EXPOSURE OF AFLATOXIN B1 (AFB1) IN INHALABLE DUST AND ITS RESPIRATORY EFFECTS AMONG RICE MILLERS

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Abstract

Introduction: Optimal humidity, temperature, improper handling and storage of rice will increase the likeliness of aflatoxin growth in the air. The most common and carcinogenic aflatoxin is Aflatoxin B1 (AFB1) that may cause lung cancer if inhaled. This study aims to associate the exposure of AFB1 in inhalable dust and its respiratory effects among rice millers.

Materials and Methods: This cross-sectional study utilised the purposive sampling method and recruited 76 rice millers as exposed subjects and 48 office workers as the control group. The total inhalable dust was collected using the filter-loaded air samplers for an eight working hours' exposure. The subjects' hands were swabbed with cotton pads wetted with 0.5 ml Phosphate buffered Saline Tween-20 solution post shift. The collected samples were analysed for AFB1 by using the ELISA kits. The questionnaire gathering information on sociodemographic, work data and respiratory symptoms were completed. The lung function test was performed for the pre- and post-shifts.

Results: The mean airborne AFB1 at the rice mill area and personal exposure were 2.22 ng/m^3 ± 0.07 and 0.25 ng/m^3 ± 0.24, respectively. The mean contamination level of AFB1 on hands was 0.25 ng/ml detected on two rice millers (2.3%) while non-detectable in non-exposed workers. The most complained symptoms among rice millers were wheezing and breathlessness (n = 6, 9.2%). There was a significant difference in the mean forced expiration volume (FEV1) for pre- and post-shifts between rice millers and the non-exposed workers, but no significant correlation between the mean AFB1 concentration and lung function. Age and work factors were confounders in lung function.

Conclusion: Despite no association being established in this study, the promotion of wearing suitable personal protective equipment (PPE) is highly recommended to prevent cumulative exposure among the rice millers.

Keywords: *Aflatoxin B1, Airborne, Rice millers, Respiratory effects*

Introduction

Among the five common mycotoxins, i.e. deoxynivalenol, zearalenone, ochratoxin, fumonisin and aflatoxin, researches on aflatoxin are widely studied due to their silent threat (1). In the various aflatoxins, Aflatoxin B1 (AFB1) is categorised as Class I carcinogen by the International Agency for Research on Cancer (IARC) (2,3).

AFB1 is produced mainly by *Aspergillus flavus*, the main fungus that can colonise airways and has the potential to cause lung cancer. It reproduces via small conidial spores capable of passing through the mucociliary clearance mechanism (4). In healthy people, inhalation of conidia might be cleared by the mucociliary mechanism but in susceptible people such as children and elderly, infection may arise (5). A previous study conducted in testing exhaled breath condensate and bronchial brushing of lung cancer patients has found that about one third of lung cancer patients were positive with fungus such as *Aspergillus niger* and *Aspergillus ochraceus* (6). The mould species were hypothesised to have released carcinogenic mycotoxins that could contribute to lung cancer progression (6).

Rice is the main staple food and it has become an important agricultural commodity in Malaysia. Nonetheless, a study on local rice found that it contained AFB1 of 1.75 ng/m^3 (7). However, a separate study detected the level of aflatoxin to be ranging from 0.19 to 3.96 ng/g (8) indicating that attention should be given to aflatoxin contamination. Note that the permissible exposure limit (PEL) set by the European Union for AFB1 in grains is at 2 ng/m^3 (9). Previous studies have established the inhalable limit of concentration of no toxicologic concern (CoNTC) to be 30 ng/m^3 (9, 10).

It is predictable that the contamination of crops by AFB1 could occur before or after harvesting, during handling and during the storage periods (2,11). Studies have shown that poultry production and rice millers who are in indirect contact with grain dust have often been exposed to AFB1 (12,13). A study among food-grain workers in India has found about 30.0% among 46 rice millers were positive with aflatoxins in broncheoalveolar lavage (BAL) (14). However, these researchers studied the biological samples of the subjects that could cause privacy or ethical issues, especially during sampling. Therefore, this current study applied a non-invasive physical sampling to determine the airborne and dermal exposure level of AFB1 and associate them with the respiratory effects among rice millers in Malaysia. This is important for the future investigation on the mechanism of the toxic responses whereby the findings on airborne and dermal exposure levels of AFB1 in rice dust will help in identifying and recommending suitable control measures to reduce the rice dust exposure at workplaces in Malaysia.

Methods

Study Design and the Recruitment of Participants

This cross-sectional study utilised the purposive sampling method and recruited a total of 124 subjects consisting of 76 rice millers and 48 administrative staff of Universiti Sains Malaysia Health Campus as the control group. The exposed subjects were identified from 12 rice mills located in Kelantan, Perak, Kedah and Perlis who voluntarily agreed to participate in this study. Ethical approval was obtained from the University's human ethical board (reference: USM/JEPeM/14090312). Prior to the sampling, the subjects had signed the informed consent forms.

