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Aspergillus hazardous problem in ceramic workers

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Abstract

Ceramic workers are at a high risk of developing respiratory problems as they are exposed to high levels of respirable dust containing silica and high microbial counts, including high *Aspergillus* counts. The aim of the study was to study the percentage of ceramic workers with positive *Aspergillus* (A.) through polymerase chain reaction (PCR) and specific IgE (slgE) for the different *Aspergillus* species. PCR and specific IgE (slgE) for the different *Aspergillus* species (*A. flavus*, *A. fumigatus* and *A. niger*) were estimated in 40 ceramic workers and 56 control subjects. Results revealed that 32.5% of the workers' sputum was PCR positive for *Aspergillus*. About 69.2% of them were *A. flavus* positive, 15.4% *A. niger* positive, 7.7% *A. fumigatus* positive and 7.7% *A. flavus* and *A. fumigatus* positive. The percentage change in slgE for *A. fumigatus* between the workers and their controls was over 100%, while less than 50% for the other two species. The slgE levels for the three *Aspergillus* species were not significantly correlated with the duration of exposure. Fungal exposure could be considered potential hazardous problem in ceramic industry. There were no significant correlations between the duration of exposure and the slgE for the different *Aspergillus* species.

Keywords

Ceramic workers, PCR, specific IgE, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*

Introduction

Fungal spores with an aerodynamic diameter of less than 5 µm can enter the lungs causing hypersensitivity and toxic pneumonitis (Hansen, 1999; Sorenson, 1999). According to Denning (1998), the presence of *Aspergillus* in air is a major risk factor for both invasive and allergic aspergillosis. However, most of the moulds do not cause toxic effects or diseases unless the exposed person is severely immunodeficient.

Additionally, invasive pulmonary aspergillosis is an emerging serious infection in patients with chronic obstructive pulmonary disease (COPD). Denning (1998) found that approximately 50% of proven or probable cases of aspergillosis had COPD. The majority of patients with aspergillosis having advanced COPD are on corticosteroid therapy (Samarakoon and Soubani, 2008).

Little attention has been paid to the presence of bioaerosols in the occupational environments. The state of knowledge about biologically originated indoor pollution in Egypt seems to be narrow. In

ceramic industry, quantitative and qualitative knowledge about airborne bacteria, fungi and actinomycetes are essential demands for the effective diagnosis of health problems, as the environment creates a suitable condition for microbial growth. The working environment in ceramic industry needs to be warm and humidified, providing sufficient condition for initial growth of fungi. In previous study, Saad-Hussein et al. (2006) found that indoor air fungi counts were high compared to the control environment ($1.0\text{--}3.0 \times 10^3$ CFU/m³ and $0.6\text{--}0.9 \times 10^3$ CFU/m³, respectively), where the

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Table 1. Primer sequence and location

Primer	DNA sequence (5'-3')	Location	Species specificity
ASPU Af3r	ACTACCGATTGAATGGCTCG CATACTTTCAGAACAGTGTTC	521-bp	<i>A. fumigatus</i>
ASPU: Fl2r:	ACTACCGATTGAATGGCTCG TTCACTAGATCAGACAGAGT	521-bp	<i>A. flavus</i>
ASPU: Nilr:	ACTACCGATTGAATGGCTCG ACGCTTTCAGACAGTGTTCG	521-bp	<i>A. niger</i>

A.: *Aspergillus*.

commonest fungi were *A. flavus*, *A. fumigatus* and *A. niger*.

Several studies proved that polymerase chain reaction (PCR) is a powerful tool for the diagnosis of pulmonary fungal infection (El-Mahallawy et al., 2006; Sugita et al., 2004). Additionally, the enzyme-linked immunosorbent assay (ELISA) for detection of specific antibodies for *Aspergillus* was developed and proved to be significant diagnostic method for *Aspergillus* infection (Eder et al., 2000; Kurup et al., 2006; Wise et al., 2008).

The present study aimed to study whether *Aspergillus* can be accused as a health problem for ceramic workers occupationally exposed to high *Aspergillus* environmental counts and identify the commonest hazardous species.

Methodology

Study design

This study was a cross-sectional study.

Subjects

The present study consisted of two comparable male groups of workers; namely, 40 ceramic workers, from a ceramic factory at industrial Saddat city, occupationally exposed to high *Aspergillus* counts and a comparable 56 control group from workers in the National Research Centre exposed to lower *Aspergillus* counts, according to Saad-Hussein et al. (2006). Years of employment in the ceramic factory were considered the duration of exposure; as all the included workers were working 8-h shifts for 6 days a week. The two groups were comparable for age, smoking habits and socioeconomic status.

After explaining the purpose of the study for the included workers, written consents were obtained. The ceramic workers employed for less than 5 years or those failed to bring morning sputum samples were excluded from the start of the study.

DNA isolation from the sputum sample

Morning sputum specimens from the workers and the controls were collected in plastic sterile containers and stored at -80°C until extracted. Genomic DNA was extracted using genomic DNA purification kit (Promega kit) from all the collected sputum samples. DNA extracts were then measured for DNA concentrations using spectrophotometer (UV 160IPC Shimadzu) at 260 and 280 nm. The average DNA concentrations in samples necessitated the precipitation and concentration of DNA to obtain optimal concentrations of DNA for accurate PCR. DNA precipitation was done according to Sambrook and Russell (2001).

