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Cut-Off Values And Pepsin Levels As Risk Factors For Laryngeal Carcinoma

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ABSTRACT

Background: Laryngeal carcinoma is the most prevalent malignancy of the larynx, with smoking and alcohol being the primary risk factors. HPV and Helicobacter pylori infections, LPR, genetic factors, and betel nut consumption are the most common risk factors. The purpose of this study is to describe the correlation between pepsin levels and laryngeal carcinoma.
Methods: This study investigated the correlation between pepsin levels and laryngeal carcinoma. ELISA is utilized to quantify pepsin values in patients' saliva. Data analysis included logistic regression, and the ROC curve was used to determine the pepsin cut-off value.

Results: This study analyzed 36 laryngeal carcinoma with a majority of male patients (100%) and 36 patients as a control group. The most prevalent age distribution for laryngeal cancer patients is 60 to 70 years, with an age range of 36 to 82 years and an average age of 59.69 years. A history of smoking was present in 88.89% of carcinoma cases compared to 52.8% in the control group, showing a strong association between smoking and laryngeal carcinoma (p=0.002, OR=9.718). Logistic regression analysis confirmed a strong correlation between elevated pepsin levels and laryngeal carcinoma (p = 0.018, OR = 1.072). Pepsin levels in patients with laryngeal carcinoma had a cut-off value of 27.4 ng/ml.

Conclusions: Elevated pepsin levels are significantly correlated with the occurrence of laryngeal carcinoma.

Keywords: *pepsin, ELISA, laryngeal carcinoma, laryngopharyngeal reflux, cancer cell*

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1. INTRODUCTION

Laryngeal carcinoma is a malignancy that arises from the tissues of the larynx, including the supraglottic, glottic, and subglottic regions. [1] Among all malignancies, laryngeal carcinoma is the 22nd most prevalent, with a global incidence of 2.76 per 100,000 population and a mortality rate of 1.66 per 100,000. There is a fivefold increase in incidences among men. The incidence of laryngeal carcinoma begins to rise after the age of 35 and reaches its maximum after the age of 65. [2] Risk factors for laryngeal carcinoma include tobacco use, alcohol consumption, HPV and H. pylori infections, laryngopharyngeal reflux (LPR), and genetic predisposition. Pepsin is synthesised by stomach parietal cells and is a primary component of gastric refluxate. [3] Pepsin significantly contributes to laryngeal carcinogenesis, with mechanisms that require further investigation.

Laryngeal carcinoma is one type of head and neck cancer that has high morbidity and mortality. Risk factors such as smoking and alcohol consumption have been proven to be highly important, but the role of LPR is still not fully understood. Various studies have demonstrated that alcohol consumption can increase the relative risk by 1.09 per 10 g of alcohol per day (World Cancer Research Fund, 2018), while research related to LPR is still limited. Pepsin, as an exclusive product of LPR, can cause inflammation in the upper aerodigestive tract, which has the potential to trigger carcinogenesis. [4,5]

Research conducted by Tasli et al. (2019) and Anis et al. (2018) concluded that pepsin is not significantly implicated in laryngeal carcinoma; conversely, findings by Zubčić et al. (2020) reveal an elevation in pepsin levels within the laryngeal mucosa experiencing malignant transformation. [6-8] The meta-analyses published by Zhang, et al. (2014) and Parsel, et al. (2019) demonstrate a significant association between LPR and laryngeal carcinoma. [9,10] Tan, et al. (2019) explained the molecular pathways implicated in the role of pepsin in the carcinogenesis of laryngeal carcinoma, showing that interleukin-8 (IL-8), Carbonic Anhydrase III (CA III), and E-cadherin are involved in this process. This indicates a significant correlation between LPR and the development of laryngeal carcinoma. [11]

2. METHOD

This research is an analytical observational study with a case-control design, conducted from June to August 2024. The laryngeal carcinoma samples were collected through consecutive sampling, while the control samples were obtained through simple random sampling. Thirty-six samples that met the inclusion and exclusion criteria were acquired. The pepsin assay was performed on saliva with the ELISA in the Clinical Pathology laboratory in Dr. Soetomo Hospital using Human Pepsin ELISA Kit E0922Hu. Logistic regression analysis to evaluate the correlation between pepsin levels and laryngeal carcinoma at a significance level of $\alpha = 0.05$. The threshold for pepsin levels was established utilising the AUC.