Area and Personal Airborne AFB1 Measurement

Health and Safety Executive's Methods for the Determination of Hazardous Substances (MDHS 14/4) on general methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols (15) was used as a guideline to assess the AFB1 exposure involving the measurement of area and the personal dust airborne levels. The sampling train set up for this procedure consisted of the Institute of Medicine (IOM) samplers loaded with 25 mm Glass Microfiber Filter (GMF) connected to an air pump (GilAir Plus Personal

Air Sampling Pump, Sensidyne) by tygon tubing, set at 2.0 litres/min. The sampling trains for the area monitoring were placed at three sampling sites namely paddy intake, processing and storage at the height between 75 – 120 cm from the floor, and not obstructing the workers especially during emergencies (16). The personal airborne assessment was done by attaching the sampling train of the air pump in a waist bag for the sake of the workers' convenience while the IOM sampler was clipped onto their collars within the breathing zone (not exceeding 30 cm from the nose and mouth area).

After eight hours of sampling, the filter with the collected inhalable dust was removed from the IOM sampling head using a tweezer into a screw-capped 15 ml tube and preserved in 2 ml of 0.1% Phosphate Buffered Saline Solution Tween-20 (PBST), pH 7.4 (17).

Dermal AFB1 Measurement

The collection of dermal wipe samples was conducted on both hands among the rice millers and the non-exposed workers after their work shift. Sterilised cotton pads (Premier, NTPM: Malaysia) wetted with 0.5 ml of 0.1% PBST (pH 7.4) were used to swab the palms of both hands beginning from the heel of the hand up to the fingertips and finger sides. The cotton pads were then kept in a 15 ml test tube with screw-cap and well labelled. Three ml of 0.1% PBST, pH 7.4 was added into the test tube to preserve the sample (17).

Sample Transportation and Storage

The collected airborne filter samples and hand swabs were transported to the laboratory in an ice box under 4°C and stored at -20°C until further analysis (within two months). Field blank samples were handled in a similar manner except that for the airborne filter samples, the sampling pump was not switched on whereas for the dermal wipe samples, the hands were not swabbed.

Measurement of Lung Function

The lung function test was performed among the subjects (rice millers and the non-exposed workers) for the pre- and post-shifts by using the Spirometer (COSMED Pony FX, Italy). The parameters measured in the lung function are forced expiratory value in the first second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio and peak expiratory flow rate (PEFR). A brief clarification and demonstration on the procedure was given. Data on height, weight, gender, race, date of birth and the smoking habit of each worker were recorded. Then, the subjects were asked to place the mouthpieces inside their mouths, to clip their noses, and to inhale deeply and blow hard into the mouthpieces. The procedures were repeated thrice where the best reading recorded by the spirometer was taken.

Questionnaires

The questionnaire was adopted from the British Medical Research Council's Committee on Environmental and Occupational Health (18) as well as the European Respiratory Health Survey II (19) was administered to the subjects. This questionnaire collected the sociodemographic and work data of the subject participants as well as to check their respiratory symptoms such as cough, phlegm, chest illness dyspnoea and other chest diseases. A piloting of the questionnaire for checking its suitability was conducted among ten rice millers, who were not included in this study.

Enzyme-Linked Immunosorbent Assay (ELISA)

Extraction of airborne filter and hand swab samples were carried out according to the previous study (20). The samples were thawed at room temperature, then rocked for two hours using a gyro-rocker STR 9 (Sigma-Aldrich, USA). All samples were centrifuged at 1000 g for 10 minutes at 25 °C. Supernatants were extracted from the filters and hand swabs, and were aliquoted into 50 ul for analysis using an enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO, USA), according to the manufacturer's manual. All standards and samples were analysed in duplicates. The samples were washed four times using a wash buffer with 30 seconds interval between each wash. Within five minutes, the optical density was determined using a microplate reader set at 450 nm. AFB1 concentration was

subsequently obtained and calculated from a standard graph plot. The unit of the results was then converted into ng/m^3 to compare with the permissible limit of inhalable mycotoxin.

Data analysis

The data was analysed using the Statistical Packages for Social Sciences (SPSS) software version 24.0 and the significant level of $p < 0.05$ was used. The data with a value lower than the limit of detection (LOD) (1.50×10^{-1} ng/m^3) were substituted with half LOD (8.0×10^{-1} ng/m^3) for the statistical analysis purpose (21). The analysis of descriptive data was presented using percentages, frequencies, means and standard error means (SEM). The symptoms association between the rice millers and the non-exposed group were analysed using the Fisher's Exact Test. The paired sample t-test and independent t-test were used to compare the lung function test values between the pre- and post-shifts as well as between the rice millers and the non-exposed workers, respectively. The correlation of AFB1 concentration (ng/m^3) in the airborne filter and lung function values was determined by using the Spearman's Rho correlation test. The Linear Regression was used to analyse the relationship between the sociodemographic and work data with the lung function.

