ENDOTOXIN EXPOSURE AND LUNG FUNCTION AMONG RICE MILLERS IN MALAYSIA

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Abstract

Introduction: The health effects of inhaling rice dust contaminated with endotoxin include respiratory problems, asthma, chronic bronchitis and chronic obstructive pulmonary disease. Thus, this comparative cross-sectional study aims to associate the concentration of endotoxin levels in inhalable rice dust and the lung function decline among rice millers.

Materials and Methods: The endotoxin level in inhalable dust for both area and personal samplings were collected using 25 mm Glass Fiber (A) filter loaded in IOM samplers connected to a pump by tygon tubing. The pump was operated at 2.0 litres/min and clipped around the breathing zone of the rice millers for eight hours. The endotoxin concentrations were analysed using Limulus Amoebocyte Lysate Chromogenic Endpoint assay at 405 nm. Lung function tests were carried out using Spirometer (Pony FX), for both the rice millers and the non-exposed groups.

Results: Findings for the mean concentration of endotoxin for areas was 0.26 (standard deviation (SD) = 0.12) EU/m^3 whereas the mean personal inhalable endotoxin level among the rice millers was 0.29 (SD = 0.15) EU/m^3. Post-shift lung function tests for FEV1/FVC measured appeared lower among rice millers (54%) compared to non-exposed workers (62%), but not statistically significant (p = 0.313). However, there were significant correlations between endotoxin concentration and post-shift LFT parameters of measured FVC, FEV1 and PEFR ($p < 0.05$).

Conclusion: Despite the low level of endotoxin exposure, proper protective measures should be applied for rice millers for long term protection.

Keywords: *Airborne exposure, Endotoxin, Lung function test, Rice miller*

Introduction

Grain dust has a complex mixture of organic and inorganic materials that may contain bacterial toxin contamination such as endotoxin. Endotoxins also known as lipopolysaccharide (LPS), are a major component of the cell wall of Gram-negative bacteria which consists of three main parts; namely Lipid A (inner core of hydrophobic segments), polysaccharide chains (the intermediate layer, the central part with hydrophilic properties) and specific O-antigen

(1,2). The toxicity of endotoxins is related to the Lipid A components which are released into the environment when the structures of the Gram-negative bacteria are destroyed (2).

Plant materials and animal faeces contaminated with endotoxins that are released into the environment can be inhaled by the workers working in related sectors such as the agricultural sector. Through inhalation, endotoxins enter the upper respiratory tract by the mucocilliary tract movement before penetrating deeper into the alveolar region which can lead to lung inflammation. The acute effects of inhaling endotoxins include symptoms such as dry cough, dyspnea, lowered lung function, and fever. Apart from that, symptoms like bronchoconstriction, headache and aching joints start to develop after several hours of endotoxin inhalation (3). Long term exposure may lead to chronic effects of respiratory disorder, asthma, chronic bronchitis and chronic obstructive pulmonary disease (COPD) (4,5,6).

Occupational health issues related to endotoxin exposure have been frequently reported (7,8,9). A recent study in Indonesia had reported a positive correlation between the personal LPS-endotoxin and a decline in lung function among rice millers (10). A previous study in Malaysia looking at respiratory symptoms and lung function among rice millers however failed to establish an association between the duration of employment and lung function impairment although this may have been as a result of their simplistic (years of exposure) method of assessing exposure (11). The study suggested that the adverse effects were associated with non-specific irritation and allergic responses, either to a protein constituent of the rice husk or to some microbiological contaminants. This current study characterizes inhalable rice dust exposure using endotoxin concentrations as exposure markers, and to relate these with lung function. It is fundamental to identify the markers associated with the irritants or allergic aetiology of respiratory symptoms, for future investigation on the mechanism of toxic responses; not only on the respiratory system but also on other target organs