3. RESULT

Research has been conducted at the ENT outpatient and oncology clinic at Dr. Soetomo General Academic Hospital in Surabaya to investigate the correlation between pepsin levels and laryngeal carcinoma. The sampling was conducted over three months, starting on June 10, 2024, until August 26, 2024. A sample size of 36 patients was used for the consecutive sampling of laryngeal carcinoma. The control sample was obtained using simple random sampling. No samples were withdrawn or dropped out of the study, and a total of 72 samples were obtained following the inclusion and exclusion criteria.

Table 1. Description of the sample in terms of gender, age, and smoking history

(n =36)

Patients Characteristics	Group		P value	OR
	Laryngeal carcinoma	Control		
Sex				
Male	36 (100)	32 (88.89)	0.123	
Female	0 (0)	4 (11.11)		
Age				
21-30	0 (0.00)	3 (8.33)		
31-40	3 (8.33)	6 (16.67)		
41-50	3 (8.33)	11 (30.56)		
51-60	9 (25.00)	5 (13.89)		
61-70	18 (50.00)	9 (25.00)		
71-80	2 (5.56)	2 (5.56)		
81-90	1 (2.78)	0 (0.00)		
Mean ± SD	59.69 ± 10.490	49.75 ± 13.812	0.001	1.096
Range	36-82	22-74	=	
Smoking history				
Yes	32(88.89)	19 (52.8)	0.002	9.178
No	4 (11.11)	17 (47.2)		
Histophatology type				

Well Differentiated SCC	29 (80.55)
Poorly Differentiated SCC	2 (5.56)
Moderately Differentiated SCC	3 (8.33)
Not otherwise specified (NOS) SCC	2 (5.56)

During the period from June to August 2024, 36 new cases of laryngeal carcinoma were identified, all of which involved male people. The predominant age group is 61 to 70 years, with 18 patients (37.5%); the youngest patient is 36 years old, while the oldest is 82 years old. The mean age is 59.69 years. A smoking history was identified in 32 individuals (88.89%) of the total patient cohort. A control group comprising 36 individuals was established, of which 19 individuals (52.8%) were smokers.

According to the findings of the analysis, there is a strong correlation between age and laryngeal carcinoma, with a p-value of 0.001 ($p<0.05$). The association is strong, according to an OR value of 1.096. Laryngeal carcinoma is also significantly associated with a history of smoking, as evidenced by the results of the logistic regression test ($p= 0.002$; $p<0.05$). The association is strong, showed by an OR value of 9.718.

The most predominant histological finding was well differentiated SCC 29 patients (80.55%), followed by moderately differentiated SCC 3 patients (8.33%), poorly differentiated SCC 2 patients (5.56%), and SCC Not Otherwise Specified (NOS) with 2 patients (5.56%).

Table 2. Results of pepsin levels in laryngeal carcinoma patients

Pepsin level	Laryngeal carcinoma	Control	*p*	OR
Mean ± SD	27.664 ± 9.5952	22.508 ± 7.6672	*p*=0.018	1.072
Range	10.7 – 44.9	4.3 – 34.1	(log reg)	

The pepsin levels in the laryngeal carcinoma patient group ranged from a high of 44.9 ng/ml to a minimum of 10.7 ng/ml. The median value is 28.9 ng/ml, whereas the mean is 27.66 ng/ml. Significant results were obtained with a p-value of 0.018 ($p < 0.05$) from the logistic regression test on pepsin levels in 36 patients with laryngeal carcinoma and 36 control patients. The strength of the relationship was further demonstrated by an OR of 1.072.

Cut-off point for pepsin levels.

The cut-off value of pepsin in patients with laryngeal carcinoma has not been reported in any publication to date. The minimum pepsin level required to accurately predict laryngeal carcinoma must be determined to establish the cut-off pepsin level with appropriate sensitivity and specificity.

