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Buku

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Buku dengan editor

Hopewell PC. Tuberculosis and other mycobacterial disease. In: Murray JF, Mason RJ, Broaddus VC, Nadel JA, editors. Textbook of respiratory medicine. 4 th edition. New York: WB Saunders Company; 2005.p.979-1043.

Jurnal

Edginton ME, Rakgokong L, Verver S, Madhi SA, Koornhof HJ, Wong ML, et al. Tuberculosis culture testing at a tertiary care hospital: options for improved management and use for treatment decisions. Int J Tuberc Lung Dis. 2008;12:786-91.

Tesis

Rassuna V. Pengamatan hasil akhir pengobatan tuberkulosis paru BTA negatif kasus baru di RS Persahabatan. Tesis Departemen Pulmonologi dan Ilmu Kedokteran Respirasi FKUI. Jakarta; 2008.

Organisasi sebagai sumber

World Health Organization. Global Tuberculosis Control 2009: epidemiology strategy financing. Geneva: WHO Press; 2009. p.11-32.

Perhimpunan Dokter Paru Indonesia. Tuberkulosis Pedoman diagnosis dan penatalaksanaan di Indonesia. Jakarta: Indah Offset Citra Grafika; 2006.p.1-8.

Materi Elektronik

The Highland Council. The Learning Environment. [Online].2010 [Cited 2011 November 28]. Available from:http://www.highlandschoolsvirtualib.org uk/ ltt/ inclusive_enjoyable/environment.htm

Sack K. With Medicaid cuts, doctors and patients drop out. The New York Times [Online]. 2010 Mar 16 [cited 2010 Mar 16]; Health:A1. Available from: http://www.nytimes.com/2010/03/16/health policy/16m edicaid.html?ref=health

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World Health Organization. Global Tuberculosis Control 2009 : epidemiology strategy financing. Geneva : WHO Press; 2009.p.11-32.

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Electronic resources

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Sack K. With Medicaid cuts, doctors and patients drop out. The New York Times [Online]. 2010 Mar 16 [cited 2010 Mar 16]; Health:A1. Available from: http://www.nytimes.com/2010/03/16/health/policy/16medicaid.html?ref=health.

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TABLE OF CONTENT

The Correlation of Microsomal Epoxide Hydrolase (EPHX1) His139ArgGene Polymorphism and Lung Cancer Incidence in H. Adam Malik General Hospital Medan

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Abstract

Background: *Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both activation and detoxification of polycyclic aromatic hydrocarbons (PAH) and aromatic amines. Polymorphism of EPHX1 His139Arg on susceptibility to lung cancer has been reported with inconsistent results. The purpose of this study was to analyze the correlation between this gene polymorphism and lung cancer incidence in smokers.*

Method: *This was a case-control study using a consecutive sampling method. Genotyping test was performed by PCR-RFLP assay. The chi-square test with* P *<0.05 was considered significant.*

Results: *Of the 84 subjects, in the case and control groups, His139His wild-type variants were found in 34 subjects (81%) and 30 subjects (71.4%), respectively, while His139Arg heterozygous variants were in 8 (19%) and 12 (28.6%) subjects. No homozygous variants of Arg139Arg were identified (*P*=0.36).*

Conclusion: *The EPHX1 His139His enzyme gene polymorphism was a common polymorphism in both groups of subjects. There was no correlation between EPHX1 His139Arg gene polymorphism of and lung cancer. (**J Respirol Indones 2022; 42 (2): 86–9)***

Keywords: *EPHX1; His139Arg; Lung cancer; PCR-RFLP; Polymorphism*

Hubungan Polimorfisme Gen *Microsomal Epoxide Hydrolase* (EPHX1) His139Arg dengan Kejadian Kanker Paru di RSUP H. Adam Malik Medan

Abstrak

Latar Belakang: Microsomal epoxide hydrolase *(EPHX1) memegang peranan penting dalam aktivasi dan detoksifikasi polisiklik hidrokarbon aromatik (PAH) dan amina aromatik. Polimorfisme gen EPHX1 His139Arg pada kerentanan terhadap terjadinya kanker paru telah dilaporkan dengan hasil yang tidak konsisten. Tujuan penelitian ini adalah untuk menganalisis hubungan antara polimorfisme gen ini dengan kejadian kanker paru pada perokok*

Metode Penelitian: *Penelitian ini merupakan studi kasus kontrol dengan metode pengambilan sampel secara* consecutive sampling*. Uji genotip dilakukan menggunakan PCR-RFLP. Uji* chi-square *dengan P<0,05 dianggap bermakna.*

Hasil: *Dari ke-84 subyek, pada masing-masing kelompok kasus dan kontrol, varian* wild-type *His139His ditemukan pada 34 subjek (81%) dan 30 subjek (71,4%), sedangkan varian heterozigot His139Arg ditemukan pada 8 (19%) dan 12 subjek (28,6%). Tidak ada varian homozigot Arg139Arg yang dijumpai (P=0,36).*

Kesimpulan: *Polimorfisme gen enzim EPHX1 His139His merupakan polimorfisme umum pada kedua kelompok subjek. Tidak terdapat hubungan antara polimorfisme gen EPHX1 His139Arg dengan kanker paru. (**J Respirol Indones 2022; 42 (2): 86–9)***

Kata kunci: *EPHX1; His139Arg; Kanker paru; PCR-RFLP; Polimorfisme*

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INTRODUCTION

Lung cancer is one of the most common cancers worldwide and has the highest mortality rate among all cancer types. Strong evidence suggests that tobacco smoking causes bronchogenic cancer in approximately 85–90% of lung cancer patients.[1]

Although several causes are associated with the development of lung cancer include smoking, history of respiratory disease, and exposure to chemical carcinogens, not all exposed people will develop lung cancer. Other causes such as genetic polymorphisms are also mentioned as a contribution to individual differences in the susceptibility to lung cancer.[2]

Exposure to carcinogens in cigarette smoke, such as polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, and aromatic amines, is a major cause of lung cancer among smokers.[3]

Microsomal epoxide hydrolase (EPHX1) is very important in various detoxification processes and metabolism of endogenous and exogenous compounds. The EPHX1 plays a dual role in the detoxification and bioactivation of PAHs and other environmental pollutants depending on their substrates. Reactive compounds such as sugar palm, alkene, and aliphatic epoxide are hydrolyzed by EPHX1, which is produced by cytochrome P450 and other phase 1 enzymes to the corresponding dihydrodiol upon addition of trans water. On the other hand, dihydrodiols which are less reactive than PAHs can be substrates for further transformation into dihydrodiol-epoxides such as benzo[a]pyrene-7,8-diol-9,10 epoxide, the most mutagenic and carcinogenic metabolites.[4]

A polymorphic form in the EPHX1 gene, amino acid residues 139 (Arg/His), has been identified. The amino acid substitution from Histidine to Arginine can cause changes in protein stability. Changes in exon 4 were associated with a 25% increase in EPHX1 activity.[5]

Many studies have investigated the association between the EPHX1 His139Arg gene polymorphism and lung cancer risk, but the impact of this polymorphism on lung cancer risk were reported with inconsistent results. This study aimed to identify the EPHX1 His139Arg gene polymorphism and its association with lung cancer in smokers.

METHODS

Eighty-four male subjects with smoking history were recruited into the study: 42 subjects with lung cancer at Adam Malik General Hospital compared with 42 healthy subjects. All subjects gave written *informed consent* and were interviewed regarding age, smoking status, history of cancer, and family history of cancer. Only individuals without a history of cancer and chronic respiratory disease were eligible for controls. Histological outcome information was searched manually in the pathology result. The Ethics Committee had approved this study protocol of the Faculty of Medicine, Universitas Sumatera Utara.[4]

Three milliliters of venous blood were collected from the median cubital vein, put into a sterile tube containing EDTA, and stored at 4°C in the refrigerator.[4]

The EPHX1 gene was amplified using a forward 5'- GGG GTA CCA GAG CCT GAC CGT-3' primer and a reverse 5'- AAC ACC GGG CCC ACC CTT GGC-3' primer (MBI Fermentas). PCR was carried out with a final volume of 25 l containing 2 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 0.2 mM dNTP (MBI Fermentas), and 1.5-unit Taq polymerase (MBI Fermentas). The DNA was denatured at 94ºC for 5 minutes. Thirty-five amplification cycles began with denaturation at 94ºC for 30 seconds, primary annealing at 62ºC for 30 seconds, and extension at 72ºC for 45 seconds, followed by a final 5-minute extension step at 72ºC. The PCR product produced a band at 357 bp. After one hour of digestion of the 15-µL PCR product with 10 U RSaI (MBI Fermentas), this product was visualized by electrophoresis on a 3% agarose gel.[4]

A Chi-square test was used to compare genotype frequencies between groups with P <0.05, which was considered significant. Odds ratio and

95%CI were calculated to determine the correlation between the variables and the risk of lung cancer in the EPHX1 His139Arg gene polymorphism. Statistical analysis was carried out using SPSS 17.0 statistical software for computer devices.

RESULT

The characteristics of the subjects based on age, Brinkman index, type of cigarette, and the distribution of EPHX1 gene polymorphism can be seen in Table 1.

Table 1. Characteristics of Study Subjects

Characteristics	Case		Control	
	n	%	n	%
Age				
<40 years	0	0	26	61.9
40–59 years	23	54.8	14	33.3
≥60 years	19	45.2	2	4.8
Brinkman Index				
Mild	-	-	4	9.5
Moderate	6	14.3	22	52.4
Severe	36	85.7	16	38.1
Cigarette Type				
Clove	23	54.8	35	83.3
White	11	26.2	4	9.5
Mixture	8	19.0	3	7.2
Polymorphism				
AG (His/Arg)	8	19.0	12	28.6
AA (His/His)	34	81.0	30	71.4

Based on Table 1, the majority of the case group were aged 40–59 years (54.8%), while the control group was mostly <40 years old (61.90%). The most common Brinkman index found in the case group was the severe category as observed in 36 subjects (85.7%), while in the control group was the moderate category as found in 22 subjects (52.4%). The mostly used cigarettes in both case and control groups was cloves as observed in 23 subjects (54.80%) and 35 subjects (83.30%), respectively.

Table 2. Correlation of Gene Polymorphisms with Lung Cancer Incidence

Gene Polymorphisms	Case		Control		*P*
	n	%	n	%	
AG (His/Arg)	8	19.0	12	28.6	0.30
AA (His/His)	34	81.0	30	71.4	

The genotype frequency of AA (His/His) was a common polymorphism in both groups of subjects. No GG (Arg/Arg) genotype was found in this study. The chi-square test stated that there were no correlation between the EPHX1 His139Arg gene polymorphism and the incidence of lung cancer in the case and control groups (*P*>0.05).

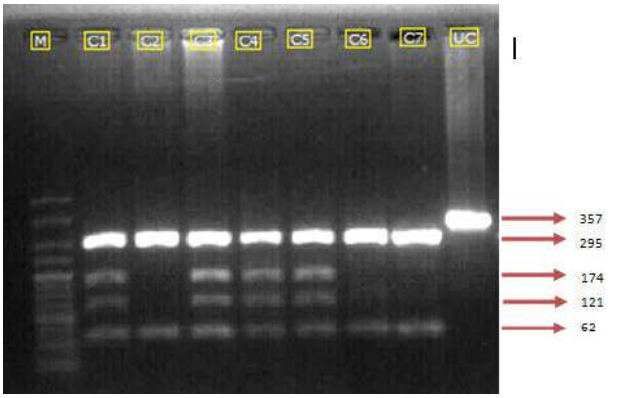

Figure 1. PCR-RFLP analysis for His139Arg polymorphism with RSaI. M: DNA markers, pathways C1, C3, C4, and C5 were genotyped with His/Arg at 295, 174, 121, and 62 bp; pathways C2, C6, and C7 were genotyped with His/His at 295 and 62 bp, respectively. UC: PCR product 357 bp.

DISCUSSION

In this study, lung cancer patients were mostly in the 40–59 years age group, which was 54.76%. These results were in line with several studies, such as the study from Erkisi et al. on 2010 in Turkey, which pointed out that the mean age of lung cancer patients was >50 years. The similar result was also obtained by Soeroso et al. in 2014, who stated that lung cancer patients who visited H. Adam Malik General Hospital Medan were mostly 51–60 years old.

Increasing age could lead to the accumulation of carcinogenic substances in the body and also genetic disorders. Increasing age also causes a decline in immunity, DNA repair and induces a loss of cell regulation that facilitates carcinogenesis.

This study noticed that severe Brinkman index was the most common category in the lung cancer group while moderate Brinkman index was the most common in the control group. Hoffman in 1997 reported that lung cancer risk factors were directly proportional to the Brinkman index. Cigarette consumption in large quantities will lead to long-term exposure to carcinogens; this condition can escalate the risk of lung cancer.[6]

Clove cigarettes were the most widely used type of cigarette by both the lung cancer group and the control group, with 54.8% and 83.3%, respectively. Clove cigarettes are the most popular and well-known type of cigarette in Indonesia. This type of cigarette is a cigarette whose raw material is tobacco added with cloves and other flavors to get a certain effect and aroma. Cloves have a pleasant aroma and secrete eugenol, which can affect the sensory effects to trigger deeper cigarette smoking.[7]

Although smoking increases a person's risk of developing lung cancer and other smoking-related malignancies, not all individuals who smoke experience lung cancer. It is thought that genetic differences or polymorphisms in genes encoding xenobiotic metabolic enzymes may influence individual susceptibility to potential carcinogens.[8]

In this study, we found no significant difference between the distribution of the EPHX1 enzyme genotype and lung cancer risk. Zhou et al. analyzed 974 Caucasian lung cancer patients and 1142 controls, in which no association was observed between EPHX1 enzyme gene polymorphisms and lung cancer risk.

The lower affinity of the enzyme could not effectively remove the epoxide compounds causing the accumulation of intermediate metabolites in lung tissue. This metabolite is lipophilic, which is very easy to react with DNA to form DNA-Adducts. The formation of these DNA-adducts can cause mismatches in DNA replication, methyl replacement, and promoter changes, resulting in inherited DNA mutations or abnormal gene expression, and ultimately the process of carcinogenesis. Reactive metabolism can also induce the formation of Protein-Adducts in cells, which affects the normal activity of these proteins. Metabolites can also trigger an increment in reactive oxygen species (ROS), that directly affect DNA, lipids, or proteins and initiate carcinogenesis.[9]

CONCLUSION

There were no significant differences between the EPHX1 His139Arg gene polymorphism and the risk of lung cancer.

REFERENCES

1. Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y. EPHX1 polymorphisms and the risk of lung cancer: a HuGE review. Epidemiology. 2006;17(1):89–99.
2. Liu H, Li H-Y, Chen H-J, Huang Y-J, Zhang S, Wang J. EPHX1 A139G polymorphism and lung cancer risk: a meta-analysis. Tumour Biol. 2013;34(1):155–63.
3. Graziano C, Comin CE, Crisci C, Novelli L, Politi L, Messerini L, et al. Functional polymorphisms of the microsomal epoxide hydrolase gene: A reappraisal on a early-onset lung cancer patients series. Lung Cancer. 2009;63(2):187–93.
4. Erkisi Z, Yaylim-Eraltan I, Turna A, Görmüs U, Camlica H, Isbir T. Polymorphisms in the Microsomal Epoxide Hydrolase Gene: Role in Lung Cancer Susceptibility and Prognosis. Tumori J. 2010;96(5):756–63.
5. Zhou W, Thurston SW, Liu G, Xu LL, Miller DP, Wain JC, et al. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. Cancer Epidemiol Biomarkers Prev. 2001;10(5):461–6.
6. Hoffmann D, Hoffmann I. The changing cigarette, 1950-1995. J Toxicol Environ Health. 1997;50(4):307–64.
7. Gondodiputro S. Bahaya tembakau dan bentuk-bentuk sediaan tembakau. Bagian Ilmu Kesehatan Masyarakat Fakultas Kedokteran Universitas Padjadjaran. 2007;1–2.
8. Gsur A, Zidek T, Schnattinger K, Feik E, Haidinger G, Hollaus P, et al. Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. Br J Cancer. 2003;89(4):702–6.
9. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer. 2003;3(10):733–44.

Differences in Levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) Fluid between The Immunocompromised and Immunocompetent Groups Patients with Suspected Lung Cancer

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Abstract

Background: *Invasive candidiasis occurs in immunocompromised individuals as an opportunistic infection in patients with lung cancer. Although culture and histopathology remain the standard diagnosis of fungal infections, other tests are still needed to provide faster results. Human 1,3-β-D-Glucan (BDG) uses ELISA to detect candidiasis. β D-Glucan level from BAL fluid marked positive with cut-off value ≥130 pg/mL. This study aims to determine differences in the levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) fluid between the immunocompromised and immunocompetent groups in suspected lung cancer patients at RSU Dr. Saiful Anwar Malang.*

Method: *A cross-sectional study was conducted on 33 lung cancer patients who had risk factors for invasive candidiasis in Dr. Saiful Anwar Hospital Malang.*

Result: *The result shown significant differences in BAL levels of Human 1,3-β-D-Glucan between 9the immunocompromised and immunocompetent groups (P=0.009). In the different tests, there was a slight difference in the levels of BAL Human 1,3-β-D-Glucan but it was not statistically significant based on age and sex between the immunocompromised and immunocompetent groups (P=0.632, P=0.338, P=0.472, P=0.667).*

Conclusion: *Patients suspected of lung cancer have risk factors for invasive candidiasis with higher BDG levels due to immunoparalysis. There were significant differences in the BAL Human levels of 1,3-β-D-Glucan between the immunocompromised and immunocompetent groups. (**J Respirol Indones 2022; 42 (2): 90–6)***

Keywords: *1,3-β-D-Glucan, lung cancer, invasive candidiasis, immunocompromised.*

Perbedaan Kadar Human 1,3-β-D-Glucan dari Cairan *Bronchoalveolar Lavage* (BAL) antara Kelompok *Imunokompromais* dan *Imunikompeten* Pasien Terduga Kanker Paru

Abstrak

Latar Belakang: *Candidiasis invasif terjadi pada individu imunikompromais sebagai infeksi oportunistik pada pasien keganasan termasuk kanker paru. Meskipun kultur dan histopatologi tetap merupakan standar diagnosis infeksi jamur, masih diperlukan pemeriksaan lainnya yang dapat memberikan hasil lebih cepat. Human 1,3-β-D-Glucan (BDG) menggunakan ELISA sebagai salah satu pemeriksaan candidiasis, kadar β D-Glucan dari cairan BAL adalah positif bila cut off ≥130 pg/mL. Penelitian ini bertujuan mengetahui perbedaan kadar Human 1,3-β-D-Glucan dari cairan Bronchoalveolar Lavage (BAL) antara kelompok imunokompromais dan imunokompeten pada pasien terduga kanker paru di RSU Dr. Saiful Anwar Malang.*

Metode*: Studi cross sectional dilakukan pada 33 pasien kanker paru yang memiliki faktor risiko candidiasis invasif di RSUD dr Saiful Anwar Malang.*

Hasil: *Dari penelitian menunjukkan perbedaan bermakna kadar BAL Human 1,3-β-D-Glucan antara kelompok imunokompromais dengan imunokompeten (P=0,009). Pada uji beda didapatkan sedikit perbedaan kadar BAL Human 1,3-β-D-Glucan tetapi tidak signifikan secara statistik berdasarkan usia dan jenis kelamin pada kelompok immunocompromised dan immunocompetent (P=0,632, P=0,338, P=0,472, P=0,667).*

Kesimpulan: *Pasien terduga kanker paru memiliki faktor risiko candidiasis invasif dengan kadar BDG lebih tinggi karena imunoparalisis. Terdapat perbedaan yang bermakna pada kadar BAL Human 1,3-β-D-Glucan pada kelompok imunokompromais dan imunokompeten.*
*(**J Respirol Indones 2022; 42 (2): 90–6)***

Kata kunci:*1,3-β-D-Glucan; kanker paru; imunokompromais; kandidiasis invasif*

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INTRODUCTION

Pulmonary mycoses are lung disorders, including the respiratory tract, caused by infection, fungal colonization, or hypersensitivity reactions to fungi. The frequency of pulmonary mycoses has increased in recent years along with the increasing number of patients with impaired immune systems such as malignancy patients, in this case, lung cancer, organ transplantation, HIV/AIDS infection, chronic systemic disease, corticosteroids, and invasive medical devices (e.g., mechanical ventilation and central venous catheters). Certain conditions, especially acute infections, pulmonary mycoses, or systemic mycoses in general, can result in high mortality rates reaching 50% or more or even up to 100%.[1,2]

One of the most studied immunological markers in patients with lung cancer is the CD4, CD8 count, and CD4/CD8 ratio. CD4 deficiency, quantitative lymphocyte changes, and the existence of other theories that suggest T cell dysfunction in lung cancer cause individuals with lung cancer to be usually immunocompromised, making it easy for infections to occur including fungal infections which can complicate healing.[3–5]

The morbidity and mortality associated with invasive fungal infections (IFI) caused by *Candida albicans* and non-*albicans Candida spp*., especially in immunocompromised hosts, is increasing. The diagnosis of pulmonary mycoses considered rather difficult often leads to delayed treatment. The diagnosis of pulmonary mycoses is based on a physical examination and supporting examinations in the form of imaging studies (radiology), certain clinical laboratory results, and mycological examinations. Direct microscopic examination of *Bronchoalveolar Lavage* (BAL) with 10% KOH can detect fungal elements in general in the form of spores and hyphae. However, the conventional identification of fungal species takes a long time, especially in fungal breeding.[1,6,7]

The mycological examination is an important diagnostic procedure for pulmonary mycoses, one of which is serological examination. Serological assays are classically used to detect host antibody reactions to fungal elements. Among the well-studied fungal antigens is *1,3-β-D-Glucan* (BDG), which has been developed to diagnose IFI. BDG is a cell wall component of many fungi, including *Aspergillus spp., Candida spp., Fusarium spp., Pneumocystis jirovecii*, and mycoses.[1,8–10]

There is still controversy regarding the method of examining lung fungal infections from BAL specimens, especially in patients with lung cancer. In addition, at RSU, Dr. Saiful Anwar Malang has not provided a rapid diagnostic test for fungal infections. Thus, research is needed on the examination with 1,3–β-D-Glucan (BDG).

METHODS

There were 33 subjects from RSU Dr. Saiful Anwar ward was enrolled. This type of research is analytic observational with a cross-sectional approach. The subjects are suspected lung cancer patient hospitalized with a risk of fungal infection in the inpatient ward of Dr. Saiful Anwar Malang. Sampling in this study was carried out by consecutive sampling, namely every patient with suspected lung cancer who was hospitalized at Dr. Saiful Anwar during the research period and passed after judging from the inclusion criteria included: 1) Age ≥18 years; 2) Patients suspected of lung cancer from clinical and radiological results; 3) Fulfilling the requirements for FOB and patient BAL specimen collection, and 4) The patient and/or the patient's family are willing and sign the informed consent. Exclusion criteria were patients who had previously received antifungal therapy, albumin/IVIG (intravenous immunoglobulin) therapy, patients undergoing hemodialysis, patients diagnosed with invasive candidiasis.

Lung cancer is a malignant tumor originating from the bronchial epithelium or bronchial carcinoma confirmed by anatomic pathology results.[3,1] Suspected lung cancer patients are those whom based on clinical and supporting examinations in the form of blood laboratories and chest X-rays that show the presence of a mass, either with a central picture of necrosis, or pulmonary parenchymal atelectasis, or

pleural effusion, all of which have been ruled out by other causes.

Immunocompromised status is one of the risk factors for invasive fungal infections, including candidiasis infection, and determined based on the examination of CD4 cut-off <500 cells/mm^3.

Bronchoalveolar Lavage (BAL) fluid is a sample obtained from an examination carried out with a fiber optic bronchoscopy (FOB) procedure performed by performed by flushing 50 mL of 0.9% saline solution. The technique of implementing BAL is done with a flexible bronchoscope that is inserted up to the subsegmental bronchus until the lumen is closed (wedged position).[11,12] As much as 50 ml of 0.9% saline solution, which is warmed to 37˚C, is inserted through a bronchoscope, then the liquid collected right away with suction from the bronchial tree with a negative pressure of 30 cmH_2O and inserted into the specimen holder. An ELISA procedure then follows the fluid resulting from BAL to check for BDG.

The required sample of BAL is about 10 ml, taken at the time of FOB, which is a diagnostic procedure for lung cancer. Samples from BAL Bwere then centrifuged at 1000–3000 rpm for 10 minutes, and then the supernatant was transferred to an aliquot tube and stored at -80^{o}C.

Human Beta-D-Glucan antigen is an antigen on the fungal cell wall which was detected using the Human Beta-D-glucan antigen ELISA kit (Sincere bio) and read with an ELISA reader according to the manufacturing procedure.

CD4+ examination was performed on blood samples from peripheral veins in lung cancer patients. The blood sampling results were examined for lymphocyte values and CD4+ count values.

The data obtained from the research results will be analyzed using IBM SPSS software version 25. All data variables are tabulated manually. Test the normality of the data using Shapiro-Wilk. The data included in the descriptive analysis were patient demographics, including age and gender, lymphocyte values, CD4 levels, and BDG levels from BAL results. The difference test is carried out using the T-independent test if the assumption of normality is met. If the normality assumption is not met, then the *Mann-Whitney* test is used.

RESULTS

The characteristics of the research subjects can be seen in Table 1. There were two age groups evaluated, less than equal to 45 years old subjects were 18.2% and more than 45 years old subjects were 81.8%. The Mann Whitney test showed that there was no significant difference in the levels of Human 1,3-β-D-Glucan between the two age groups (*P*=0.451). The subjects were male dominant (87.9%) which Mann Whitney test showed no significant difference in Human 1,3-β-D-Glucan levels between gender.

Table 1. Clinical Characteristics

Characteristics	N	%	Average BDG (pg/dl)	*P*
Age (years)				
≤45 years old	6	18.2	681.59±59	0.451
>45 years old	27	81.8	893.81± 810	
Gender				
Male	29	87.9	904.38±833.12	0.321
Female	4	12.1	498.81±374.44	
Lymphocyte Count				
≤1100	11	33.3	1147.67±1157.14	0.418
>1100	22	66.7	709.00±517.90	
CD4				
<500	21	63.6	1099.06±887.30	0.009
≥500	12	36.4	428.50±337.62	
Comorbid				
COPD	2	12.5	475.11±313.40	0.970
DM	3	18.8	602.36±352.07	
Other Cancer	5	31.3	621.63±464.93	
TB	3	18.8	1417.6±1578.26	
HF	3	18.8	1178.21±1693.25	

The cut-off point of lymphocytes count were 1100 cells/mm3, with subject less than equal to 1100 were less dominant (33.3%). The test result showed also no significant difference (*P*=0.418).

The immunological state was evaluated using CD4 counts. CD4 less than 500 subject group was 63.6% and more than equal to 500 subject group was 36.4% with a significant difference in the mean levels of Human 1,3-β-D-Glucan in CD4 group (*P*=0.009).

There were several comorbidities found from subjects such as chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM),

tuberculosis (TB), heart failure (HF), and other cancer that were not normally distributed from the Shapiro-Wilk test. For the comorbidities a non-parametric test was performed, which conclude no significant difference in BDG in patients with different comorbidities (*P*=0.970). The Shapiro-Wilk test was performed in the data of patients with comorbidities, and the data on patients with comorbidities were not normally distributed, so the Kruskal Walis test was performed. In the test, the *P*=0.970, it can be concluded that there is no significant difference. It can be concluded that age, gender, lymphocyte count, and comorbidities do not affect level of BDG. In the CD4 count, it was found that patients with low CD4 cells had significantly higher BDG levels than those with high CD4 cells.

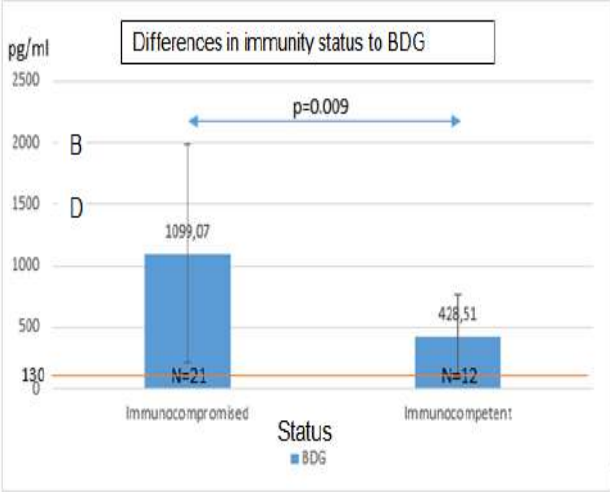

Figure 1. Comparison of BAL BDG Levels in Different Immunity Status.

The immunocompromised study subjects had an average BAL BDG level of 1099.07±887.30 pg/ml, while the immunocompetent study subjects were 428.51±337.62 pg/ml as shown in Figure 1. There was a significant difference between the mean BAL BDG in the group with different immunity status (*P*=0.009).

In the group of immunocompromised subjects, there were 19 subjects aged >45 years, with an average BDG of 1067.61±885.24 pg/ml. Meanwhile, two subjects aged 45 with an average BDG level of 1397.85±1201.25 pg/ml, as shown in Figure 2B. There was no significant difference in BDG levels between different age groups (*P*=0.632).

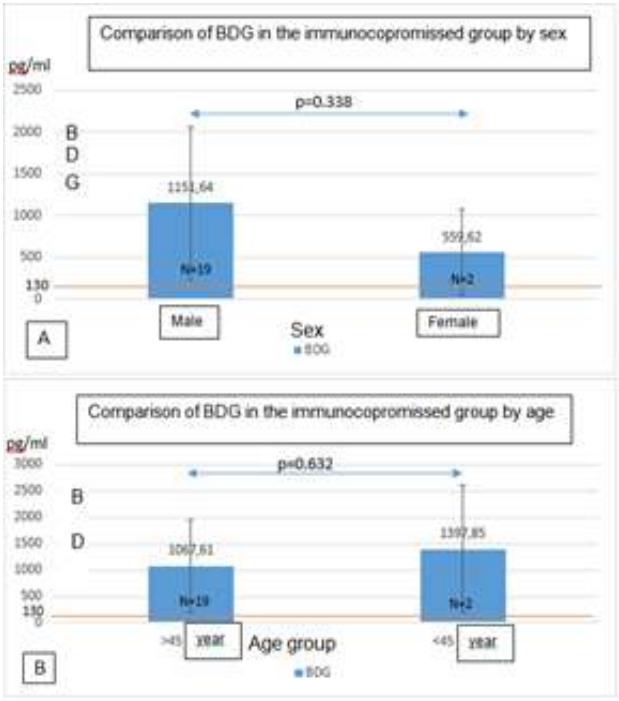

Figure 2. A) Comparison of BDG levels in male and female gender in the Immunocompromised group; B) Comparison of BDG levels at the age of ≤45 years and >45 years in the Immunocompromised group.

In immunocompetent subjects aged ≤45 years old, the mean BDG was 323.46±281 pg/ml, while in subjects >45 years old, the average was 481.03±381.54 pg/ml as shown in Figure 3A. There was no significant difference in BDG in different age groups (*P*=0.472).

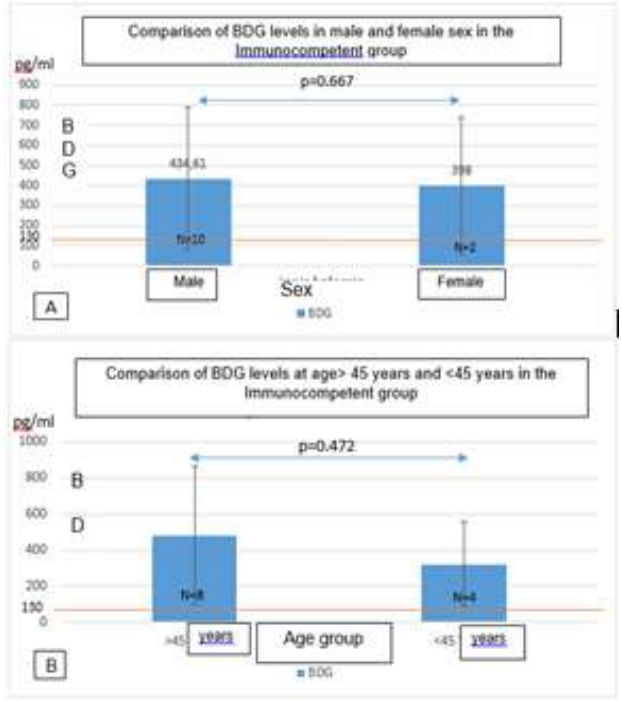

Figure 3. A. Comparison of BDG levels in male and female genders in the Immunocompetent group; B. Comparison of BDG levels at age >45 years and ≥45 years in the Immunocompetent group.

The immunocompromised group with the male gender had an average BDG level of 1151.64±910.76 pg/ml. In the female gender, the average BDG level was 599.62±513 pg/ml (figure 2A). There was no significant difference in BDG for men and women (*P*=0.338). The immunocompetent group was also divided by sex. There were 10 men with an average BDG of 434.60±355.20 pg/ml and 2 women with an average BDG value of 398±340.825 pg/ml (figure 3B). There was no significant difference between the BDG of the male and female groups (*P*=0.667).

DISCUSSION

In the characteristics of the research subjects, there was no significant difference in the age group, gender, lymphocyte count, and comorbidities on the average level of BAL Human 1,3-β-D-Glucan (*P*>0.05) between suspected lung cancer patients. This is in accordance with the literature, which states that Candida infection can be found in patients treated with lung cancer.[13]

Comorbidities found in this study included COPD, Diabetes Mellitus, tuberculosis, other cancers, and HF. These comorbidities may contribute to an increased risk of nosocomial invasive fungal infection in patients with suspected lung cancer. However, the comorbid data in this study yielded no statistically significant differences in BDG levels. Various literature states that pulmonary mycoses can occur in patients who previously had chronic lung disease as the underlying disease, such as COPD, lung and thoracic malignancies, pulmonary TB, and chronic systemic diseases such as Diabetes Mellitus.[1,2,14]

The mean lymphocyte count ≤1100 cells/mm3 in this study showed an average value of BDG levels higher than the mean lymphocyte count >1100 cells/mm3. This may also influence the risk of invasive fungal infections. Decreased lymphocyte count due to the apoptosis process causes lymphopenia and increases the risk of nosocomial infections such as candida yeast infection in lung cancer patients.[15] Although in this study, the lymphocyte count gave no statistically significant difference to the average BDG level.

This study showed a significant difference between the mean BAL BDG in groups with different immune statuses. So that the immune status is an important risk factor for BAL BDG levels. Previous studies found that the BALF BDG level may be an important predictor of mortality risk.[16] For every 100 pg/ml increase in BALF BDG, the overall 90-day risk of death increased by 5%. Thus, a patient with a BALF BDG level of 1100 pg/ml may have a 50% higher risk of dying within 90 days after bronchoscopy when compared to a patient with a BALF BDG level of 100 pg/ml. They also found that BDG levels were significantly higher among those with candida growth in BALF culture and *candida spp.* growth. Indicates that the host's immune system suppression may be associated with a higher probability of mortality risk.[16,17]

There was no significant difference in BAL BDG levels in patients suspected of lung cancer in the two different immune statuses based on the age groups >45 years and ≤45 years (*P*=0.632; *P*=0.472). However, age is a major risk factor for many cancers. Various genetic and food manipulations that slow down aging, such as studies conducted on mice, can reduce cancer incidence, and conversely, the susceptibility of host tissues to cancer increases with age. Exposure to risk factors early in life, such as infectious disease comorbid factors, may have a role in the early life peak in cancer incidence.[18] So that the above can also affect the occurrence of candidiasis in cancer patients at a younger age, and examine Human 1,3-D-Glucan meaningless by age group

In this study, showed there was no significant difference in BDG levels between male and female subjects with immunocompromised and immunocompetent status, although the mean and median were higher in the group of male subjects (*P*=0.338; *P*=0.667). The study of Lortholary et al., 2017 reported an equal chance of infection with Candida Albicans by gender in patients with malignancy.[19] Differences in gender in severity, prevalence, and pathogenesis of infections caused by viruses, bacteria, parasites, and fungi, with men

Men are generally more susceptible to these infections than women. These differences for acquired infectious diseases, observed through various routes such as individual, vector-borne, blood, food, and water, with gender differences in immunity, play a major role. Still, they may not be significant as they involve many factors.[20] This could explain why candidiasis infection examined with Human 1,3-β-D-Glucan in immunocompromised patients with suspected lung cancer by gender could not be significant.

Patients with suspected immunocompetent lung cancer by age group and gender have in common the mean value of BAL Human 1,3-β-D-Glucan levels, which are lower than patients with suspected immunocompromised lung cancer. These results are consistent with the literature, which states that invasive fungal infections occur more frequently in the immunocompromised than in the immunocompetent. In a prognostic study, Jaijakul et al., 2012 reported that the negative slope of Human 1,3-β-D-Glucan levels had a good response to antifungal therapy with PPV=90% and with a positive slope after treatment failure (NPV=90%).[21]

CONCLUSION

This study showed a significant difference in the levels of Human 1,3-β-D-Glucan from BAL fluid in the immunocompromised and immunocompetent groups. Although there was no significant difference in BAL Human 1,3-β-D-Glucan levels based on age and gender in different immune statuses, BDG levels based on age and gender in immunocompromised status had a higher positive value than immunocompetent. BDG assay as one of the diagnostic supports must be interpreted with clinical data by experienced doctors.

