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Relationship of Transforming Growth Factor-Beta, C-Reactive Protein, and Lactate Dehydrogenase in Bronchial Washing with the Diagnosis of Non-Small Cell Carcinoma Lung Cancer

Putu Kessi Vikaneswari, Ni Wayan Candrawati*, Ida Ayu Jasminarti Dwi Kusumawardani, Ni Luh Putu Eka Arisanti, I Desak Putu Agung Krisdanti, Gede Oky Aryanthana, Pande Budi Wadanta, Ida Bagus Ngurah Rai

1Department of Pulmonology and Respiratory Medicine, Prof. I.G.N.G. Ngoerah General Hospital, Faculty of Medicine, Udayana University, Indonesia, 80114

E-mail: kessi.vikaneswari@gmail.com; candrawati@unud.ac.id; jasminarti@unud.ac.id; eka.arisanti@unud.ac.id; agung.krisdanti@unud.ac.id; gedeokyaryanthana@yahoo.co.id; vandemadde@gmail.com; idabagus_ngurahrai@unud.ac.id

*Corresponding author details: Ni Wayan Candrawati; candrawati@unud.ac.id

ABSTRACT

Background: Most lung cancer cases are diagnosed at an advanced stage. Bronchial washing fluid contains many inflammatory cytokines that play a role in the malignant process. The aim of this study was to analyze the relationship between TGF-β, CRP and LDH in bronchial washing fluid with diagnosis of non-small cell lung carcinoma (NSCLC). ***Method:*** This research is an observational analytical study with a cross sectional design at Prof. Ngoerah Hospital, Denpasar (May 2023-August 2023). Descriptive analysis describes subject characteristics and research variables. Normality test with Shapiro Wilk. Bivariate analysis uses the unpaired T test with a normal distribution and the Mann-Whitney test with a non-normal distribution, data in the form of mean and standard deviation. Multivariate analysis with logistic regression. ***Results:*** The total research samples were 37 samples. 23 samples were detected as non-small cell lung carcinoma; 14 samples as non-NSCLC. The research results showed a significant relationship between TGF-β levels and diagnosis of non-small cell lung carcinoma (346.11 ± 40.21 pg/mL) vs (290.01 ± 66.22) ($p < 0.05$) and a significant relationship between LDH levels and diagnosis of non-small cell lung carcinoma (140.38 ± 98.11 U/L) vs (56.62 ± 22.68) ($p < 0.05$). There was no significant relationship between TGF-β, LDH, CRP and histopathology, bronchoscopy lesions and cancer stage of non-small cell lung carcinoma. ***Conclusion:*** There is a significant relationship between TGF-β and LDH levels and diagnosis of non-small cell lung carcinoma. TGF-β and LDH levels are increased in non-small cell lung carcinoma.

Keywords: non-small cell lung carcinoma; bronchial washing; TGF-β; CRP; LDH

INTRODUCTION

In 2018, approximately two million people were diagnosed with lung cancer worldwide, with 1.76 million dying from lung cancer. Lung cancer has a mortality rate of 18.4% of all cancer-related deaths. In Indonesia, data from Globocon in 2020, there were 34,783 new cases of lung cancer or equivalent to 8.8% of all cancer cases, placing lung cancer in the third most prevalent position in Indonesia, with a mortality rate of 13.2% which established lung cancer as the first cause of death from cancer in Indonesia. Because the symptoms of lung cancer are initially similar to those of other respiratory diseases, lung cancer is difficult to diagnose at the beginning and is usually only diagnosed at an advanced stage, where at this stage lung cancer can no longer be treated with surgical resection.

In advanced stages, lung cancer is treated with chemotherapy, immunotherapy and radiotherapy [1]. Recent data shows that the 5-year survival rate is only 18% when diagnosed at an advanced stage, whereas when diagnosed at an early stage it can reach 73%. Therefore, accurate and efficient early detection of lung cancer is very important to improve the prognosis of the disease in the future [2].

Bronchoscopy can be considered as a primary diagnostic tool in patients with suspected lung cancer. Bronchoscopy can also be an alternative treatment option to chemotherapy, radiotherapy and surgery. Bronchoscopy is less invasive than other tissue harvesting methods such as Video Assisted Thoracoscopic Surgery (VATS) or open thoracotomy, has less risk of complications and has a high specificity, albeit low sensitivity.

Bronchial washing is a useful procedure to obtain samples of cellular and humoral constituents from the lung microenvironment. Changes in the cell profile of bronchial wash fluid reflect the immunologic reaction of the lung in pulmonary malignancies. Bronchial wash has better direct contact with the tumor and tumor microenvironment compared to serum examination.