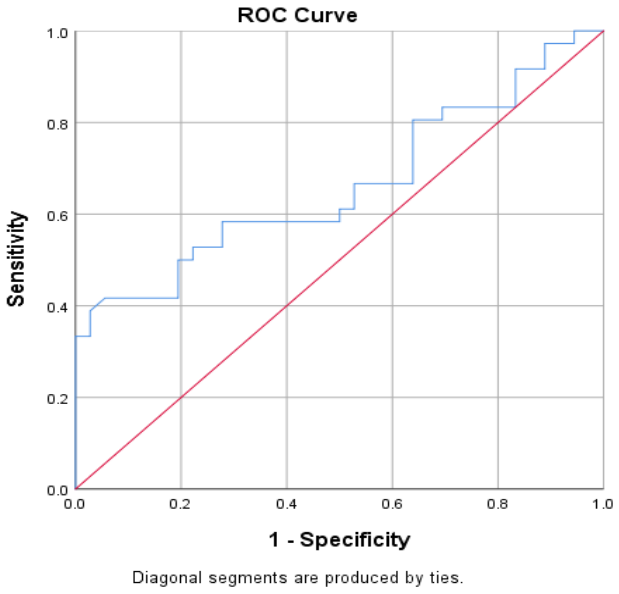

Figure 1. AUC in the analysis of the cut-off point of pepsin levels

A p-value of 0.017 and an AUC value of 0.663 were found. These findings are statistically significant, and the 95% CI of 0.535 to 0.791 suggests that the AUC was estimated relatively well. The cut-off point for pepsin levels in this study, as determined by the ROC curve test, is 27.4 ng/ml.

Table 3 Results of the analysis of the cut-off value of pepsin levels n (%)

Cut-off Pepsin level	Laryngeal carcinoma	Control	*p*	OR
> 27,40 ng/ml	21 (58,30)	10 (27.80)	p=0.02	4.322
<= 27,40 ng/ml	15 (41.70)	26 (72.20)		

According to the analysis results of the entire sample, which consisted of 72 laryngeal carcinoma patients and controls, 31 patients (43.05%) had pepsin levels that exceeded the cut-off value (27.4 ng/ml). In comparison, 41 patients (56.95%) had levels that were equal to or lower than the cut-off value. Pepsin levels in 21 out of 36 patients (58.30%) with laryngeal carcinoma exceeded the threshold limit (27.4 ng/ml), according to the study's findings. Fifteen out of 36 patients (41.70%) demonstrated pepsin levels at or below the cut-off threshold. The control group revealed that 10 of 36 patients, or 27.80%, had pepsin levels higher than the threshold. 26 of 36 patients (26.7%) demonstrated pepsin levels at or below the threshold range.

Using logistic regression, the analysis yielded a significant result ($p = 0.02$; $p < 0.05$). The association is strong, indicated by an OR value of 4.322. The findings show that exposure to pepsin significantly influences the risk of laryngeal carcinoma development.

To enhance the predictive capacity of laryngeal carcinoma occurrence, a score-card must be created by combining the three previously examined risk factors: age, smoking history, and pepsin level. The integration of these three parameters is anticipated to improve the prediction capacity for laryngeal carcinoma relative to the utilisation of a single factor independently.

Table 4. Distribution of B values and category scores based on risk factors

Variables	B score	Category	Scoring category
Age	2.832	> 50	3
Smoking	2.258	Yes	1
Pepsin level	1.994	> 24,70	1

Each variable has a different weight based on its degree of influence. The formula used is: Laryngeal carcinoma = 2.832 × age + 2.258 × smoking + 1.994 × pepsin level. In this method, age has the most significant weight (2.832), signifying its predominant contribution to risk, followed by smoking (2.258) and pepsin levels (1.994). Three categories define the score range: low risk (score 1), medium risk (score 2), and high risk (score 3), with values between 1.994 and 2.273.

Each variable is classified in an easily understood way by the score-card, with a score of 3 for age over 50, a score of 1 for smoking habit, and a score of 1 for a pepsin level greater than 24.70.