REFERENCES

1. Perhimpunan Dokter Paru Indonesia. Mikosis Paru: Pedoman Diagnosis dan Penatalaksanaan di Indonesia. 2017. p. 1–25.
2. Tille PM. Bailey & Scott's diagnostic microbiology. 14th ed. Louis S, editor. Missouri: Elsevier; 2017.
3. Sigel K, Wisnivesky J, Crothers K, Gordon K, Brown ST, Rimland D, et al. Immunological and infectious risk factors for lung cancer in US veterans with HIV: a longitudinal cohort study. Lancet. 2017;4(2):e67–73.
4. Strindhall J, Skog M, Ernerudh J, Bengner M, Löfgren S, Matussek A, et al. The inverted CD4/CD8 ratio and associated parameters in 66-year-old individuals: the Swedish HEXA immune study. Age (Dordr). 2013;35(3):985–91.
5. Warny M, Helby J, Nordestgaard BG, Birgens H, Bojesen SE. Lymphopenia and risk of infection and infection-related death in 98,344 individuals from a prospective Danish population-based study. PLoS Med. 2018;15(11):e1002685.
6. Wang Y, Hu G-F, Wang Z-H. The status of immunosuppression in patients with stage IIIB or IV non-small-cell lung cancer correlates with the clinical characteristics and response to chemotherapy. Onco Targets Ther. 2017;10:3557–66.
7. Perhimpunan Dokter Paru Indonesia. Kanker Paru: Pedoman Diagnosis dan Penatalaksanaan di Indonesia. 2015th ed. 2018. 1–37 p.
8. Delaloye J, Calandra T. Invasive candidiasis as a cause of sepsis in the critically ill patient. Virulence. 2014;5(1):161–9.
9. Kullberg BJ, Arendrup MC. Invasive Candidiasis. N Engl J Med. 2015;373(15):1445–56.
10. Theel ES, Doern CD. Point-Counterpoint: β-d-Glucan Testing Is Important for Diagnosis of Invasive Fungal Infections. J Clin Microbiol. 2013;51(11):3478–83.
11. King TE. Basic principles and technique of bronchoalveolar lavage - UpToDate [Internet]. UpToDate. 2020 [cited 2018 Mar 17]. Available from: https://www.uptodate.com/contents/basic-principles-and-technique-of-bronchoalveolar-lavage
12. Subagyo A. Bronchoalveolar lavage (BAL) [Internet]. Klik Paru. 2013 [cited 2018 Mar 17]. Available from:

https://www.klikparu.com/2013/03/bronchoalveolar-lavage-bal.html

13. Mousset S, Buchheidt D, Heinz W, Ruhnke M, Cornely OA, Egerer G, et al. Treatment of invasive fungal infections in cancer patients-updated recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol. 2014;93(1):13–32.
14. Ahmed A, Azim A, Baronia AK, Marak KRSK, Gurjar M. Risk prediction for invasive candidiasis. Indian J Crit Care Med. 2014;18(10):682–8.
15. Monneret G, Venet F, Kullberg B-J, Netea MG. ICU-acquired immunosuppression and the risk for secondary fungal infections. Med Mycol. 2011;49 Suppl 1:S17-23.
16. Reischies FMJ, Prattes J, Prüller F, Eigl S, List A, Wölfler A, et al. Prognostic potential of 1,3-beta-d-glucan levels in bronchoalveolar lavage fluid samples. J Infect. 2016;72(1):29–35.
17. Williamson DR, Albert M, Perreault MM, Delisle M-S, Muscedere J, Rotstein C, et al. The relationship between Candida species cultured from the respiratory tract and systemic inflammation in critically ill patients with ventilator-associated pneumonia. Can J Anaesth. 2011;58(3):275–84.
18. de Magalhães JP. How ageing processes influence cancer. Vol. 13, Nature reviews. Cancer. England; 2013. p. 357–65.
19. Lortholary O, Renaudat C, Sitbon K, Desnos-Ollivier M, Bretagne S, Dromer F. The risk and clinical outcome of candidemia depending on underlying malignancy. Intensive Care Med. 2017;43(5):652–62.
20. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626–38.
21. Jaijakul S, Vazquez JA, Swanson RN, Ostrosky-Zeichner L. (1,3)-β-D-glucan as a prognostic marker of treatment response in invasive candidiasis. Clin Infect Dis. 2012;55(4):521–6.

Association Between CEA Serum Level on NSCLC Patients with EGFR Mutation from Tissue and Plasma Sample

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Abstract

Background: *Lung cancer is one of the leading causes of cancer deaths in the world. The most common type of lung cancer is non-small cell lung cancer (NSCLC). Patients with NSCLC can have epidermal growth factor receptor (EGFR) mutation and increased level of CEA. Test for EGFR mutation on NSCLC has a very important role for EGFR tyrosine kinase inhibitor (TKI) therapy. CEA also can be used as a predictor for treatment efficiency of EGFR-TKI therapy. Tissue biopsy is the main diagnostic method for lung cancer but it's invasive and has some limitations. Circulating tumor DNA (ctDNA) is a new and less invasive for detecting EGFR mutation using plasma sample. In this study, we investigated the correlation between serum CEA and EGFR mutations in tissue and plasma in patients with NSCLC.*

Methods: *This cross-sectional observational study was conducted in Dr. Saiful Anwar Hospital, Malang from August 2018 until July 2019. 76 NSCLC patients underwent tests for EGFR mutation from tissue, ctDNA, and serum CEA level respectively. Extracted DNA from plasma and tissue samples from citology or biopsy was checked for the EGFR mutation. The serum CEA levels were analyzed using electrochemical luminescence.*

Results: *From 76 participants, positive EGFR mutation from tissue samples was detected on 34 subjects and ctDNA in 19 subjects. Serum level of CEA >5 ng/ml was significantly associated with EGFR mutation from tissue sample (P=0.037) with an odds ratio (OR) of 2.778 (95% CI=1.050-7.348), the area under curve (AUC) for CEA was 68,8% and the cut-off was 9.14 ng/ml. Serum level of CEA >5 ng/ml was also significantly associated with ctDNA (P=0.015) with an OR of 4.8 (95% CI=1.259-18.299), the AUC for CEA was 78,1% and cut-off=14.8 ng/ml.*

Conclusion: *Serum CEA level has poor association with EGFR mutation from tissue and moderate association with EGFR mutation from ctDNA in NSCLC patients. Patients with increased level of CEA>5 ng/ml are 2.778 times more at risk to have positive EGFR mutation and 4.8 times more at risk to have positive ctDNA mutation. (**J Respirol Indones 2022; 42(2): 97–106)***

Keywords: *lung cancer, NSCLC, EGFR, ctDNA, CEA*

Hubungan Kadar CEA dalam Serum pada Pasien KPKBSK dengan Mutasi EGFR yang didapatkan dari Sampel Jaringan dan Darah

Abstrak

Latar belakang: *Kanker paru merupakan penyebab kematian tertinggi akibat kanker di dunia. Jenis yang paling sering dijumpai adalah kanker paru karsinoma bukan sel kecil (KPKBSK). Pasien dengan KPKBSK dapat mengalami mutasi epidermal growth factor receptor (EGFR) dan peningkatan kadar CEA. Pemeriksaan mutasi EGFR pada pasien KPKBSK memiliki peranan penting untuk terapi target dengan tyrosine kinase inhibitor (TKI). CEA juga bisa digunakan untuk memprediksi efisiensi terapi EGFR-TKI. Biopsi jaringan merupakan pemeriksaan utama untuk mendiagnosis kanker tetapi metode ini invasif dan memiliki beberapa kendala. Circulating tumor DNA (ctDNA) merupakan pemeriksaan yang baru dan lebih aman untuk mendeteksi mutasi EGFR dengan sampel darah. Dalam penelitian ini, kami meneliti hubungan kadar CEA serum pada pasien KPKBSK dengan mutasi EGFR yang didapatkan dari sampel jaringan dan darah.*

Metode: *Jenis penelitian yang digunakan adalah observasional potong lintang. Penelitian dilakukan di Rumah Sakit Dr. Saiful Anwar Malang dari bulan Agustus 2018 sampai Juli 2019. Sebanyak 76 pasien dengan KPKBSK dilakukan pemeriksaan mutasi EGFR dari jaringan tumor, ctDNA dan CEA. Pemeriksaan mutasi EGFR dilakukan pada DNA yang diekstraksi dari darah dan sampel jaringan tumor dari sitologi atau biopsy. Kadar CEA dianalisis menggunakan electrochemical luminescence.*

Hasil: *Dari 76 pasien, ditemukan mutasi EGFR positif dari sampel jaringan sebanyak 34 pasien dan ctDNA positif sebanyak 19 pasien. Kadar CEA >5 ng/ml dalam serum memiliki hubungan bermakna dengan mutasi EGFR di jaringan (P=0.037) dengan odds ratio (OR)=2.778 (95% CI=1.050-7.348), area under the curve (AUC) untuk CEA 68,8% dan cut-off CEA 9.14 ng/ml. Kadar CEA >5 ng/ml juga berhubungan dengan ctDNA (P=0.015) dengan OR=4.8 (95% CI=1.259-18.299), AUC untuk CEA=78,1% dan cut-off=14.8 ng/ml.*

Kesimpulan: *Kadar serum CEA memiliki hubungan lemah dengan mutasi EGFR dari jaringan dan hubungan sedang dengan mutasi EGFR dari ctDNA pada pasien KPKBSK. Pasien dengan peningkatan CEA>5 ng/ml memiliki risiko 2.778 kali mengalami mutasi EGFR dan 4.8 kali berisiko mengalami mutasi positif ctDNA. (**J Respirol Indones 2022; 42(2): 97-106)***

Kata kunci: *kanker paru, KPKBSK, EGFR, ctDNA, CEA*

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INTRODUCTION

Lung cancer is one of the leading causes of cancer death globally. The main risk factor for lung cancer is smoking. Other factors that are also known to influence the development of lung cancer include exposure to radon gas, asbestosis, air pollution and genetic factors.[1,2]

Lung cancer is divided into two main types, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter constitutes 80% of all lung cancers, with adenocarcinoma as the most common histological type.[1,2]

Recent studies have found that genetic aberrations play a role in controlling cell survival in NSCLC. This aberration increases cell division and induces tumors. One of the pathways that can deviate is the epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase found in normal epithelial, mesenchymal and neurogenic tissues. The overexpression of EGFR is thought to play a role in the development of lung cancer.[3]

Mutations of EGFR are usually detected from tumour tissue samples obtained by biopsy or surgery. These mutations are primarily found in Asian ethnicity and lung cancer patients with adenocarcinoma type (around 51.4%), while there's no significant association with gender and smoking status. The best samples for examination of EGFR mutations were obtained surgically. However, 70–80% of NSCLC patients cannot undergo surgery at diagnosis while biopsy carries a high risk of bleeding in advanced cancer. Therefore, a more accessible and safer way is needed in estimating the occurring mutations.[4,5]

A new method to measure circulating free tumor-derived DNA (ctDNA) has been developed. In cancer patients, dead tumor cells release DNA into the bloodstream, and these DNA fragments bring about changes in tumor-specific sequences. Several studies have demonstrated the easiness and predictive value of using ctDNA to monitor tumor dynamics in various solid tumors. The degree of correlation between EGFR mutations and ctDNA is highly dependent on the detection method used, varying between 66–100%.[5–7]

Carcinoembryonic antigen (CEA) is an adhesion protein whose expression can be activated and regulated through the P13-K/Akt and STAT 3/5 signalling pathways on EGFR. Activation of the P13-K/Akt signalling pathway is involved in regulating cell differentiation, proliferation and apoptosis. CEA acts as an adhesion molecule and is involved in cell aggregation. CEA has a role in antiapoptotic and prometastatic in colon cancer. In lung cancer, CEA levels were also elevated, especially in adenocarcinoma. Overexpression of CEA levels can protect tumor cells against apoptosis induced by loss of cell contact with the extracellular matrix and inhibit cell death.[6,8,9]

EGFR mutations can cause aberrations in the P13-K/Akt and STAT 3/5 signalling pathways, thereby reducing signal transduction and causing antiapoptotic activity in tumor cells. It is suspected that the antiapoptotic signalling pathway that occurs due to aberrations in the activation of Akt and STAT 3/5 molecules can affect CEA expression. In addition, EGFR mutations are autophosphorylated in the absence of interleukin-3 without EGFR stimulation. It is suspected that continuous signalling from EGFR mutations can stimulate antiapoptotic activity; as a result, the expression of CEA protein as an antiapoptotic agent may appear to be increased in patients with EGFR gene mutations.[8–10]

One of the proteins that can be affected by activation of the EGFR pathway, an elevated serum CEA level, may be a sign of a mutated EGFR. However, this assumption requires further research. Although EGFR and CEA belong to different protein groups, studies in recent years have found a relationship between the expression of CEA levels and EGFR mutations.[11]

A study conducted by Abdurahman et al suggested a significant association between EGFR mutations and serum CEA levels. However, this study had a relatively small sample size.[12] Another study by Normawati et al regarding the association between tissue EGFR mutations and CEA levels in patients with lung adenocarcinoma at Dr. Saiful Anwar Hospital, Malang stated that lung adenocarcinoma patients with EGFR mutations had a 3.4 times

increased risk of CEA compared to patients without EGFR mutations.[13]

So far, no study has been conducted on the association between serum CEA levels and EGFR mutations in tissue and blood (ctDNA) in patients with NSCLC. Therefore, we meant to find the association between serum CEA levels with ctDNA. This study would be conducted in NSCLC patients at Dr. Saiful Anwar Hospital, Malang. It is hoped that the result of this study can be used as a consideration for patients who do not consent or are unable to undergo ctDNA EGFR mutation tests to make it easier for patients to receive appropriate therapy.

METHOD

The research was conducted in the pulmonary clinic and inpatient ward of Dr. Saiful Anwar Hospital, Malang from August 2018 to September 2019. This study was conducted using a cross-sectional observation method to determine the association between EGFR mutations in tissue and blood with serum CEA levels in newly diagnosed NSCLC patients treated at dr. Saiful Anwar Hospital, Malang who had never received chemotherapy. The inclusion criteria for this study were patients aged >18 years who were diagnosed with NSCLC through cytology and histopathology examination and had never received any kind of anti-cancer treatment. Patients whose tumor tissues did not meet the requirements for EGFR testing due to low cell counts, patients who had received anti-cancer treatment before CEA levels were examined and patients with secondary lung cancer were not included in this study.

The sampling of this study was carried out at the pulmonary clinic and the inpatient ward of Dr Saiful Anwar Hospital, Malang. Each subject signed the informed consent. Eighty patients who met the inclusion criteria and were willing to participate in the study were subjected to EGFR examination both with tissue and blood samples (ctDNA). An independent sample T-test was done to determine the association between EGFR mutations and CEA levels. Processing of data analysis using IBM SPSS software version 24.0 with a 95% confidence level, alpha = 0.05.

The area under the curve (AUC) value is obtained by analyzing the receiver operating characteristics (ROC) curve and then determining the cut-off point; after finding the cut-off point, the sensitivity, specificity, accuracy, positive predictive value and negative predictive value are calculated.

RESULTS

During the study, 80 subjects who were willing to participate in the study and signed the informed consent were obtained. In the course of the study, the examination of EGFR mutations from tissue samples could not be carried out in four patients because the number of cells was insufficient, so they were excluded from the study. At the end of the study, there were a total of 76 subjects who were included in the statistical analysis. Among them, 34 positive EGFR mutations were found in tissues and 19 in ctDNA.

The gender distribution showed that 36 (47.4%) of the subjects were female and 40 (52.6%) were male, as shown in Table 1. The association between gender and EGFR mutations was determined by chi-square test which showed *P*=0.072. It can be concluded that gender is not associated with EGFR mutations in the tissue.

Table 1. Characteristics of Gender, Smoking Status and Occupation on EGFR and ctDNA Mutations

Characteristics		EGFR Mutations				Total		*P*	ctDNA				*P*
		Positive		Negative					Positive		Negative		
		n	%	n	%	N	%		N	%	N	%	
Gender	Male	14	18.4	26	34.2	40	52.6	0.072[b]	7	9.2	33	43.4	0.111[b]
	Female	20	26.3	16	21.1	36	47.4		12	15.8	24	31.6	
Smoking Status	Smokers	11	14.5	24	31.6	35	46.1	0.031[b]	7	9.2	28	36.8	0.352[b]
	Non-Smokers	23	30.3	18	23.6	41	53.9		12	15.8	29	39.2	
Occupation	High Risk	12	15.8	16	21.1	28	36.8	0.816[b]	6	7.9	22	28.9	0.583[b]
	Low Risk	22	28.9	26	34.2	48	63.2		13	17.1	35	46.1	

Note: [*] Significant when *P*<0.05; [b] *Chi-square Test*

Table 2. Clinical Characteristics Associated with EGFR and ctDNA Mutations

Characteristics		EGFR Mutations				Total		*P*	ctDNA				*P*
		Positive		Negative					Positive		Negative		
		N	%	N	%	N	%		N	%	N	%	
Stadium	III	2	2.6	5	6.6	7	9.2	0.450[c]	0	0.0	7	9.2	0.182[c]
	IV	32	30.9	37	48.7	69	90.2	0.182[c]	19	25	50	65.8	
Histological type	Adenocarcinoma	29	28.2	35	46.1	64	84.2	Reff	16	21.1	48	63.2	Reff
	Squamous Cell Carcinoma	2	2.6	2	2.6	4	5.3	Reff	1	1.3	3	3.9	1.00[c]
	Adenosquamous	3	3.9	5	6.6	8	10.5	1.00[c]	2	2.6	6	7.9	1.00[c]
Sampling technique	FNAB	14	18.4	31	40.8	45	59.2	1.00[c]	-	-	-	-	-
	Bronchial washing/brushing	3	3.9	0	0	3	3.9	0.114[c]	-	-	-	-	-
	Pleural fluid cytology	9	11.8	8	10.5	17	22.4	0.075[c]	-	-	-	-	-
	Biopsy FOB	7	9.2	3	3.9	10	13.2	0.033[*c]	-	-	-	-	-
	TBNA	1	1.3	0	0	1	1.3	0.326[c]	-	-	-	-	-

Note=[*] Significant when *P*<0,05; [c] *Fischer's exact Test*

Another chi-square test was conducted to determine the association between gender and ctDNA results which shows *P*=0.111. It can be concluded that there is no association between gender and ctDNA results.

Smoking history was divided into smoker and non-smoker. In this study, 36 subjects were smokers (46.1%) and 41 subjects never smoked (53.9%) (Table 1). A significant association was found between non-smoker and positive EGFR mutation (*P*=0.031) but none between smoking history and ctDNA (*P*=0.352).

Among all research subjects, homemakers make up the biggest part of them with amounted to 21 people (27.6%), with 13 people (17.1%) tested positive for EGFR mutations and 8 (10.5%) were negative. Aside of them, 19 subjects work as farmers (25%), 3 as household assistants (3.9%), 7 as office employees (9.2%), 5 as carpenters (6.6%), 11 are self-employed (6.6%) and 10 have jobs other than those mentioned (13.2%). For statistical reasons, the occupations were divided into those with a high risk of lung cancer (farmers and artisans) and those with lower risk (other professions). Chi-square test shows *P*=0.816, so it can be concluded that occupation was not associated with EGFR mutations, nor was there a significant association with ctDNA results (*P*=0.583).

In this study, the majority of patients were in stage IVa, which amounted to 63 people (82.9%), while at stage IIIb there were 0 people (0%) (Table 2). EGFR mutations in tissue were also found mostly in stage IVa, with 31 people (40.8%) yielded positive results. Subjects were divided into two major groups in regards to their cancer stage for statistical analysis purposes: stage III (IIIa, IIIb and IIIc) and stage IV (IVa and IVb). Fischer's exact test showed no association between cancer stage and EGFR mutation (*P*=0.450). The association between histological cancer type and EGFR mutation was also studied, with 64 people were found to have adenocarcinoma (84.2%), 4 have squamous cell carcinoma (SCC) (5.3%) and 8 adenosquamous cell carcinoma (10.5%). Chi-square test with adenocarcinoma as a reference showed that *P*=1.000 for SCC and *P*=0.725 for adenosquamous. It was concluded that cancer type was not associated with EGFR mutations (Table 2).

Most of the materials for EGFR examination were taken by FNAB technique, which consisted of 45 samples (59.2%), while the least one came from TBNA (1 sample). A chi-square test with FNAB as a comparison showed that tissue sampling via biopsy was more likely to get positive EGFR mutation results compared to FNAB (*P*=0.033). Meanwhile, there are no positive or negative impacts on other examination materials such as bronchial washing or brushing, pleural fluid cytology and TBNA.

Positive ctDNA results were mostly found at stage IVa (Table 2), which amounted to 18 people (23.7%), while at stage III there was no positive result at all. Fischer's exact test showed no association between cancer stage and ctDNA results (p=0.182). In regards to the association between cancer type and ctDNA, *P*=1.00 both in adenosquamous and SCC type as determined by Fischer's exact test with

adenocarcinoma as reference. It was concluded that cancer type was not related to the ctDNA results (Table 2).

As mentioned before, positive EGFR mutations from tissue samples were found in 34 patients (44.74%), which consisted of mutations in exon 18 in 1 patient (2.94%), exon 19 in 26 patients (76.47%), exon 20 in 2 patients (5.88%) and exon 21 in 5 patients (14.71%). On the other hand, EGFR mutations via ctDNA were found in 19 patients (25%), consisting of mutations in exon 18 in 1 patient (5.26%), exon 19 in 14 patients (73.68%), exon 21 in 3 patients (15, 79%) and double mutations in exons 19 and 21 in 1 patient (5.26%).

On the results of the CEA examination, it was found that 46 subjects (60.5%) had increased CEA level (Table 3). Chi-square test yielded the result of p=0.037, which showed that elevated level of CEA has a significant association with the result of EGFR mutations. The value of the diagnostic Odds Ratio (OR) is 2.778 with 95% CI=1.050-7.348, meaning that with the increase in CEA level, positive EGFR mutation will be 2.778 times more likely.

From the data obtained, it can be calculated the value of the EGFR mutation trend in CEA:

Sensitivity = $\frac{25}{(25+9)}$ = 73.5%

Specificity = $\frac{21}{(21+21)}$ = 50%

Positive predictive value (PPV) = $\frac{25}{(25+21)}$ = 54.3%

Negative predictive value (NPV) = $\frac{21}{(9+21)}$= 70%

Positive Likelihood Ratio (LR+) = $\frac{0.735}{(1-0.500)}$ = 1.47

Negative Likelihood Ratio (LR-) = $\frac{1-0.735}{0.5}$ = 0.53

Accuracy = $\frac{13+39}{76}$ = 60.5%

The above results show that the sensitivity of CEA levels is 73.5%, and specificity is 50%. From the sensitivity results, it can be concluded that CEA can detect 73.5% of EGFR mutations. The specificity of CEA 50% means that the probability of CEA detecting lung cancer patients without EGFR mutation is 50%. A PPV of 54.3% means that only about 54.3% of EGFR underwent mutations. An NPV of 70% means that approximately 70% of the EGFR in patients with adenocarcinoma lung cancer are entirely unmutated. The LR+ is 1.47 and the LR- is 0.53. The accuracy of the CEA level in detecting EGFR mutations in tissue is 60.5%.

Table 4 shows that the average CEA level in people with positive EGFR mutations is 108.04; this value is higher than the group without EGFR mutations, with a mean CEA of 60.00. The Mann-Whitney test shows that *P*=0.005, so it can be concluded that there is a significant difference between the CEA values in the EGFR mutation group and the group without EGFR mutations in the tissue.

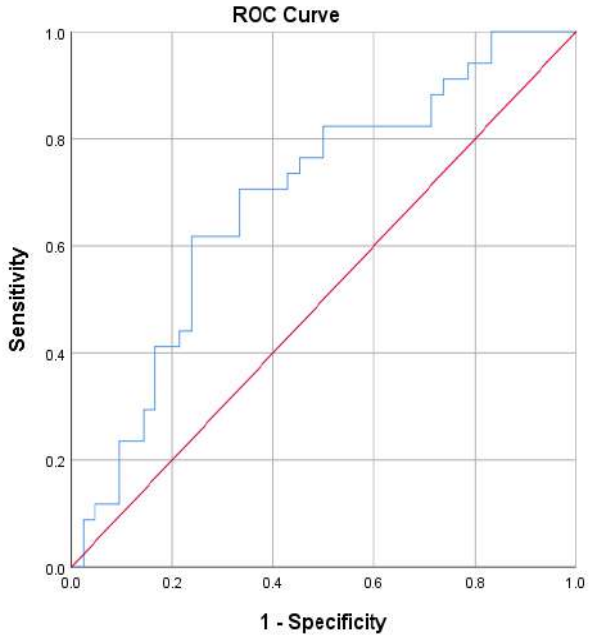

Figure 1. ROC curve of CEA value against EGFR mutations in the network

Table 3. Association between Tissue EGFR Mutations and ctDNA with CEA

Characteristics		EGFR Mutations				*P*	OR (95% CI)	ctDNA				*P*	OR (95% CI)
		Positive		Negative				Positive		Negative			
		N	%	n	%			N	%	N	%		
CEA	Elevated	25	32.9	21	27.6	0.037*b	2.778	16	21.1	30	39.5	0.015*b	4.8
	Normal	9	11.8	21	27.6		(1.050–7.348)	3	3.9	27	35.5		(1.259–18.299

Note= (* Significant when *P*<0,05; (b *Chi-square Test*

Table 4. CEA values with EGFR mutations in tissue and ctDNA

Characteristics		EGFR Mutations in tissue		Total	P-value	ctDNA		Total	P-value
		Positive	Negative			Positive	Negative		
CEA	Median	26.81	3.85	8.13	0.005[*d]	86.2	86.2	86.2	<0.001[*d]
	(Min - Max)	(1.6–872)	(0.91–951.8)	(0.91–951.8)		(1.63–870.47)	(1.63–872.1)	(1.63–872.1)	
	Mean ± SD	108.04 ± 188.22	60.00 ± 166.02	81.49 ± 176.7		189.88 ± 239.32	45.36 ± 134.56	45.36 ± 134.56	

Note= [*] Meaning when *P*< 0,05; [d] *Mann-Whitney Test*

In the ROC analysis (Figure 1), the AUC value is 0.688 or 68.8%. This means CEA level has weak accuracy in determining EGFR mutations from tissues. The interpretation value is that AUC >50.0–60.0% is very weak, >60.0–70.0% weak, >70.0–80.0% moderate, >80.0–90.0% good and >90.0–100.0% very good

Based on the plotting in the Table 3, the CEA cut-off value is 9.14. So, it can be concluded that the CEA cut-off to allow the positive EGFR mutation results in tissue to be 9.14 ng/ml.

Most subjects, amounting to 46 people (60.5%) had elevated CEA levels (Table 3), with 16 of them (21.1%) showed positive ctDNA. Chi-square test determined that there was a significant association between ctDNA results and elevated CEA levels, with *P*=0.015 and OR: 4,800 with 95% CI=1,259-18,299, which means that an increase in CEA level will yield 4.8 times the chance of positive ctDNA mutations.

From the data obtained, it can be calculated the trend value of ctDNA results in CEA:

Sensitivity = $\frac{16}{(16+3)}$ = 84.2%

Specificity = $\frac{27}{(27+30)}$ = 47%

PPV = $\frac{16}{(16+30)}$ = 34.8%

NPV = $\frac{27}{(3+27)}$= 90%

LR+ = $\frac{0.842}{(1-0.470)}$ = 1.78

LR- = $\frac{1-0.842}{0.47}$ = 0.34

Accuracy = $\frac{13+39}{76}$ = 56.6%

The above results show that the sensitivity of CEA levels to ctDNA is 84.2%, and specificity is 47%. From the sensitivity results, it can be concluded that CEA can detect EGFR mutations in plasma by 84.2%. CEA specificity of 47% means that the probability of CEA detecting lung cancer patients without EGFR mutations in plasma is 47%. A PPV of 34.8% means that only about 34.8% of ctDNA are truly mutated. An NPV of 70% means that approximately 70% of the ctDNA of lung cancer patients are completely unmutated. The LR+ is 1.78, and the LR- is 0.34. The accuracy of the CEA level for detecting EGFR mutations in plasma is 56.6%.

Table 4 shows that the average CEA in subjects with positive ctDNA was 189.88; this value is higher than the group without the EGFR mutation, with an average CEA of 45.36. Using Mann-Whitney test, *P*<0.001 was obtained, so it can be concluded that there is a significant difference between the CEA values in the group with positive and negative ctDNA.

In the ROC analysis (Figure 2), the area under curve (AUC) value is 0.781 or 78.1%. This means that CEA level has moderate accuracy in determining EGFR mutations in plasma.

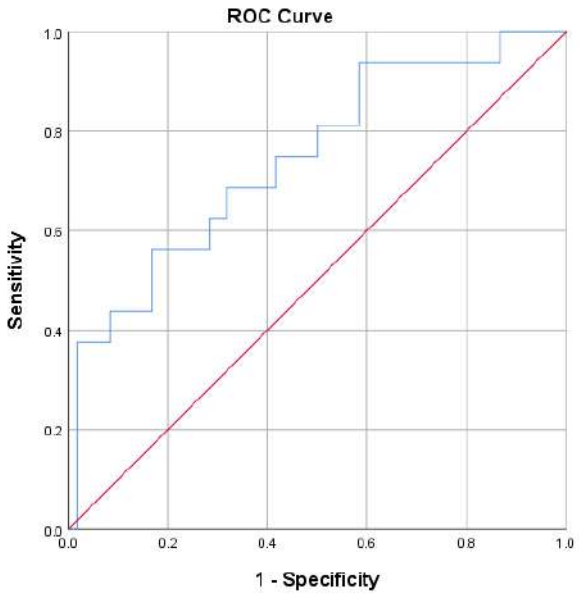

Figure 2. ROC curve of CEA value against ctDNA

Based on plotting, the cut-off value for CEA is 14.8. So, it can be concluded that the cut-off of CEA level that allows a positive result of ctDNA is 14.8 ng/ml.

DISCUSSION

In this study, the gender distribution shows that 36 subjects are female (47.4%) and 40 are male (52.6%). Positive EGFR mutations were found more in females (26.3%) than males (18.4%). Similar thing can be found for ctDNA, in which the positive result can be found in 15.8% females and 9,2% males. The results of this study is similar the ones by of Reck et al and Gao et al which found that positive EGFR mutations in tissue and plasma are more commonly found in female (*P*=0.072 and *P*=0.111 respectively).[14,15] On the other hand, a study by Feng et al revealed that the level of EGFR mutation was not related to gender.[16]

The study on smoking history found that higher percentage of positive EGFR mutations in the tissue was found in non-smokers (18.5%) than smokers (14.5%) with *P*=0.031. Positive mutations in ctDNA also were more common in non-smokers (15.8%) than smokers (9.2%) with *P*=0.352. Because smoking is one of the main risk factors for lung cancer, smoking is considered a reference for statistical analysis. This indicates that smoking history has a significant association with EGFR mutations in the tissue. This result is smilar to other studies which said that positive EGFR mutations are more common in non-smokers.[6,14]

Based on their occupations, the subjects were divided into two major groups: those with high risk of lung cancer (farmers and artisans) and those with lower risk (homemakers, office employees, entrepreneurs or traders, civil servants, etc). The results of this study are in accordance to the literature which shows that positive EGFR mutations are more commonly found in women, in this case, homemakers. However, there's no significant association between highrisk occupation and EGFR mutation status in tissue (*P*=0.816) and plasma (*P*=0.583).[6,14]

In this study, positive EGFR mutations from tissue samples were mostly found in stage IVa (40.8%) and the least in stages IIIb and IIIc (0%) but there's no significant difference (*P*=0.450). In ctDNA, positive EGFR mutations were only found at stage IVa (23.7%) and IVb (1.3%), but this isn't significantly different either (*P*=0.182). On ctDNA examination, there was no positive EGFR mutation at all for stage III. Of the 19 subjects who were tested positive for EGFR mutations from plasma, all of them were in stage IVa and IVb. Herman et al's study also found positive EGFR mutations in ctDNA in subjects with NSCLC stage IVa and IVb, with no positive ctDNA results in stage III.[17] Qui et al's study said that the level of positive EGFR mutations in ctDNA tends to be higher in more advanced cancer stage.[5] This is because ctDNA originates from tumor cells that undergo apoptosis and necrosis, thereby releasing DNA into the bloodstream. These DNA fragments then carry tumor-specific sequences (ctDNA).[5,6,17]

Based on the histological type, positive EGFR mutations in NSCLC were most commonly found in adenocarcinoma (29 subjects), and the least in SCC (2 subjects). Likewise, on ctDNA examination, positive EGFR mutations were found most in adenocarcinoma (16 people) and least in SCC (1 person). This is in accordance to the literature where it is said that EGFR mutations are most commonly found in adenocarcinoma. Therefore, for statistical calculations, adenocarcinoma was used as a reference. With adenocarcinoma as a reference, there was no significant association between cancer type and EGFR mutation status in SCC and adenosquamous tissue samples (*P*=1.000 and *P*=0.725 respectively) or ctDNA (both *P*=1.000).[6,14,18]

The largest amount of samples for tissue EGFR examination came from FNAB, which contributed to 45 samples (59.2%), compared to TBNA which only had 1 sample. With FNAB as a reference, it was found that the examination results from the biopsy were more likely to get positive EGFR mutation results compared to FNAB, with *P*=0.033. The study conducted by Guan et al compared EGFR mutations in tissue samples and pleural effusions in adenocarcinoma patients; the result was that 34% of tissue samples and 30% of pleural fluid tested positive for EGFR mutations.[19] Though the percentage of positive mutations in tissue is higher than the ones from pleural fluid, the difference isn't statistically significant.[19,20]

This study shows a significant association between tissue EGFR mutations and serum CEA levels (*P*=0.037) with an OR of 2.778. The average CEA in subjects with positive EGFR mutations is 108.04; this value is higher than in the group without EGFR mutations, which has an average CEA of 60.00 (*P*=0.005). It can be concluded that there is a significant difference between the CEA values in the two groups. This is similar to a previous study at Saiful Anwar Hospital, Malang by Normawati et al which found a significant association between tissue EGFR mutations in adenocarcinoma patients and serum CEA levels.[13] Gao et al and Lv et al also revealed that elevated serum CEA levels were significantly associated with EGFR mutations.[15,21]

Mutations of EGFR in plasma also showed a significant association with serum CEA levels in this study (*P*=0.015) with an OR of 4.8 (cut-off=5 ng/ml). The average CEA in subjects with positive ctDNA was 189.88; this value was higher than the group without the EGFR mutation, which had an average CEA of 45.36 (*P*<0.001). A study conducted by Que et al found a significant association between increased serum CEA levels and the possibility of positive ctDNA with *P*=0.034. Another study conducted by Guo et al found the same thing, while Jin et al found that lung adenocarcinoma patients who had CEA serum levels of 20 ng/mL were the ideal population to receive targeted therapy using EGFR-TKI.[6,22,23]

Many factors, both malignant and non-malignant might affect CEA levels. Aside from NSCLC, malignancies in the ovarium, breast and thyroid also caused an increase in CEA levels. In non-malignant cases, CEA levels were elevated in smokers, emphysematous lung, appendicitis, cholecystitis, cirrhosis of the liver, pancreatitis, inflammatory bowel disease and patients receiving orlistat therapy. So, in this study, there's a possibility that the increase in CEA levels on the subjects is due to the history of smoking in the patient, emphysematous lung on chest x-ray or an infectious process.[24,25]

This study also examined sensitivity, specificity, accuracy, PPV, NPV, LR+, LR- and accuracy of serum CEA levels to detect EGFR mutations in tissue and plasma. Based on the analysis, it was found that the sensitivity of CEA levels to detect EGFR mutations in tissue was 73.5% while the specificity was 50%, PPV=54.3%, NPV=70%, LR+=1.47, LR-=0.53 and accuracy 60.5%. The values obtained are almost the same as those of Pan et al and Normawati et al.[13,26] Pan et al found that the sensitivity and specificity of CEA in estimating EGFR mutations were 76% and 45% respectively, while the PPV and NPV were 52% and 71%.[26] Normawati et al found the sensitivity and specificity of CEA in estimating EGFR mutations were 77% and 50%, while PPV and NPV were 53% and 76%.[13]

In regards to the examination of CEA levels to detect EGFR mutations in plasma, it's found to have 84.2% sensitivity, 47% specificity, 34.8% PPV, 90% NPV, 1.78 LR+, 0.34 LR- and 56.6% accuracy. These results are not significantly different compared to the one to detect EGFR mutations in the tissue. A study conducted by Gao et al found that the sensitivity of CEA to detect EGFR mutations was 69.6% and the specificity was 48.8%.[15]

The ROC curve presented in Figure 1 shows an AUC value of 68.8%, so CEA is considered weak in estimating EGFR mutations in tissues. The CEA cut-off value that allows positive EGFR mutation in tissues is 9.14 ng/ml. From the results of the new CEA cut-off value of 9.14 ng/ml, it was found that the specificity, PPV, NPV, LR+ and accuracy were better than using a CEA cut-off of 5 ng/ml in determining EGFR mutations in tissues. Figure 2 shows the AUC value of 78.1%, so CEA is considered to have a moderate level of accuracy in determining EGFR mutations in plasma. The CEA cut-off value that allows positive mutations in blood is 14.8 ng/ml. From the new CEA cut-off value, it was found that the specificity, PPV, LR+, LR- and accuracy were better than using the 5 ng/ml cut off. Research by Pan et al suggested that CEA may not be an ideal predictor for EGFR mutations with a ROC curve of 0.608; this is thought to be due to the expression of CEA levels that can be influenced by various causes and from multiple pathways.[26] Research conducted by Yan Ling et al found the AUC value of 59% with a CEA cut

off of 87 ng/ml, which means CEA has a weak assocation with EGFR mutations.[21] Using the new CEA cut-off, better results of PPV, LR+ and accuracy were obtained, but this cannot be used globally because it is only limited to this research. Another study with larger number of samples is needed to prove this hypothesis.[12,21]

The limitation of this study is that it only examined the association of tissue and blood EGFR mutations with a single tumor marker, namely CEA. This study also only noted the presence or absence of EGFR mutations in tissue and blood without distinguishing common mutations and uncommon mutations or which exon the mutations happen to.