Lung cancer is one of the most common malignant neoplasms and is associated with a poor prognosis as it is usually diagnosed at an advanced stage. However, recent advances in the field of systemic medicine have changed the landscape of this disease. Much of this progress has been achieved with the discovery and use of predictive biomarkers, the detection of which can guide targeted therapies in an attempt at a more personalized treatment approach. In cancer research, biomarkers are biological molecules whose levels can be measured in tissues or fluids and indicate the presence or progress of disease or its response to specific therapies. As such, biomarkers can help in determining diagnosis, prognosis and prediction of treatment response in cancer patients. Each component of a cancer cell's metabolic pathway, starting from gene alterations and ending with various metabolites, can be tested as a biomarker according to its biological role. In recent years, the contemporary measurement of many substances that have similar levels in the metabolic cascade has been made possible with the use of technologies that enable biomarker identification.

Non-small cell carcinoma lung cancer (NSCLC) is the most common type of lung cancer, accounting for 75-80% of all cases, consisting of adenocarcinoma which accounts for 40% of lung cancer and is more prevalent in women, squamous cell carcinoma accounts for 25-30% of lung cancer cases and is most prevalent in men and the elderly and large cell carcinoma accounts for 10-15% of all lung cancer cases [3].

In the process of carcinogenesis, Transforming Growth Factor βeta (TGF- β) can function as a tumor suppressor at early stages and a tumor promoter at advanced stages. Transforming growth factor βeta inhibits the proliferation and differentiation of bronchial epithelial cells. Most non-small cell lung carcinomas (NSCLC) become resistant to the suppressor effects of TGF-β. Biological changes are fundamental to the process of malignancy that affect the role of cytokines in cell regulation and differentiation. Research by Gonzales Santiago et al found that serum TGF-β1 levels in lung cancer patients were higher than in healthy people [4]. Research by Huang et al found the value of TGF-β1 was almost twice as high in the NSCLC case group than in the normal lung tissue group and was significantly associated with TNM stage and lymph node metastasis.

Bronchial washing contains many cytokines that play a role in the process of lung malignancy. One of them is TGF- β1 which is produced directly by cancer cells or produced by the body in response to the presence of malignant cells. Bronchial washing fluid can be detected earlier than abnormalities obtained from x-rays Research by Chen Z et al, reported that TGF- β1 levels in lung cancer patients were significantly different from TGF- β1 levels in patients with lung diseases other than malignancies, so it was concluded that TGF- β1 from bronchial washing fluid could be a meaningful biological marker in lung cancer. [5] This is in line with the research of Gonzales et al who reported the same thing, namely TGF-β1 plays a role in local processes that occur in patients with lung cancer [4].

C-Reactive Protein (CRP) is secreted by hepatocytes in response to inflammatory cytokines produced by the tumor microenvironment. CRP enters the tumor microenvironment through blood circulation and binds to various types of extrinsic and autologous ligands and plays an important role in the process of tumor cell clearance. In chronic inflammation, CRP levels are associated with disease severity and response to therapy. Chronic inflammation not only plays a role in the tumor initiation phase, but also plays a role in the process of tumor invasion and progression to metastasis [6]. Research by Lee et al. reported that CRP levels before tumor resection were associated with tumor size and lymphovascular invasion in NSCLC patients [7]

The metabolic process of cancer cells requires glucose especially for cell proliferation. Glucose is then converted into pyruvate during the process of glycolysis, under anaerobic conditions, LDH will convert pyruvate into lactate. Lactate in the extracellular space causes an acidic environment. The acidic environment disrupts intercellular signaling, regulates the expression of cytokines and growth factors, causes degradation of extracellular matrix and cell membrane components, thus leading to cell invasion and metastasis. High LDH levels are associated with resistance to chemotherapy in advanced NSCLC patients [8].

Research on the levels of TGF-B, CRP, and LDH in bronchial washing in lung cancer patients, especially non-small cell carcinoma lung cancer at Prof. IGNG Ngoerah Hospital has never been done before so we are interested in doing this research.

METHOD

This study is an observational analytic study with a cross sectional design. The place of research was in the bronchoscopy room of Prof. Dr. IGNG Ngoerah Hospital. The sample was all lung cancer patients who underwent bronchoscopy procedures in the bronchoscopy room of Prof. Dr. IGNG Ngoerah Hospital in the period May 2023-August 2023 with total sampling technique. Inclusion Criteria: All adult lung cancer patients (over 18 years of age) and have never received lung cancer therapy. Exclusion Criteria: Patients who were detected suffering from other primary malignancies established from history taking, physical examination and other supporting examinations and patients who had pulmonary or non-pulmonary infections: pneumonia, HIV, active

pulmonary TB, extra-pulmonary TB. Data analysis was assisted by SPSS version 26 including descriptive data analysis, bivariate analysis with related variables using the unpaired t test if the data were normally distributed and the Mann-Whitney test if the data distribution was not normal. Multivariate analysis with logistic regression. Adjusted OR was presented with 95% confidence interval (CI). The p value that was considered significant was p value <0.05.