Methods

Design, Location and Population of Study

This comparative cross-sectional study design was conducted at 12 rice mills in different states across Peninsular Malaysia, recruiting 79 rice millers and 51 non-exposed subjects (administrative staff) from the Health Campus, Universiti Sains Malaysia. The sampling method used was purposive sampling selecting subjects based on the inclusion criteria of those who were in the age group from 18 to 60 years old for both groups (rice millers and administrative workers). However, those who were already having asthma, chronic infections of the lungs, persistent coughs, were treated recently for any respiratory illnesses, those who were pregnant and had pets at home were excluded from this study. Their participation was voluntary as all the workers were briefed on the study the sampling procedures were explained to them and the researchers obtained the signed consent forms from the subjects or participants prior to the data collection. This research was approved by the ethical committee board of the University (USM/JEPeM/145196).

Questionnaire Distribution

This study utilised a self-administered questionnaire which took about 10 to 15 minutes to complete and consisted of six parts; (i) personal details, (ii) job description, (iii) compliance with personal protective equipment, (iv) work safety practices, (v) respiratory symptoms, (vi) carrying of dust from workplaces to homes. The questions in part (v) gathering information on respiratory symptoms were based on the British Medical Research Council Questionnaire (1966) (12).

Airborne Inhalable Dust Collection

The inhalable rice dust was collected using the filter loaded sampling head (Institute of Occupational Medicine (IOM) sampler). The sampling head was loaded with 25 mm in diameter of Glass Microfiber Filter (A) (Zefon International GFA, United States) and attached via tygon tubing to a sampling pump

(GillianAir; brand: Sensidyne; United States) which operated at two litres per minute. The IOM Sampler was clipped around the breathing zone of the workers. The pump attached to the workers' belts was switched on when the workers started their work and was switched off at the end of the day. The sampling duration was approximately for eight hours. The time at which the sampler was started and ended, together with the initial and final flowrate were recorded. The field blank filter was handled similarly to the sample filters but without switching on the pump. The sampling procedure was followed according to the general methods for sampling (MDHS 14/4) of total inhalable dust (13). Whereas for area (fixed-point) sampling, the filter-loaded samplers were placed approximately at a two metre height (above head height) at the processing, storage and drying areas in the rice mills.

Filter Sample Transportation and Storage

After the sampling was completed, the filters were put in individual cassettes. Samples were stored at -20°C in two ml of 0.1% phosphate buffer saline and Tween 20, (PBS-T) in 15 ml centrifuge tube before extraction.

Extraction of Filter Dust

The filter samples were thawed and let to equilibrate at room temperature. The samples were rocked on an orbital shaker rocking apparatus for one hour. After extraction, the suspension was centrifuged; at 1000xg for ten minutes at room temperature. The supernatant was taken and stored in 1.5 ml Micro Centrifuge tubes. It was then carefully labelled, and stored at -20°C until the analysis process.

Endotoxin Analysis

The supernatants were analysed using Limulus Amoebocyte Lysate (LAL) (ACCA Pyrochrome, USA). The dilutions of the standards used in this analysis were 10 EU/ml, 1.0 EU/ml, 0.5 EU/ml, 0.25 EU/ml and 0.1 EU/ml. The thermomixer, (Eppendorf, USA) was set at 37°C, and then 50 µl of samples, standards and blanks were dispensed into the 96-well microplate. After that, 50 µl of Pyrochrome lysate was added into each of the wells. The solutions were mixed in the incubator block for ten seconds. The reaction was stopped by adding 25 µl of 50% acetic acid into each well and was read at 405 nm. A standard curve of optical density (y) against the concentration of standard endotoxin (x) was plotted. The concentrations of the samples were calculated using the equation gained.

Lung Function Test (LFT)

The LFT was performed using the spirometer (Pony FX, Italy) after the work shift. Before performing the LFT, the details of the subjects were recorded into the spirometer such as their height (cm), weight (kg), smoking status, ethnicity and date of birth. The subjects were briefed about the tests and was explained on how it would be carried out. At a standing position with a clipped nose, they were asked to breath normally for two times then to take a deep breath at the third time, and were asked to exhale within six seconds until they felt there was no more air in their lungs. The procedures were repeated for three times and the best results were recorded.