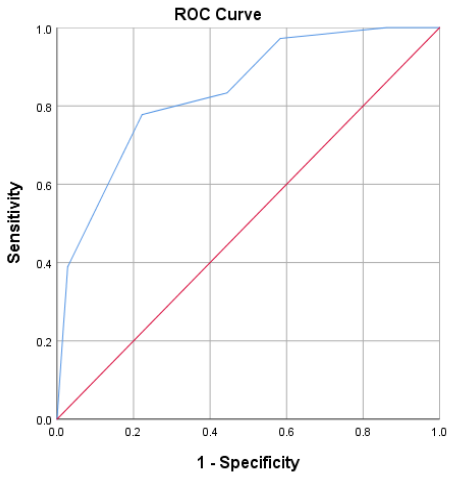

Figure 2. AUC analysis of total score of laryngeal carcinoma risk factors (age, smoking, and pepsin levels).

The "Total Risk Score" in Figure 2 has an AUC value of 0.836, indicating a substantial degree of discrimination. The standard error is 0.047, indicating the degree of variability in the AUC estimation. The significance value is p = 0.000 with a confidence interval from 0.745 to 0.927 (CI = 95%).

Based on three parameters (smoking history, age, and pepsin level), the cut-off value for the overall score of laryngeal carcinoma risk factors is 3.5. The number signifies an equilibrium between sensitivity and specificity.

Table 5. Distribution based on the cut-off value of the total risk score for laryngeal carcinoma n (%)

Cut off total risk score	Laryngeal carcinoma	Control	*p*	OR
>3,5	28 (77,8)	8 (22,2)	p=0.000	12,250
< 3,5	8 (22,2)	28 (77,8)		

There were statistically significant findings when the association between the incidence of laryngeal carcinoma and the total risk score (pepsin, age, and smoking history) was examined. Laryngeal carcinoma was diagnosed in 77.8% of the group with a risk score higher than 3.5 and in just 22.2% of the group with a score less than 3.5. With a p-value of 0.000 and an OR of 12.250, persons with a risk score over 3.5 had a 12.25-fold increased risk of being diagnosed with laryngeal carcinoma relative to those with a score of 3.5 or lower.

Table 6. Distribution by risk category of laryngeal carcinoma n (%)

Total risk score	Laryngeal carcinoma		Total	*p* value
	Yes	No		
Low	6 (16.7)	20(55.6)	26 (36.1)	0.000
Moderate	2(5.6)	8(22.2)	10(13.6)	
High	28(77.8)	8(22.6)	36(50)	

Of the 36 patients in the laryngeal carcinoma group, 28 (77.8%) were in the high-risk category, followed by 6 (16.7%) in the low-risk category and 2 (5.6%) in the moderate-risk category. On the other hand, 20 of 36 individuals (55.6%) in the non-laryngeal carcinoma group were classified into the low-risk category, while 8 out of 36 individuals (22.2%) were classified into each of the moderate- and high-risk categories. A p-value of 0.000 indicates a strong correlation between the incidence of laryngeal carcinoma and the increase in risk scores.

4. DISCUSSION

The results of this study are consistent with those of Sereg-Bahar et al (2015), as the entire laryngeal carcinoma group consists of males. [12] World Cancer Research Fund (2019) attributes this to the high prevalence of smoking and alcohol consumption among males, as well as the fact that women are less susceptible to the effects of potential carcinogens due to differences in metabolism and genetics. [4]

The mean age of the laryngeal carcinoma group is 59.69 years, which is in concordance with the reported mean age of 58.8 years. The results are similar to those of Fasunla et al. (2016), where patient ages varied from 21 to 85 years, with a mean age of 60.48 years. [12,13]

The laryngeal carcinoma group continued to have a higher percentage of patients with a smoking history in this study, which is consistent with the findings of another study conducted by Sereg et al. (2015), which indicated that 96.67% of patients had a smoking history. Well-differentiated SCC dominated 80.55% of the histopathological results. [12] This type of tumour cell exhibits a higher degree of organisation, as evidenced by the formation of keratin pearls, and a lower level of mitotic activity compared to the less differentiated type. A increased prognosis is usually associated with well-differentiated tumours, which tend to have a slower growth rate and a lower risk of metastasis in comparison to moderately or poorly differentiated tumours. [14]

Analysis of the relationship between age factors and laryngeal carcinoma shows a significant relationship with a p-value of 0.001 ($p<0.05$) and an high relationship strength with an OR value of 1.096. The findings are consistent with the research conducted by Zubčić et al. (2020), which identified a strong association between the age range of 41–77 years and laryngeal carcinoma. [8]