RESULT

The number of samples used in this study was 37 samples. The basic characteristics of the variables are presented in Table 1. In general, the mean ± SD age of the patients was 60.49 ± 10.29 years and the majority were male, with 28 patients (75.7%). More than half of the sample had no intraluminal lesions, 25 patients (67.6%). A total of 23 patients were diagnosed with non-small cell carcinoma lung cancer type malignancy (62.1%.). Most patients had adenocarcinoma cancer type, 19 patients (83.3%), with most patients detected at stage IV, 21 patients (94.6%). In terms of comorbid history, there was only 1 patient with comorbid asthma (2.7%). Meanwhile, in terms of smoking history, there were 8 patients (21.6%) with a history of heavy smoking and 3 patients (8.1%) each with a history of moderate and light smoking. Mean ± SD levels of CRP, LDH, and TGF-β were 0.18 ± 0.20 mg/dL; 110.95 ± 89.25 U/L; and 326.40 ± 56.85 pg/mL, respectively.

TABLE 1: Characteristics of basic variables.

Variables	N = 37 n (%)
Age	
Mean ± SD (years)	60,49 ± 10,29
Gender	
Male	28 (75,7)
Female	9 (24,3)
Intraluminal lesions	
Yes	12 (32,4)
No	25 (67,6)
Diagnosis	
NSCLC	23 (62,1%)
Non-NSCLC	14 (37,9%)
Cancer stage	
III	2 (5,4)
IV	21 (94,6)
Comorbid	
Asthma	1 (2,7)
Smoking History	
Light Smokers	3 (8,1)
Moderate Smokers	3 (8,1)
Heavy Smokers	8 (21,6)
CRP	
Mean ± SD (mg/dL)	0,18 ± 0,20
LDH	
Mean ± SD (U/L)	110,95 ± 89,25
TGF-β	
Mean ± SD (pg/mL)	326,40 ± 56,85

TGF-β and LDH levels were significantly associated with the diagnosis of NSCLC with p values of 0.013 and <0.001, respectively. Both TGF-β and LDH had higher levels in NSCLC compared to non-NSCLC.

Table 2 presents the results of the analysis between TGF-β, CRP, and LDH levels and NSCLC diagnosis (Table 2).

TABLE 2 : Relationship between TGF-β, CRP, and LDH levels and NSCLC diagnosis.

	n	Mean ± SD	P-value
TGF-β			
NSCLC	23	346,11 ± 40,21	0,013[a*]
Not NSCLC	14	290,01 ± 66,22	
CRP			
NSCLC	23	0,15 ± 0,13	0,415[b]
Not NSCLC	14	0,25 ± 0,29	
LDH			<0,001[b*]
NSCLC	23	140,38 ± 98,11	
Not NSCLC	14	56,62 ± 22,68	

a = T test; b = Mann-Whitney test; * = significant

ROC analysis was performed to determine the cut-off point, sensitivity, specificity, and Area Under Curve of TGF-β and LDH levels for NSCLC diagnosis. TGF-β level of 297.77 pg/mL has a sensitivity value of 87.5% and specificity of 69.2% with an AUC value of 0.750. Meanwhile, LDH level of 68.00 U/L had a sensitivity value of 91.7% and specificity of 76.9% with an AUC value of 0.904 (Table 3).