Statistical Analysis

The results were analysed using the Statistical Packages for Social Sciences, (SPSS) software Version 24 at significant level of $p < 0.05$. The descriptive data were presented in mean, standard deviation (SD), median and interquartile range (IQR). The inferential analyses used were the Mann Whitney, Spearman's Rho Correlation, Simple Linear Regression and Multiple Linear Regression.

Results

Sociodemographic Data

A total of 130 subject workers (the participants for the study) were recruited (rice millers, n=79 and non-exposed, n=51). The rice mills were dominated by male workers (98.7%; n=78), and only one female worker was recruited for this study. For the non-exposed workers, a majority of the respondents were female (62.7%, n=32) compared to males (37.3%, n=19). The age group for the population ranged from 18 years old up to more than 60 years old. For race, the majority of the subjects were Malays for both the groups (exposed: 75.9%, n=60 and non-exposed: 100%, n=51). In terms of their smoking status, a majority of the exposed workers were smokers (65.8%, n= 52) and for the non-exposed group, most of them were non-smokers (90.2%, n=46). Apart from that, for working periods of the subjects, a majority of the subjects reported to have less than ten years working experience in the premises (exposed: 74.3%, n=55; non-exposed: 70.6%, n=36). The sociodemographic data of the subjects is shown in Table 1.

Table 1: Sociodemographic of the participants

Sociodemographic	Frequency (%)		
	Exposed n=79	**Non-Exposed n=51**	**Total N=130**
Gender			
Male	78 (98.7)	19 (37.3)	97 (74.6)
Female	1 (1.3)	32 (62.7)	33 (25.4)
Age (Years)			
≤30 years	29 (36.7)	9 (17.6)	38 (29.2)
>30 years	50 (63.3)	42 (82.4)	92 (70.8)
Race			
Malay	60 (75.9)	51 (100)	111 (85.4)
Indian	1 (1.3)	0 (0.0)	1 (0.8)
Others	18 (22.8)	0 (0.0)	18 (13.8)
Smoking status			
Non-Smoker	16 (20.3)	46 (90.2)	62 (47.7)
Ex-Smoker	11 (13.9)	4 (7.8)	15 (11.5)
Smoker	52 (65.8)	1 (2.0)	53 (40.8)
Working Period (Years)*			
≤10 years	55 (74.3)	36 (70.6)	91 (72.8)
>10 years	19 (25.7)	15 (29.4)	34 (27.2)

*Missing data among exposed = 5

Airborne Endotoxin Concentration

The concentration of airborne endotoxin was calculated from a series of endotoxin dilutions per described previously. The values of the endotoxin concentration, EU/ml were then converted to EU/m^3 by dividing with the volume of air sampled (14,15). The limit of detection (LOD) was determined according to the manufacturer's kits LOD of 0.005 EU/ml. All the concentration of the samples was detected above the LOD. The results were reported as Endotoxin Unit per meter cube (EU/m^3).

Area Endotoxin Concentration

A total of 28 designated areas of rice mills in the Peninsular of Malaysia were characterised for endotoxin concentration level. The mean concentration of endotoxin for the total areas was 0.26 ± 0.12 EU/m^3 and median was 0.32 (Interquartile Range, IQR: 0.13 - 0.37) EU/m^3. The areas were categorised into three different sections; drying, processing and storage. Among all the three sections, the processing area showed the highest detectable endotoxin, mean = 0.28 ± 0.12 EU/m^3; median = 0.34 (IQR: 0.14 - 0.37) EU/m^3.

Personal Endotoxin Concentration

The personal exposure of inhalable endotoxin was measured among the rice millers; the mean was 0.29 ± 0.15 EU/m^3 and median concentration was 0.33 (IQR: 0.14 - 0.34) EU/m^3. The rice millers at Factory C appeared to have the highest mean concentration of endotoxin which was 0.48 ± 0.24 EU/m^3; median = 0.38 (0.37-0.53) EU/m^3. The lowest detectable mean endotoxin concentration was among the rice millers at Factory H and J (0.11 ± 0.01 EU/m^3). The data is shown in Table2.

