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# Analysis of Saliva Pepsin Level in Patients with Tracheoesophageal Fistula and Voice Prosthesis Complications

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## ABSTRACT

*The aim of this cross-sectional study was to investigate the relationship between pepsin concentration in saliva and the occurrence of tracheoesophageal fistula (TEF) complications and voice prosthesis (VP) complications, after total laryngectomy and VP implantation. We assessed the concentrations of pepsin in the saliva of 41 laryngectomized patients and correlated it with the incidence of TEF complications (periprosthetic leakage, atrophy, esophageal mucosa hypertrophy, granulations, fistula enlargement, and VP dislocation), VP complications (transprosthetic leakage, Candida infection) and voice quality. Pepsin levels were measured by enzyme-linked immunoadsorbent assay (ELISA). Voice quality was assessed by Harrison-Robilard – Schultz (HRS) scale. In all, 17 (42%) patients had complications. All of them had TEF complications, whereas VP complication, together with TEF was found in 9 (22%) patients. We found no significant correlation between adjuvant radiotherapy and TEF complications. Most of patients, 30 (73%), had positive pepsin level in saliva. Median value of pepsin concentration in all patients was 4.8 (range 81.7). Median pepsin concentration was higher in patients free of TEF or VP complications (6.6, range 81.7 vs. 3.2, range 19.3) but that difference was not statistically significant (Mann-Whitney test,  $Z = -1.562$ ,  $p = 0.118$ ). In addition, statistically insignificant negative correlation between pepsin levels and voice quality measured by HRS scale (Spearman's rho,  $p > 0.05$ ). Although reflux was proposed as cause of TEF complications and pepsin has been proven as a most sensitive and specific marker of extraesophageal reflux, we did not find any statistically significant correlation between pepsin levels and occurrence of TEF or VP complications.*

**Key words:** tracheoesophageal fistula, reflux, pepsin, laryngectomy

## Introduction

Voice prosthesis (VP) insertion into tracheoesophageal fistula (TEF) is a golden standard in voice rehabilitation in patients who have undergone total laryngectomy. In the last 40 years easily replaceable prosthesis with different valve design have been developed to facilitate satisfactory voice production and prevent aspiration of saliva. Still, several complications regarding VP and TEF have been described in current literature<sup>1,2</sup>. Complications related to TEF are periprosthetic leakage, atro-

phy, esophageal mucosa hypertrophy, granulations, fistula enlargement, and VP dislocation. VP complications are transprosthetic leakage and Candida infection. Preservation of TEF and VP can be very demanding not only to maintain patient's communication ability and quality of life but to prevent very serious complications due to an aspiration. It was purposed that these complications can be associated with reflux of gastric contents to upper esophagus and TEF<sup>3,4</sup>. Lorenz and al have found higher

occurrence of TEF enlargement and reduction in life span of VP in patients with more severe reflux disease confirmed by 24-hour dual-probe pH monitoring<sup>3</sup>. Some studies demonstrated that in majority of patients, the symptoms associated with TEF complications can be improved or cured by rigorous anti-reflux treatment with proton pump inhibitors (PPIs)<sup>5,6</sup>. Bock and al confirmed reflux with pepsin deposition into the TEF in majority of laryngectomy patients<sup>5</sup>. Pepsin is a reliable biological marker of extraesophageal reflux (EER)<sup>7,8</sup>. Detection of reflux with pepsin assay is noninvasive method and it is easily obtainable from fluid secretions like saliva or sputum<sup>7</sup>. The aim of this cross-sectional study was to investigate the relationship between pepsin concentration in saliva and the occurrence of tracheoesophageal fistula (TEF) complications and voice prosthesis (VP) complications in patients who have undergone total laryngectomy.

