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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.

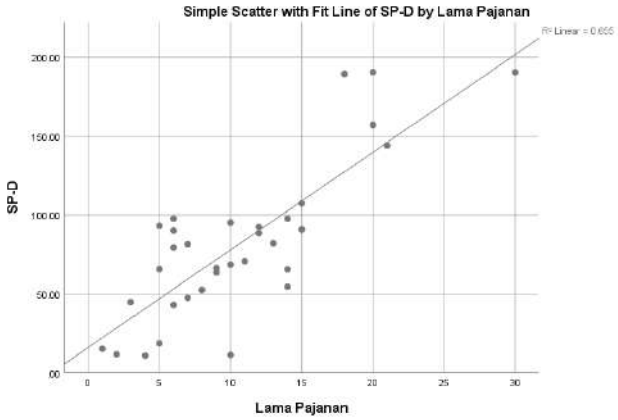

Figure 1 Scatter Plot Relationship of Exposure Time to Serum SP-D Levels

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.

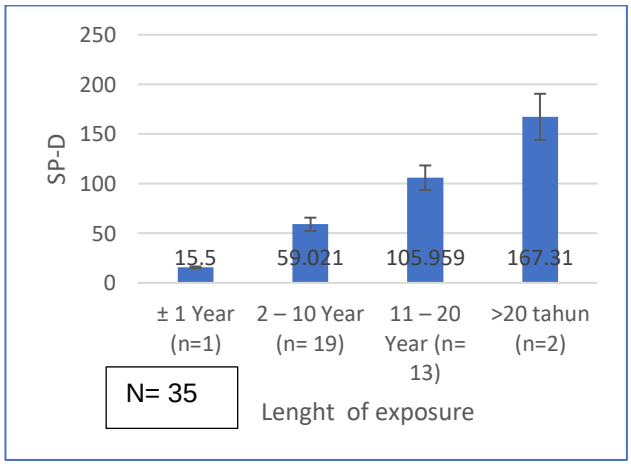

Figure 2. Graph of Effect of Long Exposure on Serum Sp-D Levels

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.

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