longitudinal incisions run towards the edge of the tympanic membrane where they are joined with a transversal incision. The upper one runs along the tympanosquamous suture line, whereas the lower one follows the tympanomastoid suture line (in left ear at position of 3 o'clock, in the right ear at 9 o'clock)[4–7].

The objective of the paper is to assess the revascularization of auditory canal skin of patients to whom TMF or VS was formed following a tympanoplasty. The objective was carried out by means of the stereology method in quantitative analysis of vascular elements (capillaries, arterioles, venulolymphatic spaces).

Material and Methods

In this brief study, three groups of nine patients each were analyzed. The first group was made of 5 males and 4 females in the age of 20–45 (average age being 36.9). The second group consisted of 4 males and 5 females in the age of 19–43 (average age being 32.3). The third group included 6 males and 3 females in the age of 23–52 (average age being 34.8). Both the first and the second group of patients were chosen among those who had already been tympanoplasty operated more than a year before (average before 2 years and 9 months). The first group was subjected to a skin incision leading to the of TMF formation in the auditory canal, whereas the second one underwent VS formation. The third group of patients was chosen among those having chronic ear inflammation and going to have tympanoplasty for the first time. All the patients had a normally wide auditory canal, without pathological changes and anatomical anomalies. Their ears were fully dry.

Three pieces of auditory canal skin were taken from each patient for analysis, one from upper, one from posterior and one from lower canal wall, at a distance of 1–2 mm from the tympanic membrane (Figure 1). The skin was taken in its full thickness, starting from epithelium to the near bone tissue. Then the skin was dipped into 10% formalin solution (pH = 7.0) for unlimited fixation at 60 °C. This was followed by a routine embedding into paraffin (70% alcohol – 6 hours, 96% alcohol – 18 hours, 100% alcohol – 4 hours, toluol – 2 hours, paraffin 18 hours).

The skin was embedded into paraffin cubes, thus enabling to obtain slices of 6 mm in depth throughout the skin thickness. Each biotic sample was cut into 5–15 slices referred to in the language of stereology as 5 – 15 cross sectional views, or referential systems (referential spaces measured by the test system). The incisions were treated at a water tempera-

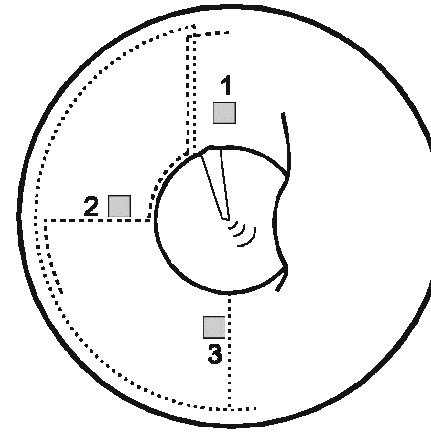

Fig. 1. The auditory canal segments where from the tissue was taken for the quantitative analysis of vascularisation: 1 = upper wall; 2 = posterior wall; 3 = lower wall. Schematic diagrams of two different canal incisions: (……) = tympanomeatal flap; (------) = vascular strip.

ture of 38–40°C. The incision staining was carried out by a standard method (hemalaun-eosin) in a Stainette (Rechert Wien) for 1 hour.

The quantitative analysis of the auditory canal skin vascularization of the three above quoted groups of patients was carried out by means of the stereological method. In the histological skin, the volume density of individual vascular elements – vascular phases (Vp), was determined. The volume density Vp, as a relative stereological variable, should show the percentage of the analyzed unit volume in the total skin volume. The volume density of capillaries (c), arterioles (a) and venulolymphatic spaces (vl) was analyzed. Since the subject matter elements are of a different volume (capillaries are much smaller than arterioles and venulolymphatic spaces), a double B 100 network system was used (Weibel). The microscopic magnification was 10 (objective) × 1.6 (intermediate lens) × 6.3 (eyepiece lens). The double network test system is made up of two kinds of test points, thick line intersections (P_t) and all line intersections (P_t'). When magnified, the capillaries were found to be present in test points corresponding to all line intersections (P_t'), whereas the arterioles and venulolymphatic spaces were identified at one to two thick line intersections. The relation between P_t and P_t' was established by formula: $P_t' = P_t \times q^2$. The test was performed starting from epithelium to the near bone tissue and, in parallel, backwards. In order to obtain the volume density of each analyzed Vp, the following formulae were used[8]:

$V_{Vc} = P_c/P_t'$, $V_{Va} = P_a/P_t$ and $V_{Vvl} = P_{vl}/P_t$

V_{Vc} = Volume density c; P_c = number of identified test points per c; $P_t' = 400$

V_{Va} = Volume density a; P_a = number of identified test points per a; $P_t = 100$

V_{Vvl} = Volume density vl; P_{vl} = number of identified test points per vl; $P_t = 100$

The average volume density of each Vp for each group of patients was calculated by means of arithmetic averages of individual patients' Vp. The arithmetic average of the total volume density of all patients' Vp within the group was used to determine the total volume density of all Vp (vascularization) of each group of patients. The average of volume density of each Vp and the total volume density of all Vp were calculated separately for upper, posterior and lower walls of the auditory canal[9].

The significant differences of the total volume density of all Vp were defined by the t-test for each of the above quoted auditory canal segments (software package STATISTICA 6.0, StatSoft, Inc.).

Results

The numeric variables of stereological variables obtained by the skin tested (average volume density of individual Vp and total volume density of upper, posterior and lower Vp walls) were contrasted. Contrasting the numeric values referring to upper, posterior and lower auditory canal regions, following results were obtained:

– The numeric value of individual Vp volume density averages is the highest in the upper region, somewhat lower in the posterior, and the lowest in the low wall region in all three groups of patients tested (Table 1);
– The numeric value of the total volume density of all Vp is also highest in the upper region, somewhat lower in the posterior, and the lowest in the low wall region, in all three groups of patients tested;

Such results correspond to the existing data of the qualitative histological analyses of the auditory canal.

The primary purpose of the study was to analyze the revascularization after TMF

TABLE 1
AVERAGE VOLUME DENSITY OF VASCULAR PHASES (CAPILLARIES, ARTERIOLES AND VENULOLYMPHATIC SPACES) OF UPPER, POSTERIOR AND LOWER AUDITORY CANAL WALL IN THREE GROUPS OF PATIENTS

Auditory canal wall		TMF	VS	Non-operated
Upper	V_{Vc}	0.2301	0.1087	0.4400
	V_{Va}	0.0156	0.0232	0.1600
	V_{Vl}	0.0044	0.0106	0.0840
Posterior	V_{Vc}	0.1100	0.0913	0.3495
	V_{Va}	0.0289	0.0111	0.0721
	V_{Vl}	0.0133	0.0023	0.0863
Lower	V_{Vc}	0.1437	0.0783	0.1976
	V_{Va}	0.0189	0.0000	0.0178
	V_{Vl}	0.0056	0.0000	0.0267

V_{Vc} – volume density of capillaries; V_{Va} – volume density of arterioles; V_{Vl} – volume density of venulolymphatic spaces

TABLE 2
TOTAL VOLUME DENSITY COMPARISON OF ALL VASCULAR PHASES OF THE UPPER, POSTERIOR AND LOWER CANAL WALLS IN BOTH THE OPERATED (FORMED TYMPANOMEATAL FLAP) AND NON-OPERATED PATIENTS

Auditory canal wall	TMF		Non-operated		t	p
	V_P	SD	V_P	SD		
Upper	0.0834	0.0361	0.2280	0.0536	6.71	0.000005
Posterior	0.0507	0.0464	0.1693	0.0327	6.27	0.000011
Lower	0.0560	0.0284	0.0807	0.0477	1.33	0.202

V_P – mean total volume density of all vascular phases (capillaries, arterioles and venulolymphatic spaces)

TABLE 3
TOTAL VOLUME DENSITY COMPARISON OF ALL VASCULAR PHASES OF THE UPPER, POSTERIOR AND LOWER CANAL WALLS IN BOTH THE OPERATED (FORMED VASCULAR STRIP) AND NON-OPERATED PATIENTS

Auditory canal wall	VS		Non-operated		t	p
	V_P	SD	V_P	SD		
Upper	0.0475	0.0524	0.2280	0.0536	7.22	0.000002
Posterior	0.0349	0.0358	0.1693	0.0327	8.31	0.0000003
Lower	0.0261	0.0336	0.0807	0.0477	2.80	0.0127

V_P – mean total volume density of all vascular phases (capillaries, arterioles and venulolymphatic spaces)

and VS tympanoplasty operation. Therefore, the total values of the volume density of all Vp of individual auditory canals were compared. The following was deduced:

– The numeric value of the total volume density of all Vp, and in all three regions of the auditory canal was statistically significant highest ($p<0.05$) with non-operated patients (Tables 2 and 3).