CONCLUSION

This study found that EGFR mutations are most commonly found in females, non-smokers and adenocarcinoma type. Elevation in CEA level is found to be significantly associated to EGFR mutations both in tissues and serum, but CEA only has weak accuracy in determining EGFR mutation in tissues and moderate in blood. A higher cut-off value of CEA is needed to allow positive EGFR mutation.

REFERENCES

1. Jazieh A-R, Al Sudairy R, Abu-Shraie N, Al Suwairi W, Ferwana M, Murad MH. Erlotinib in wild type epidermal growth factor receptor non-small cell lung cancer: A systematic review. Ann Thorac Med. 2013;8(4):204–8.
2. Siegelin MD, Borczuk AC. Epidermal growth factor receptor mutations in lung adenocarcinoma. Lab Investig. 2014;94(2):129–37.
3. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. J Thorac Dis. 2010;2(1):48–51.
4. Cote ML, Haddad R, Edwards DJ, Atikukke G, Gadgeel S, Soubani AO, et al. Frequency and type of epidermal growth factor receptor mutations in African Americans with non-small cell lung cancer. J Thorac Oncol. 2011;6(3):627–30.
5. Qiu M, Wang J, Xu Y, Ding X, Li M, Jiang F, et al. Circulating tumor DNA is effective for the detection of EGFR mutation in non-small cell lung cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2015;24(1):206–12.
6. Que D, Xiao H, Zhao B, Zhang X, Wang Q, Xiao H, et al. EGFR mutation status in plasma and tumor tissues in non-small cell lung cancer serves as a predictor of response to EGFR-TKI treatment. Cancer Biol Ther. 2016;17(3):320–7.
7. Bernabé R, Hickson N, Wallace A, Blackhall FH. What do we need to make circulating tumour DNA (ctDNA) a routine diagnostic test in lung cancer? Eur J Cancer. 2017;81:66–73.
8. Shoji F, Yoshino I, Yano T, Kometani T, Ohba T, Kouso H, et al. Serum carcinoembryonic antigen level is associated with epidermal growth factor receptor mutations in recurrent lung adenocarcinomas. Cancer. 2007;110(12):2793–8.
9. Yang Z-M, Ding X-P, Pen L, Mei L, Liu T. Analysis of CEA expression and EGFR mutation status in non-small cell lung cancers. Asian Pac J Cancer Prev. 2014;15(8):3451–5.
10. Wang W-T, Li Y, Ma J, Chen X-B, Qin J-J. Serum carcinoembryonic antigen levels before initial treatment are associated with EGFR mutations and EML4- ALK fusion gene in lung adenocarcinoma patients. Asian Pac J Cancer Prev. 2014;15(9):3927–32.
11. Pan J-B, Hou Y-H, Zhang G-J. Correlation between EGFR mutations and serum tumor markers in lung adenocarcinoma patients. Asian Pac J Cancer Prev. 2013;14(2):695–700.
12. Abdurahman A, Anwar J, Turghun A, Niyaz M, Zhang L, Awut I. Epidermal growth factor receptor gene mutation status and its association with clinical characteristics and tumor markers in non-small-cell lung cancer patients in Northwest China. Mol Clin Oncol. 2015;3(4):847–50.
13. Normawati, Pratiwi SD, Setijowati N. Epidermal Growth Factor Receptor (EGFR) and Carcinoembryonic Antigen (CEA) Relationship

of Lung Adenokarsinoma in Saiful Anwar Hospital, Malang. Berk Kedokt. 2017;13(2):173–81.

14. Reck M, Hagiwara K, Han B, Tjulandin S, Grohé C, Yokoi T, et al. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. J Thorac Oncol. 2016;11(10):1682–9.
15. Gao Y, Song P, Li H, Jia H, Zhang B. Elevated serum CEA levels are associated with the explosive progression of lung adenocarcinoma harboring EGFR mutations. BMC Cancer. 2017;17(1):484.
16. Feng Y, Zhou J, Chen S, Jiang Y, Zhou Y. Relationship between EGFR gene mutation and serum tumor biomarkers in advanced lung adenocarcinoma. Int J Clin Exp Pathol. 2016;9(1):250–5.
17. Herman, DP S, PP N. Nilai Diagnostik Pemeriksaan Mutasi EGFR Dengan Sampel Plasma Darah Dibandingkan Sampel Jaringan Pada Pasien NSCLC. Universitas Brawijaya; 2019.
18. Cheng L, Alexander RE, Maclennan GT, Cummings OW, Montironi R, Lopez-Beltran A, et al. Molecular pathology of lung cancer: key to personalized medicine. Mod Pathol. 2012;25(3):347–69.
19. Guan Y, Wang ZJ, Wang LQ, Hua DF, Liu J. Comparison of EGFR mutation rates in lung adenocarcinoma tissue and pleural effusion samples. Genet Mol Res. 2016;15(2).
20. Yang J, Lee O-J, Son S-M, Woo CG, Jeong Y, Yang Y, et al. EGFR Mutation Status in Lung Adenocarcinoma-Associated Malignant Pleural Effusion and Efficacy of EGFR Tyrosine Kinase Inhibitors. Cancer Res Treat. 2018;50(3):908–16.
21. Lv Y-L, Liu H-B, Yuan D-M, Zhou L, Jin S-X, Song Y. Carcinoembryonic antigen in pleural effusion of patients with lung adenocarcinoma: a predictive marker for EGFR mutation. Transl Cancer Res. 2019;8(4):1027–34.
22. Guo N, Lou F, Ma Y, Li J, Yang B, Chen W, et al. Circulating tumor DNA detection in lung cancer patients before and after surgery. Sci Rep. 2016;6(1):33519.
23. Jin B, Dong Y, Wang H, Huang J, Han B. Correlation between serum CEA levels and EGFR mutations in Chinese nonsmokers with lung adenocarcinoma. Acta Pharmacol Sin. 2014;35(3):373–80.
24. Asad-Ur-Rahman F, Saif MW. Elevated Level of Serum Carcinoembryonic Antigen (CEA) and Search for a Malignancy: A Case Report. Vol. 8, Cureus. 2016. p. e648.
25. Chung S. False-positive Elevations in Carcinoembryonic Antigen Levels at a Health Screening Center. Lab Med Online. 2019;9(3):146–52.
26. Pan Y, Shi W, Xu D, Xu H, Zhou M, Yan W. Associations between epidermal growth factor receptor gene mutation and serum tumor markers in advanced lung adenocarcinomas: a retrospective study. Chinese Med Sci J. 2014;29(3):156–61.

Comparison of Eutectic Mixture of Local Anesthesia Cream and Subcutaneous Lidocaine to Reduce Chest Tube Removal Pain and Willingness to Repeat Procedure

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Abstract

Background: *Patients with chest tube tend to have allodynia (pain from stimuli that is normally painless) and hyperalgesia (increased sensitivity to pain). Anesthetics has not been used routinely during chest tube removal because of the assumption that the procedure is brief and the pain is short-lived, though it could be the most painful part of chest tube procedures.*

Objective: *This study compared the effectiveness of local anesthetic eutectic mixture of local anesthesia (EMLA) cream and subcutaneous infiltration of lidocaine to reduce pain of chest tube removal, 10 minutes afterwards and its effect on the patient's willingness to repeat the procedure.*

Method: *A quasi-experimental clinical trial conducted on 28 patients undergoing chest tube removal at dr. Moewardi Hospital from September 2020. The EMLA group (n=14) received 2 grams of topical EMLA cream applied two hours before chest tube removal. The lidocaine group (n=4) received subcutaneous infiltration of 2% lidocaine five minutes before chest tube removal. Pain was measured by visual analog scale (VAS) before, during and 10 minutes after the chest tube was removed, then the patients were asked to fill out a willingness to repeat procedure questionnaire.*

Results: *Topical EMLA cream was comparable to 2% lidocaine infiltration for reducing pain during chest tube removal (P=0.679) and 10 minutes thereafter (P=0.833). EMLA cream did not increase the patient's willingness to repeat the procedure (P=0.815)*

Conclusion: *Topical EMLA cream is capable of replacing the subcutaneous infiltration of 2% lidocaine as a local anesthetic for chest tube removal but does not increase the patient's willingness to repeat the procedure. (**J Respirol Indones 2022; 42 (2): 107–14)***

Keywords: *Chest tube, pain, EMLA, lidocaine, repeat procedure*

Perbandingan Krim *Eutectic Mixture of Local Anesthesia* dan Lidokain Subkutan untuk Mengurangi Nyeri Melepas Selang Dada dan Kesediaan Mengulang Prosedur

Abstrak

Latar belakang: *Pasien terpasang selang dada akan memiliki kondisi alodinia (rasa nyeri dari rangsang yang secara normal tidak menimbulkan nyeri) dan hiperalgesia (peningkatan sensitivitas terhadap nyeri). Penggunaan antinyeri belum dilakukan secara rutin ketika melepas selang dada dengan anggapan bahwa prosedur tersebut hanya berlangsung cepat. Nyeri yang dirasakan juga dianggap terjadi dalam tempo yang singkat padahal bisa jadi merupakan bagian paling menyakitkan dari rangkaian prosedur pemasangan selang dada.*

Tujuan: *Penelitian ini membandingkan efektivitas anestesi lokal krim eutectic mixture of local anesthesia (EMLA) dan infiltrasi subkutan lidokain 2% untuk mengurangi nyeri melepas selang dada, 10 menit setelah selang dada dilepaskan dan pengaruhnya terhadap kesediaan pasien untuk mengulang prosedur.*

Metode: *Uji klinis kuasi-eksperimental dilakukan pada 28 pasien yang akan dilakukan tindakan pelepasan selang dada di RSUD dr. Moewardi mulai bulan September 2020 dengan consecutive sampling. Kelompok EMLA (n=14) mendapatkan krim EMLA topikal 2 gram yang diaplikasikan dua jam sebelum selang dada dilepas. Kelompok lidokain (n=14) mendapat infiltrasi subkutan lidokain 2% lima menit sebelum selang dada dilepaskan. Nyeri diukur dengan visual analog scale (VAS) sebelum, saat dan 10 menit setelah selang dada dilepaskan, kemudian pasien diminta mengisi kuesioner kesediaan mengulang prosedur.*

Hasil: *Krim EMLA topikal sebanding efektivitasnya dengan infiltrasi lidokain 2% untuk mengurangi nyeri saat melepas selang dada (P=0,679) dan 10 menit setelahnya (P=0,833). Krim EMLA tidak meningkatkan kesediaan pasien untuk mengulang prosedur (P=0,815).*

Kesimpulan: *Krim EMLA secara topikal mampu menggantikan infiltrasi subkutan lidokain 2% sebagai anestesi lokal dalam prosedur pelepasan selang dada tetapi tidak meningkatkan kesediaan pasien untuk mengulang prosedur. (**J Respirol Indones 2022; 42 (2): 107–14)***

Kata kunci: *Selang dada, nyeri, EMLA, lidokain, mengulang prosedur.*

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INTRODUCTION

Chest tube insertion is a procedure that is often performed in pulmonary medicine practice. An estimated 1.330.000 chest tube insertion procedures were performed in 1995 in the United States. A fully conscious patient will experience excruciating pain when the chest tube is inserted. Therefore, pain medication is always given by intravenous injection or subcutaneous infiltration before the insertion procedure.[1]

The use of painkillers has not been done routinely when removing the chest tube with the assumption that the procedure is short and the pain only occurs briefly. In truth, the patient normally experiences a moderate to a severe increase in pain during the chest tube removal procedure. Patients with chest tube tend to experience allodynia (pain from normally painless stimuli) and hyperalgesia (increased sensitivity to pain). Chest tube insertion causes sensitization of sleep nociceptors on the skin's surface, muscle tissue and parietal pleura. The release of adenosine triphosphate (ATP) and inflammatory mediators causes a decrease in the nociceptor sensitivity threshold. As a result of these two processes, other procedures performed where the chest tube is attached will induce pain in higher intensity to the patient.[1–3]

Administration of local anesthetics either topically or subcutaneously before chest tube removal has been shown to reduce pain during the procedure compared to systemic anesthesia and placebo. Topical anesthetics provide more comfort to the patient since the application is painless. Eutectic mixture of local anesthesia (EMLA) is a topical anesthetic in a mixture of 2.5% lidocaine and 2.5% prilocaine in the form of a cream. Valenzuela et al. (1999) proved that EMLA was significantly better to reduce chest tube pain than systemic opioid administration.[4,5]

This research aims to compare the effectiveness of two local anesthetic modes, namely topical EMLA cream and 2% lidocaine by subcutaneous infiltration to reduce pain during chest tube removal and 10 minutes after the chest tube is removed. The score of patient satisfaction in both groups was then measured by a questionnaire of willingness to repeat the procedure.

METHODS

This study is a quasi-experimental with the population of patients to whom chest tube removal is to be performed. The study was conducted in dr. Moewardi Hospital, Surakarta during September and October 2020 until the number of samples needed was met. The research subjects consisted of 28 patients who underwent removal of chest tube, divided into the EMLA group (n=14) and the lidocaine group (n=14).

Pain at the chest tube insertion site was measured thrice using a visual analog scale (VAS) for pain. The first VAS measurement was performed before the local anesthetic was applied. Patients in the EMLA group will receive EMLA cream spread by the radius of 2 cm from the site of chest tube insertion. The cream is then covered with a transparent occlusive dressing and left for 120 minutes before the chest tube is removed. Patients in the lidocaine group will receive 2% lidocaine infiltration at 3, 6, 9, and 12 o'clock from the chest tube insertion site five minutes before the chest tube removal procedure.

The second VAS measurement was performed when the chest tube was removed and the sutures tied, while the third one was done 10 minutes after the procedure. Patients were then asked to complete a questionnaire of willingness to repeat chest tube procedure on a scale of 0 (highly likely, not willing) to 10 (very likely willing).

The inclusion criteria in this study included patients who were to undergo a chest tube removal procedure, willing to participate in the study by signing informed consent, aged ≥18 years, could read and write and were free from short and medium-acting systemic pain medications within 24 hours or systemic long-acting painkillers within 72 hours. Exclusion criteria included patients with decreased consciousness, symptomatic central nervous system disorders, requiring re-stitching of a chest tube

closure or retainer and allergy to lidocaine or prilocaine. The criteria for discontinuation were if the patient experiences a loss of consciousness within 10 minutes after the chest tube is removed. The research was done after the researcher obtained approval and ethical clearance from the Health Research Ethics Committee of Dr. Moewardi Hospital (No.1.070/IX/HREC/2020).

Data analysis was carried out using SPSS version 19 for Windows and presented using Microsoft Office 2016. The normality test of research data was carried out using the Shapiro-Wilk test because the number of samples was less than 50 subjects. Statistical testing on paired samples was done using paired t-test if the data distribution was normal and Wilcoxon test if not. Statistical testing on unpaired samples was tested using the independent t-test if the data distribution was normal and Mann-Whitney test if not.

RESULT

This study was conducted on 28 patients undergoing chest tube removal at Dr. Moewardi Hospital, Surakarta. The number of subjects was 14 people in the EMLA group and 14 in the lidocaine group, making total number of subjects 28. There were no resignation nor discontinuation of subjects from either the first or second group. The subjects were characterized by age, gender, occupation, level of education, presence of malignancy in the lung, presence of malignancy outside the lung, presence of infection in the lung and infection at the chest tube insertion site and duration of chest tube insertion.

Table 1. Basic characteristics of research subjects

Characteristics	Group		Total	*P*
	Lidocaine	EMLA		
Age	53.43±11.78	50.07±16.81	51.75±14.35	0.546
Gender				
Female	5 (35.7%)	6 (42.9%)	11 (39.3%)	0.669
Male	9 (64.3%)	8 (57.1%)	17 (60.7%)	
Education				
Elementary	7 (50.0%)	4 (28.6%)	11 (39.3%)	0.243
Junior High School	3 (21.4%)	4 (28.6%)	7 (25.0%)	
Senior High School	4 (28.6%)	5 (35.7%)	9 (32.1%)	
University	0 (0.0%)	1 (7.1%)	1 (3.6%)	
Job				
Laborer	3 (21.4%)	2 (14.3%)	5 (17.9%)	0.808
Employee	0 (0.0%)	1 (7.1%)	1 (3.6%)	
Trader	2 (14.3%)	2 (14.3%)	4 (14.3%)	
Farmer	6 (42.9%)	5 (35.7%)	11 (39.3%)	
Civil servant	0 (0.0%)	1 (7.1%)	1 (3.6%)	
Not working	3 (21.4%)	3 (21.4%)	6 (21.4%)	
Lung Malignancy				
Yes	9 (64.3%)	11 (78.6%)	20 (71.4%)	0.678
No	5 (35.7%)	3 (21.4%)	8 (28.6%)	
Malignancy outside Lung				
Yes	0 (0.0%)	1 (7.1%)	1 (3.6%)	1.000
No	14 (100.0%)	13 (92.9%)	27 (96.4%)	
Lung Infection				
Yes	7 (50.0%)	6 (42.9%)	13 (46.4%)	0.705
No	7 (50.0%)	8 (57.1%)	15 (53.6%)	
WSD site infection				
Yes	3 (21.4%)	2 (14.3%)	5 (17.9%)	1.000
No	11 (78.6%)	12 (85.7%)	23 (82.1%)	
Duration of WSD insertion				
<7 days	0 (0%)	0 (0%)	0 (0%)	1.000
7–14 days	6 (42.9%)	6 (42.9%)	12 (42.9%)	
>14 days	8 (57.1%)	8 (57.1%)	16 (57.1%)	

Characterization of research subjects in both groups was carried out to determine the homogeneity of the two groups as a feasible clinical trial procedure. The normality of the distribution of the characteristics of the subjects in both groups was tested using the Shapiro-Wilk test.

The categorical variables included gender, occupation, level of education, presence of malignancy in the lung, presence of malignancy outside the lung, presence of infection in the lung, presence of infection at the site of chest tube insertion, and duration of chest tube insertion. Characteristics of research subjects in the form of categorical data are presented in frequency (percentage). The homogeneity test used for categorical data is the chi-square/Fisher exact test.

The numerical variables in this study, namely age and VAS score, are presented by mean±standard deviation (SD). Homogeneity test for numerical data uses an independent t-test if the data distribution is normal and Mann-Whitney test if not. The basic characteristics of the two groups of subjects are said to be homogeneous if the homogeneity test gives *P*>0.5. All data on the characteristics of subjects and research variables between the two groups had *P*>0.05, indicating that the characteristic data between control and treatment were homogeneous.

The EMLA cream group had a mean initial VAS score of 2.02±1.48 which then increased to 4.66±1.82 during the removal procedure. The average increase in pain VAS scores in the EMLA cream group was 2.63±1.67, so it was concluded that in the group of subjects receiving local anesthesia with EMLA cream there was an increase in mild pain when the chest tube was removed.

The mean initial VAS in the lidocaine group was 1.61±1.46, while the score during chest tube removal was 4.66±1.45. The mean increase in pain VAS scores in the lidocaine group was 3.05±1.70, so in this group, there was also an increase in mild pain when the chest tube was removed.

The mean initial VAS in the EMLA cream group and the lidocaine group, respectively 2.02±1.48 and 1.61±1.46, did not have a statistically significant difference with *P*=0.456. The lidocaine group had a higher increase in VAS when the chest tube was removed, namely 3.05±1.70 (mild increase) compared to 2.63±1.67 (mild increase) in the EMLA cream group, but statistically this difference was not significant either with *P*=0.679. It can be concluded that the effectiveness of topical EMLA cream is comparable to subcutaneous infiltration of 2% lidocaine in reducing pain during chest tube removal.

The mean score of VAS 10 minutes after chest tube removal in the EMLA cream and lidocaine injection groups was 1.73±1.30 and 1.79±0.97 respectively. The statistical test between the scores of the two groups got *P*=0.833. This means that there was no statistically significant difference in the VAS scores between the two groups 10 minutes after the chest tube was removed.

Table 2. Comparison of the effectiveness of EMLA cream and lidocaine infiltration for relieving chest pain

Group	Initial VAS	VAS during chest tube removal	VAS increase
Lidocaine	1.61±1.46	4.66±1.45	3.05±1.70
EMLA	2.03±1.48	4.66±1.82	2.63±1.67
P	0.456	1.000	0.679

The mean initial VAS in the EMLA cream group was 2.02 + 1.48. The mean VAS score 10 minutes after the chest tube was removed was 1.73 ± 1.30. There was a decrease in the score of VAS at 10 minutes after the chest tube was removed compared to the initial VAS with a mean decrease of -0.30 ± 1.62, which was not statistically significant with *P*=0.501.

The lidocaine group had an initial mean VAS score of 1.61±1.46. The mean VAS 10 minutes after chest tube removal in the lidocaine injection group was 1.79±0.97, so in the lidocaine injection group the VAS score 10 minutes after chest tube removal increased instead compared to the initial VAS with a mean of 0.19±1.17. The increase was not statistically significant either with *P*=0.562.

As mentioned previously, the mean changes in initial VAS and VAS 10 minutes after chest tube removal in the EMLA cream and lidocaine injection groups were -0.30±1.62 and 0.19±1.17 respectively. The statistical test showed *P*=0.713, which means that there is no significant difference in the change in

VAS score 10 minutes after the chest tube is removed in both groups. It can be concluded that topical EMLA cream is comparable to subcutaneous injection of 2% lidocaine to control pain 10 minutes after the chest tube is removed.

Table 3. Comparison of topical EMLA cream and 2% lidocaine subcutaneous injection for pain relief 10 minutes after chest tube removal

Group	Initial VAS	VAS after 10 mins	VAS change	*P*
Lidocaine	1.61±1.46	1.79±0.97	0.19±1.17	0.562
EMLA	2.03±1.48	1.73±1.30	-0.30±1.62	0.501
P	0.456	0.833	0.713	

A comparative test of the effect of EMLA cream and lidocaine injection for chest tube removal on the patient's level of willingness to repeat the chest tube procedure was carried out using the Mann-Whitney unpaired test because based on the normality test, the data were not normally distributed. The willingness to repeat the procedure in the lidocaine group had an average score of 5.57±3.08, while the EMLA cream group had an average score of 5.79±3.77. Based on statistical tests, there was no significant difference between those two (*P*=0.815), so it can be said that the use of EMLA cream to reduce pain in chest tube removal did not increase the patient's willingness to repeat the procedure when compared to the subcutaneous injection of 2% lidocaine.

Table 4. Effect of EMLA cream and lidocaine infiltration when removing the chest tube on the patient's level of willingness to repeat the chest tube procedure

Group	The level of willingness to repeat the procedure
Lidocaine	5.57 ± 3.08
EMLA	5.79 ± 3.77
P	0.815

DISCUSSION

This study is a quasi-experimental to compare two modes of local anesthetic to reduce pain in chest tube removal. The subjects in this study were patients undergoing chest tube removal. The research subjects were divided into two groups. The first group received local anesthetic cream EMLA topically and the second group received local anesthetic subcutaneous infiltration of 2% lidocaine. The dependent variable was the increase in the VAS score during and 10 minutes after the chest tube was removed, analyzing the comparison of the effectiveness of the two modes of local anesthesia to control pain during the removal procedure. Another dependent variable is the patient's willingness to repeat the entire series of chest tube insertion procedures to analyze patient's satisfaction with the overall procedure.

This research was conducted on 28 subjects at dr. Moewardi Hospital. The average age of the study subjects was 51.75±14.35 years, where patients in the lidocaine group were 53.43±11.78 years old, and the EMLA group had an average age of 50.07±16.81 years. The statistical test results got *P*=0.546 which means there is no significant difference in the age between the lidocaine and EMLA groups.

Among all the research subjects, 17 patients are male (60.7%), with 9 (64.3%) in lidocaine group and 8 (57.1%) in EMLA group. There was no significant difference between the genders in the two study groups. Most of the subjects have education level of elementary school or equivalent, amounting to 11 patients (39.3%), with 7 of them in EMLA group, which makes 50% of the group. In the lidocaine group, most have high school education or the equivalent, namely 5 patients (35,7%). Statistical test results showed *P*=0.243 which means there is no significant difference in the two groups based on education level. Farming makes up the majority of the subjects' occupations, with 11 patients (39.3%) who were divided into 6 (42.5%) in the lidocaine group and 5 (35.7%) in the EMLA group. The statistical test obtained the score of *P*=0.808, which means that there is no significant difference in the profession of the research subjects.

20 of the 28 research subjects had lung malignancy (71.4%), with 9 patients (64.3%) in lidocaine group and 11 patients in (78.6%) EMLA group. Extrapulmonary malignancy was found in one patient from the EMLA group (7.1%). The results of statistical tests concluded that there was no significant difference between the two groups based on malignancy in the lung (p=0.678) or outside the lung (*P*=1.000).

The incidence of lung infection in the subjects was 13 (46.4%), which occurred in 7 patients (50.0%)

in lidocaine group and 6 (42.9%) in EMLA group. Chest tube infection was found in 5 patients (17.9%), of which 3 (21.4%) came from the lidocaine group and 2 (14.3%) from the EMLA group. The statistical test results obtained p=0.705 for lung infection and *P*=1.000 for chest tube infection, so it can be concluded that there was no significant difference between the two groups. The longest duration of chest tube insertion was >14 days which happened in 16 patients (57.1%), with each group having 8 patients (57.1%). In another 12 (42.9%) patients, the chest tube was inserted for 7–14 days. No patient had a chest tube for less than 7 days. The statistical test got *P*=1.000, meaning there was no significant difference between the two groups.

Chest tube removal, even if it only lasts for a short time, can be the most painful part in the entire series of chest tube insertion procedures. Bruce et al in their literature review stated that there was an increase in moderate to severe pain or a VAS score of 4 to 10 experienced by the patients when the chest tube was removed, so it is highly recommended not to perform chest tube removal without prior local anesthetic administration.[5]

This study compared the effectiveness of local anesthetic with topical EMLA cream and 2% lidocaine by subcutaneous infiltration to reduce pain when the chest tube is removed. The EMLA cream group experienced an increase in mild pain with a mean increase in pain VAS score of 2.63±1.67. The lidocaine group also experienced an increase in mild pain with a mean increase in pain VAS score of 3.05±1.70. The lidocaine group experienced a higher increase in pain VAS score than the EMLA group, but the difference was not statistically significant with *P*=0.679. This study showed that EMLA cream applied topically 2 hours before chest tube removal was comparable to subcutaneous infiltration of 2% lidocaine to reduce pain from chest tube removal.

The researchers have not obtained a previous study comparing two different modes of local anesthetic in chest tube removal procedures. Research comparing infiltrative local anesthesia with systemic anesthesia was conducted by Jawad et al in Cairo, Egypt and Akrofi et alin Liverpool, England. Both studies concluded that infiltrative local anesthesia is better than systemic anesthesia with non-steroidal anti-inflammatory drugs (NSAIDs), opioids or a combination of both.[6,7]

Previous studies comparing topical local anesthetics with systemic anesthesia or placebo conducted by Watanabe et al in Akita, Japan, Singh et alin Andhra, India and Valenzuela et al in Morgantown, USA, also concluded that topical anesthesia is better compared to systemic anesthesia with opioids or placebo for pain relief when removing the chest tube.[4,8,9]

The local anesthetic mode used in the two groups in this study used an anesthetic agent from the amino amide group with differences in the application method (topically and subcutaneously). The point of action of the two anesthetic agents is also the same, namely at voltage-gated sodium channels (VGSC) to inhibit the propagation of pain impulses from nociceptors. Topical use of EMLA cream has advantages over subcutaneous infiltration of 2% lidocaine because it does not cause additional pain in its application. The biggest drawback of using EMLA cream in this study was the two-hour waiting time before chest tube removal could be performed, but given that patients with a chest tube inserted usually have been in treatment for a relatively long time, an additional two hours of waiting time shouldn't be a problem to worry about.[2,10]

Patients with chest tube insertion are in a state of allodynia or hyperalgesia around the chest tube insertion site due to sensitization of nociceptors by inflammatory agents. Chronic C nerve fiber activity will cause a long duration of pain, often for hours after the chest tube was removed. Pain is usually felt as a pull rather than a sharp prick. This study found that the mean VAS score 10 minutes after the chest tube was removed in the EMLA group was 1.73±1.30. In the lidocaine group, the mean VAS score 10 minutes after the chest tube was removed was 1.79±0.97. There was no significant difference in the VAS score 10 minutes after the chest tube was removed in the two groups with *P*=0.833.

Compared to the initial VAS, the EMLA group had a decrease in pain VAS score with a mean of -

0.30±1.62, while in the lidocaine group, there was an increase in VAS score with a mean of 0.19±1.17. These differences did not reach significance with *P*=0.713. The results of this study demonstrated that topical EMLA cream was comparable to subcutaneous infiltration of 2% lidocaine to revert pain to the initial score within 10 minutes after chest tube removal.

The results of this study are in line with the results of previous studies by Jawad et aland Singh, which stated that local anesthesia was better than systemic anesthesia or placebo in achieving quicker pain control and returning to the initial pain level. This study showed that EMLA cream reduced pain level 10 minutes after the chest tube was removed to below the initial pain level, although it did not reach significancy.[6,9]

The patient's willingness to repeat the procedure is one measure of patient satisfaction. Research conducted by Loftus et al stated that the patient's willingness to repeat the process was closely related to the absence of pain during the procedure.[11] This study showed no significant difference in the level of willingness to repeat the chest tube insertion procedure in the EMLA group and lidocaine (mean 5.79±3.77 and 5.57±3.08 respectively, *P*=0.815). This result was obtained because although the EMLA cream group did not experience pain during the application, both groups still had moderate pain when the chest tube was removed (mean 4.66±1.82 and 4.66±1.45, *P*=1.000). Pain is often associated with patients with a low level of operator proficiency, causing a reluctance to return to the same health facility, especially if the procedure has to be repeated. The series of chest tube insertion procedures is also a complex one and has many stages and complications leading to many factors that reduce patient satisfaction, such as pain during insertion, long duration of treatment and limited movement when the chest tube is inserted.[12]

CONCLUSION

Topical application of EMLA cream was as useful as subcutaneous infiltration of 2% lidocaine to reduce pain on chest tube removal and control pain within 10 minutes afterwards. On the other hand, it did not increase the patient's willingness to repeat the chest tube procedure. It can be concluded that EMLA cream can replace subcutaneous infiltration of 2% lidocaine as a local anesthetic when removing a chest tube.

REFERENCES

1. Light RW. Chest Tubes. In: Light RW, editor. Pleural Diseases. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 466–80.
2. Gold MS. Peripheral Pain Mechanisms and Nociceptor Sensitization. In: Ballantyne J, Fishman S, Rathmell JP, editors. Bonica's management of pain. 5th ed. Philadelphia: Wolters Kluwer; 2019. p. 263–304.
3. Santosa TB, Sutanto YS, Septiawan D. Efektivitas Hipnoterapi Saat Bronkoskopi terhadap Kontrol Kecemasan, Sesak Napas dan Batuk. J Respirologi Indones. 2019;39(1):21–9.
4. Valenzuela RC, Rosen DA. Topical lidocaine-prilocaine cream (EMLA) for thoracostomy tube removal. Anesth Analg. 1999;88(5):1107–8.
5. Bruce EA, Howard RF, Franck LS. Chest drain removal pain and its management: A literature review. J Clin Nurs. 2006;15(2):145–54.
6. Al Jawad MA, Ali I, Shokri H, Shorbagy MS. Systemic versus local analgesia for chest drain removal in post cardiac surgery patients: The taming of a beast. J Egypt Soc Cardio-Thoracic Surg. 2017;25(4):289–93.
7. Akrofi M, Miller S, Colfar S, Corry PR, Fabri BM, Pullan MD, et al. A randomized comparison of three methods of analgesia for chest drain removal in postcardiac surgical patients. Anesth Analg. 2005;100(1):205–9.
8. Watanabe S, Imai K, Kimura T, Saito Y, Takashima S, Matsuzaki I, et al. Effect of lidocaine cream analgesia for chest drain tube removal after video-assisted thoracoscopic surgery for lung cancer: a randomized clinical trial. Reg Anesth Pain Med. 2020;45(1):16–21.

9. Singh M, Gopinath R. Topical analgesia for chest tube removal in cardiac patients. J Cardiothorac Vasc Anesth. 2005;19(6):719–22.
10. Malamed SF. Clinical action of specific agents. In: Malamed SF, editor. Handbook of Local Anesthesia. 7th ed. St. Louis: Elsevier; 2020. p. 57–84.
11. Loftus R, Nugent Z, Graff LA, Schumacher F, Bernstein CN, Singh H. Patient Satisfaction with the Endoscopy Experience and Willingness to Return in a Central Canadian Health Region. Can J Gastroenterol. 2013;27(5):259–66.
12. Sun BC, Adams J, Orav EJ, Rucker DW, Brennan TA, Burstin HR. Determinants of Patient Satisfaction and Willingness to Return With Emergency Care. Ann Emerg Med. 2000;35(5):426–34.

Risk Factors for Mortality of Patients with COVID-19 in RSJPD Harapan Kita, Jakarta

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Abstract

Background: *The COVID-19 disease has caused significant morbidity and mortality worldwide since COVID-19 was first reported in December 2019. The more severe types of disorders leading to mortality occured more frequently in the elderly and in patients with more than one comorbidity. This study aimed to determine the risk factors associated with mortality in COVID-19 patients at RSJPD Harapan Kita.*

Methods: *In this cross-sectional study, 500 adult inpatients (≥18 years old) with laboratory-confirmed COVID-19 from RSJPD Harapan Kita between March 2020 until April 2021 were enrolled. The Demographic, clinical, and outcome data were extracted from the hospital medical record system. To explore the risk factors associated with mortality, univariate, bivariate and multivariate analysis using logistic regression were used.*

Results: *Of the 500 patients included in this study, 110 (22%) died in the hospital. The logistic regression multivariate analysis pointed out that age ≥65 years (adjusted odds ratio (aOR)=2.624; 95% CI=1.552–4.435), chronic lung disease (aOR=8.173; 95% CI=3.834–17.422), chronic kidney disease (aOR=2.523; 95% CI=1.358–4.689), and cardiovascular disease (aOR=3.149; 95% CI: 1.763–5.624) had been recognized as risk factors of mortality among COVID-19 patients.*

Conclusion: *Age ≥65 years, chronic lung disease, chronic kidney disease patients, and cardiovascular disease had been recognized as risk factors for mortality of COVID-19 patients at RSJPD Harapan Kita. Further research on the risk factors associated with COVID-19-related deaths need to be conducted to manage disease progression and to improve the treatment. (**J Respirol Indones 2022; 42 (2): 115–20)***

Keywords: *COVID-19, Risk Factors, Mortality, Comorbidities.*

Faktor Risiko Kematian pada Pasien COVID-19 di RSJPD Harapan Kita, Jakarta

Abstrak

Latar Belakang: *Penyakit COVID-19 telah menyebabkan morbiditas dan mortalitas yang bermakna di seluruh dunia sejak laporan pertama COVID-19 pada Desember 2019. Jenis gangguan yang lebih parah yang menyebabkan kematian, muncul lebih sering pada pasien usia lanjut dan pasien yang memiliki lebih dari satu penyakit penyerta. Penelitian ini bertujuan untuk mengetahui faktor risiko yang berhubungan dengan kematian pada pasien COVID-19 di RSJPD Harapan Kita.*

Metode: *Dalam uji potong lintang ini, 500 pasien rawat inap dewasa (≥18 tahun) dengan COVID-19 terkonfirmasi laboratorium dalam rentang Maret 2020 hingga April 2021 dari RSJPD Harapan Kita dimasukkan ke dalam penelitian. Data demografi karakteristik klinis dan luaran diekstraksi dari sistem rekam medis rumah sakit. Untuk mengeksplorasi faktor risiko yang terkait dengan mortalitas, analisis univariat, bivariat dan multivariat dengan regresi logistik digunakan dalam penelitian ini.*

Hasil: *Dari 500 pasien yang termasuk dalam penelitian ini, 110 subjek (22%) meninggal di rumah sakit. Analisis multivariat regresi logistik menunjukkan bahwa usia ≥65 tahun (adjusted odds ratio (aOR)=2,624; 95% CI=1,552–4,435), penyakit paru kronik (aOR=8,173; 95% CI=3,834–17,422), penyakit ginjal kronik (aOR=2,523; 95% CI=1,358–4,689), dan penyakit kardiovaskular (aOR=3,149; 95% CI=1,763–5,624) diidentifikasi sebagai faktor risiko kematian pada pasien COVID-19.*

Kesimpulan: *Usia ≥65 tahun, penyakit paru kronik, penyakit ginjal kronik, dan penyakit kardiovaskular diidentifikasi sebagai faktor risiko kematian pada pasien COVID-19 di RSJPD Harapan Kita. Penelitian lebih lanjut tentang faktor risiko terkait kematian pada COVID-19 diperlukan untuk mengendalikan perkembangan penyakit dan meningkatkan pengobatannya. (**J Respirol Indones 2022; 42 (2): 115–20)***

Kata Kunci: *COVID-19, Faktor Risiko, Mortalitas, Komorbiditas.*

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INTRODUCTION

In March 2020, the World Health Organization (WHO) officially declared COVID-19 a pandemic. COVID-19 is a disease caused by a new type of virus from one of the largest virus family, namely Coronavirus. The WHO named this virus as 2019-novel coronavirus (2019-nCoV) and finally it was named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).[1] As of 9th March 2022, 450 million confirmed cases of COVID-19, including 6.01 million deaths, had been reported to WHO.[2]

COVID-19 is a health problem that is currently a priority in various countries in the world, including Indonesia. In Indonesia, as of March 9, 2022, COVID-19 cases have reached 5.8 million cases with around 30,000 new cases reported every day.[3] The COVID-19 problem in Indonesia that has become a concern, apart from the increasing number of new cases, is the death rate of COVID-19 patients which is also increasingly concerning. The Indonesian COVID-19 Task Force reported that the COVID-19 case fatality rate in Indonesia has touched 2.6%, higher than the global mean mortality rate of 1.3%. Until March 9, 2022, the number of patients who died from COVID-19 in Indonesia had reached more than 150,000 people with an average of 300 people dying every day.[3]

The COVID-19 disease has caused significant morbidity and mortality worldwide since COVID-19 was first reported in December 2019.[1] Based on previous studies, the more severe types of disorder that caused loss of life occured more frequently in elderly, especially the older adult population group and those with more than one comorbidity.[4]

RSJPD Harapan Kita is one of the COVID-19 referral hospitals in Jakarta area especially for COVID-19 patients with cardiovascular disease. Currently, there is no study to determine the risk factors associated with mortality in COVID-19 patients at RSJPD Harapan Kita. In addition, there was a suspicion regarding the number of deaths in certain age groups. The purpose of this study was to identify risk factors associated with mortality of COVID-19 patients at RSJPD Harapan Kita, Jakarta.