## Patients and Methods

Forty one adult patients at the ENT Department, »Dr. J. Benčević« General Hospital, Slavonski Brod and ENT University Departments, Zagreb and Rijeka University Hospital Centres who underwent total laryngectomy and insertion of the voice prosthesis were recruited to participate at this study. Median age of all patients was 60 years (50–76), and proportion of males was 93%. Before participation, each study subject signed an informed, written consent that was approved by ethics committee of »Dr. J. Benčević« General Hospital, ethics committee of Zagreb University Hospital Center and ethics committee of Rije-

ka University Hospital Center. All patients used voice prosthesis either from Atos Medical Inc (Horby, Sweden) or InHealth technologies (Carpinteria, USA). Patients who were on IPP therapy were excluded from the study. Subjects were given sterile tubes and 3–5 ml of saliva was collected in the morning. Pepsin levels in saliva were assayed by Human Pepsin (PG) ELISA kit (Causabio Biotech Co., Ltd, P.R.C.) according to manufacturers protocol. Undiluted saliva samples were assayed and pepsin results were expressed in ng/ml. Positive test for pepsin was considered to be higher than 1.2 ng/ml. Concentrations of pepsin in the saliva were compared according to incidence of TEF complications (periprosthetic leakage, atrophy, esophageal mucosa hypertrophy, granulations, fistula enlargement, and VP dislocation), VP complications (transprosthetic leakage, Candida infection) and voice quality. Voice quality was assessed by Harrison-Robilard – Schultz (HRS) scale (Table 1).

Statistical analysis with chi square, non-parametric Mann-Whitney test (for data with abnormal distribution), Student T-test, and nonparametric correlation Spearman's rho test was performed using statistical software SPSS for Windows, version 11.0.3, Chicago, IL, USA. Statistical significance was set to  $p < 0.050$ .

## Results

Among 41 investigated patients, there were 17 patients (42%) with complications. All patients with complications had at least one of the TEF complications, whereas VP complication was present together with TEF

TABLE 1  
HARRISON-ROBILLARD SHULTZ SCALES USED FOR VOICE QUALITY ASSESSMENT

Scale	Answer	Points
A. Use	Never uses tracheoesophageal speech (0%)	1
Degree of use of tracheoesophageal speech	Uses tracheoesophageal speech less than 50% of the time	2
	Uses tracheoesophageal speech 50% to 80% of communicative attempts	3
	Uses tracheoesophageal speech manually occluded as main means of communication	4
	Uses tracheoesophageal speech with tracheostoma valve as main means of communication	5
B. Quality	Unable to get sound, no use of pulmonary air for speech	1
The ease of production and intelligibility of speech as determined by fluency and ability to occlude	Voice is too strained or too breathy to permit functional use in conversation (may interfere with intelligibility), includes whispered speech	2
	Stoma, more often than not, is poorly occluded with resultant air escape that interferes with intelligibility or is distraction to the listener	3
	Voice is mildly strained or mildly breathy, but continuous use in conversation is possible, occlusion is generally good, speech is intelligible.	4
	Voice is easily produced, occlusion is good, speech is intelligible	5
C. Care*	Unable to do any of 4 behaviours	1
	Independent for any 1 of 4 behaviours	2
	Independent for any 2 of 4 behaviours	3
	Independent for any 3 of 4 behaviours	4
	Independent for any 4 of 4 behaviours	5

\*Patient independence of medical care or other health care professionals (speech/language pathologist, nurse, community worker) for 4 behaviours: remove and insert prosthesis, clean and sterilize prosthesis, recognize problems and seek help immediately, and order supplies

complication in 9 patients (22%). The most common complication was granulation of the tracheal mucosa followed by transprosthetic and periprosthetic leakage and atrophy of tracheoesophageal wall (Table 2). There were no differences between patients with complications and patients free of complication regarding relevant clinical

**TABLE 2**  
FREQUENCIES OF DIFFERENT TEF AND VP COMPLICATIONS AMONG PATIENTS WITH COMPLICATIONS

Complications, N (%) patients		N (%)
TEF complications 17 (100) patients	Periprosthetic leakage	5 (24)
	TEF dilatation	1 (4.5)
	VP dislocation	2 (10)
	Granulations	7 (33)
	Atrophy	5 (24)
VP complications 9 (53) patients	Esophageal hypertrophy	1 (4.5)
	Total	21 (100)
	Candida infection	4 (40)
	Transprosthetic leakage	6 (60)
Total	10 (100)	

characteristics, except for secondary insertion of VP, miotomy and time since last VP exchange. Patients with secondary VP insertion had significantly higher proportion of TEF complications than patients with primary VP insertion (chi square,  $p=0.029$ ). Patients who underwent miotomy during laryngectomy had significantly lower proportion of TEF and VP complications (chi square,  $p=0.039$ ). In addition, patients with longer time since last VP exchange had more frequent TEF and VP complications (Mann-Whitney test,  $p=0.011$ ). More than half of patients underwent adjuvant neck radiation, but there were no differences in proportions of complications regarding referral to adjuvant radiotherapy (Table 3).