Smoking history and laryngeal carcinoma show a strong correlation, which is highly significant. Smoking is the most critical risk factor for laryngeal carcinoma, with an incidence rate of approximately 70 to 95 percent. Laryngeal carcinoma can be significantly increased by a history of smoking. [15,16] Cigarettes contain nicotine, which inhibits the apoptosis

mechanism, a mechanism that is essential for the survival of malignant cells. The production of DNA adducts, which are linked to the early stages of carcinogenesis and have the potential to cause tumor formation, is a potential way that carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons, and aromatic amines in tobacco smoke could damage DNA. [17]

In the tumor microenvironment, chronic inflammation caused by tobacco smoke exposure can result in the release of inflammatory components, including TNF-α, IL-1, IL-6, CXCL1, and CXCL8, which can promote tumor growth, invasion, angiogenesis, and immune suppression. [18] The cytochrome P450 enzymes also interact with these carcinogens, converting non-reactive carcinogens into forms that bind to DNA. This process increases the number of DNA adducts associated with early carcinogenesis. Mutations in the p53 gene, a tumor suppressor gene that inhibits tumor growth, are the primary focus of polycyclic aromatic hydrocarbons. In smokers, the G to T transversion in laryngeal tumors caused by tobacco smoke is greater than that in non-smokers. [19]

The laryngeal carcinoma group demonstrated higher average pepsin levels than the control group. These results are similar to those of Sereg-Bahar et al. and discovered a pepsin level range of 2.1-265 ng/ml, a mean of 44.6 ng/ml, and a standard deviation of 66.8 in the laryngeal carcinoma group. The pepsin levels in the control group were within the range of 2.4-39 ng/ml, with a mean of 9.6 ng/ml and a standard deviation of 8.1 ($p<0.05$) . [12]

The pepsin levels are higher; however, the ratio of pepsin levels between the malignant group and the control group is not significantly different from that of other studies. In the study conducted by Zubčić et al. (2020), pepsin levels were compared in the malignant tumor group, laryngeal benign tumor group, and control group. [8] A detection range of 3.12–200 ng/ml was employed in the investigation. The median pepsin concentration in the malignant tumor cohort was 200 ng/ml, with a range of 107–261 ng/ml. The median pepsin concentration in the benign tumor group was 175 ng/ml, with a range of 155–240 ng/ml. The median pepsin concentration in the control group was 152.5 ng/ml, with a range of 140–190 ng/ml. [8]

This study demonstrates that pepsin levels in the laryngeal carcinoma group are elevated relative to the control group. Similar with Sereg-Bahar et al., they found that the laryngeal carcinoma group's pepsin levels were considerably higher from the control group (P=0.044). This may suggest that pepsin and LPR contribute in the development of laryngeal carcinoma. [12]

Research by Zubčić et al. compared pepsin levels in groups with malignant tumors, benign laryngeal tumors, and controls. Both benign and malignant alterations in the laryngeal mucosa were associated with elevated pepsin concentrations. This suggests that benign and malignant alterations in the larynx are associated with LPR. LPR and pepsin are regarded as etiological factors in the development of these modifications. [8]

In both benign and malignant diagnoses, immunohistochemical results show that laryngeal mucosal cells contain a higher amount of pepsin, which increases the likelihood of cell injury through the direct action of pepsin within the cell cytoplasm (Zubčić et al. 2020). JJ. Tan et al. (2016) also corroborated the hypothesis that pepsin expression in laryngeal tissue increases in patients with vocal leukoplakia and laryngeal carcinoma, thereby contributing to the development of laryngeal carcinogenesis. [8,11]

Kelly et al. (2014) demonstrated that hypopharyngeal squamous cells persistently exposed to pepsin exhibited increased colony-forming ability and cell migration compared to control cells. The findings suggest that prolonged exposure to pepsin promotes tumorigenesis and metastasis in respiratory epithelial cells, indicating pepsin's involvement in laryngopharyngeal carcinogenesis associated with LPR. The meta-analysis conducted by Eells et al. (2020) identified a substantial association between LPR and laryngeal carcinoma (OR = 1.95, 95% CI = 1.33–2.86). [20, 23]

The pepsin level diagnostic test's ability to differentiate between laryngeal carcinoma and non-laryngeal carcinoma is pretty adequate, as evidenced by the AUC value of 0.663. The moderate AUC value of the pepsin level test suggests that its diagnostic accuracy can still be improved, despite producing statistically significant results.