METHODS

This was a cross-sectional observational study with a quantitative approach. The population in this study were all COVID-19 patients at RSJPD Harapan Kita, Jakarta. The sample used in this study was 500 patients who met the inclusion criteria obtained using random sampling technique. This study was conducted on 500 adults patients (≥18 years old) hospitalized with confirmed COVID-19 infection at RSJPD Harapan Kita, Jakarta who were admitted to COVID-19 Isolation Room or COVID-19 ICU between March 2020 to April 2021. Confirmed cases of COVID-19 were determined by a positive result in the polymerase chain reaction (PCR) test. This study was approved by the Institutional Review Board of RSJPD Harapan Kita (No: LB.02.01/VII/578/KEP 054/202).

The demographic characteristics examined were age and sex. Clinical features included primary comorbidities such as hypertension, diabetes, chronic lung disease, chronic kidney disease, and cardiovascular disease. Demographic, clinical, and outcome data were extracted from the hospital medical record system.

Death following COVID-19 infection was considered as the main outcome in this study. The study population was categorized into two groups: death (described as patients with an outcome labeled death) and survived (described as patients with an outcome labeled cured or discharged).

Statistical analysis was performed using SPSS (version 24). The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. In this study, continuous variables with and without normal distribution were mentioned as mean (with standard deviation) and median (with interquartile range/IQR), while categorical data were presented as frequency (percentage with 95% CI). Statistical analysis was accomplished in univariate analysis, bivariate analysis using chi-square test for categorical variables and Mann Whitney U test for continuous variables (OR with 95% CI), and multivariate analysis using logistic regression (OR with 95% CI).

RESULTS

The demographic and clinical characteristics of the patients are shown in Table 1. In terms of outcomes, 390 patients (78%) were discharged or survived and 110 (22%) died during hospitalization. The median age (IQR) was 54 (40-63), and 307 patients (61.4%) were male. The most common comorbidities were cardiovascular disease of 293 (58.6%), hypertension of 186 (37.2%), and diabetes mellitus of 146 (29.2%).

Table 1. Demographics and Clinical Characteristics of Patients with COVID-19

Variable	n	%
Mortality		
Death	110	22
Survived	390	78
Age		
≥65	102	20,.4
<65	398	79.6
Sex		
Male	307	61.4
Female	193	38.6
Comorbidities		
Hypertension	186	37.2
Diabetes Mellitus	146	29.2
Chronic Lung Disease	41	8.2
Chronic Kidney Disease	63	12.6
Cardiovascular Disease	293	58.6

Bivariate analysis was carried out to determine the variables to be included in the multivariate analysis (*P*<0.25). Based on the bivariate analysis in Table 2, the variables included in the multivariate analysis were age ≥65 years (OR=4.04; 95% CI:=2.52–6.48; *P* <0.001), hypertension (OR=1.48; 95% CI=0.96–2.28; *P*=0.090), diabetes mellitus (OR=2.30; 95% CI=1.48–3.58; *P*<0.001), chronic lung disease (OR=9.90; 95% CI=4.91–19.93; *P*<0.001), chronic kidney disease (OR=4.39; 95% CI=2.53–7.61; *P*<0.001), and cardiovascular disease (OR=4.45; 95% CI=2.61–7.59; *P*<0.001). The sex variable had *P*>0.25 so it was not included in the multivariate analysis.

The results of the multivariable analysis are summarized in Table 3. Age ≥65 years (aOR 2.624; 95% CI: 1.552–4.435), chronic lung disease patients (aOR 8.173; 95% CI: 3.834–17.422), chronic kidney disease (aOR 2.523; 95% CI: 1.358–4.689), and cardiovascular disease (aOR 3.149; 95% CI: 1.763–5.624) were identified as multicausal risk factors for mortality among COVID-19 patients.

Table 3. Final Model of Multivariate Analysis

Variables	Adjusted OR	95% CI	*P*
Age ≥65 years	2.624	1.552–4.435	<0.001
Comorbidities			
Chronic Lung Disease	8.173	3.834–17.422	<0.001
Chronic Kidney Disease	2.523	1.358–4.689	0.003
Cardiovascular Disease	3.149	1.763–5.624	<0.001

Table 2. Result of Bivariate Analysis

Variable	Death (n = 110)	Survived (n = 390)	Total (n = 500)	OR (95% CI)	*P*
Age					
≥65	45 (44.1%)	57 (55.9%)	102 (20.4%)	4.04 (2.52 – 6.48)	<0.001*
<65	65 (16.3%)	333 (83.7%)	398 (79.6%)		
Sex					
Male	63 (20.5%)	244 (79.5%)	307 (61.4%)	0.80 (0.52 – 1.23)	0.370
Female	47 (24.4%)	146 (75.6%)	193 (38.6%)		
Comorbidities					
Hypertension	49 (26.3%)	137 (73.7%)	186 (37.2%)	1.48 (0.96 – 2.28)	0.090
Non-Hypertension	61 (19.4%)	253 (80.6%)	314 (62.8)		
Diabetes Mellitus	48 (32.9%)	98 (67.1%)	146 (29,2%)	2.30 (1.48 – 3.58)	<0.001*
Non-Diabetes Mellitus	62 (17.5%)	292 (82.5%)	354 (70.8%)		
Chronic Lung Disease	28 (68.3%)	13 (31.7%)	41 (8.2%)	9.90 (4.91 – 19,93)	<0.001*
Non-Chronic Lung Disease	82 (17.9%)	377 (82.1%)	459 (91.8%)		
Chronic Kidney Disease	31 (49.2%)	32 (50.8%)	63 (12.6%)	4.39 (2.53 – 7.61)	<0.001*
Non-Chronic Kidney Disease	79 (18.1%)	358 (81.9%)	437 (87.4%)		
Cardiovascular Disease	91 (31.1%)	202 (68.9%)	293 (58.6%)	4.45 (2.61 – 7.59)	<0.001*
Non-Cardiovascular Disease	19 (9.2%)	188 (90.8%)	207 (41.4%)		

Note=*Significant (*P*<0.05)

DISCUSSION

This study identified several risk factors for mortality of COVID-19 patients in RSJPD Harapan Kita. Multivariate analysis showed that older age, chronic lung disease, chronic kidney disease, and cardiovascular disease were associated with higher odds of in-hospital death.

There was a statistically significant correlation (*P*<0.001) between older age and mortality of COVID-19 patients in RSJPD Harapan Kita. The risk analysis obtained an adjusted OR of 2.624 (95% CI=1.552–4.435), meaning that patients aged ≥65 years had a 2.624 times risk of dying compared to patients aged <65 years or in other words, patients aged ≥65 years had twice the risk of death compared to patients aged <65 years. The results of this study were in line with a study from Albitar, et al which stated that 80% of deaths associated with COVID-19 were observed in adults aged ≥65 years.[5] A proper explanation was that most of the older patients had numerous chronic diseases and poor health conditions to fight viral infections.[6]

Moreover, elderly patients were more likely to have weaker immune response; therefore, they were at greater risk to develop acute respiration distress syndrome (ARDS) and mortality.[7] Antibody production and response to viruses decrease with age, this occurs because of the reduced number of plasma cells contained in the bone marrow on the elderly population. This condition weakens the immune system so that the infection is not controlled and causes multi-organ failure, especially in organs or systems that have a lot of ACE-2 receptors such as the respiratory, cardiovascular, hepatic, and renal system.[8]

From several studies, it was mentioned that COVID-19 patients with chronic comorbidities have been associated with excessive COVID-19 situations including mortality. Multivariate analysis in this study obtained that comorbidities which had significant association with high risk of mortality were chronic lung disease, chronic kidney disease, and cardiovascular disease.

It was found that there was a statistically significant correlation (*P*<0.001) between chronic lung disease and mortality of COVID-19 patients in RSJPD Harapan Kita. The risk analysis obtained aOR value of 8.173, meaning that patients with chronic lung disease such as asthma, emphysema, bronchitis, chronic obstructive pulmonary disease (COPD), and pulmonary hypertension had eight times greater risk of death compared to patients without chronic lung disease.

Intuitively, since COVID-19 mainly affects the respiratory system, patients with chronic lung disease may be susceptible to worse outcomes from COVID-19 than patients without chronic lung disease. According to recent findings, COVID-19-related lung disease in ARDS remains the leading cause of mortality worldwide.[9] Patients with lung disease may be at greater risk for Covid-19 morbidity, over and above risks conferred by metabolic conditions alone. This was supported by evidence that patients in such situations sustained damage to their lung tissue.[10] Furthermore, patients with lung disease including COPD also have impaired innate and adaptative immune responses and express inappropriate respiratory viral clearance.[11]

There was a statistically significant correlation (*P*=0.003) between chronic kidney disease and mortality of COVID-19 patients in RSJPD Harapan Kita. The risk analysis showed aOR 2.523, meaning that patients with chronic kidney disease such as acute kidney injury (AKI), severe electrolyte imbalances, and glomerular disease had the possibility of 2.523 times greater risk of death compared to patients without chronic kidney disease. This result was consistent with study from Surendra, et al who discovered a significant correlation (*P*<0.001) between chronic kidney disease and mortality in patients with an aOR of 2.60 (95% CI=1.64–4.13).[12]

Chronic kidney disease is the most common risk factor for mortality in patients with COVID-19 worldwide, and the risk increases with higher stages of chronic kidney disease, with the highest risk occurring in those with kidney failure receiving replacement therapy and kidney transplant

recipients.[13] This might be due to the fact that in patients with chronic kidney disease, the inflammatory immune response occurs because of continuous oxidative stress, due to the presence of prolonged levels of pro-inflammatory cytokines. As a result, a compromised immune system can increase the susceptibility to bacterial and viral infections, and this may be the main reason for the extended hazard of pulmonary inflammation.[14]

It was determined that there was a significant correlation (*P*<0.001) between cardiovascular disease and mortality of COVID-19 patients in RSJPD Harapan Kita. Risk analysis received an aOR of 3.149, meaning that patients with cardiovascular disease have a three times risk of death compared to patients without cardiovascular disease. This result was in accordance to a study from Du, et al which pointed out that there was a statistically significant correlation (*P*=0,007) between cardiovascular disease and mortality in COVID-19 patients (aOR=2.464, 95% CI=1.279–4.747).

COVID-19 patients with cardiovascular disease tend to be at high risk, possibly because of the presence of ACE-2 receptors on cardiac muscle cells. Patients with cardiovascular disease have a higher risk of developing acute coronary syndromes in acute infections, which sooner or later lead to myocardial damage or infarction.[15]

This study had several limitations. First, this study used cross-sectional design in which effect sizes were reported using odds ratios even though the percentage of outcome was low, so the results were likely to be overestimated. Second, pre-existing comorbidities were retrieved from the medical record database which was obtained not only from the assessment in the treatment room and laboratory measurement but also from the patient's acknowledgment at that time.

Therefore, the severity of the comorbidities and patient adherence to medical prescriptions could not be evaluated. In addition, this study was only conducted at RSJPD Harapan Kita, which was a COVID-19 plus cardiovascular referral hospital so that the characteristics of patients treated at this hospital were very different from patients admitted to other hospitals which were non-referral for cardiovascular and COVID-19. Therefore, the results of this study might not reflect the mortality rate and risk factors for COVID-19-related mortality in the general population.

CONCLUSION

In conclusion, about 22% of hospitalized patients with COVID-19 in our study died. Age ≥65 years, chronic lung disease, chronic kidney disease patients, and cardiovascular disease were recognized as risk factors for mortality of COVID-19 patients at RSJPD Harapan Kita. Further research on the risk factors associated with COVID-19-related deaths need to be conducted to manage disease progression and to improve the treatment.

REFERENCES

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054–62.
2. World Health Organization. WHO Coronavirus (COVID-19) Dashboard [Internet]. World Health Organization. 2022 [cited 2022 Mar 9]. Available from: https://covid19.who.int
3. Kementerian Kesehatan RI. Covid-19 [Internet]. Kementerian Kesehatan RI. 2022 [cited 2022 Mar 9]. Available from: https://infeksiemerging.kemkes.go.id/dashboard/covid-19
4. Sanyaolu A, Okorie C, Marinkovic A, Patidar R, Younis K, Desai P, et al. Comorbidity and its Impact on Patients with COVID-19. SN Compr Clin Med. 2020;2(6):1069–76.
5. Albitar O, Ballouze R, Ooi JP, Sheikh Ghadzi SM. Risk factors for mortality among COVID-19 patients. Diabetes Res Clin Pract. 2020;166.
6. Imam Z, Odish F, Gill I, O'Connor D, Armstrong J, Vanood A, et al. Older age and comorbidity are independent mortality predictors in a large cohort of 1305 COVID-19 patients in Michigan, United States. J Intern Med. 2020;288(4):469–

76.

7. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated with Acute Respiratory Distress Syndrome and Death in Patients with Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934–43.
8. Wasityastuti W, Dhamarjati A, Siswanto. Imunosenesens dan Kerentanan Populasi Usia Lanjut Terhadap Coronavirus Disease 2019 (Covid-19). Respirologi Indones. 2019;40(3):182–91.
9. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033–4.
10. Ferguson J, Rosser JI, Quintero O, Scott J, Subramanian A, Gumma M, et al. Characteristics and outcomes of coronavirus disease patients under nonsurge conditions, Northern California, USA, March–April 2020. Emerg Infect Dis. 2020;26(8):1679–85.
11. Mallia P, Message SD, Gielen V, Contoli M, Gray K, Kebadze T, et al. Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation. Am J Respir Crit Care Med. 2011;183(6):734–42.
12. Surendra H, Elyazar IR, Djaafara BA, Ekawati LL, Saraswati K, Adrian V, et al. Clinical characteristics and mortality associated with COVID-19 in Jakarta, Indonesia: A hospital-based retrospective cohort study. Lancet Reg Heal - West Pacific. 2021;9:100108.
13. Ortiz A, Cozzolino M, Duivenvoorden R, Fliser D, Fouque D, Franssen CFM, et al. Chronic kidney disease is a key risk factor for severe COVID-19: A call to action by the ERA-edta. Nephrol Dial Transplant. 2021;36(1):87–94.
14. Betjes MGH. Immune cell dysfunction and inflammation in end-stage renal disease. Nat Rev Nephrol. 2013;9(5):255–65.
15. Bonow R, Fonarow G, O'Gara P, Yancy C. Association of Coronavirus Disease 2019 (COVID-19) With Myocardial Injury and Mortality. JAMA - J Am Med Assoc. 2020;5(7):751–3.

An Evaluation of Short-Acting β2-Agonist Prescriptions and Associated Clinical Outcomes in Asthma Management in Indonesia – The SABINA Indonesia Study

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Abstract

Background: *Asthma is a chronic inflammatory disease; therefore inhaled corticosteroid (ICS) should become the cornerstone of asthma treatment. However, patient tends to rely on short-acting β2-agonist (SABA) due to immediate symptom relief and underuse ICS which undertreats the underlying inflammation. As part of the study addressing multi-country SABA use IN Asthma (SABINA) III, we aimed to describe SABA prescription patterns and asthma-related clinical outcome in Indonesia.*

Methods: *Cross-sectional study in asthma patients (> 12 years old) during August 2019 to January 2020. Disease characteristic, prescribed asthma treatment in the last 12 months prior to the study, and clinical outcomes were documented in a single visit and registered into an electronic case report form.*

Results: *Of 219 patients recruited, the average number of prescribed SABA was 4 canisters annually. SABA over-prescription (≥ 3 canisters/year) was seen in 37% patients and more frequent in patients with moderate-to-severe asthma than mild case (40% to 17.9%). As much as 47.5% of patients had at least 1 severe exacerbation; and 7.3% of patients had ≥3 severe exacerbation in the past 12 months. Almost half of the patients (40.2%) were prescribed with oral corticosteroids (OCS). Overall, the well-, partly, and uncontrolled asthma symptom among patients were 41.6%, 37.4%, and 21%, respectively.*

Conclusion: *SABA over-prescription occurs in approximately one third of patients with asthma, especially among moderate-to-severe cases and almost half of patients with asthma experienced at least 1 severe exacerbation in the previous year. This highlights a public health concern and the need to improve asthma management by aligning with global recommendations including reducing SABA over-reliance in Indonesia. (**J Respirol Indones 2022; 42 (2): 121–8)***

Keywords: *asthma; SABA; over-prescription; exacerbation*

Evaluasi Peresepan B2-Agonis Kerja Singkat dan Keluaran Klinis Terkait pada Manajemen Asma di Indonesia – Studi SABINA Indonesia

Abstrak

Latar belakang: *Asma merupakan penyakit inflamasi kronik, sehingga kortikosteroid inhalasi (ICS inhaled corticosteroid) seharusnya menjadi inti pengobatan asma. Namun pasien cenderung mengandalkan β2-agonis kerja singkat (SABA short-acting β2-agonist) karena cepat menghilangkan gejala dan kurang menggunakan ICS, sehingga kurang menangani inflamasi yang mendasarinya. Sebagai bagian dari studi penggunaan SABA pada Asma multi-negara (SABINA SABA use IN Asthma) III, kami bertujuan menggambarkan pola peresepan SABA dan keluaran klinis terkait asma di Indonesia.*

Metode: *Studi potong lintang pada pasien asma (>12 tahun) Agustus 2019 – Januari 2020. Karakteristik penyakit, peresepan obat asma dalam 12 bulan sebelum kunjungan studi, dan keluaran klinis, dicatat pada satu kali kunjungan dan dimasukkan ke dalam formulir laporan kasus elektronik.*

Hasil: *Dari 219 pasien yang direkrut, rata-rata jumlah peresepan SABA adalah 4 canister per tahun. Peresepan SABA berlebihan (>3 canister/tahun) dijumpai pada 37% pasien, dan lebih sering pada asma sedang hingga berat dibanding asma ringan (40% dibanding 17,9%). 47,5% pasien mengalami sedikitnya 1 kali eksaserbasi berat dan 7,3% pasien mengalami 3 kali eksaserbasi berat dalam 12 bulan terakhir. Hampir setengah dari pasien (40,2%) mendapatkan resep kortikosteroid oral (OCS oral corticosteroid). Secara keseluruhan, pasien yang terkontrol baik, terkontrol sebagian, dan tidak terkontrol adalah 41,6%, 37,4% dan 21%.*

Kesimpulan: *Peresepan SABA berlebihan terjadi pada sekitar sepertiga pasien asma, terutama pada pasien asma sedang sampai berat dan hampir setengah dari pasien asma mengalami sedikitnya 1 kali eksaserbasi berat pada tahun sebelumnya. Hal ini menunjukkan masalah kesehatan masyarakat dan kebutuhan untuk meningkatkan tatalaksana asma selaras dengan rekomendasi global termasuk mengurangi ketergantungan SABA di Indonesia. (**J Respirol Indones 2022; 42 (2): 121–8)***

Kata kunci: *asma; SABA; peresepan berlebih; eksaserbasi*

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INTRODUCTION

Asthma is a common and potentially serious chronic disease that imposes a substantial burden on patients, their families and community. It causes respiratory symptoms, limitation of activity, and flare-ups which occasionally require urgent health care and may be fatal.[1–3] The prevalence of asthma varies between 7% in low-prevalence countries to >15% in other western societies.[4] According to Riskesdas 2018, the prevalence of asthma in Indonesia is 2.4%.[5]

The long-term goals of asthma management are to achieve good symptom control and to minimize future risk of exacerbations, fixed airflow limitation and side-effects of treatment.[6] Low-dose inhaled corticosteroids (ICS) is the cornerstone of asthma treatment.[1,7] For decades, the use of short-acting-β2-agonists (SABA) has been recommended as the first treatment step for symptomatic relief in patients across the spectrum of asthma.[8]

The use of inhaled SABA increased dramatically with the introduction of metered dose inhaler in the early 1960s.[9] In clinical practice, poor adherence to asthma medications, particularly ICS as maintenance therapy, is a major problem across all severities of asthma, leading to undertreatment of the underlying inflammation and higher risk of exacerbations.[10] The underuse of ICS, even in patients with mild asthma, is associated with severe asthma exacerbations and death.[11,12]

The Global Initiative for Asthma (GINA) provides internationally-accepted recommendations for asthma treatment and management.[2] In April 2019, GINA published new recommendations that might be considered the most fundamental change in asthma management in 30 years. For safety, GINA no longer recommends treatment of asthma in adolescents and adults with SABA alone. Instead, to reduce the risk of serious exacerbations, all adults and adolescents with asthma should receive either symptom-driven (in mild asthma) or daily ICS-containing treatment.[13]

With this knowledge, SABA overreliance is now an even greater concern. However, it will be difficult to change this overreliance, linked to decades of patient behaviour and guidelines recommendation, SABA use for immediate symptom relief and as the first treatment for newly diagnosed mild intermittent asthma.[7] The description between the number of SABA and ICS prescriptions in relation to asthma management, health status and burden of illness has not been estimated in many countries. Our study was conducted to describe the treatment pattern of asthma patients in terms of SABA prescriptions, provide patient background characteristics and determine their correlation with asthma related clinical outcomes and asthma symptom control in Indonesia.

METHODS

This observational cross-sectional study was part of the SABA use IN Asthma (SABINA), a series of global observational studies, which to evaluate prescriptions and clinical outcomes related to short-acting β2-agonist use in asthma.[14]

The study took place at 5 sites and 5 satellite sites: RSUP Persahabatan Jakarta, RS Universitas Airlangga Surabaya, RSUD Saiful Anwar Malang, RS Universitas Sumatera Utara Medan, RSUD Budhi Asih Jakarta, RSUD Lawang Malang, RS Lavelette Malang, Puskesmas Kecamatan Cawang Jakarta, Puskesmas Kendal Kerep Malang and Klinik Aviati Jamin Ginting Medan. Approval was given by the ethics committees as follows: Research Ethics Committees of Fakultas Kedokteran Universitas Indonesia Jakarta, Research Ethics Committees of Rumah Sakit Universitas Airlangga Surabaya, Research Ethics Committees of RSUD Dr. Saiful Anwar Malang, Research Ethics Committees of Fakultas Kedokteran Universitas Sumatera Utara/ RSUP H. Adam Malik Medan, and Research Ethics Committees of Rumah Sakit Umum Budhi Asih Jakarta.

The asthma patients were consecutive patients, who attended the outpatient clinic at hospitals and primary care clinics during August

2019 – January 2020. The inclusion criteria included male or female patients aged 12 years or older; documented diagnosis of asthma as per medical records; have had ≥3 consultations with the HCP at study starting date; after full explanation, a patient or legal guardian must have signed an informed consent document. The exclusion criteria were patient with a diagnosis of chronic obstructive pulmonary disease or any other chronic respiratory diseases different from asthma; and an acute or chronic condition that, in the investigator's opinion, would limit the patient's ability to complete the questionnaires or participate in this study.

At study visit, patients were asked about their asthma symptom control (defined by the 2017 GINA assessment of asthma control). The HCPs were requested to complete study electronic case report forms recording specified information about patient characteristics, asthma severity and level of symptom control (per the 2017 Global Initiative for Asthma [GINA] recommendations), asthma prescribed treatment in the last 12 months and history of exacerbations.

Every patient was categorized by their SABA prescriptions in the previous 12 months prior to study visit. The definition of SABA over prescription has been harmonized across all SABINA studies based on British Thoracic Society guidelines and GINA recommendation in place at the time of study design, over-prescription SABA has been defined as ≥3 SABA canisters per year.[14]

Descriptive analyses (n, %) assorted patients according to baseline demographic and clinical characteristics including morbidity and parameters acquired from the GINA assessment of asthma control.The study was conducted in accordance with ethical principles that are consistent with the Declaration of Helsinki, ICH GCPs, GPP and the applicable legislation on Non-Interventional Studies and/or Observational Studies.

RESULTS

Overall, 219 asthma patients (mean age 48.4 years, 76.6% female) were included. Most patients (n=187 [85.8%]) were enrolled by pulmonologist and 31 patients (14.2%) by primary care physicians. The percentage of mild and moderate-to-severe asthma treated by primary care physician were similar (6% vs 8.3%). Patients treated by pulmonologist were mostly moderate-to-severe (78.9%) compared to mild ones (6.9%) (Figure 1 and Table 1). The never smoker (87.2%) and with 1–2 (48.9%) or no comorbidities (48.9%) were the dominant groups in this study. 96.3% patients had fully-reimbursed healthcare insurance (Table 1).

Table 1. Demographic, Lifestyle and Clinical Characteristics

Demographic, Lifestyle and Clinical Characteristics	n (%)
Age (years); mean±SD	48.4±14.1
≥18–54 years	140 (63.9%)
≥55 years	79 (36.1%)
Gender	
Female	168 (76.7%)
Male	51 (23.3%)
BMI (kg/m^2); mean±SD	26.6±5.1
<18.5	7 (3.2%)
≥18.5–22.9	46 (21.0%)
≥23–24.9	39 (17.8%)
≥25	127 (58.0%)
Education level	
Unknown	1 (0.5%)
Primary school	27 (12.3%)
Secondary school	34 (15.5%)
High-school	98 (44.7%)
University and post-university	59 (26.9%)
Healthcare insurance/medication funding	
Not reimbursed	8 (3.7%)
Fully Reimbursed	211 (96.3%)
Treating physician	
Primary care physician	31 (14.2%)
Mild asthma patients (GINA steps 1–2)	13 (6.0%)
Moderate to severe asthma patients (GINA steps 3–5)	18 (8.2%)
Pulmonologist/Respiratory Physician	187 (85.4)
Mild asthma patients (GINA steps 1–2)	15 (6.8%)
Moderate to severe asthma patients (GINA steps 3–5)	172 (78.5%)
Number of missing values (classified as moderate to severe asthma patient)	1
Smoking status	
Active smoker	3 (1.4%)
Former smoker	25 (11.4%)
Never-smoker	191 (87.2%)
Number of co-morbidities	
No co-morbidities	94 (42.9%)
1–2 co-morbidities	107 (48.9%)
3–4 co-morbidities	18 (8.2%)

Note=Data are presented as n (%), unless otherwise stated

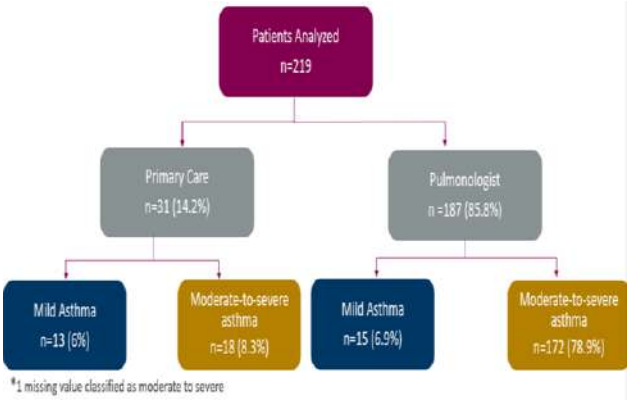

Figure 1. Patient disposition and study population by practice type and investigator-classified asthma severity

The average of asthma duration among participants was 25.7 years with percentage of GINA classification were step 1 (7.3%), step 2 (5.5%), step 3 (58.0%), step 4 (26.9%), and step 5 (2.3%) respectively.

Table 2. Asthma characteristics and clinical outcomes

Asthma characteristics and clinical outcomes	n (%)
Asthma duration years; mean±SD	25.7±17.5
GINA classification	
Step 1	16 (7.3%)
Step 2	12 (5.5%)
Step 3	127 (58.0%)
Step 4	59 (26.9%)
Step 5	5 (2.3%)
Number of severe asthma exacerbations in the last year; mean±SD	0.8±1.1
Number of severe asthma exacerbations in the last year	
0	115 (52.5%)
1	60 (27.4%)
2	28 (12.8%)
3	8 (3.7%)
4	6 (2.7%)
5	1 (0.5%)
>5	1 (0.5%)
Asthma Symptom Control	
Well-controlled	91 (41.6%)
Partly controlled	82 (37.4%)
Uncontrolled	46 (21.0%)

Note=Data are presented as n (%), unless otherwise stated

As much as 47.5% of patients had at least 1 severe asthma exacerbation (defined as a deterioration in asthma resulting in hospitalization; or emergency room treatment; or the need for intravenous or oral corticosteroid for ≥3 days or a single intramuscular corticosteroid dose in the past 12 months) and 7.3% of patients had 3 or more severe exacerbations in the past 12 months.

Table 3. SABA prescriptions in the past 12 months

SABA prescription in the past 12 months	n (%)
SABA monotherapy	
No	218 (99.5%)
Yes	1 (0.5%)
SABA in addition to maintenance therapy	
No	85 (38.8%)
Yes	134 (61.2%)
Total prescriptions in the last 12 months (canister); mean±SD	4.0±3.0
Total prescriptions in the last 12 months (canisters) by groups (n=134)	
0	0 (0.0%)
1–2	46 (37.4%)
3–5	46 (37.4%)
6–9	20 (16.3%)
10–12	9 (7.3%)
≥13	2 (1.6%)
Number of missing values	11
All asthma patients - number of SABA canister prescribed (n=208)	
0	84 (40.4%)
1–2	47 (22.6%)
3–5	46 (22.1%)
6–9	20 (9.6%)
10-12	9 (4.3%)
≥13 SABA	2 (1%)
Mild asthma patients (n=28)	
0	14 (50%)
1-2	9 (32.1%)
3-5	5 (17.9%)
6-9	0 (0.0%)
10-12	0 (0.0%)
≥13	0 (0.0%)
Moderate to severe asthma patients (n=192)	
0	70 (38.9%)
1–2	38 (21.1%)
3–5	41 (22.8%)
6–9	20 (11.1%)
10–12	9 (5.0%)
≥13	2 (1.1%)
Number of missing values	11

Note=Data are presented as n (%) unless otherwise stated.

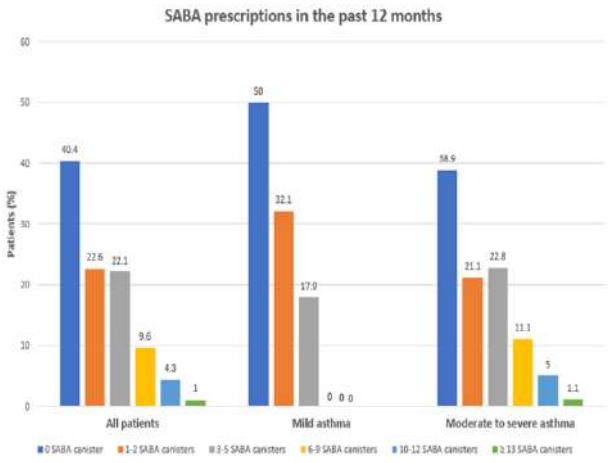

Figure 2. SABA prescriptions according to asthma severity

Of all asthma patients, 41.6% were well-controlled, 37.4% were partly controlled, and 21% were uncontrolled based on GINA 2017 criteria (daytime symptoms more than twice/week, any night waking due to asthma, reliever needed more than twice/week, and any activity limitation due to asthma) (Table 2).

Table 4. Other categories of asthma treatment in the past 12 months

Treatment	n (%)
ICS	
No	199 (90.9%)
Yes	20 (9.1%)
Total daily dose	
Low dose	15 (75%)
Medium dose	5 (25%)
High dose	0 (0.0%)
Total use in the last 12 months (canisters); mean±SD	2.0±1.8
ICS/LABA (fixed dose combination)	
No	13 (5.9%)
Yes	206 (94.1)
Total daily ICS dose	
Low dose	132 (64.4%)
Medium dose	68 (33.2%)
High dose	5 (2.4%)
Number of missing values	1
OCS treatment (short course)	
No	131 (59.8%)
Yes	88 (40.2%)
Total daily dose (mg/day); mean±SD	14.0±8.2
Number of days per prescription; mean±SD	4.4±3.3
OCS long-term/maintenance	
No	196 (89.5%)
Yes	23 (10.5%)
Total daily dose (mg/day); mean±SD	7.7±10.3
Duration of use (days); mean±SD	17.8±26.4
OCS prescribed for any reason other than asthma	
No	203 (92.7%)
Yes	16 (7.3%)
Antibiotics prescribed for asthma	
No	118 (56.7%)
Yes	90 (43.3%)
Number of missing values	11

Note=Data are presented as n (%) unless otherwise stated.

SABA monotherapy prescription was not common in our study with only one patient prescribed with SABA alone, however 61.2% (n=134) of patients received prescriptions for SABA canisters on top of any maintenance treatment; of these patients, 62.6% were prescribed >3 canisters and 8.9% were prescribed >10 canisters. The mean number of SABA canisters prescribed annually was 4 canisters. 37% of patients were prescribed >3 canisters of SABA (Table 3).

A greater percentage of patients with moderate to severe asthma (versus mild asthma) were prescribed > 3 SABA canisters in the past 12 months (40%vs17.9%) (Figure 2).

Among all asthma patients, only 9.1% were prescribed with ICS and mostly using low dose of it. On the other hand, 94.1% patients were prescribed with ICS/LABA (fixed dose combination) (LABA = long-acting-β2-agonists); 64.4% low dose, 33.2% medium dose, and 2.4% high dose. Almost half of the patients (40.2%) were prescribed with a short course of oral corticosteroid (OCS). Antibiotics for the treatment of asthma were prescribed for 90 patients (43.3%) (Table 4).

DISCUSSION

It is known that the prevalence of asthma in female is higher than in men.[5,15] In line with this fact, our study showed that 76.7% patients were female. According to SABINA (SABA use in Asthma) study in Germany, the prevalence of mild and moderate-to-severe-asthma patients in primary care was similar (48% vs 52%); while the prevalence of mild asthma in specialist care was much smaller than the moderate-to-severe patients (10% vs 90%).[15] The same patterns can be seen in our study. The percentages of mild vs moderate-to-severe asthma who visited a GP were 6% vs 8.3% (from all patients), and the numbers were 6.9% vs 78.9% in pulmonologist practice. It suggests that asthma patients with more severe symptoms were more likely to visit their specialist for management of their asthma. Almost all patients were recruited in government health care facilities, therefore 96.3% patients had healthcare insurance which fully reimbursed.

In this asthma observational cross-sectional study, approximately one-third (37%) of all asthma patients in Indonesia received over-prescriptions of SABA canisters, 22.1% were prescribed 3 to 5 canisters, 9.6% were prescribed 6–9 canisters,

4.3% were prescribed 10–12 canisters, and 1% were prescribed ≥13 canisters. This finding is consistent with other studies in a European population. It has been reported that the prevalence of SABA over-prescription was 30% in Sweden,[16] 38% in the United Kingdom,[8] 36% in Germany,[15] 15.9% in Taiwan,[17] and 29% in Spain.[7] The mean number of annual SABA canister prescriptions was 4 canisters. This is quite similar with data from European countries, e.g., 3.1 canisters in Italy, 1.6 in Germany, 3.3 in Spain, 1.9 in Sweden, and 4.2 in the UK and data from SABINA III study which showed 38% of patients were prescribed >3 SABA canisters.[7] Use of >3 SABA canisters/year is considered undesirable since it indicates over-reliance on SABA for the management of persistent symptoms, usually related to the underuse of ICS and other controllers.[18]

We also analysed the SABA over-prescriptions among asthma severity groups (Table 3). The SABA over-prescription in mild asthma patients was 17.9%, and 40% in the moderate-to-severe asthma group. This data suggest that SABA over-prescription is more pronounced in moderate-to-severe asthma patients, and most probably as add on to maintenance therapy. These results are similar with data in the UK (26% vs 58%) and Spain (22% vs 31%), but different with other European countries, such as Italy (9% vs. 9%), Germany (23% vs 14%), and Sweden (33% vs 29%).[7]

According to GINA 2021, there are 3 categories of asthma medications, including controller, reliever, and add-on therapies for patients with severe asthma. ICS is the only medication which is included into reliever and controller categories.[19] ICS act as topical anti-inflammatory agents in the bronchial passages, in particular, their ability to reduce eosinophilic inflammation within the airway with minor risk of any significant systemic exposure.[20] ICS has been shown to decrease the frequency of severe exacerbations, hospitalization, and death.