TABLE 4
TOTAL VOLUME DENSITY COMPARISON OF ALL VASCULAR PHASES OF THE UPPER, POSTERIOR AND LOWER CANAL WALLS IN THE PATIENTS UNDERGONE TWO DIFFERENT CANAL INCISIONS (FORMED TYMPANOMEATAL FLAP AND VASCULAR STRIP)

Auditory canal wall	TMF		VS		t	p
	V_P	SD	V_P	SD		
Upper	0.0834	0.0361	0.0475	0.0525	1.69	0.111
Posterior	0.0507	0.0464	0.0349	0.0358	0.81	0.429
Lower	0.0560	0.0284	0.0261	0.0336	2.04	0.058

V_P – mean total volume density of all vascular phases (capillaries, arterioles and venulolymphatic spaces)

– Further statistical analysis could not prove any statistically significant difference ($p>0.05$) in total volume density of all Vp, for each of the analyzed auditory canal segments, between the TMF patients and patients with VS (Table 4).

Discussion

The differences of internal body structure between the experimental and control target groups are often small and unclear due to their particular structure. In such cases, a reliable establishment of distinctions, based only upon the qualitative methods seems to be very hard, unreliable or completely impossible. Stereology offers a multidisciplinary methodology on the basis of which it is viable to estimate quantitatively the three-dimensional internal body structure taking into consideration two dimensional cross-sections and geometrical probability. Such minor differences can be quantitatively described and analyzed by means of stereological methods[10,11].

The auditory canal skin does not have only a protective task. Its physiological activity is demanding and logically dependent upon the vascular network condition. The vascular elements of the auditory canal take part in irrigation of the middle ear conductive structures and in particular of the tympanic membrane[12–13].

The auditory canal functions may be lowered down by a middle ear surgery operation.

The idea of different incisions has not originated only from the necessity to have an easier surgery access to the ear, but also because of a more complete and quicker auditory canal recovery, and in particular of its revascularization.

A successful revascularization of the auditory canal should take place most intensively in the first month following the operation, bringing about an enhanced vascularization of the transplanted tympanic membrane. In this way a condition is met for a successful result of tympanoplasty.

For several decades various ear surgery schools (European schools on the one side and American on the other) have been in existence[14]. Their scientific research and extensive experience have enabled them to use their specific tympanoplasty methods, i.e. specific incisions of the auditory canal skin. Ultimate results of the different tympanoplasty methods do not significantly differ. Therefore, different auditory canal skin incisions should be more or less equally satisfactory. However, minor structural differences in the auditory canal skin can be detected. This may be manifested in the density of vascular elements after applying different types of incisions. Those dif-

ferences should have a practical value in the reconstructive surgery of the middle ear.

Conclusion

Since no statistical differences in the skin revascularization following the two auditory canal incisions (TMF, VS) were established, it could be concluded that both of them can be equally applied in ear surgery. No papers dealing with revascularization of auditory canal applying quantitative histology were found. Therefore, no analysis comparison could be made. The number of tested persons is insufficient for drawing ultimate conclusion, but it is assumed that the quantitative analysis applied in the above test could be successively applied in further analyses of this and similar otological matters.

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KVANTITATIVNA ANALIZA VASKULARIZACIJE NAKON DVIJE TEMELJNE INCIZIJE KOŽE ZVUKOVODA

SAŽETAK

U radu se prikazuju tri grupe od devet ispitanika. U prve dvije grupe su ispitanici kod kojih je prethodno, radi kronične upale srednjeg uha, učinjena timpanoplastika. Prilikom timpanoplastike su korištene dvije standardne, ali u principu različite incizije kože zvukovoda. Kod prve grupe je oblikovan timpanomeatalni režanj (TMF), a kod druge vaskularni strip (VS). Trećoj kontrolnoj grupi pripadaju neoperirani ispitanici. Kod svih ispitanika je učinjena kvantitativna analiza vaskularizacije kože zvukovoda stereološkom metodom. Korišten je dvostruki mrežasti sistem B 100 (Weibel). Određuje se volumna gustoća kapilara, arteriola i venulolimfatičnih prostora te ukupna volumna gustoća svih vaskularnih elemenata. Statistički značajne razlike u vaskularizaciji kože zvukovoda između dvije grupe operiranih ispitanika nisu primijećene. Značajne razlike u vaskularizaciji nisu dokazane ni između grupa operiranih bolesnika i kontrolne grupe ispitanika.