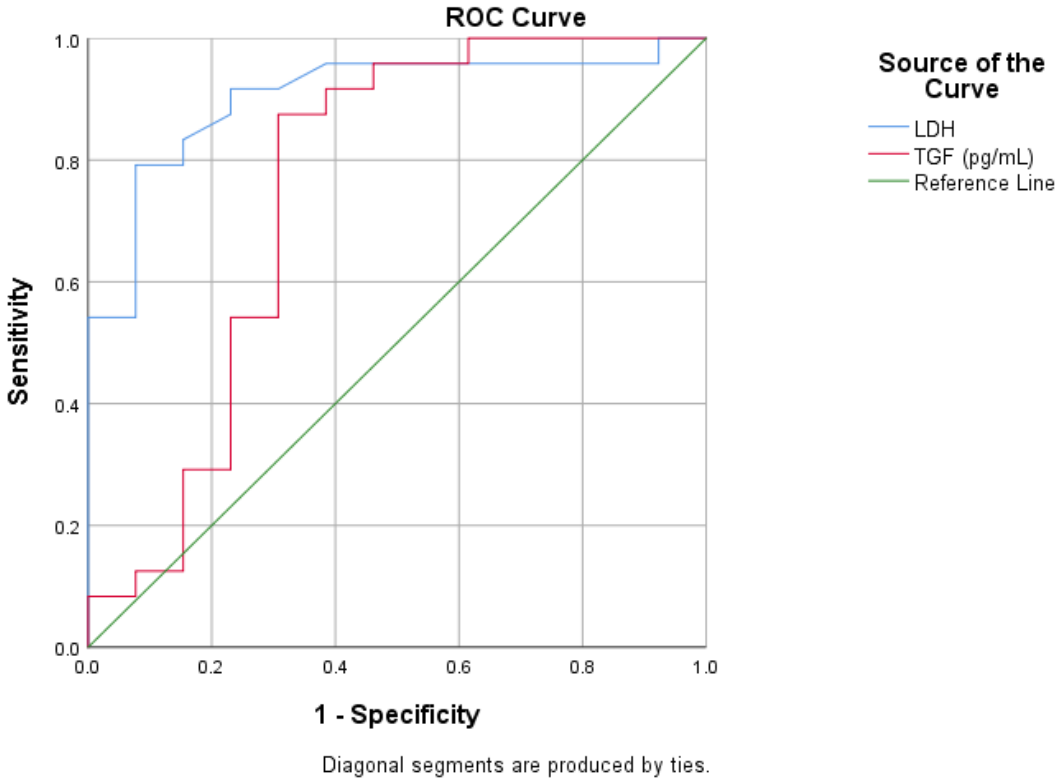

FIGURE 1: ROC analysis results.

TABLE 3: Cut-off values, sensitivity, specificity, and AUC.

	Cut-Off	Sensitivity (%)	Specificity (%)	AUC
TGF-β	297.77 pg/mL	87,5	69,2	0,750
LDH	68.00 U/L	91,7	76,9	0,904

AUC = Area Under Curve.

TGF-β, CRP, and LDH levels were not significantly related to NSCLC histopathology with p values > 0.05. Both TGF-β and LDH had higher levels in the non-squamous group. Meanwhile, CRP had higher levels in the squamous group. Table 4 presents the results of the analysis between TGF-β, CRP, and LDH levels and NSCLC histopathology.

TABLE 4: Relationship between TGF- β, CRP, and LDH levels with NSCLC histopathology.

Histopathology	n	Mean ± SD	P-value
TGF-β			
Squamous	4	319,84 ± 51,66	0,354[a]
Non-Squamous	19	349,86 ± 38,40	
CRP			
Squamous	4	0,16 ± 0,14	0,145[b]
Non-Squamous	19	0,05 ± 0,02	
LDH			
Squamous	4	94,67 ± 80,43	0,310[b]
Non-Squamous	19	146,90 ± 100,33	

a = T test; b = Mann-Whitney test; * = significant

TGF-β, CRP, and LDH levels were not significantly related to NSCLC stage with p values >0.05. Both TGF-β and LDH had higher levels in stage IV NSCLC.

Table 5 presents the results of the analysis between TGF-β, CRP, and LDH levels and NSCLC stage.

TABLE 5 : Relationship between TGF- β, CRP, and LDH levels and NSCLC stage.

Stadium	n	Mean ± SD	P-value
TGF-β			
III	2	341,71±40,52	0,154[a]
IV	21	385,44 ± 26,03	
CRP			
III	2	0,32 ± 0,23	0,170[b]
IV	21	0,13 ± 0,11	
LDH			
III	2	66,50 ± 3,53	0,380[b]
IV	21	148,52 ± 102,11	

a = T test; b = Mann-Whitney test; * = significant

TGF-β, CRP, and LDH levels were not significantly associated with bronchoscopic lesions of NSCLC with p values > 0.05. Both TGF-β and CRP had higher levels in the extralumenal group.

Meanwhile, LDH had higher levels in the intralumen group. Table 6 presents the results of the analysis between TGF-β, CRP, and LDH levels and bronchoscopic lesions of NSCLC.

TABLE 6: Relationship between TGF- β, CRP, and LDH levels and bronchoscopic lesions of NSCLC.

Bronchoscopic lesions	n	Mean ± SD	P-value
TGF-β			
Intralumen	12	325,29 ± 62,47	0,936[a]
Extralumen	25	326,93 ± 55,30	
CRP			
Intralumen	12	0,15 ± 0,10	0,896[b]
Extralumen	25	0,20 ± 0,24	
LDH			
Intralumen	12	126,00 ± 75,95	0,249[b]
Extralumen	25	103,72 ± 95,58	

a = T test; b = Mann-Whitney test; * = significant

The association between LDH, CRP, and TGF-β with NSCLC diagnosis was analyzed using multivariate analysis of logistic regression test because they are categorical variables.

The analysis showed that TGF-β and LDH were significantly associated with NSCLC diagnosis. TGF-β was found to be significantly associated with a p value = 0.013 and OR (95% CI) = 1.019 (1.010 - 1.043).

Meanwhile, LDH was found to be significantly associated with NSCLC diagnosis with a p value = 0.015 and OR (95% CI) = 1.070 (1.013 - 1.130).

Multivariate analysis is presented in Table 7 Smoking and comorbid variables were excluded due to the large number of missing variables (Complete comorbid variables: 4 samples; Smoking: 14 samples).

TABLE 7: Multivariate analysis of the association between TGF-β and LDH with NSCLC diagnosis.