Results

Sociodemographic and Work Data

The sociodemographic and work data of the subject workers were tabulated in Table 1. The majority of the rice millers were males (n = 75, 98.70%), Malays (n= 60, 78.90%), with a mean age of 34.61 years ± 1.57 (standard error mean -SEM). The mean work years was 6.61 years ± 1.10. Mean work hour was 7.41 hours ± 0.40. About half of the workers worked a normal shift (n = 47, 73.40%). Most of them were smokers (n = 46, 71.90%). On the other hand, the non-exposed workers were more evenly distributed in gender with almost half of them being males (n = 21, 43.80%) and 27 being females (n = 27, 56.30%). Almost all the non-exposed workers were Malays (n = 47, 97.90%). The mean age for the non-exposed workers was 7.50 years ± 0.85. The mean work hour was 8.28 hours ± 0.13. Almost all of them worked normal shifts (n = 47, 97.90%). The majority of them were non-smokers (n = 43, 89.60%).

Table 1: Sociodemographic and work data

Sociodemographic Variable	Rice millers (n = 76)	Non-exposed (n = 48)	Total (N = 124)
Gender (Frequency, %)			
Male	75 (98.70)	21 (43.80)	96 (77.40)
Female	1 (1.30)	27 (56.30)	28 (22.60)
Race			
Malay	60 (78.90)	47 (97.90)	107 (86.30)
Indian	1 (1.30)	1 (2.10)	2 (1.60)
Others	15 (19.70)	0 (0.00)	15 (12.10)
Age (Years)			
Mean ± SEM	34.61 ± 1.57	38.56 ± 1.20	36.14 ± 1.08
Work Years			
Mean ± SEM	6.61 ± 1.10	7.50 ± 0.85	6.96 ± 0.75
Work Hour			
Mean ± SEM	7.41 ± 0.40	8.28 ± 0.13	7.74 ± 0.25
Work Shift (Frequency, %) (n = 112)			
Normal	47 (73.40)	47 (97.90)	94 (83.90)
Night	3 (4.70)	0 (0.00)	3 (2.70)
Others	14 (21.90)	1 (2.10)	15 (13.40)
Smoking Status (Frequency, %) (n=112)			
Yes	46 (71.90)	1 (2.10)	47 (42.00)
No	10 (15.60)	43 (89.60)	53 (47.30)
Ex-smoker	8 (12.50)	4 (8.30)	12 (10.70)

The mean concentration of AFB1 in area and personal airborne filters are shown in Table 2. The mean for the total area airborne filters was 2.22 ng/m^3 ± 0.07 with the highest mean level being recorded at the storage area (2.28 ng/m^3

± 0.13) while the lowest level was at the paddy intake area (2.01 ng/m^3 ± 0.06). The mean total for the personal airborne filters was 0.25 ng/m^3 ± 0.24 with the distribution ranging from 0.14 to 1.00 ng/m^3. From Table 2, Factory I showed the highest detectable airborne AFB1 (0.53 ng/m^3 ± 0.37) followed by Factory L (0.48 ng/m^3 ± 0.26) while Factory E had the lowest detectable airborne AFB1 (0.15 ng/m^3 ± 0.00). In this study, the mean concentration of AFB1 for hand swabs was 0.25 ng/ml, detectable in 2.63% (n=2) of the rice millers and all of the non-exposed subjects giving negative results for hand swabs; thus, the results were not tabulated.

Table 2: Table of mean AFB1 concentration of area and personal filter at different rice mills

Airborne Filters	n	AFB1 Concentration (ng/m^3)# Mean ± SEM	Range
Area (All)	32	2.22 ± 0.07	1.84 – 3.19
Intake	3	2.01 ± 0.06	1.90 – 2.11
Process	17	2.22 ± 0.10	1.88 – 3.19
Storage	12	2.28 ± 0.13	1.84 – 3.17
Personal (All)	73	0.25 ± 0.24	0.14 – 1.00
Factory A	9	0.43 ± 0.10	0.15 – 0.87
Factory B	8	0.16 ± 0.00	0.15 – 0.16
Factory C	6	0.21 ± 0.04	0.16 – 0.43
Factory D	12	0.25 ± 0.05	0.14 – 0.65
Factory E	5	0.15 ± 0.00	0.14 – 0.15
Factory F	7	0.19 ± 0.03	0.15 – 0.33
Factory G	4	0.15 ± 0.00	0.15 – 0.16
Factory H	2	0.17 ± 0.00	0.17 – 0.17
Factory I	2	0.53 ± 0.37	0.16 – 0.90
Factory J	2	0.17 ± 0.02	0.15 – 0.19
Factory K	13	0.23 ± 0.04	0.15 – 0.64
Factory L	3	0.48 ± 0.26	0.17 – 1.00

#Results were replaced with half of Limit of Detection for analysis purpose

Frequency of Reported Symptoms among Rice millers and Non-Exposed Subjects

The frequency of symptoms reported among the rice millers and the non-exposed subjects were tabulated in Table 3. The most complained symptoms among the rice millers were wheezing and breathlessness (n = 6, 9.20%) whereas the majority of the non-exposed subjects reported the symptoms of cough and phlegm (n=5, 10.90%). However, none of the symptoms were significantly associated between the two groups (p > 0.05).

