Occupational exposure to *Aspergillus* and aflatoxins among food-grain workers in India

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Background: Aflatoxins are a metabolite of *Aspergillus* molds and are widespread in the natural environment. Workers who handle food grains are at increased risk of exposure to aflatoxins and subsequently certain respiratory conditions. In India, more than half of the employed population is engaged in some type of agricultural work, yet little known about the respiratory problems as a result of exposure to aflatoxins among workers who handle food grains in India.
Objectives: The aim of this study was to determine the risk of occupational exposure to aflatoxins in food-grain workers compared to workers who are not occupationally exposed to food grains.
Methods: Bronchoalveolar lavage (BAL) and serum samples from 46 food-grain workers and 44 non-food-grain workers were analyzed for the presence of aflatoxins. Microscopy and culture of BAL samples were performed to detect *Aspergillus* species.
Results: Aflatoxins were detected in 32.6% of the food-grain workers and 9.1% of non food grain workers ($P<0.01$). A significant difference was also found in BAL culture for *Aspergillus* ($P<0.01$) between the two groups. About 47.8% of the food-grain workers and 11.4% of non-food-grain workers had chronic respiratory symptoms.
Conclusion: Occupational exposure to aflatoxins in food-grain workers was found to be associated with the increased presence of respiratory symptoms.

Keywords: Aflatoxins, *Aspergillus*, India, Respiratory health, Occupational health, Food-grain workers

Introduction

Aspergillus molds are widespread in the environment and human exposure is common. The molds are found in soil, dust, and decomposing organic matter, and the conidia are often found in outdoor air.[1] Over the past 20 years, fungal infections of the lung, especially bronchopulmonary aspergillosis, have become increasingly common.[2,3]

Aflatoxins are secondary metabolites of certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* and grow rapidly on grains, seeds, and other foodstuffs, contaminating an estimated one-quarter of agricultural products worldwide, with maize, cereals, and groundnuts being the most susceptible.[4,5] Previous epidemiological studies have found that the ingestion and inhalation of aflatoxins poses a serious occupational health risk worldwide.[6–9] Exposure to aflatoxins via inhalation most commonly results from the handling of contaminated food grains (wheat, maize, etc.).[10]

Prior research has found the presence of aflatoxins in corn and corn dust and an increased risk of *A. flavus* infestations during drought conditions.[7,11] However the full extent of aflatoxin exposure is still unknown. It is also unknown whether aflatoxin exposure varies by demographic and/or ecologic factors. This information is critical for determining the disease burden attributed to occupational exposure to aflatoxins and for the subsequent implementation of effective public health interventions to prevent exposure. While, pulmonary aspergillosis in the context of bronchogenic carcinoma has been previously reported, aflatoxin production in such patients has largely remained unresearched.[2]

In India, agriculture is the most common occupation, with approximately 53% of the Indian population employed in some sort of agricultural activity.[12] The aim of this study was to determine the risk of occupational exposure to aflatoxins in food-grain workers in India compared to workers who are not occupationally exposed to food grains. This is the first study of its kind to assess the presence of aflatoxins in both bronchoalveolar lavage (BAL) as well as serum samples and correlate it with

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Aspergillus growth. The secondary aim of the study was to measure the prevalence of chronic respiratory illness among these workers.

Methods
Participants
The study population was composed of 90 workers, 46 employees who were occupationally exposed to food grains and 44 employees not occupationally exposed to food grains, matched by age and sex to the exposed group. The occupationally exposed group consisted of 40 farmers, 4 food-grain laborers, and 2 flour-mill shopkeepers. The unexposed group members were employed in various occupations including non-grain shopkeepers, teachers, and construction workers. All food-grain workers had been employed for a minimum of 6 months in order to be eligible to participate in the study.