The growing concern about the negative effects of SABA has led to research into alternative treatments options for providing quick relief of asthma symptoms either for occasional symptom relief or when symptoms indicating an approaching severe exacerbation. The anti-inflammatory reliever approach which contains combination of a rapid-onset bronchodilator and ICS has been shown to be highly effective in mild asthma, where it may be used without maintenance dosing, and in moderate to severe asthma with fixed daily dosing of the same combination as maintenance treatment.[18] Low dose ICS/formoterol as reliever in mild asthma and on top of ICS/formoterol regular daily treatment in moderate and severe asthma has become the preferred approach in the GINA report.[19]

Despite advances in disease understanding, asthma management guidelines and the availability of effective treatment, poor asthma control remains a major problem. Poor asthma control is associated with an increased risk of exacerbations, poor quality of life, reduced productivity for individuals and increased healthcare utilization. In a cross-sectional observational study in Australia, 17.6%, 35.5%, and 46.9% of participants had controlled, partially controlled and uncontrolled asthma, respectively.[21] Another study among Asia – Pacific countries reported that Singapore had the highest proportion of well-controlled asthma (14%), while India and China had the lowest (0% and 2.0%, respectively). Furthermore, the partly-controlled and uncontrolled asthma rates were 61% and 26%, respectively, in Singapore.[22] However, the asthma symptom control data in our study showed a different pattern. Overall, patients in Indonesia have control levels as follows, 41.6% well-controlled; 37.4% partially controlled and 21.0% uncontrolled.

Exacerbations of asthma are episodes characterized by a progressive increase in symptoms of shortness of breath, cough, wheezing or chest tightness and progressive decrease in lung function, that require a change treatment.[19] A report showed that the overall mean annual exacerbation (defined as a worsening of asthma requiring an ED/hospital admission or OCS treatment) rates per patient in the US and the UK were 0.16/year and 0.11/year.[23] The mean number of severe exacerbations in our study is 0.8 in the last 12

months, which is quite high compared to the study in the US or the UK. 47.5% of patients had at least 1 severe exacerbation; and 7.3% of patients had ≥3 severe exacerbations, in the past 12 months.

In spite of the fact that ICS/LABA was prescribed to a majority of the patients (64.4% low dose, 33.2% medium dose and 2.4% high dose) less than half of the patients had well-controlled asthma. There are some factors which may contribute to patients' asthma control level that was not addressed in this study, i.e., adherence to treatment which is essential to optimize the benefits of therapy.[6]

A short course of OCS was prescribed to 40.2% patients with average duration 4.4 days. OCS long-term treatment was prescribed to 10.5% patients and there were 7.3% patients received OCS for any reason other than asthma.

There were some limitations in our study. Data input into the electronic case report form relied on physicians; finding may be affected by misinterpretation of instruction, incorrect patient classification and differences in local treatment practices with GINA recommendations. In addition, SABA prescription data may not always reflect actual dose. Furthermore, the number of patients recruited in this study was relatively small and only 14.2% of patients were recruited by primary care, which is lower than expected as we were targeting an accurate representation of how asthma patients are being treated in Indonesia. As 85.8% of patients were treated by a pulmonologist, most likely we see here a "better case scenario".

CONCLUSION

We have reported for the first time that SABA over-prescription occurs in Indonesia in over one third of all asthma patients, especially among moderate-to-severe patients and almost half of asthma patients experienced at least 1 severe exacerbation in the previous year. This highlights a public health concern and the need to improve asthma care by aligning with global recommendations including reducing SABA over-reliance in Indonesia.

REFERENCES

1. Global Initiative for Asthma. Pocket Guide for Asthma Management and Prevention. Global Initiative for Asthma. 2019.
2. Ställberg B, Lisspers K, Hasselgren M, Janson C, Johansson G, Svärdsudd K. Asthma control in primary care in Sweden: a comparison between 2001 and 2005. Prim Care Respir J. 2009;18(4):279–86.
3. Global Asthma Network. The Global Asthma Report 2018. Global Asthma Network. Auckland; 2018.
4. Bårnes CB, Ulrik CS. Asthma and adherence to inhaled corticosteroids: current status and future perspectives. Respir Care. 2015;60(3):455–68.
5. Kementerian Kesehatan RI. Hasil Utama Riskesdas 2018. Kementrian Kesehatan RI. 2018.
6. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention 2017. Global Initiative for Asthma. 2017.
7. Janson C, Menzies-Gow A, Nan C, Nuevo J, Papi A, Quint JK, et al. SABINA: An Overview of Short-Acting β(2)-Agonist Use in Asthma in European Countries. Adv Ther. 2020;37(3):1124–35.
8. Bloom CI, Cabrera C, Arnetorp S, Coulton K, Nan C, van der Valk RJP, et al. Asthma-Related Health Outcomes Associated with Short-Acting β2-Agonist Inhaler Use: An Observational UK Study as Part of the SABINA Global Program. Adv Ther. 2020;37(10):4190–208.
9. Pearce N, Hensley MJ. Epidemiologic studies of beta agonists and asthma deaths. Epidemiol Rev. 1998;20(2):173–86.
10. O'Byrne PM, FitzGerald JM, Bateman ED, Barnes PJ, Zhong N, Keen C, et al. Inhaled Combined Budesonide-Formoterol as Needed in Mild Asthma. N Engl J Med. 2018;378(20):1865–76.

11. Bateman ED, Reddel HK, O'Byrne PM, Barnes PJ, Zhong N, Keen C, et al. As-Needed Budesonide-Formoterol versus Maintenance Budesonide in Mild Asthma. N Engl J Med. 2018;378(20):1877–87.
12. Sadatsafavi M, Tavakoli H, Lynd L, FitzGerald JM. Has Asthma Medication Use Caught Up With the Evidence?: A 12-Year Population-Based Study of Trends. Chest. 2017;151(3):612–8.
13. Reddel HK, FitzGerald JM, Bateman ED, Bacharier LB, Becker A, Brusselle G, et al. GINA 2019: a fundamental change in asthma management: Treatment of asthma with short-acting bronchodilators alone is no longer recommended for adults and adolescents. Vol. 53, The European respiratory journal. England; 2019. p. 1901046.
14. Cabrera CS, Nan C, Lindarck N, Beekman MJHI, Arnetorp S, van der Valk RJP. SABINA: global programme to evaluate prescriptions and clinical outcomes related to short-acting β(2)-agonist use in asthma. Eur Respir J. 2020;55(2):1901858.
15. Worth H, Criée C-P, Vogelmeier CF, Kardos P, Becker E-M, Kostev K, et al. Prevalence of overuse of short-acting beta-2 agonists (SABA) and associated factors among patients with asthma in Germany. Respir Res. 2021;22(1):108.
16. Nwaru BI, Ekström M, Hasvold P, Wiklund F, Telg G, Janson C. Overuse of short-acting β(2)-agonists in asthma is associated with increased risk of exacerbation and mortality: a nationwide cohort study of the global SABINA programme. Eur Respir J. 2020;55(4):1901872.
17. Wang C-Y, Lai C-C, Wang Y-H, Wang H-C. The prevalence and outcome of short-acting β2-agonists overuse in asthma patients in Taiwan. NPJ Prim care Respir Med. 2021;31(1):19.
18. Bateman ED, Price DB, Wang H-C, Khattab A, Schonffeldt P, Catanzariti A, et al. Short-acting β(2)-agonist prescriptions are associated with poor clinical outcomes of asthma: the multi-country, cross-sectional SABINA III study. Eur Respir J. 2021;2101402.
19. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention 2021. 2021.
20. Cusack RP, Satia I, O'Byrne PM. Asthma maintenance and reliever therapy: Should this be the standard of care? Ann allergy, asthma Immunol. 2020;125(2):150–5.
21. Azzi EA, Kritikos V, Peters MJ, Price DB, Srour P, Cvetkovski B, et al. Understanding reliever overuse in patients purchasing over-the-counter short-acting beta(2) agonists: an Australian community pharmacy-based survey. BMJ Open. 2019;9(8):e028995.
22. Gold LS, Thompson P, Salvi S, Faruqi RA, Sullivan SD. Level of asthma control and health care utilization in Asia-Pacific countries. Respir Med. 2014;108(2):271–7.
23. Suruki RY, Daugherty JB, Boudiaf N, Albers FC. The frequency of asthma exacerbations and healthcare utilization in patients with asthma from the UK and USA. BMC Pulm Med. 2017;17(1):74.

Increased Serum SP-D Level, Neutrophils and Lymphocytes Sputum in Malang Splendid Bird Market Workers

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Abstract

Background: *Workers in the bird market have a high risk of exposure in the form of air pollution, pollutant particles including debris / organic dust, loose feathers, insects/ticks, aerosol particles in food, bird excreta (ammonia), various kinds of gram bacteria, fungi and virus. Exposure to particles will stimulate the immune system against harmful pathogens in the form of inflammatory response. An infection or injury will stimulate the secretion of Surfactant protein-D (SP-D) which is a group of collectin (collagen-lectin) with subgroups of the C-type lectin superfamily, together with bovine coagglutinin, mannose-binding lectin (MBL), and CL43 protein. This aim of this study was to determine serum SP-D and neutrophil and sputum lymphocyte levels in workers at splendid bird market.*

Methods: *A cross sectional analytic observational study on 35 subjects, analyzed the characteristics of the workers, calculated neutrophil types, lymphocytes on induced sputum and serum SP-D levels using sandwich ELISA.*

Results: *Mean SP-D serum levels in workers in the bird market environment increased (81.39±47.656 ng/ml) from normal levels (60±3 ng/ml). There was a significant positive correlation between length of exposure and serum SP-D levels (r=0.693; P<0.001). There was an average increase in the percentage of neutrophils and sputum lymphocytes (90.71±4.04% and 9.17±4.42%) compared to normal limit (50.3±23.5% and 2.6±5.2%).*

Conclusion: *Inhalation exposure in the Bird Market environment can cause an increase in the percentage of neutrophils, sputum lymphocytes and serum SP-D levels in workers that indicate an airway inflammation process, as well as an alleged increase in alveolar wall permeability, damage and regeneration of type II alveolar epithelial cells (Type II AEC). (**J Respirol Indones 2022; 42 (2): 129–35)***

Keywords: *Bird Market; type II AEC; lymphocytes; neutrophils; serum SP-D*

Peningkatan Kadar SP-D Serum, Neutrofil dan Limfosit Sputum pada Pekerja di Pasar Burung Splendid Malang

Abstrak

Latar belakang: *Pekerja di pasar burung memiliki risiko tinggi terhadap pajanan berupa pencemaran udara, partikel polutan termasuk debris/debu organik, bulu yang terlepas, serangga/kutu, partikel aerosol pada makanan, eksreta burung (amonia), berbagai macam bakteri gram, jamur serta virus. Pajanan terhadap partikel tersebut akan merangsang sistem imun yang berupa respon inflamasi salah satunya adalah sekresi Surfactant protein-D (SP-D) yang merupakan kelompok collectin (collagen-lectin) dengan sub kelompok superfamili lectin tipe-C, bersama dengan bovine coglutinin, mannose-binding lectin (MBL), dan protein CL43. Tujuan peniltian ini adalah untuk mengetahui kadar SP-D serum dan neutrofil dan limfosit sputum pada pekerja di pasar burung splendid malang.*

Metode: *Desain penelitian ini adalah observasional analitik-cross sectional pada 35 subjek, menganalisa karakteristik pekerja, hitung persentase neutrofil, limfosit pada sputum induksi serta pengukuran kadar SP-D serum menggunakan ELISA sandwich.*

Hasil: *Kadar rerata SP-D serum pada pekerja di lingkungan pasar burung meningkat (81,39±47,656 ng/ml) dari kadar normal (60±3 ng/ml). Terdapat korelasi positif yang signifikan antara lama pajanan dengan kadar SP-D serum (r=0,693; P<0,001). Terdapat peningkatan rerata hitung persentase neutrofil dan limfosit sputum (90,71±4,04% dan 9,17±4,42%) dibandingkan pada nilai normal (50,3±23,5% dan 2,6±5,2%).*

Kesimpulan: *Pajanan inhalasi di lingkungan Pasar Burung dapat menyebabkan peningkatan hitung persentase neutrofil, limfosit sputum dan kadar SP-D serum pada pekerja hal ini menunjukan adanya proses inflamasi jalan nafas, serta diduga terdapat peningkatan permeabilitas dinding alveolar, kerusakan dan regenerasi sel alveolar tipe II (AEC tipe II). (**J Respirol Indones 2022; 42 (2): 129-35)***

Kata kunci*: AEC tipe II; limfosit; neutrophil; pasar burung; SP-D serum*

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INTRODUCTION

Poultry sellers in the bird market can experience respiratory complaints and lung function disorders. Workers whom contacted with birds have a high risk of occupational exposure in the form of airborne contamination in the cage, including organic debris/dust on the skin, loose feathers, insects/fleas, aerosol particles in food, bird excreta, high ammonia in poultry feces, and a wide variety of bacteria, viruses and fungi. Air pollutants that can cause problems in the respiratory tract are Nitrogen Dioxide (NO_2), Sulfur Dioxide (SO_2), Hydrogen Sulfide (H_2S), and ozone (O_3). Particle pollutants get into the human body through the respiratory system and irritate the tract, which can cause lung function disorders.[1–4]

In workers exposed to inhalation exposure, the body will activate an immune response caused by various microbial products or particles that enter the respiratory tract. Particles measuring 1µm or smaller can enter the alveolar surface and interact with surfactant proteins and alveolar macrophages. The surfactant, Surfactant protein-D (SP-D), is produced and secreted in alveolar type 2 pneumocytes.[5,6] This study aims to determine whether inhalation exposure in the avian market can increase serum SP-D levels in response to cell damage, particularly in airway inflammation and lung disease.

METHOD

The research design was carried out by analytical observation with a cross-sectional approach. Subjects were workers exposed to inhalation in the Splendid Bird Market Malang with the age of 18–50 years (productive working age), workers exposed to the work environment at the Splendid Bird Market Malang >6 months with working hours a day >8 hours. The exclusion criteria in this study were workers who had been diagnosed with malignancy in both lung and extrapulmonary cancer, pneumonia and pulmonary TB with or without treatment, and also diabetes mellitus based on clinical data and treatment history explained by the workers during the history taking and physical examination.

This research was conducted at the Splendid Bird Market, Microbiology and Biomedical Laboratory, RSU dr. Saiful Anwar Malang/FK Universitas Brawijaya Malang on November – December 2018. The ethics committee has approved the study and procedures of FK Universitas Brawijaya Malang. Subjects who participated in the study had signed informed consent. The subjects underwent physical analysis and examination, spirometry examination to assess lung function (FVC, FEV_1, FEV_1/FVC), count the types of neutrophils, eosinophils and lymphocytes in sputum-by-sputum induction and serum SP-D levels using quantitative sandwich ELISA.

As a sputum induction procedure, subjects were asked to rinse their mouth with boiled water before sputum induction, and FEV1 measurements were taken before sputum induction. Sputum induction procedure was performed using a 3% hypertonic saline solution given at 5-minute intervals, maximum of 15 minutes via a jet nebulizer (NE-C28, Omron Co., Kyoto, Japan) with an output of 0.3 ml/minute. In addition, FEV1 measurements were taken after sputum induction. Sputum is stored in a phlegm bottle; specimens must be sent to the laboratory for <2 hours; if >2 hours the media/placed in a sterile container, it is not allowed to store specimens >24 hours. All sputum samples were processed in the laboratory.

The collected sputum is separated from the saliva contamination by using disposable forceps—mix sputum with 0.1% dithiothreitol solution in a ratio of 1:1. Vortex for 15 minutes and centrifuged at 2000 rpm for 10 minutes, discard the supernatant, and the remaining cell pellets are remixed with RPMI media in a 4:1 ratio, then centrifuged. At 1500 rpm for 10 minutes. The supernatant was discarded, and the remaining cell pellets were mixed with 200µl PBS solution. Take about 10 l and then prepare on a clean object glass and stained with Giemsa or Wright. The slides were examined for cell count using a binocular microscope and a cell counter by a laboratory analyst to find the percentage of neutrophils, eosinophils, and lymphocytes.

Pulmonary function tests were carried out following the 2005 ATS/ ERS TASK FORCE standard protocol on pulmonary function tests, using the CHEST HI-101 Spirometer. As much as 3 ml blood specimens from workers exposed to inhalation at the Malang Splendid Bird Market met the inclusion criteria. The enzyme-linked immunosorbent assay (ELISA) Kit (Cusabio) measured SP-D serum levels.

Processing and data analysis using IBM SPSS software version 20.0. Serum SP-D levels and other variables in workers exposed to inhalation were analyzed using the Shapiro-Wilk test to assess the normality of the data distribution. To assess the correlation between variables, the Pearson test or Spearman test was used and to assess the effect, the independent T-test and ANOVA test were used if the data were normally distributed or the Mann-Withney test or the Krusal-Walis test if the data were not normally distributed, with 95% confidence degree, α=0.05. Value means if *P*<0.05.

RESULTS

This study was followed by 35 subjects who met the inclusion criteria. Characteristic data and supporting clinical data are described in Tables 1 and 2. Based on the data on the characteristics of the research subjects, they were between 19 and 50 years old with an average age distribution of 36.6±9.6 years.

The gender of the research subjects are 80% male and 20% women. The subject's education level consists of 8 elementary schools, 9 junior high schools, and 18 high school students. The subjects' occupations consisted of 32 bird/feed traders and 3 non-bird traders: cleaners, market managers, and permanent food stall traders.

Most of the research subjects had an exposure of 2–10 years (54.3%), and 11–20 years (37.1%) with mean duration of exposure 10.6 ± 6.2 years. The number of subjects who smoked was 22 people (62.9%), and 18 people (51.4%) had no respiratory symptoms. Almost all study subjects had average BMI values, with the mean BMI value being 25.3 ± 4.9 kg/m^2.

Table 1 Characteristics of Research Subjects (n=35)

Characteristic	n	%
Gender		
Male	28	80
Female	7	20
Age (Years Old)		36.6 ± 9.6 *
Education		
Elementary School	8	22.9
Junior High School	9	25.7
Senior High School	18	51.4
Job		
Bird trader	32	91.5
Non - Bird trader	3	8.5
Exposure Time		10,6 ± 6,2 *
≤1 years	1	2.9
2–10 years	19	54.3
11–20 years	13	37.1
>20 years	2	5.7
Smoking status		
Non-Smoker	12	34.3
Smoker	22	62.9
Ex-Smoker	1	2.9
Index Brinkman (n=22)		
Mild (0–199)	8	36.4
Moderate (200–599)	7	31.8
Weight (≥600)	7	31.8
No respiratory symptoms	18	51.4
No respiratory symptoms		
Chronic cough	17	48.6
Phlegm	3	17.6
Hard to breathe	2	11.7
Physical examination		
Weight (Kg)	35	65(45–94)**
Height (cm)	35	163(145–180)**
BMI (Kg/m^2)	35	25.19 (35.79–15.21)**
Blood Sample		
Hemoglobin (gr/dl)	35	15.1 (11.8–16.9)**
Leukocyte (/µl)	35	8,342.86 (4,700–134,000)**
Basophil (%)	35	1 ± 1.0*
Eosinophil (%)	35	0.4 ± 0.65 *
Neutrophil (%)	35	55.83 ± 9.46*
Limfosit (%)	35	33.57 ± 8.69*
Monosit (%)	35	8 (5–12)**
Lung Function		
FEV_1 prediction (%)	35	81 (64.1–99.7)**
FVC prediction (%)	35	84 (64.3–97)**
FEV_1/FVC (%)	35	84.5 (73–99)**
CXR		
Normal	34	97.1
Bronchitis	1	2.9

Note=*Mean±SD; **Median (Min–Max); BMI: Body mass index
FEV: Forced expiratory volume; FVC: Forced vital capacity

Hemoglobin levels in research subjects mainly were normal, with an average Hb value of 15.01±1.25

gr/dl. The number of leukocytes the percentage of basophils, eosinophils, neutrophils, lymphocytes and monocytes mainly were average.

Table 2. Data on mean serum SP-D levels and total percentage Neutrophils, Eosinophils and Sputum Lymphocytes Research Subjects

Parameter (n=35)	Mean±SD	Median (Min–Max)
Blood Sample		
SP-D	81.39±47,656	-
Sputum sample		
Neutrophil sputum (%)	90.71±4.4	-
Eosinophil sputum (%)	0	0
Lymphocyte sputum (%)	-	9 (3–18)

Note= SP-D: *Surfactant protein-D*

The lung function results in research subjects using spirometry showed that the mean FEV1 value was average, 81.52±8.02% of the predicted value. Most of the FVC values were standard, with the mean value being 84.02±8.49% of the expected value. 9 subjects with FVC value <80% of the predicted value (Restriction). The percentage value of FEV_1/FVC was standard in all topics with a mean value of 84.81±6%. On chest x-ray examination, there were 34 regular patients and 1 patient with bronchitis.

The overall neutrophil and lymphocyte count percentage increased from average values in the induced sputum sample. However, the total number of neutrophil and lymphocyte count percentages in the blood was standard. The number of neutrophil percentages increased from the average value (40–60%) with a mean number of 90.71±4.04%. The mean number of lymphocytes in the induced sputum samples increased with a mean number of 9.17±4.42%.

The mean serum SP-D level in research subjects was found to be increased (81.39±47.66 ng/ml) from normal levels in healthy individuals (Range: 60±3 ng/ml). The Spearman correlation tested the relationship between duration of exposure and serum SP-D levels. In this test, there was a significant relationship between the length of exposure to SP-D levels, which had a positive correlation (r=0.693) with *P*<0.001, described in Figure 1.

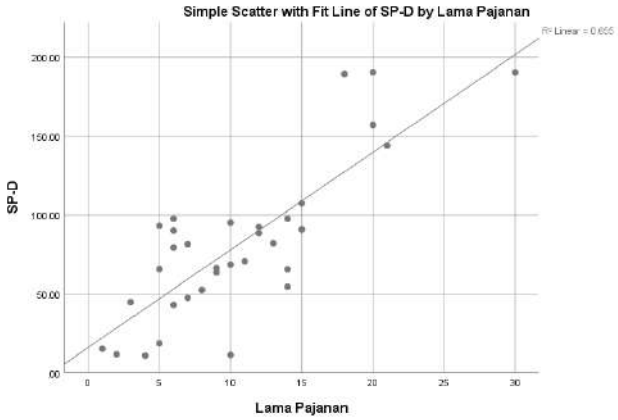

Figure 1 Scatter Plot Relationship of Exposure Time to Serum SP-D Levels

The mean level of SP-D in the group with duration of exposure less than or equal to 1 year was 15.05 ng/ml, duration of exposure more than 2–10 years was 59.02±29.56 ng/ml, length of exposure 11–20 years was 105.96±44.62 ng/ml, duration of exposure more than 20 years was 167.30±32.87 ng/ml. In the Kruskall-Walis test, there was a significant difference in serum SP-D levels in each group of exposure time (r=0.693) with *P*=0.001, described in Figure 2.

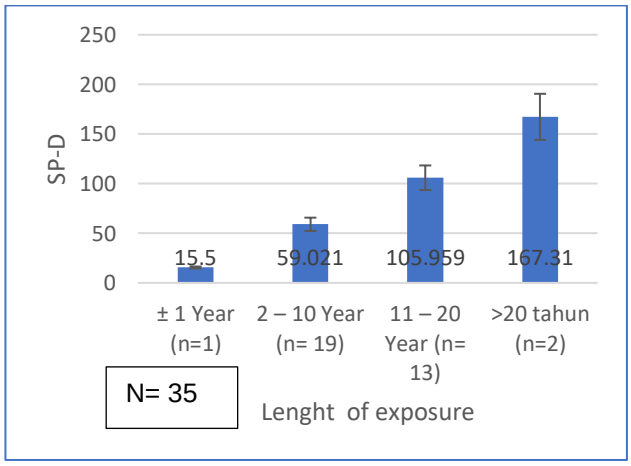

Figure 2. Graph of Effect of Long Exposure on Serum Sp-D Levels

In the induced sputum sample, the mean percentage of neutrophils was 90.71±4.04%. In the Spearman correlation test, there was a negative correlation (r = - 0.092) but it was not significant with *P*=0.601. The average number of lymphocyte percentage is 9.17±4.42%. There was a positive correlation (r=0.102) but not significant with the value of *P*=0.562.

Examination of lung function in research subjects using spirometry showed the mean value of FEV_1 was 81.52±8.02% prediction. The mean value of FVC is 84.02±8.49% prediction. There are 9 subjects with FVC value < 80% of the predicted value (Restriction). The percentage value of FEV_1/FVC obtained the mean value is 84.81±6% where all subjects have normal percentage values.

DISCUSSION

Based on the data on the characteristics of the subjects in this study, they were between 19 and 50 years old, with a mean age distribution of 36.6±9.6 years, and dominated by male (80%). Most subjects have a senior high school education level. The subjects are commonly bird/feed traders. In line with study from Cramer et al showed that the Danish Racing Pigeon Association was dominated by male poultry or pigeon breeders (93.5%).[7,8]

Most of the subjects did not experience respiratory symptoms, but 17 people (48.6%) had respiratory symptoms in coughing, 17.6% were phlegm, and 11.7% were accompanied by shortness of breath. This shows that some workers have clinical signs due to an inflammatory process. they are caused by cigarette smoke, both active and passive smokers, and exposure to inhalation in the bird market environment.[7,8] Poultry kept or sold freely is known to be at risk of contracting the influenza virus due to increased contact with wild birds, poultry and other poultry introduced to or returning from live poultry markets and other environmental exposures.[7] In the study, respiratory symptoms consisted of chronic cough, cough with phlegm, and shortness of breath. These symptoms are not specific to influenza, but exposure to poultry itself is a risk factor.

In the subgroup analysis of exposed stable patients, the avian antigen was lower than the population with worsening disease.[9] This is consistent with studies showing clinical symptoms and chest radiographs that did not significantly affect workers.

Hemoglobin levels in research subjects mainly were normal, with an average Hb value of 15.01±1.25 gr/dl. The number of leukocytes the percentage of basophils, eosinophils, neutrophils, lymphocytes and monocytes mainly were standard. The following studies and case reports show no significant value in hematological parameters due to the diagnosis made in the study of zoonoses by serological examination. Deterioration of hematological parameters occurs in a disease that is already widely manifest.[10]

The lung function results in research subjects using spirometry showed that the FEV1 average value was primarily standard, 81.52±8.02% of the predicted value. Most of the FVC values were moderate, with the mean value being 84.02±8.49% of the expected value. 36% of subjects suffered with restrictive disorder with FVC value less than 80% (Restriction). The percentage value of FEV_1/FVC was normal in all issues, with a mean value of 84.81±6%. Most of the subjects' lung function are within normal limit proved by normal clinical symptoms, physical examination and chest X-ray. In the bird market, the most common agent that is harmful to the body are those caused by microorganisms, so that restrictive disorders dominate with pulmonary function disorders.[11]

Overall serum SP-D levels in workers exposed to inhalation in the bird market environment were found to be higher than normal levels in healthy individuals (60±3 ng/ml). In this study, the researchers measured serum SP-D levels in 4 healthy subjects without inhalation exposure to the bird market environment, where the average serum SP-D level was 6.44 ng/ml. The mean value of SP-D levels in 35 worker subjects with inhalation exposure in the bird market environment was 81.39±47.66 ng/ml. The levels were higher than healthy subjects without inhalation exposure in the bird market environment. Surfactant Protein D (SP-D) expression varies depending on the season. In a study conducted on patients with bird-related hypersensitivity pneumonitis (BRHP) in Japan, surfactant protein D (SP-D) levels were highest in winter and lowest in summer (median, 217 ng/ml vs 182 ng/ml, *P*=0.007). It is thought that the increase in serum Surfactant Protein D (SP-D) levels in winter is thought to be due to increased exposure to avian

antigens found in insulated bird feather products such as divets, pillows, and jackets used for protection from cold temperatures.[5] This is by the theory, which states that an increase in SP-D is associated with an increase in inflammation when surfactant protein D (SP-D) binds to LPS and increases the effect of LPS on lung fluid.[12]

The overall percentage of neutrophils and lymphocytes increased from average values in the induced sputum sample, although the rate of neutrophils and lymphocytes in the blood was standard. The percentage of neutrophils increased from the average matter (40–60%) with a mean number of 90.71±4.04%.

In the study of Ishikawa et al, there was a positive correlation between the number of neutrophils and SP-D levels in chronic obstructive pulmonary disease (COPD) patients, but it was not statistically significant (r=0.216; *P*=0.198).[13] Research by Cao et al in asthma and COPD patients showed differences in the expression of inflammatory mediators in sputum and serum, where the term was higher in sputum.[14] This indicates that sputum samples are more sensitive than serum in assessing airway inflammatory response. The study of Kolsum et al linked the presence of bacterial infections and the presence or absence of exacerbations to the number of sputum types and serum in COPD patients, where the number of neutrophils was higher in sputum samples than in serum.[15]

The relationship between duration of exposure and serum SP-D levels found a significant association which is a positive correlation (r=0.693; *P*=0.001). From this study concluded that higher levels of SP-D corresponding with longer duration of exposure. The average level of SP-D in the group with a duration of exposure less than or equal to 1 year was 15.05 ng/ml, exposure duration of more than 2–10 years was 59.02±29.56 ng/ml, exposure duration of 11–20 years was 105.96±44.62 ng/ml, duration of exposure more than 20 years was 167.30±32.87 ng/ml. Significant differences were found in the three groups where the length of exposure was 1 year and 2–10 years with an exposure duration of 11–20 years (*P*=0.001), 1 year of exposure and 2-10 years with an exposure duration of >20 years (*P*=0.009). There was no significant difference between the combined group of 11–20 years of exposure and the group of >20 years of exposure (*P*=0.171). In line with meta-analysis from Wang et al showed that higher levels of SP-D differentiated patients with interstitial lung disease of any cause only compared with healthy controls. SP-D can predict the prognosis in these patients. Patients with interstitial lung disease and elevated SP-D levels had a 2.11-fold (95% CI=1.60-2.78) risk for a poor prognosis.[16]

The total percentage of neutrophils in the study subjects as a whole increased from the normal value (40–60%), with a mean value of 90.71 ± 4.04%. The Spearman correlation test assessed the effect of exposure time on the number of sputum neutrophils, negative correlation (r = - 0.092) but not significant (*P*=0.601). This study showed an increase in neutrophils and lymphocytes in the sputum. The number of lymphocyte percentages in the induced sputum samples increased with a mean value of 9.17±4.42%, wherein in healthy subjects, the average number of lymphocytes was 2.6% but not significant (r=0.102' p=0.562). Similar with study from Okamoto et al, where the percentage of lymphocytes in sputum was significantly higher in acute pneumonitis and significantly lower in interstitial lung disease.[5] Study from Chiba and Takahashi, showed a correlation between SP-D levels with alveolar lymphocytes in cases of hypersensitivity pneumonitis. Chronic lung conditions in bird sellers show increased SP-D associated with lymphocytes in the sputum.[17]

CONCLUSION

The mean serum SP-D levels of workers exposed to inhalation in the Malang Splendid Bird Market increased from the mean normal range. The longer the exposure time, the higher the serum SP-D level. There was an increase in the percentage of neutrophils and sputum lymphocytes in workers exposed to inhalation in the Splendid Bird Market Malang. An increase in neutrophils, lymphocytes in

sputum and serum SP-D levels indicates an inflammatory process in the airways. It is suspected that there is an increase in the permeability of the alveolar walls, damage and regeneration of AEC type II due to inhalation exposure in the Splendid Bird Market.

REFERENCES

1. Wardhana WA. Dampak Pencemaran Lingkungan. Revisi. Yogyakarta: Andi; 2004. 35–38 p.
2. Cardona C, Yee K, Carpenter T. Are live bird markets reservoirs of avian influenza? Poult Sci. 2009;88(4):856–9.
3. Arganata FZ. Status Faal Paru dan Faktor yang Mempengaruhinya pada Penjual Unggas di Pasar Burung Kupang, Surabaya. Universitas Airlangga; 2015.
4. Swiderska-Kiełbik S, Krakowiak A, Wiszniewska M, Dudek W, Kowalczyk M, Walusiak-Skorupa J, et al. Work-related respiratory symptoms in bird zoo keepers--questionnaire data. Int J Occup Med Environ Health. 2009;22(4):393–9.
5. Okamoto T, Fujii M, Furusawa H, Tsuchiya K, Miyazaki Y, Inase N. The usefulness of KL-6 and SP-D for the diagnosis and management of chronic hypersensitivity pneumonitis. Respir Med. 2015;109(12):1576–81.
6. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, et al. Surfactant proteins SP-A and SP-D: structure, function and receptors. Mol Immunol. 2006;43(9):1293–315.
7. Cramer C, Schlünssen V, Bendstrup E, Stokholm ZA, Vestergaard JM, Frydenberg M, et al. Risk of hypersensitivity pneumonitis and interstitial lung diseases among pigeon breeders. Eur Respir J. 2016;48(3):818–25.
8. Tjiptoherijanto P. Proyeksi penduduk, angkatan kerja, tenaga kerja, dan peran serikat pekerja dalam peningkatan kesejahteraan. Maj Perenc Pembang. 2001;23:1–10.
9. Tsutsui T, Miyazaki Y, Kuramochi J, Uchida K, Eishi Y, Inase N. The amount of avian antigen in household dust predicts the prognosis of chronic bird-related hypersensitivity pneumonitis. Ann Am Thorac Soc. 2015;12(7):1013–21.
10. Daut EF, Lahodny G, Peterson MJ, Ivanek R. Interacting Effects of Newcastle Disease Transmission and Illegal Trade on a Wild Population of White-Winged Parakeets in Peru: A Modeling Approach. PLoS One. 2016 Jan;11(1):e0147517.
11. Sperandio EF, Arantes RL, Matheus AC, Silva RP da, Lauria VT, Romiti M, et al. Restrictive pattern on spirometry: association with cardiovascular risk and level of physical activity in asymptomatic adults. J Bras Pneumol. 2016;42(1):22–8.
12. Forbes LR, Haczku A. SP-D and regulation of the pulmonary innate immune system in allergic airway changes. Clin Exp Allergy. 2010;40(4):547–62.
13. Ishikawa N, Mazur W, Toljamo T, Vuopala K, Rönty M, Horimasu Y, et al. Ageing and long-term smoking affects KL-6 levels in the lung, induced sputum and plasma. BMC Pulm Med. 2011;11:22.
14. Cao Y, Gong W, Zhang H, Liu B, Li B, Wu X, et al. A Comparison of Serum and Sputum Inflammatory Mediator Profiles in Patients with Asthma and COPD. J Int Med Res. 2012;40(6):2231–42.
15. Kolsum U, Donaldson GC, Singh R, Barker BL, Gupta V, George L, et al. Blood and sputum eosinophils in COPD; relationship with bacterial load. Respir Res. 2017;18(1):88.
16. Wang K, Ju Q, Cao J, Tang W, Zhang J. Impact of serum SP-A and SP-D levels on comparison and prognosis of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. Medicine (Baltimore). 2017;96(23):e7083.
17. Chiba H, Takahashi H. What Is the Significance of KL-6, SP-A and SP-D. In: Nakamura H, Aoshiba K, editors. Specific Serum Markers of IPF. Tokyo: Springer Japan; 2016. p. 67.