Table 3: Percentage of symptoms; association of AFB1 exposure with symptoms

Symptoms		Frequency (%) Rice Millers (n=65)	Non-exposed (n=48)	p-value
Coughing	Yes	1 (1.5)	1 (2.2)	0.804
	No	64 (98.5)	45 (97.8)	
Phlegm	Yes	2 (3.1)	4 (8.7)	0.197
	No	63 (96.9)	42 (91.3)	
Cough and Phlegm	Yes	3 (4.6)	5 (10.9)	0.209
	No	62 (95.4)	41 (89.1)	
Breathlessness	Yes	1 (1.5)	2 (4.3)	0.369
	No	64 (98.5)	44 (95.7)	
Wheezing	Yes	3 (4.6)	1 (2.2)	0.497
	No	62 (95.4)	45 (97.8)	
Wheezing and breathlessness	Yes	6 (9.2)	2 (4.3)	0.327
	No	59 (90.8)	44 (95.7)	
Chest Pain	Yes	2 (3.1)	0 (0.0)	0.230
	No	63 (96.9)	46 (100.0)	

Comparison of Lung Function between Pre- and Post-Shifts

The mean difference in the lung function test values between the pre- and post-shifts utilising the paired sample t-test were compiled in Table 4. There were significantly lower measured FEV1 for the post-shifts compared to the pre-shifts for both groups; rice millers (p = 0.022) and the non-exposed (p = 0.044) group.

Table 4: Comparison of lung function between pre- and post-shift among rice millers and non-exposed workers, respectively

LFT Parameters (Measured)	Rice millers (Mean ± SEM)		p-value	Non-exposed (Mean ± SEM)		p-value
	Pre-shift (n = 76)	Post-shift (n = 76)		Pre-shift (n = 48)	Post-shift (n = 48)	
FVC (L)	2.78 ± 0.11	2.79 ± 0.11	0.892	2.56 ± 0.10	2.52 ± 0.10	0.503
FEV1 (L)	1.82 ± 0.10	1.66 ± 0.09	0.022*	1.72 ± 0.10	1.56 ± 0.08	0.044*
FEV1/FVC (%)	59.79 ± 2.95	56.91 ± 2.75	0.230	67.65 ± 2.90	63.35 ± 2.83	0.150
PEF (L)	2.66 ± 0.24	2.38 ± 0.22	0.114	2.40 ± 0.16	2.23 ± 0.23	0.291

*Significant different at p<0.05; statistical test – Paired t-test, FVC - Forced Vital Capacity, FEV1 – Forced Expiration Volume, FEV1/FVC – FEV1/FVC ratio, PEF – Peak Expiratory Flow

The independent sample t-test was used to compare the lung function test (LFT) parameters between the rice millers and the non-exposed group; nonetheless, no significant difference was observed (results were not tabulated).

Correlation between Mean AFB1 Concentration (ng/m^3) in Airborne Filters and Decline in Lung Function Values of Rice Millers.

Table 5 depicts the correlation between AFB1 in airborne filters, and lung function values among rice millers. There was an inverse but a weak correlation between the AFB1 concentration with LFT parameters such as pre- and post-FVC, post-FEV1 as well as post-PEF. No significant correlations between the variables were observed (p > 0.05).

Table 5: Correlation between mean AFB1 concentration (ng/m^3) in personal airborne filters and lung function values among rice millers.

Measured LFT Parameters	Shift		Airborne AFB1 Concentration (ng/m^3)
FVC	Pre	p-value	0.795
		r	-0.03
	Post	p-value	0.219
		r	-0.15
FEV1	Pre	p-value	0.583
		r	0.07
	Post	p-value	0.773
		r	-0.03
FEV1/FVC	Pre	p-value	0.968
		r	0.01
	Post	p-value	0.453
		r	0.09
PEF	Pre	p-value	0.345
		r	0.11
	Post	p-value	0.803
		r	-0.03

[a]Significant difference at p<0.05; statistical test – Pearson's correlation

Sociodemographic and Work Factors as Potential Confounders in Lung Function Tests

Table 6 shows the relationship between the sociodemographic and work characteristics with lung function tests among all the subject participants. There was a significant relationship between race and pre-shift FEV1 (p = 0.019). Similarly, significant relationships were shown between age with post-shifts of FEV1 (p = 0.002), FEV1/FVC (p = 0.018) and PEF (p = 0.014), respectively.