Participants were recruited from the outpatient department and medical ward of Jawaharlal Nehru Medical College and Hospital, India. A detailed clinical history was obtained by the study authors from all participants. Information collected in the history included occupation, work activities, residence, diet, smoking history, use of broad spectrum antibiotics, corticosteroid therapy, and history of other immunosuppressant, chemotherapy, or transplantation. The presence and duration of other respiratory symptoms such as cough, shortness of breath, chest pain, fever, and hemoptysis was also obtained. This study was approved by the Institutional Ethics Committee at the Aligarh Muslim University in India and informed consent obtained from subjects before participation.

Specimen: collection, transport, and storage
Bronchoalveolar lavage and serum samples were collected from all study participants. The detection of aflatoxins in BAL was used to confirm the route of aflatoxin exposure as through inhalation versus ingestion.

Bronchoalveolar lavage samples were collected in three sterile vials using fibrotic bronchoscopy for direct microscopy and culture and aflatoxin detection. Direct microscopy and cultures were performed immediately and BAL specimens, for the detection of aflatoxins, were stored at −20°C until tested. Five milliliters of blood were collected from each participant by venipuncture in order to detect aflatoxins and the collected specimens were immediately transported to the laboratory for processing. Serum was separated and stored at −20°C until further processed. Each specimen of BAL was homogenized using vortex and subjected to the following laboratory procedures.

Direct microscopy and culture
Direct mount and lactophenol cotton blue (LPCB) mount were prepared according to the standard procedure for detecting fungal elements such as fungal hyphae, vesicle, and *Aspergillus* species spores.

Bronchoalveolar lavage was streaked on two sets of Sabouraud's Dextrose Agar (SDA) plain and SDA containing chloramphenicol (0.05 mg/ml) and on two sets of Czapek Dox Agar. One set was incubated at 25°C and the other at 37°C. The fungal isolates were confirmed and characterized according to the standard technique.[13]

Detection of human aflatoxin albumin adduct in BAL and serum by ELISA
Aflatoxin albumin adduct was detected in BAL and serum of workers and controls using Human Aflatoxin albumin adduct Elisa kit (Glory Science Co. Ltd, USA). This kit uses a direct competitive ELISA method, with Aflatoxin albumin adduct coupled antigen-coating microplate. Tests were performed according to the manufacturer's instructions and absorbance was read at 450 nm. The sample absorbance value is inversely proportional to the sample Aflatoxin albumin adduct content and calculated through the standard curve.

Assay procedure
The location of B0 (first standard 0 ng/ml), standards and samples were labeled on microtiter plate. Sample diluent ($10 \times$) and wash solution ($20 \times$) were diluted with the working fluid (distilled or deionized water). About 50 μl of 0.0 ng/ml standard solution was added to the well of B0. Fifty microliters of rest of the standard solutions were added to their respective standard wells. Fifty microliters of sample solutions were added to each sample well. Next, 50 μl of anti-aflatoxin albumin adduct antibody conjugate was added to each well. The plate was shaken gently for several seconds and incubated at 37°C for 30 minutes. The liquid in the wells was removed and the microplate was washed five times with wash solution. After completing the washing process, 50 μl of chromogenic reagent A and 50 μl chromogenic reagent B were immediately added to each well, mildly shaking the plate to mix thoroughly. The plate was incubated at 37°C for 10 minutes and then 50 μl stop solution was added to each well and mixed. Absorbance was read at 450 nm within 5 minutes of adding the stop solution.

Albumin estimation
Albumin was estimated quantitatively in all BAL and serum samples and controls by Albumin BCG (RFCL Ltd., Uttarakhand, India).

Statistical analysis
Data were analyzed using SPSS version 8.0 for Windows (SPSS, Inc., Chicago, IL, USA) or were manually calculated. Odds ratios (OR) were calculated and variables were compared using the Fisher exact test. The P values of <0.05 were considered statistically significant.