Table 2: Area and personal airborne endotoxin concentration

Airborne Exposure	n	Concentration of Endotoxin in EU/m^3			
		Mean	SD	Median	IQR
Work Area					
Drying	4	0.24	0.14	0.23	0.12 - 0.37
Processing	13	0.28	0.12	0.34	0.14 - 0.37
Storage	11	0.25	0.13	0.32	0.11 - 0.37
Total	28	0.26	0.12	0.32	0.13 - 0.37
Personal					
Factory A	9	0.34	0.03	0.34	0.33 - 0.35
Factory B	8	0.34	0.01	0.34	0.33 - 0.35
Factory C	6	0.48	0.24	0.38	0.37 - 0.53
Factory D	12	0.33	0.01	0.33	0.31 - 0.34
Factory E	5	0.33	0.01	0.33	0.33 - 0.36
Factory F	7	0.41	0.15	0.33	0.33 - 0.40
Factory G	4	0.33	0.01	0.33	0.32 - 0.34
Factory H	8	0.13	0.01	0.13	0.32 - 0.03
Factory I	2	0.11	0.01	0.11	0.11
Factory J	2	0.11	0.01	0.11	0.11
Factory K	13	0.19	0.14	0.13	0.12 - 0.18
Factory L	3	0.30	0.27	0.17	0.12
Total	79	0.29	0.15	0.33	0.14 - 0.34

Lung Function Test

Table 3 summarises the comparison of the lung function parameters for rice millers and their control group. The parameter of post-shift FEV1/FVC measured showed a lower value among the exposed workers compared to the non-exposed workers with a median of 54 (IQR: 40 - 79) versus 62 (IQR: 50 - 76), but it was not statistically significant. However, there was a significant different in the predicted FEV1/FVC between exposed and non-exposed, p = 0.03.

Table 3: Comparison between lung function between rice millers and non-exposed workers

Post-shift LFT Parameters	Median (IQR)		p-value
	Rice millers (n=79)	Non-Exposed Workers (n= 51)	
FVC (L)			
Measured	2.99 (2.30 – 3.35)	2.50 (2.05 – 2.86)	0.001*
Predicted	3.95 (3.54 – 4.28)	3.23 (2.95 – 3.69)	0.001*
FEV1 (L)			
Measured	1.64 (1.10 – 2.33)	1.54 (1.09 – 1.82)	0.195
Predicted	3.32 (2.96 – 3.64)	2.75 (2.55 – 3.09)	0.001*
FEV1/FVC (%)			
Measured	54 (40 - 79)	62 (50 - 76)	0.313
Predicted	81 (79 - 82)	81 (80 - 83)	0.037*
PEFR (L)			
Measured	2.04 (1.09 – 3.31)	1.85 (1.31 – 2.28)	0.664
Predicted	8.89 (8.15 – 9.14)	6.79 (6.22 – 8.27)	0.001*

*Significant difference at p<0.05; Statistical test – Mann Whitney, LFT – lung function test, L-litre, FVC- Force Vital Capacity, FEV1- Force Expiratory Volume in first second, PEFR- Peak Expiratory Flow Rate.

Correlation between Personal Inhalable Endotoxin and Lung Function

The correlation between personal inhalable endotoxin with lung function is shown in Table 4. In the post-shift measurements, the parameters showed a significant correlation, p<0.05 for FVC measured, FEV1 measured, FEV1/FVC predicted and PEFR measured. These variables were linearly related, nevertheless none of the parameters showed a strong correlation between the concentration of endotoxin with the lung function tests.