Table 6: Sociodemographic and work factors as potential confounders in Lung Function Tests

Variables		Pre				Post				R^2	Adjusted R^2
		FVC	FEV1	FEV1/ FVC	PEF	FVC	FEV1	FEV1/ FVC	PEF		
Gender	B	-0.030	0.12	0.003	-0.041	-0.019	-0.143	0.009	-0.060	0.233	0.180
	p	0.779	0.938	0.258	-0.973	0.869	0.460	0.050	0.121		
Race	B	0.139	-0.917	0.006	0.100	0.282	0.402	-0.009	-0.070	0.154	0.095
	p	0.608	0.019*	0.413	0.438	0.335	0.408	0.469	0.469		
Age	B	-1.735	0.505	0.096	-1.331	6.586	-18.347	0.318	2.780	0.201	0.145
	p	0.582	0.911	0.283	0.278	0.054	0.002*	0.018*	0.014*		
Working Years	B	-1.167	4.030	-0.025	-0.986	-0.758	-9.013	0.092	2.702	0.202	0.146
	p	0.594	0.199	0.689	0.246	0.748	0.023*	0.318	0.001*		
Working Hour	B	-0.449	0.887	-0.011	-0.148	2.907	-5.606	0.102	0.864	0.246	0.193
	p	0.535	0.393	0.605	0.597	0.001*	0.001*	0.001*	0.001*		
Working Shift	B	0.187	-0.291	-0.005	0.142	-0.374	0.348	-0.008	-0.031	0.078	0.006
	p	0.383	0.336	0.351	0.130	0.228	0.494	0.550	0.685		
Smoking Status	B	-0.378	0.631	-0.007	-0.059	-0.223	-0.176	-0.007	0.046	0.066	0.001
	p	0.054	0.023*	0.158	0.483	0.425	0.702	0.601	0.513		

*Significant difference at p<0.05; Statistical test – Linear Regression, mode: Enter, FVC - Forced Vital Capacity, FEV1 – Forced Expiration Volume, FEV1/FVC – FEV1/FVC ratio, PEF – Peak Expiratory Flow

Working years also showed a significant relationship with post-shift FEV1 (p = 0.023) and post-shift PEF (p = 0.001). In addition to that, working hours displayed a significant positive relationship with post-shifts of FVC (p = 0.001), FEV1/FVC (p = 0.001) and PEF (p = 0.001) but inversely related with FEV1 (p = 0.001). Lastly, the smoking status showed a significant relationship with the pre-shift FEV1 (p = 0.023).

Discussion

In this study, the mean concentration of AFB1 in airborne filters did not exceed the CoNTC of 30 ng/m^3. The half LOD was used in this study to replace the left-censored data to estimate the mean concentration of AFB1. Note that, this method is only acceptable to be used with data that only has one LOD (21). In a previous study conducted to determine whether respiratory tract exposure to AFB1 suppresses pulmonary and systemic host defenses of rats, the authors had calculated and estimated that the pulmonary dose rate was 2.80 x 10^{-1} µg AFB1/kg/min or 5.6 ng/m^3 for 20-minute dose among rats (22). The generic permissible limit of inhalable mycotoxin including AFB1 is 30 ng/m^3 was subsequently established by Hardin and colleagues (10). This value was used in our study since it takes into consideration all the studies of inhalable mycotoxins including AFB1 (10).

Using our experimental parameters, the mean concentration of AFB1 for hand swabs were detectable in 2.63% (n=2) of rice millers but was non-detectable among the non-exposed subjects, as expected. The dermal uptake could be influenced by factors such as skin condition and moisture, ambient temperature as well as humidity. The spores containing AFB1 on the skin could be removed by sweating or washing (23). It is worth noting that the dermal exposure limit has yet to be established despite some indications that skin penetration to human body is possible (24).

In this study, the storage area gave the highest AFB1 concentration (2.28 ng/m^3) indicating that the storage area could have provided optimal environmental growth parameters for AFB1. It is known that moisture content and temperature play big roles in *Aspergillus sp.* growth. In industry practice, grains are stored at low water activity (a_w) which is below 0.65 a_w or 12% to avoid fungal growth (25,26). A

study done on 111 rice samples found that 61.3% of the samples were positive with aflatoxins indicating the effect of humidity on its growth (27). It has been previously reported that the water activity of 0.99 a_w and temperature of 30°C and high relative humidity (70-80%) were optimal for the growth of *Aspergillus flavus* (22) while the moisture content of less than 12% prevents fungal growth (27–29). Therefore, a more comprehensive data to include temperature and ambient water activity will be useful for future studies.

In this study, wheezing with breathlessness was the predominant complaint among the rice millers. Contrary to this study, cough was found to be the most complained among the food-grain workers in India and Ethiopia (13, 29). Some people are very sensitive to mould exposure and they present symptoms such as a wheezing cough and others that are related to asthma (30). A study conducted in South Finland found that the risk of asthma was related to the presence of mould in workplaces and not related to water damage or damp stains (30, 31). Despite no visible water damage or stains at the premises, our observation showed that there were some water puddles mixed with rice husks near the drying machine in certain rice mills which could contribute to *Aspergillus spp.* growth.