Table 1 Demographic characteristics of study participants

Demographics			No. of workers (%)
Age (years)		20–30	2 (2.2)
		31–40	12 (13.3)
		41–50	16 (17.7)
		51–60	36 (40)
		61–70	22 (24.4)
		>70	2 (4.4)
Gender		Males	84 (93.3)
		Females	6 (6.7)
Occupation Type	Food-grain workers (*n*=46)	Farmers	40 (44.4)
		Food grain laborer	4 (4.4)
		Flour-mill shopkeeper	2 (2.2)
	Non-food-grain workers (*n*=44)	Construction workers	18 (20)
		Businessmen	7 (7.7)
		Non-grain shopkeeper	6 (6.6)
		Teachers	5 (5.5)
		Factory workers	4 (4.4)
		Unemployed	4 (4.4)

Results

Descriptive statistics for study participants are presented in Table 1.

Respiratory symptoms among workers

Thirty per cent of all study participants reported experiencing respiratory symptoms in the previous 6 months. Cough was the predominant complaint (24.4%), followed by breathlessness (13.3%) and chest pain (7.8%). Respiratory symptoms were found to be positively associated with food-grain workers compared to non-food-grain exposed workers (OR=35.285 (10.859–114.651)) (Table 2).

BAL culture for *Aspergillus*

Aspergillus species were found in 32 of the study participants (35.5%), and was more prevalent in food-grain workers (*n*=24, 52.1%) compared to non-food-grain workers (*n*=8; 18.2%) (Table 2). The difference in the presence of *Aspergillus* was statistically significant ($P<0.01$) (Table 3). Among participants testing positive for *Aspergillus*, *Aspergillus fumigatus* was the most common species isolated (*n*=16, 17.8%), followed by *A. flavus* (*n*=12; 13.3%), and *Aspergillus niger* (*n*=4; 4.4%).

Detection of aflatoxins

Among the 46 exposed workers, 15 (32.6%) showed the presence of aflatoxins in BAL (Table 3). Of the 15 food grain workers exposed to aflatoxins, 11 had

aflatoxins in both BAL and serum and 4 had aflatoxins in BAL fluid only. Among the non-exposed workers, only four (9.1%) had aflatoxins in their samples, three in their serum samples, and one in both their BAL and serum samples. There was a statistically significant association of aflatoxin positivity ($P<0.01$) among exposed food-grain workers when compared to those not exposed to food grains (Table 3). Among farmers, those involved in farming of wheat and maize had an increased (although not statistically significant) risk of exposure to aflatoxins compared to farmers of other food grains (OR=2.500 (CI: 0.618–10.112)).

Discussion

Our study collected and analyzed the BAL and serum samples of workers occupationally exposed to food grains and workers not occupationally exposed to food grains. Samples were analyzed for aflatoxin albumin adducts and compared. We found a significantly higher presence of aflatoxins in grain-exposed workers compared to those workers not occupationally exposed to grains ($P<0.01$). We also found a statistically significant risk of *Aspergillus* infection in grain-exposed individuals ($P<0.05$).

Respiratory symptoms were found to be more prevalent among food-grain workers (47.8%) than workers of other occupations (11.4%). Various epidemiological and clinical studies have contributed to the identification of associations between respiratory

Table 2 Chronic respiratory symptoms among workers

	Number of workers *n* (%)			
	Food-grain workers	Non-food-grain workers	Total	
Symptoms	19 (41.3)	3 (6.8)	22 (24.4)	
Cough	10 (21.7)	2 (4.5)	12 (13.3)	
Breathlessness	6 (13)	1 (2.3)	7 (7.8)	
Chest pain	3 (6.5)	–	3 (3.3)	
Fever	1 (2.2)	–	1 (1.1)	
Hemoptysis	19 (41.3)	3 (6.8)	22 (24.4)	
Any respiratory symptom	39 (84.7)	6 (13.6)	45 (50)	OR=35.285 (CI: 10.859–114.651)

Table 3 Distribution of *Aspergillus* and aflatoxins among participants

| | | | | Positive for aflatoxins | | |
	N	*Aspergillus* isolation from BAL* (%)	Positive for aflatoxins† (%)	BAL	Serum	BAL and Serum
Occupational group						
Food-grain worker (*n*=46)						
Farmer	40	19	12	2	0	10
Food-mill laborer	4	4	2	2	0	0
Flour-mill shopkeeper	2	2	1	0	0	1
Non-food-grain worker (*n*=44)	44	8	4	0	3	1