	P-value	OR	95%CI (Lower - Upper)
TGF-β	0,013*	1,019	1,010 - 1,043
LDH	0,015*	1,070	1,013 - 1,130
Constant	0,024	0,016	

OR = odds ratio; CI = confidence interval; * = significant (logistic regression test).

DISCUSSION

The mean age of non-small cell carcinoma lung cancer patients in this study was 60.49 ± 10.29 years. Most of the non-small cell carcinoma lung cancer patients in this study were male. Non-small cell carcinoma lung cancer is the most common type of lung cancer, covering 75-80% of all cases, consisting of adenocarcinoma which covers 40% of lung cancer and appears more in women, squamous cell carcinoma covers 25-30% of lung cancer cases and is most common in men and the elderly and large cell carcinoma covers 10-15% of all lung cancer cases [3] The same thing was also conveyed by Hannun et al. (2015), that almost 85% of newly diagnosed lung cancer cases are classified in non-small cell lung cancer, which consists of adenocarcinoma, squamous carcinoma, and large cell carcinoma, according to histological analysis. Adenocarcinoma and squamous cell carcinoma represent approximately 50%o and 40%o of non-small cell carcinoma lung cancer cases, respectively [9].

The average age of diagnosis of lung adenocarcinoma is 71 years, and the cancer is extremely rare in someone younger than 20 years old. Adenocarcinoma has replaced squamous cell cancer of the lung as the most common non-small cell cancer in the last two decades [10]. In this study, only 1 patient was found to have comorbidities, namely 1 patient suffering from asthma. However, there were 14 patients with a history of smoking where a history of heavy smoking was found in 8 patients while moderate and light smokers were found with a frequency of 3 people each. This is consistent with the theoretical basis which states that smoking is the biggest risk factor for lung cancer [11]. However, the percentage of smokers in this study is still relatively low (37.8%).

The most common histopathologic type of non-small cell carcinoma lung cancer found in patients in this study was adenocarcinoma. In previous studies, the most common histopathologic type found was adenocarcinoma [12]. Another study also reported similar results, where the most common type found was adenocarcinoma (66.9%) [13]. Meanwhile, another study reported that the most common type found in non-small cell carcinoma lung cancer patients was squamous cell carcinoma (43.6%) followed by adenocarcinoma (35.2%) [14].

Most of the non-small cell carcinoma lung cancer patients in this study did not have intraluminal lesions and had stage IV. Only very few patients had stage III (IIIC/2 samples). Most cases of non-small cell carcinoma lung cancer in the clinic already present with unresectable stage III disease [9]. Other studies have also reported similar findings, with most non-small cell carcinoma lung cancer patients having stage 3B (38%) followed by stage 3A (19.7%) [14]. Meanwhile, in another study it was reported that non-small cell carcinoma lung cancer patients were mostly found to be at stage IV [12]. The study by Yang et al. (2019) also reported the same thing, namely the majority of non-small cell carcinoma lung cancer patients were at stage IV (55.5%) [13].

In this study, the mean TGF-β of patients was 326.40 ± 56.85 pg/mL. Research by Gonzales Santiago et al. (2011) obtained results, namely serum TGF-β1 levels in lung cancer patients were higher than in healthy people (37,225 ± 9,436 vs. 28,416 ± 9,324 pg/mL) [4]. Similar results were also reported in a study by Hou et al. (2013), where TGF-β1 serum levels were found to be significantly higher in patients with lung cancer compared to healthy samples [15]. Kumar et al. (2010) also reported that there was an increase in TNF-α and TGF-β1 levels in patients with non-small cell carcinoma lung cancer compared to controls [16]. Research by Huang et al. (2014) found the value of TGF-β1 was almost twice as high in the group of non-small cell carcinoma lung cancer cases than in the group with normal lung tissue [17] TGF-β concentration in Bronchoalveolar Washing Fluid (BALF) was also found to be significantly higher in non-small cell carcinoma lung cancer patients compared to healthy controls (p=0.047) [18]. The level of TGF-β1 in BALF was also reported to be significantly higher in patients with lung cancer compared to patients with non-malignant disease (p=0.003) and suggested that TGF-β1 in BALF could be a potential biomarker for lung cancer [5].

TGF-β released by cancer cells, stromal fibroblasts, and other cells in the tumor microenvironment further stimulates cancer progression and suppresses the anti-tumor activity of immune cells, resulting in immunosuppressive conditions. High TGF-β levels found in NSCLC are associated with lymph node metastasis and tumor angiogenesis.

Tumor cells from NSCLC express TGF-β ligand. β. TGF- β induces the process of epithelial to mesothelial transition (EMT) which plays a role in the progressivity of advanced NSCLC. TGF--mediated epithelial to mesothelial transition β has the potential to be aggressive, resistant to apoptosis, and chemotherapy.