Positive test for pepsin was found in 30 patients (73%). There were no significant differences in proportions of complications between pepsin negative and pepsin positive patients (Table 4). Median pepsin value in all investigated patients was 4.8 ng/ml (range <1.2 and 81.7 ng/ml). Median pepsin value was higher in patients free of complications (6.6 ng/ml vs. 3.2 ng/ml) but that difference was not statistically significant (Mann-Whitney test,  $Z=-1.562$ ,  $p=0.118$ ). There were no differences in median pepsin value between patients in regard to miotomy, alcohol abuse and radiotherapy. Voice quality measured with HRS scale A and C correlated negatively with pepsin concentration in saliva, whereas weak positive correlation was found for HRS scale B results and pepsin levels. Correlations were not statistically significant (Spearman's rho, -0.151, -0.125, and 0.071, respectively,  $p>0.050$ ). There were no significant differences in voice quality measured by HRS scales between patients with complications and patient free of complications (Table 5), except for 2 patients with hypertonicity who had very poor voice quality.

**TABLE 3**  
DIFFERENCES IN RELEVANT CLINICAL CHARACTERISTICS AMONG 41 INVESTIGATED PATIENTS REGARDING PRESENCE OF TEF AND VP COMPLICATIONS

Clinical characteristic	Patients, N (%)		
	Complications	No complications	Total
Age, years, median (range)	63 (51–76)	61 (50–75)	60 (50–76)
Male gender	15 (40)	23 (60)	38 (100)
History of alcohol abuse	7 (41)	10 (59)	17 (100)
Current smoker	2 (100)*	0 (0)	2 (100)
Diabetes mellitus	2 (50)	2 (50)	4 (100)
Radical neck dissection	5 (35)	9 (65)	14 (100)
Selective neck dissection	4 (80)	1 (20)	5 (100)
Miotomy	2 (15)*	11 (85)	13 (100)
Adjuvant chemotherapy	1 (33)	2 (67)	3 (100)
Adjuvant radiotherapy	9 (36)	16 (64)	25 (100)
Time since TEF creation, months, median (range)	29 (3–83)	25.5 (3–84)	25.5 (3–84)
Time since last VP exchange, months, median (range)	3 (1–8)†	1 (0.3–8)	2 (0.3–8)
Secondary VP insertion	8 (73)*	3 (27)	11 (100)

\*  $\chi^2$ ,  $p<0.050$

† Mann-Whitney test,  $Z=-2.557$ ,  $p<0.050$

**TABLE 4**  
RELATIONSHIP BETWEEN HUMAN PEPSIN ELISA TEST AND PROPORTION OF COMPLICATIONS

Complications	Human Pepsin ELISA test, N (%)*	
	Negative	Positive
No	5 (21)	19 (79)
Yes	6 (35)	11 (65)
Total	11 (27)	30 (73)

\*  $\chi^2$ ,  $p=0.476$

## Discussion

Simple surgical technique of insertion of VP into TEF and rapid and successful voice rehabilitation makes this method a treatment of choice in voice rehabilitation after total laryngectomy. However, there is still significant number of complications reported in the literature to date and the incidence varies from 10–50%<sup>1,3,9</sup>. We have found 42 % of patients with TEF and VP complications. Periprosthetic leakage in association with moderate fistula enlargement was reported as the most common complication<sup>3</sup>. In our study, the most common complication was granulation of the tracheal mucosa followed by transprosthetic and periprosthetic leakage and atrophy of tracheoesophageal wall. There are several risk factors that are contributing to TEF and VP complications described in the literature, such as arterial hypertension, diabetes mellitus, miotomy during total laryngectomy