The ROC curve analysis results for the cut-off point of pepsin levels in this study are 27.4 ng/mL. The value signals a harmonious equilibrium between specificity and sensitivity. In the meta-analysis conducted by Jing et al., 16 research results were identified. Six studies employed a cutoff value of 16 ng/mL, while four studies employed a cutoff value of 50 ng/mL. In comparison to 16 ng/mL, the subgroup analysis results indicate that the 50 ng/mL cut-off value provides preferable diagnostic data. Therefore, implementing a higher threshold value can result in a greater degree of specificity, which in turn enhances the quality of diagnostic data . [21]

Several patients in the control group had high salivary pepsin levels that were higher than the cut-off value of 27.4 ng/ml. Although pepsin is frequently used as a marker for LPR, many asymptomatic people can also have positive pepsin levels in their saliva. The amounts of pepsin in the saliva of healthy people can vary greatly. Dietary factors, particularly the consumption of high-fat and high-protein foods, can impact test results by stimulating gastric secretion and increasing pepsin production. Additionally, the concentration of pepsin in the saliva may be elevated as a result of the sample collection's schedule, such as immediately following a meal . [22]

Compared to smoking history or pepsin levels, age had the most significant correlation with laryngeal carcinoma. When compared to each risk factor alone, the combination of these three demonstrates a significantly stronger correlation with the development of laryngeal carcinoma. The study of the overall risk score reveals that the group with a risk score exceeding 3.5 shown a laryngeal carcinoma incidence rate of 77.8%, significantly higher than the 22.2% incidence rate found in the group with a risk score of 3.5 or less. [23]

The interaction and accumulation of risk factors between smoking, age, and pepsin have a greater impact than if each item were examined separately, according to the highly significant p-value (p=0.000) obtained when the risk factors are combined into a single overall score. The risk of developing laryngeal carcinoma is more than 12 times higher for individuals with a score of >3.5 than for those with a score of ≤3.5, as shown by the Odds Ratio (OR) value of 12.250.

According to the outcome of this study, the risk of laryngeal carcinoma is independently increased by age as a physiological factor, smoking history as an environmental factor, and pepsin as a predictive biomarker. The combined analysis of these three factors demonstrates that the predictive sensitivity and specificity have increased. This is because the total risk score includes information from multiple pathophysiological pathways that mutually reinforce by initiating or accelerating the process of laryngeal carcinogenesis. [24]

According to Table 7, the risk of laryngeal carcinoma is higher when compared to that of the control group. With a p-value of 0.000, this data suggests a significant relationship between the incidence of laryngeal carcinoma and the increase in risk scores. This indicates that the "Total Risk Score" effectively predicts laryngeal carcinoma, making it a valuable instrument for clinical screening of the disease. [25]

The levels of pepsin in laryngeal carcinoma vary across various publications. This may be due to variations in the reagents used for pepsin examination. There has been no research in the field of laryngeal carcinoma that has reported the cut-off level of pepsin. Due to the restricted research time and the number of patient visits to the hospital, saliva specimen collection was only performed once in this study. Different times may have various results (morning, postprandial, afternoon, and evening). Collecting saliva samples at specific times may provide more accurate test results regarding pepsin concentrations.

Dietary factors, including the consumption of spicy, acidic, or fatty foods, are among several variables that may affect pepsin levels. These are variables outside the researcher's control. An additional factor that could impact the results is the patient's inability to recall the precise time, particularly if the sample is obtained immediately following a substantial meal. Caffeine and alcohol consumption can induce reflux and trigger the production of gastric acid.

5. CONCLUSION

Pepsin levels and laryngeal carcinoma are strongly correlated. The development of a scoring card that incorporates age, smoking history, and pepsin levels is highly beneficial for predicting laryngeal carcinoma.

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