Expression of Immune Checkpoint Marker PD-L1 in Surgical Lung Cancer Specimens

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Abstract

***Background**: Currently, immune checkpoint pathways of PD1-PD-L1 are being used to treat lung cancer and PD-L1 serves as a predictive biomarker. Investigations of PD-L1 expression as targeted immunotherapy in lung cancer specimens in Indonesia are needed. This study evaluated PD-L1 expression in resected lung cancer specimens using immunohistochemistry techniques.*

***Methods**: Thirty surgically resected samples from lung cancer patients were obtained. The whole specimens were stained using immunohistochemistry techniques automatically in BOND-MAX Autostainner (Leica, Germany). PD-L1 polyclonal antibody (Genetex) at 1:500 dilution was applied to the immunohistochemistry staining procedure. Clinicopathological characteristics were acquired from the hospital registry database.*

***Result:** A total of 30 surgical specimens were assessed from lung cancer patients. Twenty-four (80%) of them had the histological type of adenocarcinoma (AC), 1 (3,33%) were adenosquamous carcinoma, 2 (6,67%) squamous cell carcinoma (SSC), 1 (3,33%) large cell carcinoma, 1 (3,33%) neuroendocrine carcinoma and 1 (3,33%) adenoid cystic carcinoma. The expression of PDL-1 positive reactivity were detected in 16 of 30 (53.3%) specimens. Samples were categorized into strong positivity (>50%) in 7 specimens, medium (50%) in 0 specimen and low positivity (<50) in 9 specimens.*

Conclusion:** PD-L1 expression could be detected in lung cancer specimens using polyclonal antibody. Further investigation is needed to determine the clinical correlation between this examination and lung cancer. (**J Respirol Indones 2022; 42 (2): 136-40)

***Keywords:** non-small cell lung cancer, PD-L1 expression, polyclonal antibody*

Ekspresi Petanda Hayati “Immune Check Point” PD-L1 pada Sediaan Jaringan Bedah Kanker Paru

Abstrak

***Latar belakang:** Imunoterapi dengan intervensi “check point system” seperti PD1-PD-L1 saat ini sudah menjadi salah satu pilihan terapi kanker paru. Ekspresi PD-L1 merupakan salah satu petanda hayati kanker paru. Penelitian ekspresi molekul PD-L1 pada kanker paru di Indonesia saat ini masih jarang, terutama yang bersumber dari jaringan bedah. Oleh karena itu, penelitian ini bertujuan menilai ekspresi PD-L1 pada jaringan reseksi kanker paru menggunakan teknik imunohistokimia.*

***Metode:** 30 jaringan reseksi kanker paru di RS Persahabatan Jakarta dilakukan pemeriksaan imunohistokimia mengunakan sistem BOND-MAX Autostainner (Leica, Germany). Antibodi yang digunakan adalah PD-L1 polyclonal antibody (Genetex) dengan pengenceran 1:500. Karakteristik klinis subjek dianalisis lebih lanjut.*

***Hasil**: Dari 30 sampel jaringan reseksi kanker paru, 24 kasus (80%) merupakan adenokarsinoma (AC), 1 kasus (3.33%) adenoskuamosa, 2 kasus (6.67%) karsinoma sel skuamosa serta masing-masing 1 kasus (3,33%) karsinoma sel besar, karsinoma neuroendokrin dan karsinoma adenoid kistik. Ekspresi PD-L1 ditemukan pada 16 dari 30 spesimen (53,3%) dan dikategorikan menjadi positif kuat (>50%), medium (50%) dan positif rendah (<50%) dengan komposisi masing-masing 7, 0 dan 9 spesimen. Selain itu, ditemukan 14 spesimen tanpa ekspresi PD-L1.*

Kesimpulan:** Ekspresi PD-L1 dapat ditemukan pada sediaan kanker paru dengan menggunakan pemeriksaan imunohistokimia. Korelasi klinis pemeriksaan ini pada kanker paru perlu diteliti lebih lanjut. (**J Respirol Indones 2022; 42 (2): 136-40)

***Kata kunci:** KPKBSK, ekspresi PD-L1, antibodi poliklonal.*

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INTRODUCTION

Lung cancer is one of the leading cause of cancer related death in the world. Based on data from WHO, it was estimated that 9.6 million cases of death in 2018 were caused by cancer and 2.09 million of them were attributed to lung cancer. On 2012, WHO categorized Indonesia's lung cancer incidence as medium. Lung cancer incidence rate in Indonesian males was 25.8 with mortality rate of 23.2, meanwhile in female was 8.1 with mortality rate of 7.3.[1–3]

Improvements in lung cancer management have been achieved in many ways. Radiotherapy, chemotherapy and surgery all contribute greatly to patients' survival, but more advanced and novel therapy is needed to increase quality of life and survival. Nowadays, targeted therapy based on specific genes is known to improve patient survival and has become a standard therapy in lung cancer. But newer therapeutic option such as immunotherapy that targets immune checkpoint mechanism may also have potential benefit in lung cancer management. Data from clinical trials have shown proven antitumor efficacy that resulted in better survival and durable response compared to standard therapy.[4,5]

Immune checkpoint is a protein located on the surface of immune cells, notably on cytotoxic T-cells. Upon binding to a specific ligand, it's capable of transmitting the stimulatory or inhibitory signal.[6] The primary role of immune checkpoint is to protect tissue damage during severe active inflammation.[7]

One of the potential immune check point molecules is programmed cell death protein 1 (PD1) and its ligand (programmed death ligand 1/PD-L1). Tumor exploited this interaction between PD1 and PD-L1 by increasing their activation, thus limiting immune cells recognition, reducing immune cells activation and reducing elimination of tumor cells. Clinical trials using PD1 or PD-L1 inhibitor so far have shown durable response and better survival in lung cancer.[8] PD1 or PD-L1 expression in lung cancer tissue are considered as potential biomarker, but further investigation is needed especially in Indonesia.

METHODS

Surgical specimens were obtained from 30 patients diagnosed with primary lung cancer at Department of Anatomical Pathology, Persahabatan Hospital, Jakarta. The specimens were collected and stored at the Laboratory of Anatomical Pathology at the same hospital. Clinical data were taken from the hospital medical records. This research was conducted according to the Ethics Committee of the Faculty of Medicine University of Indonesia No: 534/UN2.F1/ETIK/2017.

This study used formalin-fixed, paraffin embedded (FFPE) specimens of lung cancer. FFPE blocks were sliced at 4 µm sections. Immunohistochemistry (IHC) staining performed on the whole tissue used primary antibody PDL-1 Rabbit polyclonal GTX 104763 (Genetex, USA) at a dilution of 1:500. The IHC staining for PDL-1 antibody was performed automatically on BOND-MAX Autostainer M495401 (Leica, Germany).

Antigen retrieval and secondary antibody used Leica Detection System. The percentage of PD-L1 expression was evaluated and ranging from 0-100% as reviewed by pathologist. The staining result of PD-L1 showed positive reaction in cytoplasm. Villi was used as reactive positive control. The positive expression of PD-L1 in the specimens ranged from 0–100%. They were categorized into strong positivity (>50%), medium (50%), low (<50%) and negative (0%)

RESULT

A total of 30 specimens from lung cancer patients were examined. Among all of them, 24 (80%) had the histological type of adenocarcinoma (AC), 1 (3,33%) was adenosquamous carcinoma, 2 (6,67%) squamous cell carcinoma (SSC), 1 (3,33%) large cell carcinoma, 1 (3,33%) neuroendocrine carcinoma and 1 (3,33%) adenoid cystic carcinoma. The majority of samples came from male patients, amounting to 20 (66,67%), while the rest were female (10/33,3%).

We found that PD-L1 was tested positive in 16 of 30 samples (53.5%). Strong positivity of PD-L1 expression was found in 7 samples, consisting of 1

adenocarcinoma sample with 70% positivity, 3 adenosquamous carcinoma/SCC samples with 80% positivity and 3 adenocarcinoma samples with 90% positivity.

Table 1. The expression of PDL-1 positivity in surgically resected lung cancer specimens.

PDL1 expression	N	Histology
Strong positivity (>50%)	7	SCC, AdenoCa
Medium (50%)	0	-
Low positivity (1-49%)	9	adenoCA, SCC, LC-NEC, ACC
Negative (0%)	14	adenoCa

Note=SCC: squamous cell lung cancer; adenoCA: adenocarcinoma; LC-NEC: Large cells neuroendocrine cells; ACC: adenoid cystic carcinoma.

No medium positivity of PD-L1 were found. Low positivity of PD-L1 expression was found in 9 samples, consisting of 2 adenocarcinoma samples with 1% positivity, 1 adenocarcinoma with 2% positivity, 1 large cell carcinoma with 3% positivity, 1 adenocarcinoma with 5% positivity and each 2 samples of neuroendocrine carcinoma and adenocarcinoma with 20% positivity. PD-L1 expression was completely absent in 14 samples. The results are listed in on Table 1 and PD-L1 positive reaction can be seen in Figure 1.

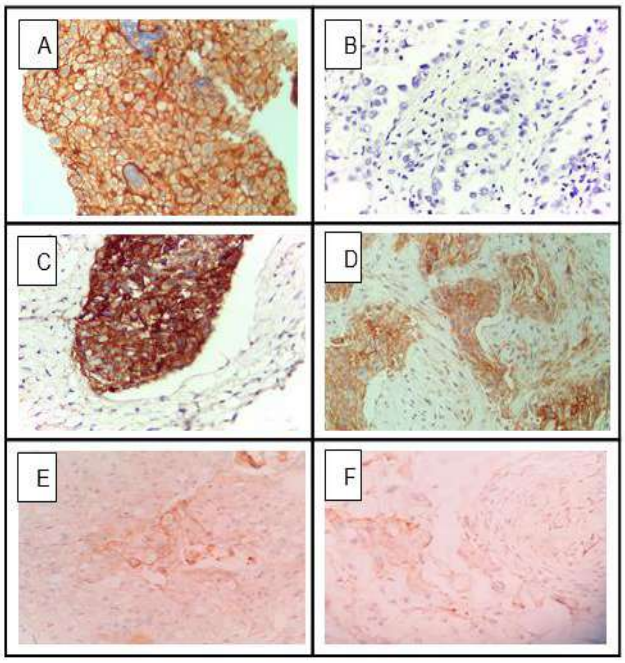

Figure 1. Representative images of PD-L1 staining. A) positive control from villi tissue showed strong membrane staining of PD-L1; B) Negative control showed no cells with PD-L1 staining; C) Strong positive expression of PD-L1 (90% of tumor cells were stained) in adenocarcinoma; D) Strong positive expression of PD-L1 (70% of tumor cells were stained); E) Low positive expression of PD-L1 (5% of tumor were stained) in adenocarcinoma; F) Low positive expression of PD-L1 (3% of tumor cells were stained) in large cell carcinoma

DISCUSSION

PD-L1 is an important immune checkpoint inhibitor expressed by the tumor cells (TCs) or tumor-infiltrating immune cells (ICs) and determined as the major membrane inhibitory ligand.[6,7] PD-L1 binds to PD1 as a key immune checkpoint receptor activated by the T cells. This PD1 and PD-L1 interaction decreases the ability of the activated T cells to produce an effective immune response and inhibit the immune system to destroy the tumor. This interaction has been studied as a target for lung cancer treatment.[7]

PD-L1 expression in lung cancer is varied according to the histological type of the specimen and the selection of the tumor sample location, which mainly influence the positivity rate of PD-L1 expression in the specimens.[9,10] The prevalence of PD-L1 expression in the population of patients with non-small cell lung cancer (NSCLC) ranges from 24% to 60%, even with a cutoff for positivity set at 5%.[11,12] Surgical specimen from lung cancer were used in this study.

Expression of PD-L1 also ranged widely in the NSCLC sample because of the variation of antibody and platform.[9] PD-L1 has a dynamic expression related to the heterogeneity in many tumors.[10] The evaluation of PD-L1 using IHC techniques also needs more validation regarding the protocol, since different ones might also affect its positivity. The positivity indicates immune active tumor that could be sensitive to anti PD-1 therapy and serves as predictive biomarker. In some clinical trials, high positivity of the PD-L1 expression, i.e., >50% correlates with better clinical outcomes during anti-PD-1 treatment.[12,13]

There are various methods and platforms to evaluate PD-L1 expression in lung cancer. The commonly used clones are 22C3, SP263, SP142, 28-8, E1L3N, E1J2J and 5H1 with DAKO, Benchmark and BONDMAX as the platforms.[8] This study used BOND-MAX Autosteiner platform from Leica with PD-L1 Rabbit polyclonal antibody GTX 104763 (Genetex, USA) at a dilution of 1:500.

This study shows that PD-L1 was dominantly expressed in non-small cell lung carcinoma

(NSCLC). High positivity of PD-L1 was majorly found in SCC and adenocarcinoma. From 30 samples included in this study, low positivity of PD-L1 mostly came from neuroendocrine carcinoma, large cell carcinoma and adenoid cystic carcinoma. Meanwhile, the lowest positivity was found in adenocarcinoma. A study from Heymann et al showed that out of 102 samples from surgical lung cancer resection, 26% had high positive staining (>50% of tumor cells) using IHC-based, 22C3 pharmDx assay.[14] Another study by McLaughlin et al using conventional chromogenic IHC with E1L3N and SP142CV antibody in histological lung cancer specimen showed variability/heterogeneity of result.[15]

As stated previously, this study showed conflicting result of PD-L1 staining which might be attributed to the small sample size, different protocols of IHC application and different PD-L1 antibodies.[8] Hence, no precise standard for FFPE unstained slide cut-off could be applied in the IHC staining procedure. Despite the vast differences with other studies, this IHC protocol proved to be feasible, but its correlation with the clinical value warrant further investigation.

CONCLUSION

New therapy to treat lung cancer is currently being developed and immunotherapy is one promising approach to be investigated to reach the goals of better survival. As more treatment options are available, evaluating PD-L1 expression in lung cancer will become more relevant and served as prognostic marker. Characterizing tumor and immune cells via PD-L1 protein by IHC may be helpful to identify the patients who potentially benefit with anti PD1 or anti PD-L1 agents. PD-L1 is a predictive biomarker in lung cancer and its positivity presents an opportunity to administer the agents that prevent PD-1 and PD-L1 pathway interaction either in advanced lung cancer or metastatic lung cancer.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
3. Rafiemanesh H, Mehtarpour M, Khani F, Hesami SM, Shamlou R, Towhidi F, et al. Epidemiology, incidence and mortality of lung cancer and their relationship with the development index in the world. J Thorac Dis. 2016;8(6):1094–102.
4. Kerr KM, Nicolson MC. Non-Small Cell Lung Cancer, PD-L1, and the Pathologist. Arch Pathol Lab Med. 2016;140(3):249–54.
5. Janzic U, Kern I, Janzic A, Cavka L, Cufer T. PD-L1 Expression in Squamous-cell Carcinoma and Adenocarcinoma of the Lung. Radiol Oncol. 2017;51(3):357–62.
6. Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? J Immunother Cancer. 2016;4(1):48.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
8. Tsao MS, Cancer IA for the S of L, Kerr KM, Dacic S, Yatabe Y, Hirsch FR. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer. Editorial Rx Press; 2017.
9. Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther. 2016;9:5023–39.
10. Kluger HM, Zito CR, Turcu G, Baine MK, Zhang H, Adeniran A, et al. PD-L1 Studies Across Tumor Types, Its Differential Expression and Predictive Value in Patients Treated with Immune Checkpoint Inhibitors. Clin Cancer Res. 2017;23(15):4270–9.
11. Brody R, Zhang Y, Ballas M, Siddiqui MK, Gupta P, Barker C, et al. PD-L1 expression in advanced NSCLC: Insights into risk stratification and treatment selection from a systematic literature review. Lung Cancer. 2017;112:200–15.

12. Yu H, Boyle TA, Zhou C, Rimm DL, Hirsch FR. PD-L1 Expression in Lung Cancer. J Thorac Oncol. 2016;11(7):964–75.
13. Teixidó C, Karachaliou N, González-Cao M, Morales-Espinosa D, Rosell R. Assays for predicting and monitoring responses to lung cancer immunotherapy. Cancer Biol Med. 2015;12(2):87–95.
14. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, Haghighi M, et al. PD-L1 expression in non-small cell lung carcinoma: Comparison among cytology, small biopsy, and surgical resection specimens. Cancer Cytopathol. 2017;125(12):896–907.
15. McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorf D, Pelekanou V, Rehman J, et al. Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. JAMA Oncol. 2016;2(1):46–54.

The Effect of Roflumilast on Absolute Neutrophil Count, MMP-9 Serum, %FEV_1 Value, and CAT Scores in Stable COPD Patients

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Abstract

Background: *Chronic obstructive pulmonary disease is the leading cause of morbidity and mortality worldwide. Cigarette smoke and noxious agent cause oxidative stress to activate nuclear factor-κB which increases inflammatory cell releases, including neutrophil and matrix metalloproteinase-9 (MMP-9). Roflumilast possesses anti-inflammatory effect which may be used as additional therapy for stable COPD.*

Methods: *Pre-test and post-test experimental clinical trial was conducted on 40 patients with stable COPD in pulmonology outpatient clinics of Dr. Moewardi Surakarta and dr. Soehadi Prijonegoro Sragen hospital from 6 January to 6 March 2020. Forty participants were assigned into treatment group (n=20) who received standard therapy along with roflumilast 500 mg/day and placebo group (n=20) who received only standard therapy for 28 days. Decline in inflammation was measured by ANC and MMP-9 serum, improvement in obstruction was measured by %FEV_1, and clinical improvement was measured by CAT score.*

Results: *Our finding revealed a decrease in ANC and MMP-9 serum among the treatment group, although statistically insignificant (P=0.449), (P=0.195) respectively. %FEV_1 value also increased insignificantly in the treatment group (P=0.189). Chronic obstructive pulmonary disease assessment test (CAT) score decreased significantly in the treatment group (P=0.0001).*

Conclusion: *Roflumilast administration reduced inflammation as indicated by insignificant lower level of ANC, MMP-9 serum, and insignificantly increased %FEV_1, and improved clinical condition of patients with stable COPD as suggested by decrease in CAT score. (**J Respirol Indones 2022; 42 (2): 141–50)***

Keywords: *Roflumilast, stable COPD, absolute neutrophil count, MMP-9 serum, and CAT score.*

Pengaruh Pemberian Roflumilast Terhadap Jumlah Neutrofil Absolut Darah, MMP-9 Serum, Nilai %VEP_1, Dan Skor CAT pada Penderita PPOK Stabil

Abstrak

Latar belakang: *Penyakit paru obstruktif kronik merupakan penyebab utama morbiditas dan mortalitas di dunia. Asap rokok dan partikel berbahaya menyebabkan stres oksidatif yang mengaktivasi nuclear factor-Kb meningkatkan pengeluaran sel inflamasi antara lain neutrofil dan matrix metalloproteinase-9 (MMP-9). Roflumilast mempunyai efek antiinflamasi yang dapat digunakan sebagai terapi tambahan pada PPOK stabil.*

Metode: *Uji klinis eksperimental pretest dan postest design dilakukan terhadap 40 penderita PPOK stabil di poliklinik paru RSUD Dr. Moewardi Surakarta dan RSUD dr. Soehadi Prijonegoro Sragen tanggal 6 Januari sampai 6 Maret 2020. Subyek kelompok perlakuan (n=20) diberikan roflumilast 1 x 500 mg per hari selama 28 hari, kelompok kontrol (n=20) hanya mendapatkan terapi standar PPOK stabil. Penurunan derajat inflamasi diukur dengan pemeriksaan neutrofil darah dan MMP-9 serum, perbaikan derajat obstruksi diukur dengan %VEP_1, dan perbaikan klinis diukur dengan skor CAT.*

Hasil: *Hasil penelitian menunjukan adanya penurunan jumlah neutrofil absolut darah pada kelompok perlakuan tetapi tidak signifikan terlihat dari nilai P=0,449. Kadar MMP-9 serum menunjukkan adanya penurunan tetapi tidak signifikan terlihat dari nilai P=0,195. Nilai %VEP_1 pada kelompok perlakuan terdapat peningkatan tetapi tidak signifikan terlihat dari nilai p= 0,189. Skor CAT menunjukan adanya penurunan secara signifikan pada kelompok perlakuan terlihat dari P=0,0001.*

Kesimpulan: *Pemberian roflumilast 1x500mg/hari mampu menurunkan inflamasi berdasarkan penurunan tidak signifikan jumlah neutrofil absolut darah, MMP-9 serum, meningkatkan tidak signifikan %VEP_1, dan dapat memperbaiki klinis penderita PPOK stabil yang terlihat pada penurunan skor CAT secara signifikan. (**J Respirol Indones 2022; 42 (2): 141–50)***

Kata kunci: *Roflumilast, PPOK stabil, Jumlah Neutrofil Absolute Darah, MMP-9 Serum, dan Skor CAT.*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable lung disease characterized by persistent airflow limitation and generally progressive, associated with an exaggerated chronic inflammatory response in the airways and lung parenchyma due to toxic gases or particles.[1,2] Chronic airflow limitation in COPD is caused by a combination of small airways disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), where the contribution varies from individual to individual. In 2015, 3.17 million people died from COPD; this number is equivalent to five per cent (%) of all global deaths. The epidemiology of COPD is predicted to be the third leading cause of death and the fifth leading cause of disability in the world by 2020.[1–3]

COPD lung tissue damage is a complex interaction between oxidative stress, extracellular matrix proteolysis, inflammation, and apoptosis. Cigarette smoke and other harmful particles cause airway inflammation within minutes or hours of exposure. One of the early manifestations of COPD is the withdrawal of systemic inflammatory cells into the airway. Cigarette smoke and harmful particles cause oxidative stress that activates the nuclear factor kappa (NF-κβ), increasing the release of inflammatory genes.[4,5]

NF- dimers are inactivated in the cytoplasm of cells bound by kappa B (Iκβ) inhibitors. Phosphorylation by the Iκβ kinase complex (IKK) causes degradation of Iκβ so that the bond between NF-κβ and Iκβ is broken, causing NF- to be free and translocated into the nucleus and transcription of inflammatory genes, including tumour necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-8 cause airway inflammation in COPD. The inflammatory process that occurs continuously will cause more severe airway damage. The degree of airway inflammation correlates with the severity of existing obstruction. Therefore, the measurement of inflammatory cells count in airway can describe the degree of airway obstruction. An increase in the number of neutrophils in airway is related to the severity of COPD. Neutrophils increase during exacerbations. Neutrophils secrete serine proteases, namely matrix metalloproteinase-9 (MMP-9), which play a role in alveolar destruction, causing emphysema and activating tumour growth factor-α (TGF-α), triggering mucus hypersecretion.[4–7]

Suppressing inflammation to prevent these complications is among the objectives of COPD therapy. Long-term anti-inflammatory treatment is expected to reduce disease progression.[8–11] Roflumilast is a potent and selective phosphodiesterase 4 (PDE4) enzyme inhibitor that targets systemic inflammation. Roflumilast has various anti-inflammatory effects, including reducing inflammatory mediators, expression of cell surface markers, and inhibition of apoptosis. Phosphodiesterase 4 plays a role in the pathophysiology of COPD by increasing levels of cyclic adenosine monophosphate (cAMP). The increase in cAMP levels by roflumilast then inhibits the activity of binding deoxyribonucleic acid (DNA) sequences with NF- by preventing phosphorylation and degradation of Iκβ as an inhibitory factor of NF-.[12,13]

Based on the description above, authors are intrigued to identify and prove the role of roflumilast administration at 500 milligrams (mg) dose per day as an adjunct therapy to standard therapy for patients with COPD in reducing inflammation and improving symptoms of airway obstruction. Inflammation subsidence is indicated by the decline in absolute number of neutrophils in blood and serum MMP-9, reduced airway obstruction is marked by an improvement in the percentage of forced vital capacity in one second (%FEV_1) to the predicted value. In addition, clinical improvement is characterized by an increase in lung function and quality of life (QOL) in COPD patients, as evidenced by the decrease in COPD assessment test (CAT) score.

METHOD

This study was a quasi-experimental clinical trial with pre and post-test design for treatment and

control groups. The study was conducted at Dr Moewardi Surakarta Hospital and dr. Soehadi Prijonegoro Sragen Hospital from January 6 to March 6 2020. Study sample was patients with stable COPD who had been registered as outpatients at pulmonary clinic of RSUD dr. Moewardi Surakarta and RSUD dr. Soehadi Prijonegoro Sragen on January 6 to March 6 2020. Stable COPD in this study was described as COPD which did not undergo acute exacerbation, worsening respiratory symptoms characterized by increased tightness, sputum production, and changes in sputum colour. Consecutive selection was applied by selecting participants based on inclusion criteria to be included in the study until the required number was met.

The inclusion criteria were group C and D stable COPD patients aged 40 years or older, willing to participate in the survey and to sign the consent form. The exclusion criteria were patients with stable COPD who had pulmonary or extrapulmonary malignancies, pneumonia, severe hepatic dysfunction, severe renal function impairment, and gastroenteritis. The termination criteria were patients resigning, passing away, or experiencing severe side effects of roflumilast.

Patients with stable COPD who met the inclusion criteria were explained about the aims and objectives of the study. Patients who agreed were asked to sign an informed consent. Forty patients with stable COPD were included and assigned into control and treatment group (20 patients each). The treatment group received standard therapy for stable COPD and roflumilast 1 x 500 mg for 28 days, while the control group only received standard treatment for 28 days. The control and treatment groups were examined for absolute neutrophil counts, serum MMP-9, and asked to fill out CAT questionnaire and spirometry at the beginning and end of the study. The homogeneity test of research characteristics in the form of qualitative variables with a categorical scale (nominal/ordinal) was carried out by using chi-square test. The homogeneity test for quantitative variables with a numerical scale was carried out by using a 2-mean difference test whose type of test was based on the data distribution of sample characteristic variables. Data on all variables were analyzed using SPSS 21 for windows. Analysis of normally distributed data was carried out by using the paired t-test and independent-sample t-test. In contrast, the data with abnormal distribution was carried out by using Wilcoxon signed-rank test for the paired group or the Mann-Whitney test for the unpaired group.

RESULTS

Forty participants who met the inclusion and exclusion criteria were assigned into two groups, 20 people in the treatment and control group respectively. The treatment group received standard therapy for stable COPD and roflumilast 1 x 500 mg/day for 28 days. The control group only received standard treatment for stable COPD.

Characteristics of research subjects, including age, sex, education, occupation, degree of smoking with Brinkman Index (BI), body mass index (BMI), degree of obstruction (GOLD), group score, frequency of exacerbations in one year, and comorbid diseases were measured and compared between the treatment and control group. Qualitative characteristic variables with categorical scale include gender, education, occupation, degree of smoking (IB), BMI, degree of obstruction, frequency of exacerbations in one-year, comorbid diseases, and group scores, while quantitative characteristic variable with numerical scale is age. The characteristics of research subjects are demonstrated in table one.

The homogeneity test results between the control and treatment group for quantitative characteristic variables, namely the age variable, obtained P=0.537, it's indicated that the control and treatment group have the same mean or average. The homogeneity test result of qualitative characteristic variables, including gender, education, occupation, IB, BMI, degree of obstruction, group scores, and comorbidities, showed that these variables had a homogeneous distribution between the control and treatment group.

Tabel 1. Characterisics of Study's Subjects

Variable	Groups		*P*
	Control (n=20)	Treatment(n=20)	
Age (mean±SD)	63.15±1.73	64.80±2.00	0.537
Gender			
Male	15 (75.0%)	17 (85.0%)	0.435
Female	5 (25.0%)	3 (15.0%)	
Education			
Elementary School	15 (75.0%)	17 (85.0%)	0.404
Junior High School	0 (0%)	1 (5.0%)	
Senior High School	1 (5.0%)	0 (0%)	
Bachelor's degree	4 (20.0%)	2 (10.0%)	
Job			
Farmer	10 (30.0%)	11 (55.0%)	0.079
Laborer	2 (10.0%)	4 (20.0%)	
Retired Civil Servants	1 (5.0%)	0 (0%)	
Trader	1 (5.0%)	0 (0%)	
Housewife	1 (5.0%)	2 (10.0%)	
Driver	1 (5.0%)	0 (0%)	
Seamstress	1 (5.0%)	0 (0%)	
Teacher	2 (10.0%)	2 (10.0%)	
Fisherman	0 (0%)	1 (5.0%)	
Carpenter	0 (0%)	1 (5.0%)	
Indonesian National Armed Forces	1 (5.0%)	0 (0%)	
Smoking Degree (IB)			
Nonsmokers	7 (35.0%)	5 (25.0%)	0.407
Light	7 (35.0%)	6 (30.0%)	
Moderate	5 (25.0%)	9 (45.0%)	
Heavy	1 (5.0%)	0 (0.0%)	
Body Mass Index (BMI)			
Underweight	6 (30.0%)	9 (45.0%)	0.232
Normal	12 (60.0%)	8 (40.0%)	
Overweight	2 (10.0%)	3 (15.0%)	
Frequency of Exacerbations			
1 time	15 (75.0%)	18 (90.0%)	0.218
2 times	5 (25.0%)	2 (10.0%)	
Degree of Obstruction			
Light	2 (10.0%)	0 (0.0%)	0.873
Moderate	0 (0%)	4 (20.0%)	
Severe	15 (75.0%)	11 (55.0%)	
Very Severe	3 (15.0%)	5 (25.0%)	
Group Score			
C	6 (30.0%)	1 (5.0%)	0.091
D	14 (70.0%)	19 (95.0%)	
Comorbid			
No Comorbidity	12 (60.0%)	7 (35.0%)	0.179
Hypertension	7 (35.0%)	13 (65.0%)	
Hypertensive heart disease (HHD)	1 (5.0%)	0 (0%)	
Neutrophil pretest (mean±SD)	612.2±1658.3 µL	981.2±2315.0 µL	0.797
MMP-9 pretest (mean±SD)	364.85±274.07 ng/mL	346.30±179.62 ng/mL	0.665
%FEV_1 pretest (mean±SD)	45.80±15.5341	39.15±11.85	0.136
CAT pretest (mean±SD)	16.20±3.750	17.10±2.936	0.540

Table 2. Absolute neutrophil count blood pretest, posttest, and changes in the control and the treatment group

Group	Absolute neutrophil count of blood			
	Pretest	Posttest	*P*	Δ (Postest-Pretest)
Treatment	5981.15±2315.08	5740.10±2090.84	0.303	114.50
Control	5612.20±1658.37	5998.45±2221.51	0.618	80.0
P	0.646	0.707	-	0.449

Table 3. The value of MMP-9 serum pretest, posttest, and changes in the control group and treatment group

Group	MMP-9 serum			
	Pretest	Posttest	*P*	Δ (Posttest-Pretest)
Treatment	346.30±179.62	293.00±126.89	0.167	-53.30±181.82
Control	364.85±274.07	402.15±225.47	0.520	-37.30±247.18
P	0.665	0.123	-	0.195

Table 4. Percentage of FEV_1 pretest, posttest, and changes in the control group and treatment group

Group	%FEV_1			
	Pretest	Postest	*P*	Δ (Postest-Pretest)
Treatment	39.5±11.85	41.60±12.72	0.122	4.45±11.13
Control	45.80±15.53	46.25±15.50	0.472	0.45±7.037
P	0.136	0.306	-	0.189

Table 5. CAT scores pretest, posttest, and changes in the control group and the treatment group

Group	CAT Score			
	Pretest	Postest	*P*	Δ (Postest-Pretest)
Treatment	17.10±2.93	16.65	0.0001	-1.950±1.23
Control	16.20±3.75	24.35	0.112	0.40±1.046
P	0.778[a]	0.069[a]	-	0.0001

The *P*>0.05. The result revealed that all qualitative characteristic variables, namely gender, education, occupation, IB, BMI, GOLD, and group scores had similar proportions between control and treatment group.

The result of calculating the absolute number of blood neutrophils before and after treatment in the control and treatment groups was described in Table 2. T-test was applied to examine two different means of absolute neutrophil count between pretest and posttest in the control group. The result obtained *P*=0.618, it's suggested an insignificant increase in absolute neutrophil count in the blood after standard therapy.

Wilcoxon test was applied to examine two different means of absolute neutrophil count between pretest and posttest in the treatment group. The result obtained *P*=0.303, it's indicated no significant decrease in absolute neutrophil count in blood post-treatment.

The result concurs this study hypothesis which stated that roflumilast decreases absolute neutrophil counts in stable COPD patients. The total number of blood neutrophils in pre and post-treatment group decreased but was not statistically significant (*P*=0.449).

The calculation result of serum MMP-9 values between pretest and posttest in the control and treatment group is described in Table 3. Wilcoxon test was used to examined two different tests mean of MMP-9 serum between pretest and posttest in the control group and obtained *P*=0.520, it's indicated no significant increase in serum MMP-9 following standard therapy. T-test was applied to examine two different means of MMP-9 serum between pretest and posttest in the treatment group and obtained *P*=0.167, it's implied no significant decrease in serum MMP-9 following the treatment.

The result also concurs this study hypothesis which stated that roflumilast decreases serum MMP-9 in stable COPD patients. The mean of serum MMP-9 between pre and post-treatment groups decreased but was not statistically significant (*P*=0.195).

The results of value of %FEV_1 value between pretest and posttest in the control and treatment group is demonstrated in Table 4. Different tests of 2 means of %FEV_1 between pretest and posttest in the control group using the Wilcoxon test obtained *P*=0.472, it's implied insignificant increase in %FEV_1

value after receiving standard therapy. The difference test of 2 mean of %FEV_1 between pretest and posttest in the treatment group using the t-test obtained *P*=0.122, it's signified that there was an increase in %FEV_1 value following the treatment though statistically insignificant.

This result also agrees the research hypothesis which stated that roflumilast leads to an increase in %FEV_1 in patients with stable COPD. The number of %FEV_1 in pre and post-treatment group increased but was not statistically significant (*P*=0.189).

The results of CAT score before and after receiving treatment in control and treatment group is detailed in Table 5. The CAT score of control group using paired t-test between pretest and posttest treatment obtained *P*=0.112, it's mean that the control group's CAT score after receiving standard therapy did not increase significantly. The treatment group used 2 different test mean t-test for paired samples and obtained *P*=0.0001, it's indicated that there was a significant decrease in CAT score in the group following the treatment. The result also agrees the study hypothesis stating that roflumilast lowers CAT score in patients with stable COPD.

DISCUSSION

The result reveals that majority of participants were male, 17 people (85.0%) in the treatment group and 15 people (75.0%) in the control group. The Indonesian Society of Respirology (ISR) in 2016 explained that the prevalence of COPD patients mainly affected men. It is associated with smoking habits and because most men work outside which increases risk of exposure to outdoor air pollution. WHO in 2017 explained that increased risk of exposure to outdoor and indoor air pollution increases the risk of COPD in men and women.[1-3] The average age (years) of subjects in the treatment group was (64.80±2.00) years old, while in the control group was (63.150±1.73) years old. Chronic Obstructive Pulmonary Disease treatment guideline by ISR in 2016 and GOLD 2019 revealed that COPD prevalence increases with age and is highest at >60 years of age. The increased risk of COPD is 2–3 times in old age. Two hypotheses are believed about the increased risk of COPD in old age: age is associated with lung structure and function changes. In old age, lung structure and function decrease, increasing susceptibility to COPD. Another factor that plays a role is the accumulation of exposure to harmful gases and particles during life, causing damage to the lungs and making it easier to develop COPD.[14,15]

The smoking status based on Brinkman index (IB) among participants was mostly moderate in the treatment group (9 people or 45.0%) and light smoker in the control group (7 people or 35.0%). Smoking is the leading cause of COPD; 85% of COPD cases are smokers. Cigarette smoke is a risk factor that plays an essential role in COPD and causes more than 90% of COPD in Western countries. Cigarette smoke is one of the leading causes of respiratory symptoms and impaired lung function. The 2016 Indonesian Lung Doctors Association explained that smoking and COPD is a dose-response relationship; the more cigarettes smoked and the longer the smoking habit, the higher the risk of suffering from COPD. Secondhand smoke with repeated exposure, exposure to environmental pollutants and exposure to particulate matter in the workplace also affects COPD. The results showed that the majority of COPD patients had moderate IB; according to Barnes in 2004 that cigarette smoke is a vital risk factor for the occurrence of COPD.[1,15–17]

Participant's nutritional status in the treatment group mostly had average values (40.0%) and 60.0%in the control group. The highest education level among participants in treatment and control groups was elementary school (SD), at 85.0% and 75.0% respectively. Most participants in the treatment group worked as farmers (5.0%) and as labourer (30.0%) in the control group. Educational and occupational status affect the development of COPD. Lower level of education causes common knowledge of the dangers of cigarette smoke or particle exposure to health. Lower education also causes lack of control and learning about the disease and its treatment. Employment history may determine an individual's socioeconomic status.

According to GOLD 2019, low socioeconomic status is a risk factor for COPD. Low socioeconomic status is associated with an increased risk of COPD, but the causative component is unclear. There is a strong relationship between the risk of developing COPD inversely related to socioeconomic status.[2,16]

The most common type of obstruction was severe, affecting 11 patients (55.0%) in the treatment group and 15 patients (75.0%) in the control group. According to GOLD 2019, Roflumilast combined with LABA either with or without inhaled corticosteroids or long-acting muscarinic antagonists is an excellent alternative management option for patients with COPD with severe to very severe obstruction associated with chronic bronchitis and a history of repeated exacerbations.[2]

The most common comorbidities was hypertension, affecting 13 people (65%) in the treatment group and 7 people (35.0%) in the control group. Airflow limitation in COPD has effects on cardiac function and air exchange, leading to systemic consequences. COPD inflammation causes the spill-over of inflammatory mediators into the systemic circulation leading to systemic manifestations. Systemic inflammation also worsens comorbid diseases, including ischemic heart disease, heart failure, osteoporosis, and depression. Comorbid conditions in COPD lead to increased hospitalization, mortality, and costs.[16,17]

The results showed no significant effect of giving roflumilast 500 mg on reducing the absolute number of neutrophils in stable COPD patients (P=0.449). However, the administration of roflumilast 500 mg descriptively was better in reducing the absolute neutrophil count in the blood compared to the control.