The lung function test was used to measure the obstructive or restrictive lung function between the two subject groups. In this study, FEV1 among the rice millers showed a significant decline when the values were compared between the pre- and post-shifts. FEV1 is the maximum volume of air exhaled in the first second of a forced exhalation from a full inhalation (32). An obstructive change can usually be seen among the grain dust workers (33, 34). A decline in lung function could also be due to an elevated temperature at rice mill surroundings since a high temperature could affect the lung function by straining the airways (35).

In this study, there was no significant correlation between the airborne AFB1 concentration and lung function values. This could be due to the low concentration of AFB1 in the area and personal airborne filters (ng/m^3) or that ailing workers were absent during the lung function assessment. Similarly, Demeke and his colleagues also did not find any significant decline in the FEV1/FVC values in a study done among the grain workers in Ethiopia (36).

The potential confounding factors in LFT were examined and it was manifested that race showed a significant relationship with the post-shift FEV1. A study done in Singapore among the Chinese, Malays and Indians showed that the differences in lung function could be due to a variation in the upper body length and lung volume (37). In a separate study done among similar races, a difference in FVC was shown, proving that there were variations in respiratory muscle strength and lung volumes (38).

It is evident that post-shift FEV1, FEV1/FVC and PEF seemed to be affected by age among the rice millers. With age, the human respiratory system changes functionally and structurally in the volume of thoracic cavity, lung and muscles involved in respiration (39). Kyphosis or hunched back can also reduce FEV1 especially if the angle exceeds 55° that causes a decreased thoracic cavity volume, reduction of rib space that eventually shortens the intercostal muscle length (40, 41). At cellular level, the lungs will experience cell senescence, a state of unalterable growth cycle arrest together with an increased reactive oxygen species, pro-inflammatory signalling and expression of senescence-related molecules. Previous studies had proven that AFB1 has the ability to generate reactive oxygen species in broiler and rats (42-44). This implied that AFB1 could potentially worsen the declining lung function of older adults.

Working years displayed a significant relationship with post FEV1 and post PEF. Similar results were shown where PEF declined in long-term workers in India (34). The decline in PEF can be due to an obstructive lung function caused by mucosal plugs. Grain dust

can irritate mucosal cells, thereby increasing mucous secretion. Eventually, mucosal plugs build-up will lead to an obstructive lung function (45,46). On the other hand, working hours displayed a significant relationship with all post-shift LFT parameters. A study in Nigeria also confirmed that working duration affects lung function (47). Contradictory to the current study, another Nigerian study did not find any significant association of employment duration with lung function probably due to the small sample size or healthy worker effect as claimed by the authors (48).

When the relationship between smoking and LFT was evaluated, only pre-shift FEV1 shows a significance. Most non-exposed subjects did not smoke probably due to the nature of their work. In a study on smoking effects on chest expansion, lung function and respiratory muscle strength among youths, found that FVC and chest expansion in non-smokers are higher and larger than smokers (49). In the current study, smoking did not show a significant relationship with other LFT parameters probably due to the small sample size because most studies stated that smoking affects lung function (49-51).

Conclusion

This study shows that the mean AFB1 concentration in both area and personal airborne filters was below the CoNTC (30 ng/m^3) with a mean AFB1 concentration that showed no correlation with LFT values among the rice millers. The majority of the rice millers complained of having wheezing and breathlessness. Despite the low level, this study has shown evidence of detectable/presence of AFB1 in the airborne filter as well as 2.3% of hand swab samples among the rice millers. Lastly, race, age, working years, working hours and smoking status might have affected the parameters of lung function among the subject participants.

The exposure effect on health is usually cumulative over the years; so, a prolonged exposure to AFB1 may lead to serious respiratory effects. There is an urgent need for a longitudinal study to observe the respiratory effects among the workers at risk in the future. Proper control measures such as hygiene practices and wearing suitable personal protective equipment (PPE) are highly recommended in preventing further exposure and to reduce the levels of AFB1 among the workers.

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References

1. Kensler TW, Roebuck BD, Wogan GN & Groopman JD. Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. Toxicol Sci. 2011 Mar;120(Suppl_1):S28-48. DOI: 10.1093/toxsci/kfq283.
2. Pasqualotto AC. Differences in pathogenicity and clinical syndromes due to Aspergillus fumigatus and Aspergillus flavus. Med Mycol. 2009;47(Suppl_1):261-270. DOI: 10.1080/13693780802247702.
3. Hamid AS, Tesfamariam SG, Zhang Y & Zhang ZG. Aflatoxin B1-induced hepatocellular carcinoma in developing countries: geographical distribution, mechanism of action and prevention (Review). Oncol Lett. 2013;5(4):1087-1092. DOI: 10.3892/ol.2013.1169.
4. Philippe B, Boleti H, Grenet D, Stern M & Latge JP. Phagocytosis and intracellular fate of Aspergillus fumigatus conidia in alveolar macrophages. 2003;71(2):891-903. DOI: 10.1128/IAI.71.2.891.