**TABLE 5**  
VOICE QUALITY MEASURED BY HRS SCALES REGARDING  
PRESENCE OF VP COMPLICATIONS

Voice quality test	Complications		No complications		Total		
	N (%)	Median score	N (%)	Median score	N (%)	Median score	
HRS A	1	0 (0)	1 (4)		1 (2)		
	2	3 (18)	2 (8)		5 (12)		
	3	0 (0)	4	2 (8)	4	2 (5)	4
	4	11 (18)		16 (62)		27 (66)	
	5	3 (64)		3 (13)		6 (14)	
HRS B	1	1 (6)	1 (4)		2 (5)		
	2	2 (12)	3 (12)		5 (12)		
	3	1 (6)	5	0 (0)	5	1 (2)	5
	5	3 (17)		4 (17)		7 (17)	
	4	10 (59)		16 (67)		26 (64)	
HRS C	1	0 (0)	2 (8)		2 (5)		
	2	0 (0)	0 (0)		0 (0)		
	3	0 (0)	4	3 (13)	4	3 (7)	4
	4	16 (94)		19 (79)		35 (85)	
	5	1 (6)		0 (0)		1 (3)	

and TEF formation, postoperative radiotherapy, trauma during prosthesis replacement and EER<sup>1,3,6,9</sup>. In our study, there were no differences between patients with and without complications regarding arterial hypertension and diabetes mellitus. Patients who did not undergo myotomy had higher proportion of complications which was probably a consequence of periprosthetic leakage and EER caused by pressure peak in hypopharyngeal area during swallowing<sup>9</sup>. Patients who underwent secondary VP insertion had higher proportion of complications probably because they also did not undergo myotomy.

Loss of the larynx and resection of the laryngeal nerves cause changes in esophageal motility reflux barriers<sup>10, 11</sup>. Patients who have undergone total laryngectomy have higher incidence of gastroesophageal reflux and EER<sup>12</sup>. Although it has been proven that postoperative radiotherapy reduces esophageal clearance and decreases neutralizing effects of saliva<sup>13</sup> we did not find any differences in complication rates in regard to postoperative radiotherapy. There are also several research groups that have reported similar results<sup>1,3,9,14</sup>. Esophageal mucosa is resistant to physiological reflux of gastric fluid but pathological reflux can cause different changes from erosion to adenocarcinoma. Pharyngeal, laryngeal and tracheal mucosa has no natural barriers to gastric fluid and even small amounts of gastric content, 6–10 reflux events in 24 hours, can cause a massive damage to pharyngeal or tracheal mucosa<sup>15,16</sup>. Mucosa can be damaged by low pH values of gastric acid, pepsin, pancreatic enzymes and bile acid<sup>15–17</sup>. Reflux of gastric fluid into upper esophagus after total laryngectomy would not present a problem if there would not be any communication between esophagus and airway. But, with TEF formation