Neutrophils are found in the bronchial epithelium, bronchial glands, and bronchial smooth muscle, which have increased production during a stimulus. They are found in the sputum and BAL of COPD patients. The mechanism of systemic neutrophilia is the spill-over of airway neutrophils into the systemic circulation; besides, the inflammatory stimulus directly triggers an increase in the production of neutrophils by the bone marrow. COPD patients have an increased migration of neutrophils into the airways triggered by strong chemoattractants, including IL-8 and LTB4.[18,19]

Neutrophilic inflammation is the key to pathogenesis of asthma and corticosteroid-resistant COPD. In vitro studies of Hatzelmann et al., quoted from 20 in 2010, showed that roflumilast and roflumilast N-oxide could inhibit neutrophil release from interleukin 8 (CXCL8), LTB4, MMP-9, and neutrophil elastase (NE). In addition, these PDE4 inhibitors inhibit neutrophil degranulation with a PDE4-selective effect that PDE3 inhibitors or theophylline do not have.[20,21]

The study result indicates that the administration of roflumilast 500 mg reduced the absolute blood neutrophil count lower than standard therapy alone, albeit not statistically significant. The causes were not substantial; first, the included patients were stable COPD who had average absolute blood neutrophil counts resulting in an overall mean difference between treatment and control, which was not significant for various cellular inflammatory markers. Second, according to several studies that the window period for evaluating the anti-inflammatory effect of roflumilast treatment on COPD is at least 6 months; a decrease in sputum neutrophils can occur within four weeks, while the neutrophils studied are whole blood, neutrophil counts. Third, the effect of possible confounders such as smoking status and previous use of inhaled steroids on roflumilast treatment could not be assessed.[22]

The results showed no significant effect of giving roflumilast 500 mg on the decrease in serum MMP-9 of stable COPD patients (P=0.195). However, descriptively giving roflumilast 500 mg was better in lowering serum MMP-9 compared to controls. Matrix metalloproteinase-9 is a major elastolytic MMP, responsive to tissue remodelling and repair through basement membrane degradation of type IV collagen and other matrix proteins. Macrophages and neutrophils are the primary cells that secrete MMP-9, but other cells can also secrete MMP-9, including epithelial cells and lymphocytes. In COPD, MMP-9

can be used as a biomarker measured from peripheral blood to describe disease progression.[20,23]

Roflumilast can reduce ROS formation after exposure to cigarette smoke and consequently inhibit PI3Kδ activation and increase HDAC2 activity. In addition, Roflumilast inhibits NF- translocation (p65). The effect shown by roflumilast allows the reduction of inflammatory proteins, such as MMP-9.[24]

This study indicates that administration of roflumilast 500 mg can reduce serum MMP-9 lower than standard therapy alone, and the serum MMP-9 value in control patients is still higher than usual but not statistically significant. Insignificant causes include roflumilast being able to inhibit critical cells such as neutrophils and macrophages but unable to stop MMP-9 synthesis by lymphocytes, smooth muscle cells, and airway endothelial cells triggered by inflammation. Most of the population in this study had comorbid diseases, which can be a confounding factor for serum MMP-9 levels since comorbid disorders can also induce inflammation.[20]

The results showed no significant effect of giving roflumilast 500 mg on the increase in %FEV_1 in stable COPD patients (*P*=0.189). However, roflumilast 500 mg descriptively on standard therapy is better than standard therapy alone (control). Administration of roflumilast 500 mg as an anti-inflammatory led to a decrease in airway proinflammatory cytokines in stable COPD patients. Reduction of inflammation will reduce airway obstruction through the improvement of %FEV_1 from spirometry results. Mucus hypersecretion, emphysema, and small airway fibrosis lead to a decrease in the %FEV_1 value. In addition, roflumilast can increase intracellular cAMP levels, thereby inhibiting the release of ROS from neutrophils and eosinophils. This pathway leads to decreased myofibroblast levels and enhanced fibrosis repair through inhibition of epithelial-to-mesenchymal transition.[12,25,26]

This study indicates that the administration of roflumilast 500 mg increased the value of %FEV_1 better than standard therapy alone, though statistically insignificant. The reason is that the administration of roflumilast has not been able to ultimately reduce the degree of inflammation because phosphodiesterase 4 acts on macrophages (NF-), epithelial cells, and fibroblasts, while the inflammatory process of lymphocytes, endothelial cells, smooth muscle cells continue.[20]

This study showed a significant effect of giving roflumilast 500 mg on the decrease in CAT score of patients with stable COPD (*P*=0.0001). The CAT score is a score for detecting and measuring COPD symptoms on the patient's clinical health status.[2] One of the roles of phosphodiesterase 4 in COPD is as an anti-inflammatory. The anti-inflammatory effect of phosphodiesterase 4 is to suppress NF- activation by inhibiting IKK activity, thereby suppressing the production of proinflammatory cytokines. Decreased cytokine production causes a decrease in airway inflammation and mucus hypersecretion. Therefore, it will reduce airflow resistance and lessen symptoms, which can assess a reduction in the CAT score. (*P*=0.0001). The decline in CAT score in the treatment group was more significant than in the control group.

This study results and some previous research evidence that many factors influence the effect of roflumilast on the respiratory tract. The limited-time of giving roflumilast due to the limited availability of roflumilast in Indonesia as well as side effects leading to gastroinstestinal disorders can be considered for further research. In addition, it is necessary to examine sputum neutrophils to determine the decrease in the degree of airway inflammation, but sputum neutrophil examination is currently not available in Indonesia. Laboratory and radiology examinations for screening patients with impaired liver, kidney function, pneumonia, and lung cancer may support further study.

CONCLUSION

Additional therapy of roflumilast 1 x 500 mg/day for 28 days in stable COPD patients could significantly reduce the CAT score of stable COPD patients. Reducing blood absolute neutrophil levels, serum MMP-9, increased %FEV_1 although statistically insignificant, descriptively giving

roflumilast 500 mg caused a decrease in total neutrophil count, serum MMP-9, CAT score, and an increase in %FEV_1. At the same time, in the control group, there was an increase in the absolute number of blood neutrophils and serum MMP-9, so it can be concluded that the administration of roflumilast is recommended for patients in group C and D of stable COPD with high score CAT.

REFERENCES

1. Amin M, Yunus F, Antariksa B, Djajalaksana S, Wiyono WH, Sutoyo DK, et al. PPOK (Penyakit Paru Obstruktif Kronik) : diagnosis dan Penetalaksanaan. 1st ed. Jakarta: UI Press; 2016. 1–111 p.
2. Global Initiative for Chronic Obstructive Lung (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2019. p. 1–139.
3. World Health Organization. Chronic obstructive pulmonary disease (COPD) [Internet]. World Health Organization. 2017 [cited 2019 Mar 18]. Available from: www.who.int/respiratory/copd/burden
4. Tuder RM, Petrache I. Pathogenesis of chronic obstructive pulmonary disease. J Clin Invest. 2012;122(8):2749–55.
5. Fooladi AAI. Lung and Systemic Inflammation in COPD. In: Yazdani S, editor. Chronic Obstructive Pulmonary Disease. Rijeka: IntechOpen; 2012. p. Ch. 1.
6. Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. Respir Med. 2009;103(8):1231–8.
7. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(1):16–27.
8. Emami Ardestani M, Zaerin O. Role of Serum Interleukin 6, Albumin and C-Reactive Protein in COPD Patients. Tanaffos. 2015;14(2):134–40.
9. Schuliga M. NF-kappaB Signaling in Chronic Inflammatory Airway Disease. Biomolecules. 2015;5(3):1266–83.
10. Cazzola M, Page CP, Calzetta L, Matera MG. Emerging anti-inflammatory strategies for COPD. Eur Respir J. 2012;40(3):724–41.
11. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. Clin Chest Med. 2014;35(1):71–86.
12. Zhang X, Chen Y, Fan L, Ye J, Fan J, Xu X, et al. Pharmacological mechanism of roflumilast in the treatment of asthma-COPD overlap. Drug Des Devel Ther. 2018;12:2371–9.
13. Marin L, Colombo P, Bebawy M, Young PM, Traini D. Chronic obstructive pulmonary disease: patho-physiology, current methods of treatment and the potential for simvastatin in disease management. Expert Opin Drug Deliv. 2011;8(9):1205–20.
14. Fukuchi Y. The aging lung and chronic obstructive pulmonary disease: similarity and difference. Proc Am Thorac Soc. 2009;6(7):570–2.
15. Perhimpunan Dokter Paru Indonesia (PDPI). Pengaruh Pemberian Quercetin Terhadap Kadar Interleukin 8 (IL- 8) Dan Nilai COPD Assessment Test (CAT) Pasien Penyakit Paru Obstruktif Kronik (PPOK) Stabil. J Respirologi Indones. 2019;39(2):104.
16. Brashier BB, Kodgule R. Risk factors and pathophysiology of chronic obstructive pulmonary disease (COPD). J Assoc Physicians India. 2012;60 Suppl:17–21.
17. Stockley RA, Mannino D, Barnes PJ. Burden and pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2009;6(6):524–6.
18. Barnes PJ. Chronic obstructive pulmonary disease: Effects beyond the lungs. PLoS Med. 2010;7(3):1–4.
19. Bailey KL, Goraya J, Rennard SL. The Role of Systemic Inflammation in COPD. In: Nici L, ZuWallack R, editors. Chronic Obstructive Pulmonary Disease: Co-Morbidities and Systemic Consequences. Totowa, NJ: Humana Press; 2012. p. 15–30.

20. Kawamatawong T. Roles of roflumilast, a selective phosphodiesterase 4 inhibitor, in airway diseases. J Thorac Dis. 2017;9(4):1144–54.
21. Hatzelmann A, Morcillo EJ, Lungarella G, Adnot S, Sanjar S, Beume R, et al. The preclinical pharmacology of roflumilast--a selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. Pulm Pharmacol Ther. 2010;23(4):235–56.
22. Grootendorst DC, Gauw SA, Verhoosel RM, Sterk PJ, Hospers JJ, Bredenbröker D, et al. Reduction in sputum neutrophil and eosinophil numbers by the PDE4 inhibitor roflumilast in patients with COPD. Thorax. 2007;62(12):1081–7.
23. Linder R, Rönmark E, Pourazar J, Behndig A, Blomberg A, Lindberg A. Serum metalloproteinase-9 is related to COPD severity and symptoms - cross-sectional data from a population based cohort-study. Respir Res. 2015;16(1):28.
24. Milara J, Armengot M, Bañuls P, Tenor H, Beume R, Artigues E, et al. Roflumilast N-oxide, a PDE4 inhibitor, improves cilia motility and ciliated human bronchial epithelial cells compromised by cigarette smoke in vitro. Br J Pharmacol. 2012;166(8):2243–62.
25. Bateman ED, Bousquet J, Aubier M, Bredenbröker D, O'Byrne PM. Roflumilast for asthma: Efficacy findings in non-placebo-controlled comparator and dosing studies. Pulm Pharmacol Ther. 2015;35 Suppl:S11-9.
26. Yeadon M, Clarke N, Ward J. Phosphodiesterase type 4 (PDE4) inhibition: The search for effective therapy with minimal side effects. In: Progress in Respiratory Research. 2011. p. 269–78.

Surfactant Protein D (SP-D) Serum Levels In Limestone Mining Worker

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Abstract

Background: *Limestone is sedimentary rocks consisting of the minerals calcite and aragonite, often containing silica and fossils, and commonly used in building materials. Limestone mining workers are at high risk of developing pneumoconiosis. Surfactant protein D (SP-D) is part of the collectin family, and functions as the innate immune system pathogen recognition receptor (PRR). The SP-D levels are usually elevated in fibrotic lung disease. In this study, we looked for serum levels of SP-D as a marker of early pneumoconiosis in limestone worker in Indonesia.*

Method: *This study was cross-sectional observational study. Study subjects were 65 limestone workers, willing to participate in this study by signing informed consent, were interviewed, and their blood samples were collected to measure SP-D level using ELISA.*

Results: *The study subjects were dominated by male with a median age of 42 years and had low education level. The majority had worked <6 years and worked >8 hours per day, did not wear personal protective equipment, had normal weight and had smoked. The mean SP-D level among study subjects was 66.3±5.5 ng/mL, slightly higher than normal subjects. Smoking status, gender, and working hour were correlated with higher SP-D levels.*

Conclusion: *Serum SP-D levels in limestone mining workers could be used as monitoring for early screening for pneumoconiosis although it was not statistically significant. (**J Respirol Indones 2022; 42 (2): 151–5)***

Keywords: *Biomarkers; levels; limestone miners; pneumoconiosis; serum surfactant protein D (SP-D)*

Kadar Serum Protein Surfaktan-D (SP-D) Pada Pekerja Tambang Batu Kapur

Abstrak

Latar belakang: *Batu kapur (limestone) merupakan batuan sedimentasi terdiri dari mineral kalsit dan aragonit, sering mengandung silika dan fosil, dan biasa digunakan dalam bahan bangunan. Pekerja tambang batu kapur berisiko tinggi terkena pneumokoniosis. Protein Surfaktan-D (SP-D) merupakan bagian dari rumpun* collectin*, dan berfungsi sebagai reseptor pengenalan patogen dari sistem imun bawaan. Kadar SP-D sering didapatkan meningkat pada penyakit paru fibrotik. Pada penelitian ini, kami mencari kadar SP-D serum sebagai penanda awal pneumokoniosis pada pekerja tambang batu kapur di Indonesia.*

Metode: *Penelitian ini bersifat observasional potong lintang. Subjek penelitian adalah 65 pekerja tambang batu kapur yang bersedia berpatisipasi dalam penelitian ini dengan menandatangani* informed consent*, lalu diwawancarai dan diambil sampel darahnya untuk diukur kadar SP-D menggunakan metode ELISA*.

Hasil: *Subjek penelitian didominasi laki-laki dengan nilai tengah usia 42 tahun dan tingkat pendidikan rendah. Mayoritas telah bekerja <6 tahun dan waktu bekerja dalam sehari >8 jam per hari, tidak memakai alat pelindung diri, memiliki berat badan normal, dan pernah merokok. Rerata kadar SP-D pada subjek penelitian adalah 66,3±5,5 ng/mL, sedikit lebih tinggi dari subjek normal. Status merokok, jenis kelamin dan jam kerja berkorelasi dengan kadar SP-D yang lebih tinggi.*

Kesimpulan: *Kadar SP-D serum pada pekerja tambang batu kapur dapat digunakan sebagai pemantauan untuk penapisan awal pneumokoniosis meskipun secara statistik tidak bermakna. (**J Respirol Indones 2022; 42 (2): 151–5)***

Kata kunci: *penanda hayati, kadar, penambang batu kapur, pneumoconiosis, protein surfaktan-D serum*

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INTRODUCTION

Pneumoconiosis is a disorder that occurs due to the accumulation of dust in the lungs, which causes a reaction of the tissues to the dust.[1] Pneumoconiosis can be caused by a variety of mineral dust. Asbestos, silica, and coal dust are the main causes of pneumoconiosis. Classification of dust causing pneumoconiosis includes inorganic dust such as silica, asbestos, and lead; coal mine dust; as well as organic dust such as cotton. Silicosis is a pneumoconiosis caused by chronic exposure to silica dust will later manifest into progressive pulmonary fibrosis even after exposure has ceased. Limestone is a sedimentary rock consisting of the minerals calcite and aragonite, often containing silica and fossils, which are commonly used in building materials.[1]

Surfactant-D (SP-D) is part of the innate immune system, as a pattern-recognition receptor from the collectin family (collagen-containing C-type lectins), which functions to bind, opsonize and cleanse bacteria, viruses, fungi, and parasites.[2] SP-D also binds to other biological particles, allergens, genomic DNA, apoptotic materials, and particulate matter cleared from the airways.[3]

Surfactant-D is a hydrophilic molecule, its level in BAL or circulating blood[4] may be associated with the development, progression, and severity of lung diseases, such as idiopathic pulmonary fibrosis, interstitial lung diseases, ARDS, tuberculosis, and COPD.[5,6] Data regarding the diagnosis of pneumoconiosis such as ILO chest X-ray, and SP-D as possible biomarker of pneumoconiosis have been mentioned.[7–10] In this study we tried to find out whether serum SP-D levels might be a candidate of biomarker to diagnose early lung disease in limestone workers.

METHODS

This was a cross-sectional study using total sampling to determine the serum SP-D levels of limestone mining workers in Citatah Village, West Bandung Regency. Since the prevalence of pneumoconiosis in West Bandung Regency was unknown, we calculated a sample size of at least 90 subjects. The inclusion criteria in this study were limestone mining workers and were willing to participate by signing informed consent form. Exclusion criteria were subjects with history of chronic lung diseases (asthma, COPD, pulmonary TB) or history of thoracic surgery. This study was conducted with the approval of the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia. After obtaining informed consent, the subjects were interviewed and the blood samples were collected.

Serum SP-D was measured using the ELISA method at the Laboratory of Respiratory Immunology, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Indonesia-Persahabatan Hospital, Jakarta. A total of 5 mL of blood was taken from one arm of the subject, then put into a vacutainer tube, centrifuged at 3000 rpm for 10 minutes. Serum was then diluted 11 times, and serum SP-D levels were determined by ELISA method using the Human Surfactant Protein D catalog kit ELISA Number RD194059101 (BioVendor). SP-D levels were read on a microplate reader (iMark® BioRad) at 450 nm, and measured using the provided control SP-D concentration.[4–6]

RESULTS

A total of 80 subjects participated in this study, 7 subjects were excluded due to treatment for pulmonary TB, 2 subjects had chest injuries, another 2 subjects refused to participate, while 4 samples were damaged during serum preparation.

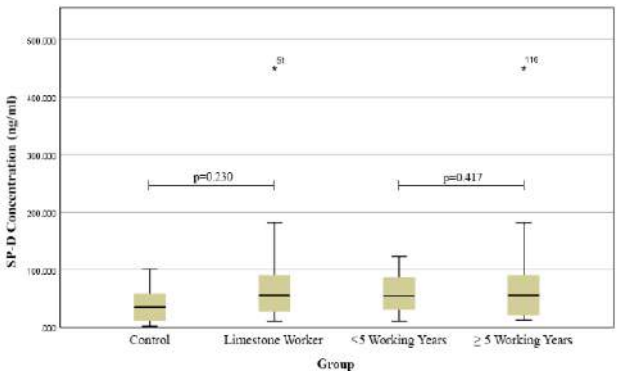

Figure 1. SP-D levels in limestone mine workers and length of work

A total of 65 participants met the study criteria, conducted a questionnaire study, and underwent blood sampling for SP-D measurement. Previously, we had measured serum SP-D concentration in ten healthy subjects, as SP-D measurements have not been carried out in Indonesia. Among these healthy subjects, the mean concentration was 40.2±5.5 ng/ml (data in file). Seventy limestone workers were mostly male (89.2%), with a median age of 42.2±16.5 (18–85) years.

Table 1. Limestone mining workers characteristics

Variables	Subjects (n=65)	
	N	%
Sex		
Male	58	89.2
Female	7	10.8
Former Education		
Elementary School	42	64.6
Junior High School	10	15.4
Senior High School	13	20.0
Smoking History		
Yes	47	72.3
No	18	27.7
Brinkman Index		
Mild	22	33.8
Moderate	23	35.4
Severe	2	3.1
Non-smoker	18	27.7
Work Duration per Day		
<8 Hours per day	13	20.0
≥8 Hours per day	52	80.0
Body Mass Index		
Underweight	4	6.2
Normal	36	55.4
Overweight	11	16.9
Obese grade I	12	18.5
Obese grade II	2	3.1
Age		
<40 years	28	43.1
≥40 Years	37	56.9
Use of PPE (mask)		
Yes	14	21.5
No	51	78.5
Length of Work		
<5 years	24	36.9
≥5 years	41	63.1

Duration of working was 7.8±1.2 hours per day and the mean length of workwas 7.8±1.2 years. Most subjects had low education level (64.6% had former education of elementary school), were smokers (72.3%) and did not use personal protective equipment/PPE (78.5%), as shown in Table 1 and Table 2. Mean serum level of SP-D in limestone workers was 66.3±5.5 ng/mL.

Table 2. Subjects' characteristics

Variables	Data distribution (mean)	Control Subjects (n=9)
Age (years)	42.2±16.5	31.±2.5
Length of work (years)	7.7±7.9	n/a
Height (cm)	162.9±8.3	166.7±10.4
Weight (kg)	60.3±12.2	74.2±19.6
BMI (kg/m^2)	21±1.4	26.7±7.1
Work duration per day (hours)	7.8±1.2	n/a
SP-D level (ng/ml)	66.3±5.5*	40.2±5.6*

Note: *P=0.230

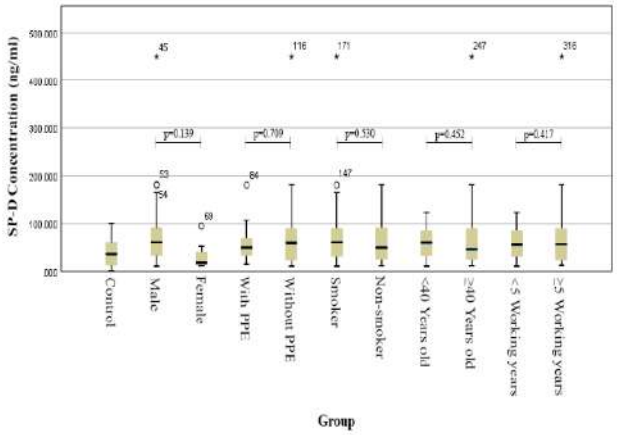

Figure 2. SP-D level in subgroup subjects

Table 3. Correlation of SP-D values with several study variables

Variables	N	SP-D±SE (ng//mL)	*P*
Sex			
Female	7	32.8±8.5	0.139
Male	58	70.4±11.7	
PPE Use			
No	51	67.9±9.5	0.709
Yes	14	60.7±11.6	
Smoking History			
No	18	45.1±10.7	0.530
Yes	47	69.0±10.1	
Working time (hour)			
<8	13	94.9±32.1	0.293
>8	52	59.2±5.5	
Age (years)			
<40	28	59.5±6.3	0.452
>40	37	71.5±12.9	
Length of Work (years)			
<5	24	57.9± 6.6	0.417
>5	41	75.6±11.8	

DISCUSSION

This was the first study to evaluate serum SP-D levels in limestone miners in Indonesia. Serum level of SP-D in limestone workers was 66.3±5.5 ng/mL, slightly higher than normal controls based on

our previous data (40.2±5.5 ng/mL) although this was not a comparative study. This might be due to the limited number of subjects and duration of occupational exposure. Honda et al stated that normal SP-D levels were 66.3±3 ng/ml in serum and 880±130 ng/ml in BAL.[4] The normal cut-off value of SP-D in Indonesian population still have to be determined.

In this study, although limestone miners were mostly male, probably due to the economic burden, female miners accounted for 10% of workers. The median age of the subjects was 42 years with the youngest of 18 years old and the oldest of 85 years old. SP-D levels in male workers (70.4±11.7 ng/mL) were higher than female workers (32.8±8.5 ng/mL), although this difference was not statistically significant.

SP-D levels were slightly higher in the older age group (>40 years) of 71.5±12.9 ng/mL. This was in accordance with our previous study which stated that there were no significant difference between serum SP-D levels and age.[7–10] In this study, 47 subjects had a history of smoking and 18 subjects had never smoked. SP-D concentrations were higher in individuals with smoking history, although not statistically significant.

Length of work >5 years showed a higher level of SP-D compared to <5 years, although it was not statistically significant. This suggested that SP-D levels might be useful in monitoring disease progression/disease detection in limestone workers. Wang et al found that SP-D levels in workers exposed to silica for 21 years were higher (47.26 ng/ml) compared to the unexposed control group (29.16 ng/ml).[7] In another study, Xue et al observed a mean SP-D of 9.9 ng/ml.[7] This data showed higher levels of SP-D in limestone workers compared to previous studies.

As an innate immune receptor, SP-D is capable of binding to silica, thereby enhancing direct phagocytosis of alveolar macrophages, regulating ROS production and alveolar macrophage clearance. Silica-exposed population had higher serum SP-D level, the reasons were thought to be caused by low clearance of alveolar macrophage, effect of silica to AT-II and Clara cells which increased level SP-D in the alveolar fluid, and leakage of SP-D level in the alveolar fluid into circulation. Chronic silica exposure will result in proliferation of AT-II cells thus increased SP-D level.[2–4]

The SP-D levels were inversely related with PPE (masks) use, this possibly due to the low adherence to PPE, although further study is needed for confirmation.[5–12] Kondo et al reported that mean serum SP-D level on 8 patients with pneumoconiosis was 121.9±92.8 ng/ml and it was not statistically different with control group (57.6±38.4 ng/mL), and several lung diseases.

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CONCLUSION

The mean SP-D level in limestone workers in West Java was 66.3±5.5ng/mL. Although this study was not designed to be compared with normal subjects, SP-D level in limestone workers were slightly higher than in normal subjects. Smoking status, male, length of work were associated with higher concentrations of SP-D in limestone workers. Serum SP-D examination might be important as a biomarker for screening of early pneumoconiosis in limestone workers.

REFERENCES

1. Susanto AD, Yunus F, Ikhsan M. Bunga rampai penyakit paru kerja dan lingkungan. 1st ed. Jakarta: Balai Penerbit FKUI; 2009. 27–38 p.
2. Sorensen GL. Surfactant Protein D in Respiratory and Non-Respiratory Diseases. Front Med. 2018;5:18.
3. Han S, Mallampalli RK. The Role of Surfactant in Lung Disease and Host Defense against Pulmonary Infections. Ann Am Thorac Soc. 2015;12(5):765–74.
4. Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, et al. Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. Am J Respir Crit Care Med. 1995;152(6 Pt 1):1860–6.
5. Kondo A, Oketani N, Maruyama M, Taguchi Y, Yamaguchi Y, Miyao H, et al. [Significance of serum surfactant protein-D (SP-D) level in patients with pulmonary tuberculosis]. Kekkaku. 1998;73(10):585–90.
6. Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, et al. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. Am J Respir Crit Care Med. 2000;162(3 Pt 1):1109–14.
7. Xue C, Wu N, Li X, Qiu M, Du X, Ye Q. Serum concentrations of Krebs von den Lungen-6, surfactant protein D, and matrix metalloproteinase-2 as diagnostic biomarkers in patients with asbestosis and silicosis: a case-control study. BMC Pulm Med. 2017;17(1):144.
8. Wang S-X, Liu P, Wei M-T, Chen L, Guo Y, Wang R-Y, et al. Roles of serum clara cell protein 16 and surfactant protein-D in the early diagnosis and progression of silicosis. J Occup Environ Med. 2007;49(8):834–9.
9. Tolinggi S, Nakoe MR, Gobel A, Sengke J, Keman S, Sudiana K, et al. Effect Inhaling of Limestone Dust Exposure on Increased Level of IL-8 Serum and Pulmonary Function Decline to Workers of Limestone Mining Industry. Int Ref J Eng Sci. 2014;3(8):66–72.
10. Zhou Y, Wang H, Xing J, Liu Y, Cui X, Guo J, et al. Expression Levels of Surfactant-Associated Proteins and Inflammation Cytokines in Serum and Bronchoalveolar Lavage Fluid Among Coal Miners. J Occup Environ Med. 2014;56(5):484–8.
11. Nalapraya WY, Susanto AD, Ikhsan M, Mansyur M, Marliana C. Proportion of Pneumoconiosis in Limestone Mining Workers In Citatah Village, West Bandung District. Respir Sci. 2021;1(2):98–115.
12. Mankar P, Mandal BB, Chatterjee D. Monitoring and Assessment of Airborne Respirable Limestone Dust and Free Silica Content in an Indian Mine. J Heal Pollut. 2019;9(23):190904.

Gastro-Esophageal Reflux Is Not a Common Cause of Chronic Cough: A Singapore Case Series

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Abstract

Background: *Gastro-esophageal reflux disease (GERD) is believed to be one of the common causes of chronic cough. There is a paucity of data on GERD-related cough (GERC) from Singapore. Our aim was to examine the prevalence, demographics and clinical features of GERC patients visiting a large teaching hospital in Singapore.*

Methods: *We did a retrospective review of patients referred to the respiratory clinics of Changi General Hospital for evaluation of chronic cough (≥6 weeks in duration) during a 6-year period (March 2010 to June 2016). All patients diagnosed with GERC were further classified into 2 groups based on the likelihood of esophageal reflux being the cause of cough, 1) Likely GERC and 2) Possible GERC. We describe the demographics, clinical characteristics and the outcomes of these patients.*

Results: *Of the 330 chronic cough patients seen over a 6 years period, 45 patients (13%) were diagnosed with GERC. Most were women (69%), the median age was 53 years and the median duration of symptoms was 26 weeks. Of all subjects, 14 patients were in the Likely group and 31 in the Possible group. Throat symptoms or signs were found in 77 % of the patients.*

Conclusions: *Amongst patients referred for cough to a specialist clinic, GERD was not seen as a common cause. Throat signs and symptoms were common and could add weight to the diagnosis of GERC. There was no particular timing for the cough with regards to day or night.* ***(J Respirol Indones 2022; 42 (2): 156-60)***

Key Words: *chronic cough; GERD; GERC; PPI*

Refluks Gastroesofagus Bukan Penyebab Umum Batuk Kronik; Seri Kasus Singapura

Abstrak

Latar Belakang: *Penyakit refluks gastroesofagus (gastro-esophageal reflux disease/GERD) diyakini sebagai salah satu penyebab umum batuk kronik. Saat ini data yang tersedia mengenai batuk terkait GERD (GERD-related cough/GERC) dari Singapura masih terbatas. Tujuan kami adalah untuk mengetahui prevalens, demografi dan gambaran klinis pasien GERC yang berobat di rumah sakit pendidikan besar di Singapura.*

Metode: *Kami melakukan tinjauan retrospektif terhadap pasien yang dirujuk ke klinik respirasi Rumah Sakit Umum Changi untuk evaluasi batuk kronik (durasi ≥6 minggu) selama 6 tahun (Maret 2010 hingga Juni 2016). Semua pasien yang didiagnosis dengan GERC selanjutnya diklasifikasikan menjadi dua kelompok berdasarkan kemungkinan refluks esofagus menjadi penyebab batuk, yaitu 1) Likely GERC dan 2) Possible GERC. Kami menggambarkan demografi, karakteristik klinis dan hasil dari pasien ini.*

Hasil: *Dari 330 pasien batuk kronik yang datang berobat selama 6 tahun, 45 pasien (13%) didiagnosis dengan GERC. Sebagian besar adalah perempuan (69%) dengan median usia 53 tahun dan median durasi gejala 26 minggu. Dari keseluruhan subjek, 14 termasuk dalam kelompok Likely dan 31 dalam kelompok Possible. Tanda atau gejala klinis pada tenggorokan ditemukan pada 77% pasien.*

Kesimpulan: *Di antara pasien yang dirujuk karena batuk di klinik spesialis, GERD tidak dilihat sebagai penyebab umum. Tanda dan gejala klinis pada tenggorokan biasa terjadi dan dapat memperkuat diagnosis GERC. Tidak ada waktu khusus untuk batuk baik pada siang maupun malam hari.* ***(J Respirol Indones 2022; 42 (2): 156-60)***

Kata Kunci: *Batuk kronik; GERD; GERC; PPI*

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INTRODUCTION

Gastro-esophageal reflux disease (GERD) has long been believed to be a common cause of chronic cough, especially in non-smokers with a normal chest radiograph. The exact prevalence is not known and estimates have varied widely from 0 to 40%.[1] Recently however, cough experts have admitted that the association is more complex than previously believed and the high prevalence of GERC reported in older studies was probably exaggerated.[2–4]

There is no good diagnostic test to prove GERC. 24-hour esophageal multichannel intraluminal impedance monitoring combined with pH-metry, which is the gold standard test for GERD, is not helpful for diagnosing GERC, even when used with symptom indices.[5] Current guidelines do not recommend using diagnostic testing for management of GERC.

There are a few factors adding to the controversy. One is the relatively high prevalence of GERD in the general population, reported to be as high as 20% in Western countries and 6–18% in Southeast Asia.[6] Therefore, even in patients with proven GERD, one cannot be certain that it is the reflux that causes the cough. Another reason is that GERD can sometimes present only with extra-esophageal symptoms (atypical GERD) and chronic cough is believed to be one of them. However, this is very difficult to prove and empiric treatment with proton pump inhibitors (PPI) in patients with non-specific cough has not shown benefit.[7,8]

A third controversial area is the possible association of cough with 'weakly acid' or 'non-acid' reflux. These patients do not respond well to PPI and may benefit better with the usage of promotility agents or Baclofen (which inhibits relaxation of the lower esophageal sphincter) to treat the cough.[9]

With all these uncertainties, probably the best way to make a clinical diagnosis of GERC is to see improvement in cough to therapy in patients who have esophageal symptoms of GERD. To our knowledge, no studies on GERC have been done in Southeast Asia and our main objective was to examine our local population.

METHODS

The study was done in Changi General Hospital, which is a 1100-bedded teaching hospital in Singapore. The study was done on patients referred to the respiratory clinics from March 1, 2010 to June 30, 2016. In our two clinics, all referrals for chronic cough were subjected to a cough questionnaire during their first visit. We used these questionnaires and review of the charts to identify and collect data on patients who met the criteria of GERC. GERC was defined as cough ≥6 weeks and at least one esophageal symptom (heartburn or acid regurgitation) as per the 2006 Montreal definition of GERD. Exclusion criteria were 1) Age <21 years; 2) Prisoners and 3) Pregnant women. We classified GERC patients into two groups based on the likelihood that reflux was the primary cause of the cough.

A subject was classified as likely GERC when there was improvement in symptoms after therapy and the absence of any other etiologies for chronic cough, while possible GERC was defined as when someone had esophageal symptoms, but there was uncertainty about whether GERD is the cause of cough. These included patients who did not respond to GERD treatment OR who defaulted follow-up visits OR were non-compliant to treatment OR had other etiologies that also could cause cough (asthma, upper airway cough syndrome, smoking). We examined the demographics and clinical characteristics of these patients. For patients diagnosed with likely GERC, we examined the medications used and the duration of treatment. We followed these patients up until June 30, 2018. The study was conducted in accordance of the amended Declaration of Helsinki. Singhealth Centralized Institutional Review Board approval was obtained prior to the commencement of the study (CIRB Reference number 2016/2421).

RESULTS

Over the 6-year period, 330 patients were referred for chronic cough (Figure 1). Among all of them, 45 (13%) were diagnosed with GERC.

Table 1. Patients' Characteristics

Parameter	N = 45	Median	Range
Age		50 years	21–79 years
Gender			
Male	15		
Female	33		
Race			
Chinese	33		
Malay	7		
Indian	5		
Others	3		
Duration of cough		26 weeks	6–30 years
Timing of the cough			
Any time	18		
Evening/night time	22		
Day time	5		
Throat symptoms or signs	35 (77%)		

Most of them (69%) were middle aged women with median age 53 years (Table 1). The median duration of cough was 26 weeks. Three patients had a past history of GERD (including one already diagnosed with GERC) and one had pre-existing hiatal hernia. We excluded three patients who had been given a diagnosis of GERD secondary to laryngopharyngeal reflux (LPR). These cases were diagnosed by ENT specialists either because their cough had responded to empiric PPI therapy or had features of LPR on endoscopy. All 45 patients received high dose PPI in the form of omeprazole 40 mg BD. All except one patient received additional domperidone (for promotility action).

Domperidone was used in varying doses (10–20 mg tds) for varying periods (2 to 6 weeks). All patients were given a pamphlet which had advice on lifestyle modifications. There was no particular timing for the cough (day or night). In all 45 cases, the cough improved or was absent altogether when sleeping. 77% of patients had associated throat symptoms or signs (itchy sore throat, globus, constant throat clearing, hoarseness, cobblestone appearance of the posterior pharynx).

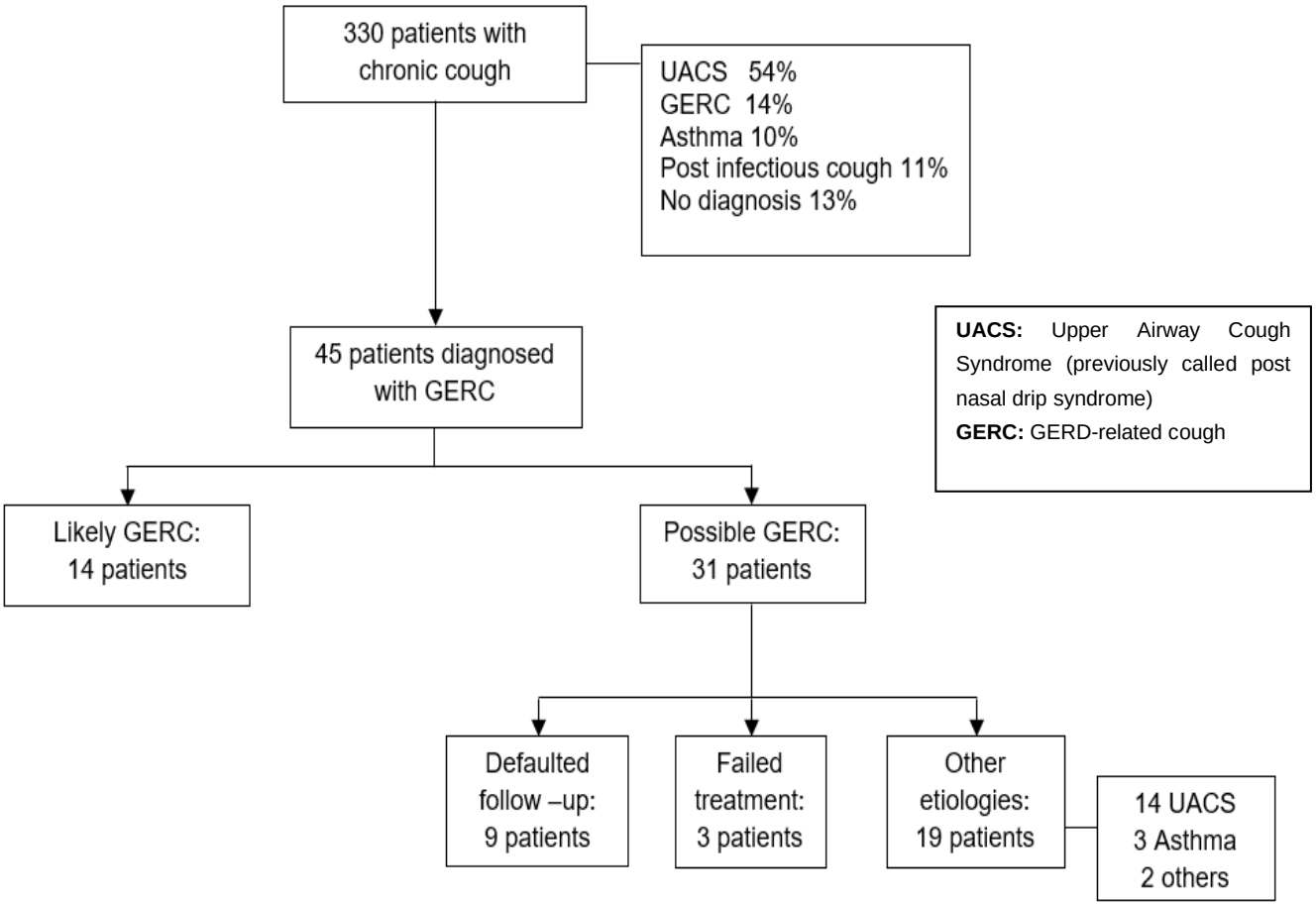

Figure 1. Flowchart of subject selection

The median duration for the next follow-up visit was 6 weeks (range 4 to 8 weeks). In the likely GERC group, most of the patients were middle-aged Chinese women with a median cough duration of one year. Throat signs/symptoms were seen in 7 patients. The median duration of initial therapy was 5.5 weeks (Table 2).