5. Fukahori S, Matsuse H, Tsuchida T, et al. Clearance of Aspergillus fumigatus is impaired in the airway. Ann Allergy Asthma Immunol. 2014.
DOI: 10.1016/j.anai.2014.05.011.
6. Carpagnano GE, Lacedonia D, Palladino GP, et al. Aspergillus spp. colonization in exhaled breath condensate of lung cancer patients from Puglia Region of Italy. BMC Pulm Med. 2014;14(1):1-5.
DOI: 10.1186/1471-2466-14-22.
7. Reddy KRN, Farhana NI & Salleh B. Occurrence of Aspergillus spp. and Aflatoxin B1 in malaysian foods used for human consumption. J Food Sci. 2011.
DOI: 10.1111/j.1750-3841.2011.02133.x.
8. Soleimany F, Jinap S & Abas F. Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. Food Chem. 2012.
DOI: 10.1016/j.foodchem.2011.07.131.
9. The Commission of the European Communities. Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Off J Eur Union. 2010.
10. Hardin BD, Robbins CA, Fallah P & Kelman BJ. The concentration of no toxicologic concern (CoNTC) and airborne mycotoxins. J Toxicol Environ Heal - Part A Curr Issues. 2009;72(9):585-598.
DOI: 10.1080/15287390802706389
11. Lai H, Mo X, Yang Y, He K & Xiao J. Association between aflatoxin B1 occupational airway exposure and risk of hepatocellular carcinoma : a case-control study. 2014;9577-9584.
DOI: 10.1007/s13277-014-2231-3.
12. Desai MR & Ghosh SK. Occupational exposure to airborne fungi among rice mill workers with special reference to aflatoxin producing A. flavus strains. Ann Agric Environ Med. 2003;10(2):159-62.
13. Viegas S, Veiga L, Malta-Vacas J, et al. Occupational exposure to aflatoxin (AFB1) in poultry production. J Toxicol Environ Heal - Part A Curr Issues. 2012;75(22-23):1330-1340.
DOI: 10.1080/15287394.2012.721164.
14. Malik A, Ali S, Shahid M & Bhargava R. Occupational exposure to Aspergillus and aflatoxins among food-grain workers in India. Int J Occup Environ Health. 2014;20(3):189-193.
DOI: 10.1179/2049396714Y.0000000055.
15. Health and Safety Laboratory. General methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols. 2005.
16. Department of Occupational Safety and Health. Industry code of practice on indoor air quality. Minist Hum Resour Dep Occup Saf Heal. 2010.
17. Tagiyeva N, Anua SM, Semple S, Dick F & Devereux G. The 'take-home' burden of workplace sensitisers: flour contamination in bakers' families, Environment International. 2012;46:44-49. Available from: http://dx.doi.org/10.1016/j.envint.2012.04.014.
18. Medical Research Council (MRC). Respiratory symptoms. 1986:4. Available from: https://mrc.ukri.org/documents/pdf/questionnaire-on-respiratory-symptoms-1986/. Accessed April 27, 2019.
19. Burney P, Jarvis D & Knox J. European community respiratory health survey II Instructions and coding to main questionnaire. Available from: www.ecrhs.org. Accessed April 27, 2019.
20. Douwes J, Versloot P, Hollander A, Heederik D & Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol. 1995 May;61(5):1763-1769.
21. Helsel D. Much ado about next to nothing: Incorporating nondetects in science. Ann Occup Hyg. 2010 Apr;54(3):257-262.
DOI: 10.1093/annhyg/mep092.
22. Jakab GJ, Hmieleski RR, Zarba A, Hemenway DR & Groopman JD. Respiratory aflatoxicosis: suppression of pulmonary and systemic host defenses in rats and mice. Toxicol Appl Pharmacol. 1994;125(2):198-205.
DOI: 10.1006/taap.1994.1065.