The mean CRP level in this study was 0.18 ± 0.20 mg/dL. High circulating serum CRP levels are associated with poor response and worse survival in patients with non-small cell carcinoma lung cancer. The CRP cut-off value in the study was 5 mg/L and all patients were divided into three groups, namely groups with low (102 people), intermediate (50 people), and high (40 people) CRP levels [12]. Serum CRP levels were also reported to be significantly higher in non-small cell carcinoma lung cancer patients compared to the control group (114.2 ± 60.1 mg/mL vs. 13.4 ± 8.6 mg/mL) [14].

LDH levels in patients in this study had a mean of 110.95 ± 89.25 U/L. High LDH levels are associated with resistance to chemotherapy in patients with advanced non-small cell carcinoma lung cancer [8]. In a previous study, it was reported that elevated LDH levels were found in the majority of patients with LDH2 being most commonly elevated in non-small cell lung cancer patients [19]. Lung cancer patients with metastatic stage group showed a statistically significant increase in serum LDH levels compared to non-metastatic stage group with serum LDH levels of non-metastatic lung cancer patients and metastatic group (350.73±80.40 vs 520.49±210.92 IU/L) [20].

In this study, there was no statistically significant relationship between TGF-β levels and histopathology of non-small cell carcinoma lung cancer patients with bronchial washing preparations. The results of this study cannot be compared with previous studies given the lack of similar studies that discuss the direct relationship between TGF-β levels and histopathological variables, stage, and bronchoscopic lesions, especially in bronchial rinse sample preparations so that this study has novelty value that can be used as a reference for further research on evaluating the relationship between inflammatory markers and clinicopathological aspects in patients with lung cancer through non-invasive sampling.

In this study, there was no significant relationship between bronchoscopic lesions and cancer stage. However, both intraluminal and extraluminal lesions had high levels of TGF-β, as well as cancer stage, with stage IV having increased TGF-β levels. This may be related to the role of TGF-β which has an essential role in cancer development. Most carcinoma cells have inactivated their epithelial antiproliferative response and benefit from increased TGF-β expression, as well as autocrine TGF-β signaling through effects on gene expression, immunosuppressive cytokine release and epithelial plasticity. This can trigger invasion, spread of cancer cells, and increased stem cell properties.

TGF-β released by cancer cells, stromal fibroblasts and other cells in the tumor microenvironment further stimulates cancer progression and suppresses the anti-tumor activity of immune cells, resulting in immunosuppressive conditions. However, tumorigenesis depends on the ability of tumor cells to generate and condition a tumor microenvironment containing various types of stromal cells that allow cancer cells to thrive, and stimulate cancer progressivity [21].

Based on several studies that discuss the role of TGF-β on the prognosis of lung cancer patients, there is a significant relationship between increased TGF-β levels in the serum of lung cancer patients with a worse prognosis. In a meta-analysis conducted by Li et al., 2019 involving eight studies with a total of 579 lung cancer patients, it was found that increased TGF-β expression in non-small cell carcinoma lung cancer patients could indicate a poor prognosis with an HR value = 2.17; p-value <0.05 [22] The results of this meta-analysis were then studied further by evaluating each study included in it, where all studies also showed similar results, where these studies showed significant results between increased TGF-β expression and poor prognosis in non-small cell carcinoma lung cancer patients. In these studies, it was found that increased TGF-β expression tends to be found in patients with advanced stages and tends to be found in the presence of spread to the lymph nodes, which indirectly supports the findings in this study [16,17,23].

Based on several studies, TGF-β plays a role in the formation of tumor micro-environment (TME) which has an important role in tumor cell development. In tumor tissue, TGF-β, which is generally produced by fibroblasts and mononuclear cells, acts as a growth factor that triggers the formation of extracellular matrix. In addition, TGF-β also plays a role in changing the behavior of epithelial cells through cellular processes where epithelial cells behave like mesenchymal cells, deregulation of epithelial characteristics known as the Epithelial-to-Mesenchymal Transition (EMT) process [22]. In this condition, changes in the phenotype of epithelial cells will cause changes in the polarity of epithelial cells so that in this condition, epithelial cells lose their ability to bind to each other through cell-to-cell adhesion junctions so that they turn into cells that have the ability to invade and migrate. In cancer, increased expression of TGF-β is known to be caused through a paracrine process by tissue components around the tumor or autocrine due to the differentiation of tumor cells that can eventually express TGF-β for themselves[24]. Both processes have a similar impact on tumor progression through an increase in extracellular matrix components, inhibition of the immune system, and increased angiogenic activity through various pathways, including the process of migration of vascular epithelial (endothelial) cells that trigger changes in blood vessel structure, the process of inflitration and invasion of tumor cells through the EMT process, increased expression of Matrix Metalloproteinase-2 (MMP-2) and Matrix

Metalloproteinase-9 (MMP-9) which play a role in the degradation of extracellular matrix structures for the formation of new blood vessel structures towards tumor tissue, and induction of other growth factors such as Vascular Epithelial Growth Factor (VEGF) and Interleukin-1 (IL-1) which play an important role in the angiogenesis process [16,17,23].