Table 2. Likely GERC

Parameter	N = 14	Median	Range
Age		48 years	28–79 years
Gender			
Male	5		
Female	9		
Race			
Chinese	9		
Malay	2		
Indian	1		
Others	2		
Duration of cough		39 weeks	6–15 years
Timing of the cough			
Any time	5		
Evening/ nighttime	5		
Day time	4		
Throat symptoms or signs	7 (50%)		
Duration of initial therapy		5.5 weeks	3–13 weeks

As much as 69% were in the possible GERC group (see Figure 1). The most common reason to be included in this group was the presence of other etiologies that could have also caused the cough.

DISCUSSION

Our study found that GERD was not a common cause (13%) of cough. If we were to infer the prevalence only from the Likely group, it would be much lower at 3%. A previous study done in Singapore on patients with unexplained chronic cough suggested that GERD was a common cause.[10] But these patients had no esophageal symptoms and were diagnosed either by response to empiric therapy or features of LPR on laryngoscopy. In the light of the recent literature, it is quite possible that the study overestimated the prevalence of GERC. In our study, most of our GERC patients were middle-aged women, which is in accordance to past studies which have shown that most patients with chronic cough belong to this demographic.[11]

Throat manifestations (sore throat, itchy throat, throat clearing, globus, cobblestoned pharynx) are well recognized in GERD and have been attributed to the refluxate bypassing the upper esophageal sphincter.[12] These were seen in majority (77%) of our patients. In our study, we did not include patients with endoscopic diagnosis of LPR, since this association is controversial.[11] Also, patients with severe cough might suffer from traumatic inflammatory changes in the larynx and there is a lack of agreement in literature about the laryngeal signs of LPR.[13] Although esophageal symptoms of GERD are mostly nocturnal, we found no time preference in our study group. This finding has also been seen in previous studies.[14]

Recent evidence suggests that PPI agents are not as efficacious as previously believed and is probably best used only in patients with esophageal symptoms.[8] Lifestyle modifications are possibly more beneficial and are recommended strongly in the 2016 CHEST guidelines.[4] Past guidelines had recommended a 3-month empiric therapy for non-specific cough to treat atypical GERD but evidence does not support such a practice and the latest guidelines advise against this practice.[3,4] Also, it may be difficult to convince patients to agree to a 3 months trial therapy. We did not prescribe empiric therapy in our patients. The median duration of initial therapy was 5.5 weeks. All cases except one were given the combination of PPI + domperidone, referred as “maximal medical therapy” by the 2016 ACCP guidelines.[4]

The rationale for adding a promotility agent like domperidone was to treat any non-acid reflux. Four patients did not respond to therapy. It is postulated that the cough might not have been due to esophageal reflux and just happened to co-exist with GERD or these were cases of GERD which were refractory to treatment. Another possibility is that the treatment period in these patients was inadequate (median 4 weeks, range 4–9 weeks). However, there is no evidence proving that the 3 months recommended by experts in the past is the optimum duration.

CONCLUSION

In conclusion, the prevalence of GERC was low in our study. Since guidelines now use stricter criteria to diagnose and treat GERC, our findings may be just a reflection of the global population. Most of our patients had associated throat signs or symptoms. Although these are not specific for GERC, they may serve as adjuncts to the diagnosis. There is no nocturnal preference for GERC as is commonly believed.

REFERENCES

1. Morice AH, Members C. The diagnosis and management of chronic cough. Eur Respir J. 2004;24(3):481–92.
2. Li X, Lin S, Wang Z, Zhang H, Sun X, Li J, et al. Gastroesophageal reflux disease and chronic cough: A possible mechanism elucidated by ambulatory pH-impedance-pressure monitoring. Neurogastroenterol Motil. 2019;31(12):e13707.
3. Morice A, Millqvist E, Stravinskaite Bieksiene K, Birring S, Dicpinigaitis P, Domingo C, et al. ERS guidelines on the diagnosis and treatment of chronic cough in adults and children. Eur Respir J. 2019;55(1):1901136.
4. Kahrilas PJ, Altman KW, Chang AB, Field SK, Harding SM, Lane AP, et al. Chronic Cough Due to Gastroesophageal Reflux in Adults: CHEST Guideline and Expert Panel Report. Chest. 2016;150(6):1341–60.
5. Vaezi MF. Chronic cough and gastroesophageal reflux disease: how do we establish a causal link? Vol. 143, Chest. United States; 2013. p. 587–9.
6. Jung H-K. Epidemiology of gastroesophageal reflux disease in Asia: a systematic review. J Neurogastroenterol Motil. 2011;17(1):14–27.
7. Chang AB, Lasserson TJ, Gaffney J, Connor FL, Garske LA. Gastro-oesophageal reflux treatment for prolonged non-specific cough in children and adults. Cochrane database Syst Rev. 2011;2011(1):CD004823.
8. Kahrilas PJ, Howden CW, Hughes N, Molloy-Bland M. Response of chronic cough to acid-suppressive therapy in patients with gastroesophageal reflux disease. Chest. 2013;143(3):605–12.
9. Xu X, Yu L, Chen Q, Lv H, Qiu Z. Diagnosis and treatment of patients with nonacid gastroesophageal reflux-induced chronic cough. J Res Med Sci. 2015;20(9):885–92.
10. Poulose V, Bin Mohd I. Prolonged cough presenting with diagnostic difficulty: a study of aetiological and clinical outcomes. Singapore Med J. 2011;52(4):267–70.
11. Smith JA, Woodcock A. Chronic Cough. N Engl J Med. 2016;375(16):1544–51.
12. Michaudet C, Malaty J. Chronic Cough: Evaluation and Management. Am Fam Physician. 2017;96(9):575–80.
13. Branski RC, Bhattacharyya N, Shapiro J. The reliability of the assessment of endoscopic laryngeal findings associated with laryngopharyngeal reflux disease. Laryngoscope. 2002;112(6):1019–24.
14. Mello CJ, Irwin RS, Curley FJ. Predictive values of the character, timing, and complications of chronic cough in diagnosing its cause. Arch Intern Med. 1996;156(9):997–1003.

Impact of Underweight on the Unsuccessful Treatment Outcome Among Adults with Drug-Resistant Tuberculosis: A Systematic Review

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Abstract

Background: *The emergence of drug-resistant (DR) strains of Mycobacterium tuberculosis has disrupted the control of tuberculosis (TB) problem worldwide. Most of DR-TB patients present with underweight problems. Prior studies showed that body mass index affects sputum conversion and could be a predictor of treatment outcome, but the causal relationship has not been established yet. This systematic review aimed to determine the impact of underweight on the unsuccessful treatment outcome among adults with DR-TB.*

Methods: *Systematic literature search and handsearching were done in four databases: Cochrane, Proquest, Pubmed, and ScienceDirect. Filtering process by using selection criteria yielded 4 eligible articles (2 prospective cohort and 2 retrospective cohort studies) for answering the clinical question. Critical appraisal was conducted by using the Newcastle-Ottawa Quality Assessment Scale (NOS).*

Results: *All four studies were assessed as having high quality according to the NOS score. The findings of all eligible studies were consistent in revealing the impact of underweight on the unsuccessful treatment outcome in DR-TB, with the relative risk: 2.194 (95% confidence interval [CI]:=1.134–4.246), 1.771 (95% CI=1.069–2.931), 3.465 (95% CI=1.114–2.712), and 4.703 (95% CI=1.709–12.947), consecutively. The number needed to harm (NNH) of 3–7 indicated the clinically meaningful harm of the exposure. This systematic review showed that one poor outcome incidence could be found with only a few underweight DR-TB patients.*

Conclusion: *Underweight increased the risk of unsuccessful treatment outcome among adults with DR-TB. Low baseline body weight (<40 kg) could be another considerable factor in anticipating the poor treatment outcome.* ***(J Respirol Indones 2022; 42 (2): 161–9)***

Keywords: *drug resistant; tuberculosis; underweight; unsuccessful treatment outcome*

Dampak *Underweight* terhadap Luaran Buruk Pengobatan pada Pasien Dewasa dengan Tuberkulosis Resisten Obat: Telaah Sistematis

Abstrak

Latar belakang: *Kemunculan strain resisten obat (RO) dari Mycobacterium tuberculosis telah menghambat upaya pengedalian masalah tuberculosis (TB) di seluruh dunia. Underweight sering dijumpai pada pasien TB-RO. Studi-studi sebelumnya menunjukkan bahwa IMT dapat memengaruhi konversi sputum dan dapat sebagai prediktor luaran pengobatan, namun hubungan kausalnya belum ditetapkan hingga saat ini. Tinjauan sistematis ini bertujuan untuk menentukan dampak underweight terhadap luaran buruk pengobatan di antara pasien dewasa dengan TB-RO.*

Metode: *Penelusuran literatur tersistematis dan handsearching dikerjakan pada empat basis data: Cochrane, Proquest, Pubmed, dan ScienceDirect. Proses penyaringan dengan menggunakan kriteria seleksi menghasilkan 4 artikel yang memenuhi syarat (2 studi kohort prospektif dan 2 studi kohort retrospektif) untuk menjawab pertanyaan klinis. Telaah kritis dilakukan dengan menggunakan Newcastle-Ottawa Quality Assessment Scale (NOS).*

Hasil: *Keempat studi dinilai memiliki kualitas tinggi berdasarkan skor NOS. Temuan dari semua studi konsisten dalam memperlihatkan dampak underweight pada luaran buruk pengobatan TB-RO, dengan risiko relatif sebagai berikut: 2,194 (interval kepercayaan [IK] 95%=1,134—4,246), 1,771 (IK 95%=1,069—2,931), 3,465 (IK 95%=1,114—2,712), dan 4,703 (IK 95%=1,709—12,947), berturut-turut. Number needed to harm (NNH) sebesar 3—7 mengindikasikan bahaya yang signifikan secara klinis dari pajanan underweight karena hanya sedikit saja pasien dengan status underweight yang dibutuhkan untuk memperoleh satu insidens tambahan luaran buruk pengobatan TB-RO.*

Kesimpulan: *Underweight meningkatkan risiko luaran pengobatan yang tidak berhasil di antara pasien dewasa dengan TB-RO. Berat badan dasar yang rendah (<40 kg) dapat menjadi faktor lain yang perlu dipertimbangkan dalam mengantisipasi luaran buruk pengobatan.* ***(J Respirol Indones 2022; 42 (2): 161–9)***

Kata kunci: *luaran pengobatan buruk; resisten obat; tuberculosis; underweight*

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INTRODUCTION

Amidst the era of COVID-19 pandemics, tuberculosis (TB) remains an unsolved public health problem with high burden of morbidity and mortality. World Health Organization (WHO) reported that in 2019, 10 million (range, 8.9–11.0 million) people globally developed TB and this number has only been declining very slowly in recent years. In 2019, TB led to 1.4 million deaths worldwide, one of the top 10 causes of death overall and the leading cause of death from a single infectious agent. In 2020, predictably the global number of TB death increased by 0.2–0.4 million due to the sharp decrease in TB case notification between January – June 2020 in four contributing countries, i.e. India, Indonesia, Philippine, and South Africa (44%).[1]

Attempts to control TB threat become more challenging due to the emergence of drug-resistant (DR) strains of *Mycobacterium tuberculosis*, which is resistant to at least rifampicin and isoniazid.[2] In 2019, an estimated 500,000 (95% uncertainty interval, 400,000–535,000) people had rifampicin-resistant TB (RR-TB), of which 78% developed DR-TB.[1] Compared to the drug-susceptible ones, TB strains with drug-resistance are more difficult to treat.[3] According to the latest WHO report, only 57% patients with DR/RR-TB showed successful treatment outcome. The treatment for DR-TB demands longer time, more drugs, higher cost and more toxic.[1] Among the top 30 high DR-TB burden countries, Indonesia ranked fifth globally in 2018.[4]

The magnitude of problem in DR-TB treatment demands an understanding of factors that can interfere with the treatment response. Previous studies demonstrated that underweight is common among DR-TB patients prior to the initiation of treatment.[5,6] Several studies also reported the potential role of underweight in predicting the outcome of DR-TB treatment.[7–9] However, the causal relationship between underweight and the unsuccessful outcome of DR-TB treatment has not yet been established. Up until now, there has been no single systematic study in this field. Therefore, this systematic review aimed to determine the impact of underweight on the unsuccessful treatment outcome among adults with DR-TB.

METHODS

This systematic review was carried out based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement. The literature search was performed from February 6–8, 2021 in four databases: Cochrane, Proquest, Pubmed, and ScienceDirect; along with manual handsearching. Keywords of “multidrug resistant”, “tuberculosis”, “underweight”, and “unsuccessful treatment outcome” as well as their synonyms and related terms were used during the search. The keyword of “adult” was omitted due to the lack of results, but it did not necessarily impact the finding and study analysis. The terms applied in each of the databases are shown in Table 1.

Literature selection was conducted based upon inclusion criteria: (1) study on human; (2) comparative study; (3) observational study; and (4) journal/ research articles/ meta-analysis/ systematic reviews. The selection was done without any time or language restriction. The exclusion criteria were: (1) not relevant to the clinical question; (2) the reported results were not complete to be analysed in this systematic review; and (3) cross-sectional studies.

The data from each yielded study was extracted into a table, compiling study citations, baseline characteristics of subjects, operational definition of the determinant and the outcome, and the relevant finding. Study citations included the principal author’s name and year of publication. Baseline characteristics of subjects in each study involved the study location, number of subjects, age, and gender. The relevant finding comprised the odds ratio (OR)/hazard ratio (HR) from each study.

Table 1. Search Queries

Database	Terms	Articles Found	Articles Used
Cochrane	multidrug resistant OR multidrug-resistant OR MDR OR multi drug resistant OR multi-drug resistant OR drug-resistant OR drug-resistant in Title Abstract Keyword AND Tuberculosis OR Tuberculoses OR kochs disease OR koch s disease OR koch disease OR mycobacterium tuberculosis infection OR lung tuberculosis OR lung tuberculoses OR pulmonary tuberculosis OR pulmonary tuberculoses in Title Abstract Keyword AND underweight OR leanness OR thinness OR body weight OR body mass index OR BMI OR low body mass index OR low bmi in Title Abstract Keyword AND treatment failure OR failure to treat OR lost to follow up OR lost to follow-up OR death OR died OR mortality OR default OR unsuccessful treatment outcome OR poor treatment outcome in Title Abstract Keyword - (Word variations have been searched)	23	0
Proquest	ab(multidrug resistant OR multidrug-resistant OR MDR OR multi drug resistant OR multi-drug resistant OR Drug-Resistant OR Drug-Resistant) AND ab(Tuberculosis OR Tuberculoses OR kochs disease OR koch s disease OR koch disease OR mycobacterium tuberculosis infection OR lung tuberculosis OR lung tuberculoses OR pulmonary tuberculosis OR pulmonary tuberculoses) AND ab(underweight OR leanness OR thinness OR body weight OR body mass index OR BMI OR low body mass index OR low bmi) AND ab(treatment failure OR failure to treat OR lost to follow up OR lost to follow-up OR death OR died OR mortality OR default OR unsuccessful treatment outcome OR poor treatment outcome)	38	2
Pubmed	(((((((((multidrug resistant[Title/Abstract]) OR (Multidrug-Resistant[Title/Abstract])) OR ("MDR[Title/Abstract])) OR (multi drug resistant[Title/Abstract])) OR (multi-drug resistant[Title/Abstract])) OR (Drug-Resistant[Title/Abstract])) OR (Drug-Resistant[Title/Abstract])) AND ((((((((((Tuberculosis[Title/Abstract]) OR (Tuberculoses[Title/Abstract])) OR (Kochs Disease[Title/Abstract])) OR (koch's disease[Title/Abstract])) OR (koch disease[Title/Abstract])) OR (mycobacterium tuberculosis infection[Title/Abstract])) OR (lung tuberculosis[Title/Abstract])) OR (pulmonary tuberculosis[Title/Abstract])) OR (pulmonary tuberculoses[Title/Abstract])) OR (lung tuberculoses[Title/Abstract]))) AND (((((((((Underweight[Title/Abstract]) OR (Leanness[Title/Abstract])) OR (Thinness[Title/Abstract])) OR (body weight[Title/Abstract])) OR (body mass index[Title/Abstract])) OR (BMI[Title/Abstract])) OR (low body mass index[Title/Abstract])) OR (low bmi[Title/Abstract]))) AND ((((((((((treatment failure[Title/Abstract]) OR (failure to treat[Title/Abstract])) OR (lost to follow up[Title/Abstract])) OR (lost to follow-up[Title/Abstract])) OR (death[Title/Abstract])) OR (died[Title/Abstract])) OR (mortality[Title/Abstract])) OR (default[Title/Abstract])) OR (unsuccessful treatment outcome[Title/Abstract])) OR (poor treatment outcome[Title/Abstract]))	67	2
ScienceDirect	(Multidrug resistant OR multi drug resistant OR MDR) AND tuberculosis AND (underweight OR low body mass index OR BMI) AND (treatment failure OR unsuccessful treatment outcome)	4	0
Handsearching	multidrug resistant AND tuberculosis AND underweight AND unsuccessful treatment outcome	6	0

The quality assessment of the studies was performed by two reviewers independently. The eligible studies were critically appraised using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies or the NOS for case-control studies. A minimum NOS score of 7 was required to define that the study was of high quality. Any discrepancy in the NOS score between reviewers were discussed to draw a conclusion. The flow of the study selection is summarized in the PRISMA flowchart, as shown in Figure 1.

RESULTS

The literature search yielded 51 studies that met the inclusion criteria and screening of duplication. From these studies, only ten articles passed the title and abstract screening.

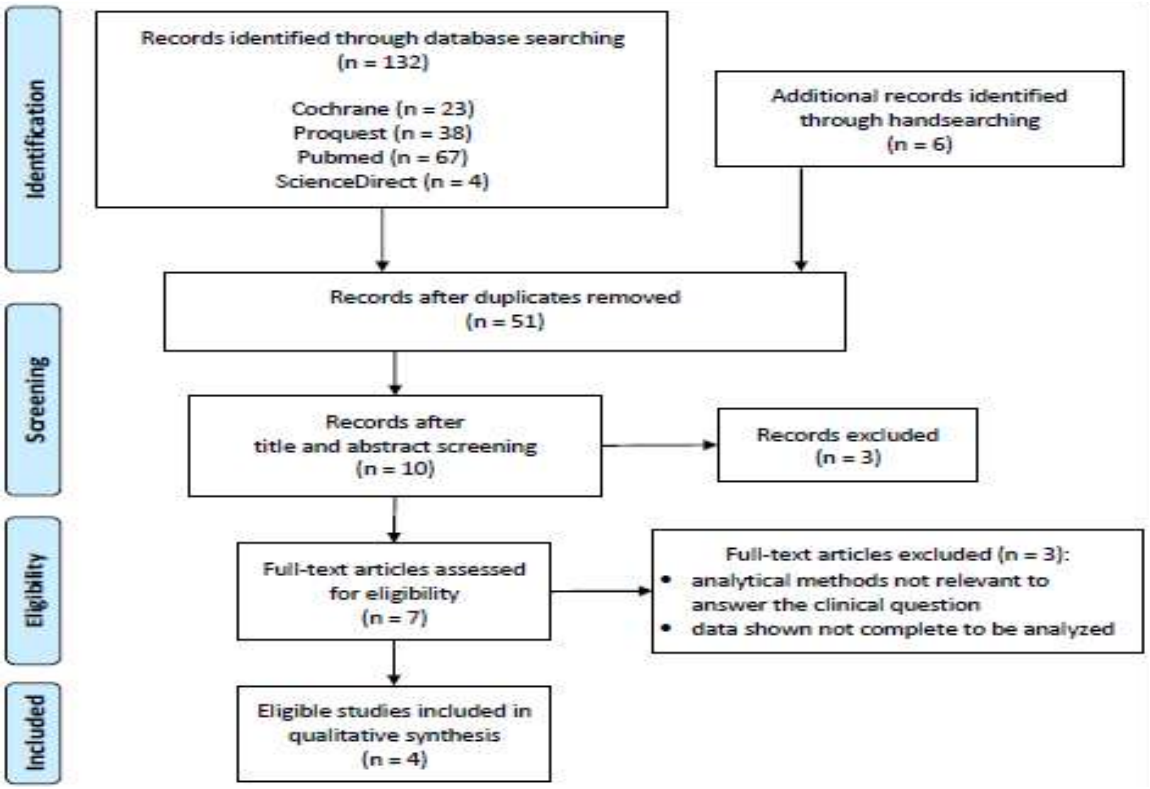

Figure 1. PRISMA flowchart of literature selection

Three of them were excluded due to irrelevant analytical method to answer the clinical questions or incomplete data. In the final analysis, there were four studies that met the eligibility criteria, i.e. Jaber et al[10], Ahmad et al[11], Khan et al[12], and Mitnick et al[13]

A summary of the baseline characteristics of each study is presented in Table 2. All eligible studies used cohort design and were categorized as level 2b based on the Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence.

Jaber et al[10] conducted a prospective cohort study aiming to evaluate the risk factors associated with DR-TB and to explore the poor TB management in Yemen. They recruited 115 adult patients with DR-TB from the four major TB centers in Yemen, namely Al-Hudaydah, Taiz, Aden, and Sana'a. The enrollment was between January 1, 2014 and December 31, 2016. In this study, an overall success rate of 77.4% was reported. The relative risk (RR) of obtaining unsuccessful treatment outcomes among DR-TB patients with baseline body weight of ≤40 kg compared with those with baseline body weight of >40 kg was 2.194 (95% confidence interval [CI]=1.134–4.246) and the number needed to harm (NNH) was 5.088 (rounded up to 6). They confirmed that a baseline weight of ≤40 kg was associated with unsuccessful treatment outcomes among adult patients with DR-TB.

A prospective cohort study by Ahmad et al[11] aimed to evaluate management and predictors of unsuccessful treatment outcomes among DR-TB patients. This study was carried out at the Programmatic Management Unit for Drug-Resistant TB of Lady Reading Hospital, Peshawar, Pakistan, between 1 January 2012 and 28 February 2013. There were 196 DR-TB patients were consecutively enrolled for treatment and followed until January 31, 2015 or any outcome has been reported. In the final analysis, there were 181 DR-TB patients included since 15 others were excluded due to unknown treatment outcome. In this study, the overall treatment success rate was 75.1%. The RR of unsuccessful treatment outcomes among DR-TB patients with baseline body weight of <40 kg compared with those with baseline body weight of ≥40 kg was 1.771 (95% CI=1.069–2.931) and the NNH was 6.797 (rounded up to 7). This study suggested a higher risk of poor treatment outcomes in DR-TB patients with low baseline body weight.

Table 2. Characteristics and Relevant Outcome of the Yielded Studies

Article	Country	Study Design	Participants (mean/median age [years old]; male %)	Definition of Underweight	Definition of Unsuccessful Treatment Outcomes	Relevant Findings
Jaber et al[10]	Yemen	Prospective cohort (follow up time: at least 20 months after culture conversion, may be extended to 24 months for patients with extensive pulmonary damage)	115 subjects (NA; 56.5)	Baseline body weight of ≤40 kg	Treatment failure, died during treatment from any cause, lost to follow-up, or not evaluated	Multivariate analysis revealed that a baseline body weight of ≤40 kg was associated with unsuccessful treatment outcomes (AOR=25.09 [95% CI=1.80–34.9], *P*=0.016).
Ahmad et al[11]	Pakistan	Prospective cohort (follow up time: a minimum of 18 months after culture conversion, defined as two consecutive negative sputum cultures taken at least 30 days apart following initial positive culture)	181 subjects (31.5±14.7; NA)	Baseline body weight of <40 kg	Death, treatment failure and default	A higher risk of poor treatment outcomes was observed in patients with low baseline body weight (OR=2.966 [95% CI=1.186–7.419], *P*=0.02).
Khan et al[12]	Pakistan	Retrospective cohort (follow up time: at least 18 months after culture conversion, defined as two consecutive negative sputum cultures taken at least 30 days apart following initial positive culture)	186 subjects (37.07±16.34; 38.7)	Baseline body weight of ≤40 kg	Died, treatment failure or lost to follow up	In multivariate analysis, a baseline weight of >40 kg had statistically significant negative associations with the outcome of death and treatment failure (OR=0.256 [95% CI=0.109–0.602], *P*=0.002). The baseline body weight of ≤40 kg showed statistically significant association with lost to follow-up in univariate analysis (OR=9.816 [95% CI=1.256–76.728], *P*=0.029), but it did not reach the level of significance in multivariate analysis (OR=6.601 [95% CI=0.809– 53.868], *P*=0.078).
Mitnick et al[13]	Peru	Retrospective cohort (median follow up time: 40 months (range, 7 to 66) after therapy which lasted at least 18 months)	75 subjects (26.8 [11.8–65.1); 49)	Low BMI (the weight in kilograms divided by the square of the height in meters), defined as <18.5 for women and <20 for men	Treatment failure or death	Low BMI was found as a risk factor associated with the time to a poor outcome with adjusted HR of 3.23 (95% CI=0.90–11.53), *P*=0.004.

Note=*AOR*: adjusted odd ratio; *HR:* hazard ratio; *BMI*: body mass index; *NA:* not available/not known/not stated; *CI*: confidence interval; *OR*: odds ratio

Table 3. Quality assessment of studies based on Newcastle-Ottawa Quality Assessment Scale

Study	Selection	Comparability	Outcome	Total
Jaber et al[10]	****	**	***	9
Ahmad et al[11]	****	**	***	9
Khan et al[12]	****	**	***	9
Mitnick et al[13]	****	**	***	9

Table 4. Critical Appraisal of Cohort Studies

Characteristics	Jaber et al[10]	Ahmad et al[11]	Khan et al[12]	Mitnick et al[13]
Year	2019	2015	2019	2003
Study Design	Prospective cohort	Prospective cohort	Retrospective cohort	Retrospective cohort
Level of Evidence	2b	2b	2b	2b
RR (95% CI)	2.194 (1.134–4.246)	1.771 (1.069–2.931)	3.465 (1.114–2.712)	4.703 (1.709–12.947)
EER	0.361	0.338	0.550	0.438
CER	0.165	0.191	0.159	0.093
RRI	1.194	0.771	2.465	3.703
ARI	0.197	0.147	0.391	0.344
NNH	5.088	6.797	2.556	2.903

Note=*ARI*: absolute risk increase; *CER*: control event risk; *CI*: confidence interval: *EER*: experimental event risk; *NNH:* number needed to harm; *RR:* relative risk; *RRI*: relative risk increase.

A retrospective cohort study by Khan et al.[12] aimed to evaluate information regarding drug resistance pattern, detailed management, treatment outcomes and factors associated with unsuccessful outcomes in DR-TB patients at Baluchistan, Pakistan. They conducted this study at the Programmatic Management of Drug Resistant TB unit in Fatimah Jinnah General and Chest Hospital (FJGCH) Quetta, Baluchistan, Pakistan. In this study, 186 DR-TB patients receiving treatment at the study site from January 1, 2012 to April 30, 2016 were enrolled and followed until the treatment outcomes were reported. The overall treatment success rate in this study was 71.6%. This study found that the RR of unsuccessful treatment outcomes in patients with baseline body weight of ≤40 kg compared with those with baseline body weight of >40 kg was 3.465 (95% CI=1.114–2.712) and the NNH was 2.556 (rounded up to 3). They concluded that baseline weight of <40 kg emerged as a risk factor for unsuccessful outcomes in DR-TB patient.

Mitnick et al.[13] carried out a retrospective study with objective of identifying risk factors associated with poor outcomes and predictors of the time to death among DR-TB patients. They included 75 patients who enrolled in the community-based therapy for DR-TB in a poor section of Lima, Peru between August 1, 1996, and November 30, 1998. In this study, the overall treatment success rate was 76.0%. This study reported that the RR of poor treatment outcomes in patients with low BMI (<18.5 kg/m^2 for women and <20 kg/m^2 for men), compared to those with normal BMI, was 4.703 (95% CI=1.709–12.947) and the NNH was 2.903 (rounded up to 3). They drew a conclusion that low BMI was one of the predictors of the time to treatment failure or death in DR-TB patients.

All four studies were assessed as having high quality according to the NOS score. Details of the quality assessment of studies are shown in Table 3. Results of the critical appraisal of the studies are provided in Table 4.

DISCUSSION

Underweight has been thought to link with the poor outcome of DR-TB treatment. In this systematic review, four included studies with a total of 557 cumulative participants were consistent in highlighting the catastrophic impact of underweight on the outcome of DR-TB treatment, including death, treatment failure, default, and loss to follow up. From the four studies, we obtained the NNH ranging from 3 to 7, indicating that we only need to have 3 to 7 DR-TB patients with pretreatment underweight status to find a new incidence of unsuccessful treatment outcome. In line with our findings, study by Putri et al[14] with retrospective cohort study of 212 DR-TB patients in Persahabatan Hospital, Indonesia found that severely underweight patients (BMI of <16 kg/m^2), compared with normal weight or overweight (BMI ≥18 kg/m^2), was associated with longer time to initial sputum conversion (adjusted HR=0.55; 95% CI=0.37–0.84) and lower probability of sputum culture conversion within 4 months (adjusted RR=0.67; 95% CI=0.54–0.83).

However, a careful interpretation of studies should be made due to the use of different definition of unsuccessful treatment outcome among studies. Death and treatment failure were included as part of

observed outcomes in all studies, whereas lost to follow-up was only reported by Jaber et al[10] and Khan et al[12]. Ahmad et al[11] was the only study to report default as one of the poor treatment outcomes. Moreover, Khan et al[12] did not consider underweight as an independent risk factor for lost to follow-up because the statistical significance was not reached in the multivariate analysis. It was considered that the rate of lost to follow-up (7.5%) in study by Khan et al[12] was lower than the range reported by prior studies (18.3–27%).[15,16]

Another vigilant interpretation should also be applied regarding the use of baseline bodyweight, instead of BMI, in studies by Jaber et al[10], Ahmad et al[11], and Khan et al[12] to classify participants' nutritional status. As demonstrated by those studies, a cutoff baseline body weight of 40 kg could be suggested as a risk factor for unsuccessful outcomes in DR-TB patients. This cutoff value was based on the logistic regression model, with a fair discrimination power according to the receiver operating characteristic (ROC) curve analysis by non-parametric method (area under curve [AUC]=0.762; 95% CI=0.676–0.847, *P*<0.001).[12] No specific upper bound of age interval was mentioned by any study in their criteria of participant selection. This implied that the cutoff value of either BMI or baseline body weight could be applicable to all adults with DR-TB.

Possible mechanisms underlying the causal relationship between underweight and the unsuccessful outcome of DR-TB treatment have been provided by previous studies. Firstly, both innate and adaptive immune responses against *Mycobacterium tuberculosis* are blunted by undernutrition.[17,18] As demonstrated by a study of 56 individuals with latent TB, low BMI was significantly associated with decreased circulating levels of proinflammatory cytokines (IFN-γ, TNF-α, IL-22, IL-1α, IL-1β, IL-6), yet increased circulating levels of type 2 and regulatory cytokines (IL-10, TGF-β, IL-5, IL-13). It revealed that low BMI caused diminished protective cytokine response and increased risk of developing active TB.[19]

Secondly, underweight patients were more likely to present with more advanced disease. Podewils et al.[5] reported that DR-TB patients who were underweight, compared to normal or overweight, were at increased risk of having a higher culture colony count (≥3 colonies) with an OR of 2.7 (95% CI=1.6–4.5; *P*<0.001) and bilateral cavitation evident on chest X-ray with an OR of 2.8 (95% CI=1.8–4.3; *P*<0.001). Thirdly, underweight was believed to be linked with low serum drug levels. In undernourishment, the anti-TB medications are poorly absorbed through the gastrointestinal tract due to morphological changes and altered enzymatic activities.[20,21] Moreover, the renal clearance of unbound drugs also increases. All these factors subsequently result in sub-therapeutic serum levels of anti-TB drugs in underweight patients who are administered fewer dose of anti-TB medications according to their weight.[20] These may lead to high incidence of death and treatment failure among underweight patients with DR-TB.[12,20]

The limitations of this systematic review came up from the use of less accurate measure of the patients' nutritional status in three included studies, which used body weight instead of BMI in defining underweight since the participants' height was not measured. Hence, the BMI of the patients was unable to be calculated. Moreover, there was heterogenous use of the control group between studies. While one of the yielded studies limited the comparator group to only consisting of patients with normal BMI, other studies included patients with no upper bound of body-weight interval. The use of heterogeneous analytical variable made this systematic review could not be proceeded to a quantitative analysis (meta-analysis).

Therefore, we recommended future studies to use BMI as a standardized measure in classifying the nutritional status of patients, so the impact of underweight on the poor treatment outcome of DR-TB could be portrayed more clearly. Overall, this is the first systematic review to affirm underweight as the risk factor for the unsuccessful treatment outcome among adults with DR-TB. The results of this systematic review could be critical for the disease management as well as prevention.

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CONCLUSION

Among adults with DR-TB, underweight increased the risk of unsuccessful treatment outcome. Only in certain circumstances where the measurement of patients' height cannot be done simultaneously, low baseline body weight (<40 kg) could also be another factor to consider in anticipating the poor treatment outcome. Therefore, assessment of nutritional status (e.g., screening for malnutrition and identification of its causes) as well as nutritional care and support (e.g., nutrition counselling, dietary program, micronutrient supplementation as indicated) for DR-TB patients may play a pivotal role in improving the outcome of DR-TB treatment.

REFERENCES

1. World Health Organization. Global tuberculosis report 2020. Geneva; 2020.
2. Iacobino A, Fattorini L, Giannoni F. Drug-resistant tuberculosis 2020: where we stand. Appl Sci. 2020;10:2153–70.
3. World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. World Health Organization. Geneva; 2019.
4. United States Agency International Development. Indonesia tuberculosis roadmap overview, fiscal year 2021. Jakarta; 2021.
5. Podewils LJ, Holtz T, Riekstina V, Skripconoka V, Zarovska E, Kirvelaite G, et al. Impact of malnutrition on clinical presentation, clinical course, and mortality in MDR-TB patients. Epidemiol Infect. 2011;139:113–20.
6. Gler MT, Guilatco R, Caoili JC, Ershova J, Cegielski P, Johnson JL. Weight gain and response to treatment for multidrug-resistant tuberculosis. Am J Trop Med Hyg. 2013;89:943–9.
7. Leimane V, Riekstina V, Holtz TH, Zarovska E, Skripconoka V, Thorpe LE, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. Lancet. 2005;365:318–26.
8. Verdecchia M, Keus K, Blankley S, Vambe D, Ssonko C, Piening T, et al. Model of care and risk factors for poor outcomes in patients on multi-drug resistant tuberculosis treatment at two facilities in eSwatini (formerly Swaziland), 2011-2013. PLoS One. 2018;13:2011–3.
9. Parmar MM, Sachdeva KS, Dewan PK, Rade K, Nair SA, Pant R, et al. Unacceptable treatment outcomes and associated factors among India's initial cohorts of multidrug-resistant tuberculosis (MDR-TB) patients under the revised national TB control programme (2007–2011): evidence leading to policy enhancement. PLoS One. 2018;13:1–24.
10. Jaber AAS, Ibrahim B. Evaluation of risk factors associated with drug-resistant tuberculosis in Yemen: data from centres with high drug resistance. BMC Infect Dis. 2019;19:464–74.
11. Ahmad N, Javaid A, Basit A, Afridi AK, Khan MA, Ahmad I, et al. Management and treatment outcomes of MDR-TB: results froma setting with high rates of drug resistance. Int J Tuberc Lung Dis. 2015;19:1109–14.
12. Khan I, Ahmad N, Khan S, Muhammad S, Ahmad Khan S, Ahmad I, et al. Evaluation of treatment outcomes and factors associated with unsuccessful outcomes in multidrug resistant tuberculosis patients in Baluchistan province of Pakistan. J Infect Public Health. 2019;12:809–15.
13. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, Alcantara F, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. N Engl J Med. 2003;348:119–28.
14. Putri FA, Burhan E, Nawas A, Soepandi PZ, Sutoyo DK, Agustin H, et al. Body mass index predictive of sputum culture conversion among MDR-TB patients in Indonesia. Int J Tuberc

Lung Dis. 2014;18:564–70.

15. Alene KA, Yi H, Viney K, McBryde ES, Yang K, Bai L, et al. Treatment outcomes of patients with multidrug-resistant and extensively drug resistant tuberculosis in Hunan Province, China. BMC Infect Dis. 2017;17:1–11.
16. Javaid A, Shaheen Z, Shafqat M, Khan AH, Ahmad N. Risk factors for high death and loss-to-follow-up rates among patients with multidrug-resistant tuberculosis at a programmatic management unit. Am J Infect Control. 2017;45:190–3.
17. Chandrasekaran P, Saravanan N, Bethunaickan R, Tripathy S. Malnutrition: modulator of immune responses in tuberculosis. Front Immunol. 2017;8:1–8.
18. Sinha P, Davis J, Saag L, Wanke C, Salgame P, Mesick J, et al. Undernutrition and tuberculosis: public health implications. J Infect Dis. 2019;219:1356–63.
19. Anuradha R, Munisankar S, Bhootra Y, Kumar NP, Dolla C, Kumaran P, et al. Coexistent malnutrition is associated with perturbations in systemic and antigen-specific cytokine responses in latent tuberculosis infection. Clin Vaccine Immunol. 2016;23:339–45.
20. Byrd RP, Mehtra JB, Roy TM. Malnutrition and pulmonary tuberculosis. Clin Infect Dis. 2002;35:634–5.
21. Maratus Sholihah, Suradi JA. The correlation of body mass index with sputum conversion time in MDR-TB patients undergoing treatment with short term regimen at H. Adam Malik Medan General Hospital. J Respirol Indones. 2019;40:225–31.

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