Table 4: Correlations between post-shift LFT and personal endotoxin concentration

Post-shift LFT		Endotoxin Concentration (EU/m³)
FVC measured	p-value	0.002*
	r	0.345
FVC predicted	p-value	0.300
	r	0.096
FEV1 measured	p-value	0.003*
	r	0.332
FEV1 predicted	p-value	0.252
	r	0.131
FEV1/FVC measured	p-value	0.058
	r	0.214
FEV1/FVC predicted	p-value	0.049*
	r	0.222
PEFR measured	p-value	0.006*
	r	0.306
PEFR predicted	p-value	0.107
	r	0.183

*Significant difference at p<0.05; Statistical test – Spearman's rho Correlation, LFT – lung function test, FVC- Force Vital Capacity, FEV1- Force Expiratory Volume in first second, PEFR- Peak Expiratory Flow Rate.

A further relationship between the significantly correlated parameters of lung function and the concentration in personal endotoxins was tested using the Simple Linear Regression as shown in Table 5. The information provided showed that about 6.0%-11.0% concentration in personal endotoxin affects the lung function of the workers. This contribution was quite low. This suggests that other factors might be contributing to the decline in the lung function.

Table 5: Relationship between personal endotoxin concentrations with decline in lung function

Post-Shift LFT		Endotoxin Concentration (EU/m³)
FVC measured	R	0.279
	R^2	0.078
	p-value	0.017*
	Constant	2.462
	β	0.279
FEV1 measured	R	0.333
	R^2	0.111
	p-value	0.004*
	Constant	1.156
	β	0.333
FEV1/FVC predicted	R	0.119
	R^2	0.014
	p-value	0.315
	Constant	79.71
	β	0.119
PEFR measured	R	0.315
	R^2	0.099
	p-value	0.007*
	Constant	1.145
	β	0.315

*Significant difference at p<0.05; Statistical test – Simple Linear Regression, FVC- Force Vital Capacity, FEV1- Force Expiratory Volume in first second, PEFR- Peak Expiratory Flow Rate

Association between other Confounding Factors and Lung Function

The decline in lung function contributable by potentially confounding variables is presented in Table 6. There was a linear relationship between the factors of personal endotoxin concentration (β_1 = 1.769, p=0.006) and the duration of employment (β_2 = -0.037, p=0.006) that contributed to about 35.5% to the reading of post-FVC measured.

The relationship between post–FVC measured and the duration of employment was an inverse relationship which implied that any increase in the duration of employment would result in a decline of lung function. The equation for the reading of post-FVC measured can be summarised as follows:

$$Reading\ of\ post-FVC\ measured$$
$$= 3.147$$
$$+ 1.769\ (Concentration\ \ of\ personal\ endotoxin)$$
$$- 0.0037\ (duration\ of\ employment)$$

Table 6: Confounding factors with LFT

Dependent Variable (y)	Independent Variables (x)	Coefficient Value (β)	p-value
Post-FVC measured	Constant	3.147	0.001
	Endotoxin (EU/m^3)	1.769	0.006*
	Age (years)	-0.010	0.299
	Duration of Employment (years)	-0.037	0.006*
	Smoking Status	-0.095	0.678
	Smoking Index (rolls/day)	-0.007	0.649
R^2	0.355		
Adjusted R^2	0.299		
Post-FEV1/FVC measured	Constant	68.956	0.001
	Conc. Endotoxin (EU/m^3)	24.278	0.238
	Age (years)	-0.308	0.318
	Duration of Employment (years)	0.830	0.058
	Smoking Status	-10.530	0.167
	Smoking Index (rolls/day)	-0.127	0.813
R^2	0.167		
Adjusted R^2	0.095		

*Significant difference at $p<0.05$; Statistical test – Multiple Linear Regression, mode: Enter, FVC-Force Vital Capacity, FEV1- Force Expiratory Volume in first second, PEFR- Peak Expiratory Flow Rate