*$P<0.01$ for food-grain versus non-grain exposed workers, †$P<0.01$ for food-grain versus non-grain exposed workers.

disorders and agricultural exposures and agricultural work environments hold the potential for exposure to a variety of respiratory biohazards. The most prevalent airborne substances include grain dust and its constituents, bacteria and their metabolites (endotoxin), fungi and their metabolites, and storage mites. Airborne contaminants frequently occur in concentrations and compositions that challenge the defense mechanisms of the lung.

Grain dust is composed of many materials including grains and their disintegration products, pollens, and fungi.[14] In a study on the impact of grain dust on respiratory health, Chan-Yeung *et al.* reported that the microflora of grain dust change during the process of harvesting, storage, and handling and that during growth, grains become colonized with saprophytic fungi. During storage, the predominant fungi on contaminating grains are *Ustilago*, *Aspergillus*, and *Mucor* commonly.[15]

In most cases, human exposure to airborne aflatoxins has been determined by estimating aflatoxins levels in food or in respirable dust.[10,16,17] However, these measurements do not provide information on the potential biological effect of exposure. Few studies have been conducted which detect the presence of aflatoxin albumin adduct in human serum samples.[18] Quantification of a carcinogenic adduct to protein or DNA is a measure of the amount of the active metabolite formed in the organism.[19] Inside the human body aflatoxin is converted to its reactive epoxide and this epoxide has an affinity for DNA. Plasma proteins, especially albumin, are then able to form aflatoxin DNA adduct and aflatoxin albumin adduct, respectively.[20,21] The average half-life of albumin in people is approximately 20 days. One of the advantages of using serum albumin rather than for DNA adduct is that the measured level represents the accumulated dose during the half-time of the serum albumin. Therefore, an accumulated dose of aflatoxin will be present in albumin long after the dietary exposure has ceased. This is a property not found for DNA adduct because the half-life of DNA adduct is approximately 12 hours, after which it is rapidly excreted in urine.[18]

Occupation as a risk factor for aflatoxin exposure via inhalation has been considered in a few studies.

Saad-Hussein *et al.* have demonstrated the effect of occupational exposure to aflatoxins on liver tumor markers in textile workers.[22] Viegas *et al.* report occupational exposure to aflatoxin B1 in workers involved in poultry and swine production.[6,10] Hayes *et al.* conducted a follow-up study on 71 Dutch oil-press workers exposed to aflatoxin primarily via the respiratory route and observed that the mortalities for total-cancer and respiratory cancer were higher than expected in the aflatoxin-exposed group.[9]

However, there have been few studies that describe the role of pulmonary aspergillosis and aflatoxins in food-grain workers, an important occupation in India. We argue that dearth of research in this area has contributed to an underestimated burden of aflatoxin exposure among food-grain workers. A major strength of this study was the measurements of toxic adduct formed inside the body.

Future studies should further investigate the role of aflatoxin exposure as a public health concern among food-grain workers. Wide-scale, evidence-based interventions are needed to decrease exposure and the subsequent negative health effects. Strategies to assist farmers with the appropriate drying and storage techniques of food grains may help reduce aflatoxin exposure. Moreover, results from this study indicate that farmers are not the only exposed population. Any occupational group handling food grains may be at risk for negative health effects. Therefore, preventive measures should also be applied during grain loading, harvesting, and grinding. Aflatoxicosis surveillance programs should be initiated for at-risk populations to detect exposure in the early stages so that it can be contained. Future studies should also investigate variations in aflatoxin exposure by geographic region. Results from future research will help in the development of interventions for specific occupational groups at risk of aflatoxin exposure.

Disclaimer Statements

Contributors AM supervision of work, paper writing, assisted in microbiological diagnosis, SA sample and data collection, putting up tests, paper writing, MS supervision of work, and RB bronchoscopy and clinical diagnosis.

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