C-Reactive Protein (CRP) is one of the proteins that play a role in inflammation, both in the acute phase and in the chronic phase. C-Reactive Protein (CRP) is a pentameric protein that is commonly produced by the liver in response to the acute inflammatory phase [25]. CRP production in the liver is generally induced through increased expression of Interleukin-6 (IL-6) as a signaling pathway that plays a role in CRP transcription in the acute inflammatory phase. CRP itself is a marker that has high sensitivity when inflammation occurs in the body with a rapid increase in CRP levels. This rapid increase in CRP can be an early sign of infection, inflammation, and tissue damage in the body, and can also be a marker of chronic pathology processes that occur, one of which is malignancy. Various studies have examined elevated CRP levels in various types of malignancies, with the findings indicating that high inflammatory conditions may be one of the risk factors for cancer [26,27].

Just as the role of TGF-β is dualistic, both as pro-tumor and anti-tumor, CRP is also known to have a dual role, both as anti-inflammatory and pro-inflammatory. Recent research suggests that this phenomenon is caused by the existence of variants of the protein structure of the CRP protein called isomers [26–28]. Several studies have identified two types of functional isomers of CRP, namely modified-monomeric CRP or mCRP and pentameric-CRP or pCRP. Both isomers have different functions in terms of inflammatory reactions, where mCRP has more anti-inflammatory properties while on the other hand, pCRP is known to have a strong pro-inflammatory role [28,29]. Several previous studies that examined the role of CRP in acute inflammatory reactions basically evaluated and successfully described pCRP to be formed as a soluble pentameric isoform in blood serum. When dissociated, pCRP will then confuse into different isoforms with lower water solubility and form mCRP with its anti-inflammatory role [25].

In this study, there was no statistically significant relationship between CRP levels and histopathology, stage, and bronchoscopic lesions of non-small cell carcinoma lung cancer patients with bronchial rinse preparations. This may be due to the difference in sample type (bronchoscopic washing fluid) from the general sample (serum) analyzed using a chemical bioanalyzer machine. Furthermore, CRP is generally found in high concentrations in serum and due to its peptide/protein structure, tends to have little diffusion ability across the bronchial epithelial membrane. However, similar to the findings of the relationship between TGF-β and dependent variables in this study, there is no similar study that discusses the relationship between CRP levels and histopathology, stage, and bronchial lesions in non-small cell carcinoma lung cancer, especially in bronchial rinse preparations, so this study has novelty value that can be used as a reference for further research on evaluating the relationship between inflammatory markers and clinicopathological aspects in patients with lung cancer through non-invasive sampling.

Tumor cells are abnormal cells that have an uncontrolled proliferation rate. To compensate for this unlimited and uncontrolled cell proliferation, tumor cells have a different metabolic process compared to normal cells by increasing the process of glycolysis without the need for oxygen through anaerobic metabolism known as the Warburg effect. Lactate dehydrogenase (LDA) is an enzyme that plays a role in the conversion of pyruvate to lactate in the anaerobic glycolysis process in tumor cells which can provide biomolecular components needed by tumor cells to meet their needs quickly [30].

In this study, a significant association was found between elevated LDH levels and histopathology of non-small cell carcinoma lung cancer. No previous studies have also discussed the relationship between elevated LDH levels and the clinicopathological aspects of lung cancer, especially in bronchial washing. However, some studies have found an association between elevated LDH levels as a prognostic marker. In a study conducted by Tjokrowidjaja et al., 2022 involving 1327 patients with a diagnosis of non-small cell carcinoma lung cancer, a statistically significant association was found between elevated LDH levels and worse overall survival (OS) of non-small cell carcinoma lung cancer patients [31]. The results of another study conducted by Wang et al., 2022 also found a significant relationship between elevated LDH and worse OS in non-small cell carcinoma lung cancer patients (p-value <0.05) [30]. These two findings are also in line with the meta-analysis study conducted by Zhang et al., 2022. This study involved twenty-six studies with a total of 3,429 patients diagnosed with non-small cell carcinoma lung cancer. In this meta-analysis study found statistically significant results (p-value <0.05) in the relationship between gross increase in LDH and patient OS, but when viewed from the Hazard Ratio value in the results of this study (HR = 1.19) shows results that cannot be used in concluding that increasing LDH levels has a significant impact in determining patient survivability [32].

The role of LDH in cancer cell metabolism is a vital part of cancer cell progressivity to fulfill the cell's need to support its rapid proliferation rate [2]. In the metabolic process, cancer cells require a strong anabolic process to be able to fulfill the nature of cancer which has an unlimited proliferation rate. In this process, cancer cells not only need ATP as an energy source to be able to carry out their intracellular processes, but also by-products of biological components to maintain cell growth.