direct communication between esophagus and trachea is made. Influence of reflux on TEF and VP has been demonstrated by only three working groups to date<sup>2,4,6</sup>. The gold standard for diagnosis of EER today is still 24 hour double – probe pH monitoring, although it has been shown to be lacking in reproducibility<sup>18–21</sup>. It is also incapable of detecting nonacidic gastric reflux, which is associated with airway disease<sup>22,23</sup>. After gastric acid, pepsin is the most studied gastric component. Pepsin is thought to cause damage by its proteolytic activity digesting the structures that maintain cohesion between cells. It is maximally active at pH 2.0, but it can also cause damage with higher pH up to 6.5 (mean pH of laryngopharynx is 6.8), when is completely inactivated but not irreversibly<sup>24</sup>. Thus it can be reactivated by a decrease in pH. Pepsin is not irreversibly inactivated until pH 8<sup>24</sup>. Recent studies by Johnston and all have demonstrated that pepsin is also taken up by hypopharyngeal and laryngeal epithelial cells by receptor-mediated endocytosis<sup>25,26</sup>. They have also shown that pepsin at pH 7 significantly alters the expression levels of multiple genes implicated in stress and toxicity, induces pro-inflammatory cytokine gene expression, and alters the expression of 27 genes implicated in carcinogenesis<sup>16</sup>. Pepsin has also been proven to be the most sensitive and specific marker of EER<sup>8</sup>. It can be detected with several different methods such as ELISA, enzymatic pepsin assay or Western blot analysis<sup>8</sup>. Detection of pepsin is a noninvasive method and effective in diagnosis of EER from easily obtainable fluid secretions like saliva or sputum<sup>8,27</sup>. Bock et al. have found that reflux with subsequent pepsin deposition into TEF tract occurs in majority of laryngectomy patients<sup>4</sup>. We have found positive test for pepsin in saliva in 73% of patients which proves EER in majority of patients after total laryngectomy. The expected life span of VP recommended by manufactures is 3 months. There is evidence that supraesophageal or EER reflux is associated with reduced life span of voice prosthesis, with fistula enlargement, increased granulation tissue formation, periprosthetic leakage and increased cricopharyngeal stenosis or spasm<sup>2,6</sup>. Our study has demonstrated that the median life span of VP in patients without complications was only 1 month which could probably mean that patients require more frequent VP exchange. We did not find any significant difference in proportions of complications between pepsin positive and pepsin negative patients. In addition, median pepsin value did not differ significantly between patients with and without complications. These results were unexpected but suggest that further studies of the impact of pepsin and EER on TEF and VP are necessary. Voice quality measured by HRS scale was more than satisfactory and complications or pepsin levels did not influence voice quality.

## Conclusion

We demonstrated that pepsin was present in saliva of majority of patients, suggesting ongoing reflux in laryngectomized patients. Although reflux was proposed to be associated with TEF complications, and pepsin proven as a most sensitive and specific marker of EER, we did not

find any statistically significant correlation between pepsin levels and occurrence of TEF or VP complications so

further studies of the impact of pepsin and EER on TEF and VP are warranted.

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## RAZINA PEPSINA U SLINI BOLESNIKA S KOMPLIKACIJAMA TRAHEOEZOFOGALNE FISTULE I GOVORNE PROTEZE

### SAŽETAK

Cilj ovog istraživanja bio je proučiti odnose koncentracije pepsina u slini i učestalosti pojave komplikacija traheoezofagelane fistule (TEF) i komplikacija govorne proteze (GP) kod bolesnika s postavljenom GP nakon totalne laringektomije. U istraživanje je bio uključen 41 bolesnik čije su razine koncentracije pepsina u slini uspoređene s pojavom TEF komplikacija (curenje oko proteze, atrofija, hipertrofija sluznice jednjaka, granulacije, povećanje fistule i dislokacija GP), komplikacija GP (curenje kroz GP, infekcija Candidom) i kvalitetom glasa. Koncentracija pepsina u slini mjerena je »enzyme-linked immunoadsorbent assay« (ELISA) metodom. Kvaliteta glasa određena je prema Harrison-Robilard – Schultz (HRS) skali. Ukupno 17 (42%) bolesnika imalo je komplikaciju. Svi su imali komplikaciju TEF, dok je 9 (22%) imalo i komplikaciju GP. Nismo našli značajnu razliku u pojavi komplikacija TEF među bolesnicima koji su primili adjuvantu radioterapiju i koji nisu primili adjuvantu radioterapiju. Većina bolesnika, 30 (73%), ima pozitivnu razinu pepsina u slini. Medijan koncentracije pepsina u slini svih bolesnika je 4,8 (raspon 81,7). Medijan koncentracije pepsina bio je viši kod bolesnika bez komplikacije TEF i GP (6,6, raspon 81,7 vs. 3,2, raspon 19,3) ali razlika nije statistički značajna (Mann-Whitney test,  $Z = -1,562$ ,  $p = 0,118$ ). Također je nađena negativna korelacija između razine pepsina i kvalitete glasa, ali nije bila statistički značajna (Spearman's rho,  $p > 0,05$ ). Iako se smatra kako ekstrezofofagealni refluks želučanog sadržaja, čiji je najosjetljiviji marker razina pepsina, doprinosi pojavi komplikacija TEF i GP ovim istraživanjem nije nađeno značajne veze između razine pepsina i učestalosti komplikacija TEF i GP.