23. Frasch HF, Dotson GS, Bunge AL, et al. Analysis of finite dose dermal absorption data: implications for dermal exposure assessment. J Expo Sci Environ Epidemiol. 2014;24(1):65-73. DOI: 10.1038/jes.2013.23.
24. Boonen J, Malysheva SV., Taevernier L, Diana Di Mavungu J, De Saeger S & De Spiegeleer B. Human skin penetration of selected model mycotoxins. Toxicology. 2012 Nov 15;301(1-3):21-32. DOI: 10.1016/j.tox.2012.06.012.
25. Iglesas HA CJ. Handbook of food isotherms: water sorption parameters for food and food components. 1st Ed. New York: Academic Press; 2016. DOI: 10.1016/b978-0-12-370380-4.x5001-4.
26. Pitt JI. Mycotoxins. Fourth Ed. Elsevier Inc.; 2013. DOI: 10.1016/B978-0-12-416041-5.00030-5.
27. Thi B, Huong M, Danh L, et al. Aflatoxins and fumonisins in rice and maize staple cereals in Northern Vietnam and dietary exposure in different ethnic groups. Food Control. 2016;70:191-200. DOI: 10.1016/j.foodcont.2016.05.052.
28. Hettlarachchi GHCM, Gooneratne J & Hirimburegama WK. Effect of initial moisture content and relative humidity on the accumulation of aflatoxin in maize grains (Zea Mays) during storage. J Natl Sci Found Sri Lanka. 2001;29(1&2):29-34.
29. Bernáldez V, Córdoba JJ, Magan N, Peromingo B & Rodríguez A. The influence of ecophysiological factors on growth, aflR gene expression and aflatoxin B1 production by a type strain of Aspergillus flavus. LWT - Food Sci Technol. 2017;83:283-291. DOI: 10.1016/j.lwt.2017.05.030.
30. Enginnering Services Division Ministry of Health. Guideline on mould control and remediation in healthcare facilities. Available from: http://www.engineering.moh.gov.my/v4/get file.php?i=212. 2016. Accessed April 27, 2019.
31. Jaakkola MS, Nordman H, Piipari R, et al. Indoor dampness and molds and development of adult-onset asthma: a population-based incident case-control study. Environ Health Perspect. 2002;110(5):543-547.
32. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-338. DOI: 10.1183/09031936.05.00034805.
33. Essen SV. The role of endotoxin in grain dust exposure and airway obstruction. Curr Opin Pulm Med. 199;3(3):198-202.
34. Ghosh T, Gangopadhyay S & Das B. Prevalence of respiratory symptoms and disorders among rice mill workers in India. Environ Health Prev Med. 2014;19(3):226-233.
35. Arteaga VE, Mitchell DC, Matt GE, et al. Occupational exposure to endotoxin in $PM_{2.5}$ and pre- and post-shift lung function in California dairy workers. J Environ Prot (Irvine, Calif). 2015;06(05):552-565.
36. Demeke D & Haile DW. Assessment of respiratory symptoms and pulmonary function status among workers of flour mills in Addis Ababa, Ethiopia: Comparative Cross-Sectional Study. Pulm Med. 2018;2018:9521297.
37. Yap WS, Chan CC, Chan SP & Wang YT. Ethnic differences in anthropometry among adult Singaporean Chinese, Malays and Indians, and their effects on lung volumes. Respir Med. 2001;95(4):297-304.
38. Johan A, Chan CC, Chia HP, Chan OY & Wang YT. Maximal respiratory pressures in adult Chinese, Malays and Indians. Eur Respir J. 1997;10(12):2825-2828. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9493668. Accessed August 4, 2019.
39. Lowery EM, Brubaker AL, Kuhlmann E & Kovacs EJ. The aging lung. Clin Interv Aging. 2013;8:1489-1496.
40. Culham EG, Jimenez HA & King CE. Thoracic kyphosis, rib mobility, and lung volumes in normal women and women with osteoporosis. Spine (Phila Pa 1976). 1994;19(11):1250-1255. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8073317. Accessed July 22, 2019.

41. Lombardi I, Oliveira LM, Mayer AF, Jardim JR & Natour J. Evaluation of pulmonary function and quality of life in women with osteoporosis. Osteoporos Int. 2005;16(10):1247-1253.
42. Liu Y & Wang W. Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis and involves Nrf2 signal pathway in primary broiler hepatocytes. Anim Sci J. 2016;87(12):1490-1500.
43. Wang W, Xu Z, Yu C & Xu X. Effects of aflatoxin B1 on mitochondrial respiration , ROS generation and apoptosis in broiler cardiomyocytes. 2017;(August 2016):1-8.
44. Rotimi OA, Rotimi SO, Goodrich JM, Adelani IB, Agbonihale E & Talabi G. Time-course effects of acute aflatoxin b1 exposure on hepatic mitochondrial lipids and oxidative stress in rats. Front Pharmacol. 2019;10:467.
45. Taytard A, Tessier JF, Vergeret J, et al. Population, material and methods. Eur J Epidemiol. 1988;4(1):104-109.
46. Dhillon SK, Bassi R & Kaur H. A study of lung function abnormalities in workers of rice mills. Indian J Fundam Appl Life Sci. 2011;1(3):217-220.
47. Ige OM & Awoyemi OB. Respiratory symptoms and ventilatory function of the bakery workers in Ibadan, Nigeria. West Afr J Med. 2002;21(4):316-8.
48. Oghuvwu SO, Egbagbe EE, Aigbirior JO, Oniovokukor BE & Erhabor GE. Respiratory health status of workers in a bottling factory in Benin city, Nigeria. Int J Environ Res Public Health. 2018;15(9):1-9.
49. Tantisuwat A & Thaveeratitham P. Effects of smoking on chest expansion, lung function, and respiratory muscle strength of youths. J Phys Ther Sci. 2014;26(2):167-170.
50. Jaakkola JJK, Hernberg S, Lajunen TK, Sripaijboonkij P, Malmberg LP & Jaakkola MS. Smoking and lung function among adults with newly onset asthma. BMJ Open Respir Res. 2019;6(1):1-10.
51. London SJ. Methylation, smoking, and reduced lung function. Eur Respir J. 2019;54(1):1900920.