Therefore, cancer cells are famous for The Warburg Effect which can provide an abundant supply of carbon for cancer cells through various pathways where the carbon component here is needed in the process of forming cell components, fatty acids, and amino acid synthesis pathways [33]. LDH is an enzyme that plays an important role in glucose metabolism. Under aerobic conditions where oxygen supply is normal, pyruvate in normal cells will be transported to the mitochondria for further processing through the tricarboxylic acid cycle which is then degraded into carbon dioside and water. In the cycle, the precursor of ATP is produced, namely NADH which then goes through the oxidation process through oxidative phosphorylation so that 36 molecules of ATP are produced in 1 molecule of glucose processed. However, in hypoxic conditions, where cells are forced to metabolize under anaerobic conditions, pyruvate transport to the mitochondria does not occur, but is directly converted to lactate by LDH [30].

In hypoxic conditions, this process is used to produce ATP in low oxygen availability. However, in malignant conditions, the role of LDH is actually used by tumor cells to produce ATP regardless of the level of oxygen availability due to the high rate of glycolysis through the intermediary of LDH and the residual organic components needed by cells to be able to produce larger amounts of cell components and parts used to support the material needs of cells to proliferate rapidly [33].

In this study, there was no significant association between LDH levels and bronchoscopic lesions. However, LDH levels in intraluminal lesions were higher than those in extraluminal lesions, which may be related to central hypoxia in the tumor tissue. As mentioned earlier, tumor cells have the ability to proliferate rapidly which is not accompanied by adequate vascularization so that the central part of the tumor tissue will have lower vascularization compared to the outer part of the tumor tissue. This results in central hypoxia which eventually causes damage or death of tumor cells in the central part of the tumor tissue. Therefore, LDH can also be a marker of tissue damage or necrosis in tumor tissue due to nutrient and oxygen insufficiency [33].

In this study, there was no significant association between LDH levels and stage. Due to sample limitations, almost all samples of this study were diagnosed at an advanced stage. This is in accordance with the research of Gupta et al 2019 who found an increase in serum LDH levels in the lung cancer group with metastases compared to the group without metastases (520.49 ± 210.92 vs 350.73 ± 80.40). Furthermore, based on the results of multivariate analysis, it was reported that TGF-β levels of 297.77 pg/mL had a sensitivity value of 87.5% and specificity of 69.2% with an AUC value of 0.750. Meanwhile, LDH level of 68.00 U/L had a sensitivity value of 91.7% and specificity of 76.9% with an AUC value of 0.904. These results indicate that both TGF-β and LDH have diagnostic value for NSCLC incidence.

This finding is in line with a previous study which reported that TGF-β1 levels were significantly higher in patients with lung cancer compared to patients with benign disease (p = 0.003). However, no significant difference between TNF-α (P = 0.72) was observed between malignancy and non-malignancy groups. As for the cut-off value of 10.85 pg/ml, TGF-β1 showed a sensitivity of 62.2% and specificity of 60.6% in predicting the malignancy of lung disease suggesting that TGF-β1 may be a significant biomarker in lung cancer [5].

Unfortunately, there are no studies reporting the diagnostic value of LDH levels for NSCLC incidence. A previous study reported that the proportion of patients with abnormal serum LDH levels was statistically higher in the high metastasis score group than in the low metastasis score group (65.3% vs 50.4%; p = 0.001). Another study reported that the cancer ratio, defined as the ratio of serum lactate dehydrogenase (LDH) to pleural fluid adenosine deaminase (ADA) reported significant diagnostic value in cases of malignant pleural effusion. The participants in the study came from a prospective cohort (SIMPLE cohort, n=199) and a retrospective cohort (BUFF cohort, n=158) with AUC in the SIMPLE and BUFF cohorts being 0.60 (95% CI 0.52-0.68) and 0.63 (95% CI 0.54-0.71), respectively. [16,17,23].

Judging from the findings obtained in this study, this study is the first study in Bali for TGF-β examination and the first time in Indonesia for CRP and LDH examination to report a significant association between LDH and TGF-β with the diagnosis of NSCLC in the lung cancer population. The association was based on the significant difference of LDH and TGF-β levels in bronchoscopic fluid rinses between patients with NSCLC and patients without NSCLC in patients with suspected lung cancer. Judging from the diagnostic value obtained from ROC analysis, LDH and TGF-β have high sensitivity and potential as predictors for diagnosing NSCLC.

However, despite these findings, there are some shortcomings that must be considered and followed up in future research. The design of this study was cross-sectional, which does not allow for equal comparison between groups of the variables under study. This is different from case-control or cohort studies, which require at least the same number of samples in the two groups being compared. Then, CRP is diagnosed using photometric tools that generally use blood serum samples. Examination using an antibody-based analysis kit (ELISA) can be used as a substitute with a higher level of sensitivity and a standardized protocol.

CONCLUSION

There is a significant relationship between TGF-β and LDH levels and diagnosis of non-small cell lung carcinoma. TGF-β and LDH levels are increased in non-small cell lung carcinoma.

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