Discussion

A study on measuring airborne personal exposure to endotoxin among rice millers in Malaysia has not been previously reported. Therefore, the exposure limit for endotoxins was referred to a Dutch Expert Committee on Occupational Exposure (DECOS) in the Netherlands. DECOS recommended that the exposure limit for endotoxins is 90 EU/m^3 or 0.009µg/m^3 (eight-hour time-weighted average) (3). This current study has shown that both the area and personal exposure to endotoxins among rice millers in Malaysia were approximately 350 times below the recommended limit. In comparing to previous studies conducted in Indonesia, the mean endotoxin containing rice dust levels reported were 232.22 EU/m^3 (10) and 91.1 EU/m^3 (16). In another broad range study of endotoxins involving the agricultural sector reported a mean level endotoxin of 1110 EU/m^3 (17) in the rice hulling plant and 4460 EU/m^3 (14) among the textile processing workers. The concentration of endotoxins among the Malaysian population appeared to be very low as compared to other studies. Aside from intensity, it is well known that factors such as duration of exposure also plays an important role in the exacerbations of health effects. Moreover, inhalations to LPS even at a dose of 15 µg in healthy human subjects may lead to systemic effects (18).

In this study, a variation in the mean and median concentration of endotoxins was measured among the rice millers across the 12 factories which can be explained may have been due to the seasonal factor of paddy harvesting in Malaysia. The sampling process was carried out during the paddy season at Factory A to G that may have resulted in a higher mean concentration of airborne endotoxins detected, as compared to Factory H to L. However, such a difference was not statistically analysed.

In characterising the endotoxins exposure based on work activities, the processing work section had the highest detectable airborne endotoxins as compared to the drying and storage sections. Since the processing section usually occupies the largest area in rice mill factories involving a major part of the milling process, cleaning, polishing and grading of the rice; hence this could explain the higher mean level. During processing, the rice millers are more likely to be exposed to the rice dust especially among those who are in charge of the milling process. However, the limitation in this current study was that the rice millers could not be categorised into different work processes or tasks as they were usually involved in all areas due to their rotation-based task; hence, the homogenous exposure. Another limitation was that the total inhalable dust was not measured in this study; yet, the endotoxin concentration serves as the marker of exposure. As several reports suggested, exposure to rice dust affects the lung function as well as increases the respiratory symptoms among rice millers (19) and leads to respiratory morbidity (20). Although there were no significant differences in the lung function test (LFT) between the rice millers and the non-exposed groups, the median FEV1/FVC parameters of rice millers showed a decline of about 8% compared to the non-exposed workers.

Apart from that, the correlation between the concentration of personal endotoxin with the post-shift LFT measured were significant ($p < 0.05$) even though the results showed a weak correlation, $r < 0.5$. Theoretically, the relationship between the concentration in personal endotoxins and lung function expected to be inversely correlated. Higher concentrations of endotoxins lead to a decline in lung function. Previous studies among dairy workers in California also found a higher endotoxin exposure being associated with a reduction in lung function but only in taller workers (21).

Further statistical analysis was conducted to identify the possible confounding factors that contribute to the decline in lung function. Among all the predictors, a concentration in personal endotoxins and the duration of employment appeared to be significant, $p < 0.05$ for post-FVC measured. For post-FEV1/FVC measured, none of the parameters were significant ($p < 0.05$). However, the smoking status of the subjects resulted in a decline in their lung function. Previous studies among cotton workers found that a greater difference in ΔFEV1 among smokers compared to non-smokers explained that smoking caused a slight increase in the airway reactivity although it was not really significant (22). Smokers in dusty workplaces were prone to result in respiratory disorders compared to those who were non-smokers but in the same environment (16). For normal and healthy people who never smoked, it was expected that FVC could decrease the volume of their lungs of about 0.2 litres per decade (23).

Conclusion/Recommendation

The current study has established that the endotoxin level detectable among rice millers in Malaysia was very low compared to the recommended exposure limit, indicating a safe level. However, despite the low detectable level, a proper protective measure should be recommended in order to protect the rice millers and avoid them from inhaling rice dust contaminated with endotoxins. This is due to the observation that the workers only covered their face by wrapping their face with old clothes while working. Ultimately, this can serve as a one-step forward for a long-term precaution since even after the cessation of exposure, the effect to occupational endotoxins can still appear to continue.

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