Oligonucleotide primers, primer sequences and nested PCR assay

The primer sequences, with courtesy of Sugita et al., 2004, were used for specific detection of the genus *Aspergillus*. The primer sequences for the first amplification step were as follows:

ASAP1:5\CAGCGAGTACATCACCTTGG3\
ASAP2:5\CCATTGTTGAACTTTTAACTGATT\

Three primer sets (Table 1) for second amplification step based on specific regions within the ASAP1 and ASAP2 were designed for use singly in identifying *A. fumigatus*, *A. flavus* and *A. niger* (Sugita et al., 2004).

Collection of blood samples

About 3 ml blood were collected in sterile tubes, left to clot for 30 min at 37°C and then centrifuged at 3000 rpm for 10 min. The separated serum was kept frozen at -80°C until estimation of specific IgE for *Aspergillus* species.

Specific IgE (sIgE) for *Aspergillus* species

Specific IgE (sIgE) for *Aspergillus* species (sIgE for *A. flavus*, *A. fumigatus* and *A. niger*) were estimated in the sera of the included subjects using ELISA kit

(r-biopharm Kit), according to the method used by Bernstein et al. (1990).

Statistical analysis

Data entry and statistical analysis were done using statistical package for the social sciences (SPSS) package system version 14.0. The quantitative results were expressed as means $\pm$ standard deviation (SD). The skewed quantitative results were expressed as median and range. The qualitative results were expressed as number (No.) and percentage (%).

The used statistical tests were independent *t* test for comparing the normally distributed quantitative variables/data between the two groups. Pearson's correlation coefficient was used to find out the statistical associations between the different quantitative variables. If the SD was more than 25% of the means, the nonparametric measure of Mann–Whitney *U* test was used for comparing quantitative variables/data between the two groups. Statistical analysis was considered significantly different when *p* value was ≤ 0.05 .

Results

The ceramic workers and their controls were comparable for age (35.8 ± 5.4 and 37.3 ± 9.8 years, respectively), for the socioeconomic status and for smoking habits (40% and 35.7%, respectively, were smokers).

All the included ceramic workers were supposed to be exposed to high *Aspergillus* counts for 5–19 years, with average 10.7 ± 3.0 years.

The first PCR amplification detected 13 (32.5%) positive sputum samples for *Aspergillus* among the ceramic workers, and 67.5% of the ceramic workers were negative (Figure 1a). Although all the sputum samples of the control group were PCR negative for *Aspergillus*.

The second PCR amplification detected that out of the 13 PCR-positive samples, 9 (69.2%) samples were positive for *A. flavus*, 2 (15.4%) for *A. niger* and 1 (7.7%) for *A. fumigatus* and 1 (7.7%) mixed *A. flavus* and *A. fumigatus* (Figure 1b).

The first and the second PCR amplification bands are shown in Figures 2 and 3.

However, there were no significant differences between the workers and their controls according to the sIgE for the different *Aspergillus* species (Table 2). The percentage changes in the levels of sIgE for the three *Aspergillus* species in the ceramic workers

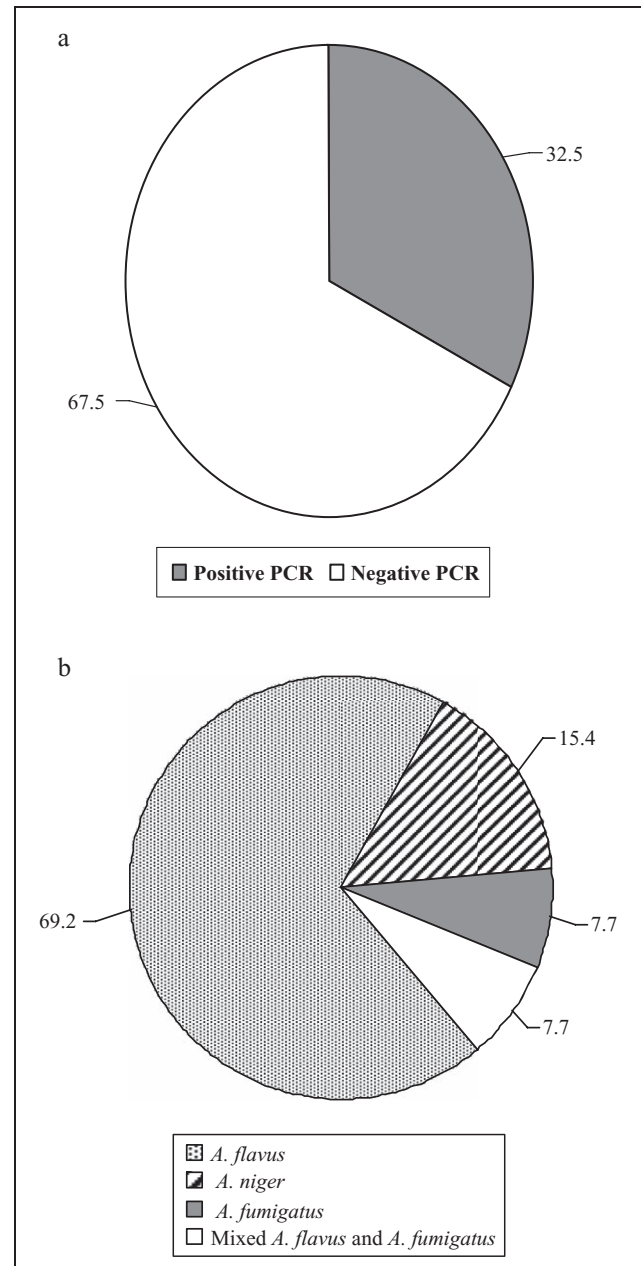


Figure 1. (a) Percentage of ceramics workers according to the results of the first amplification of polymerase chain reaction (PCR) in the sputum specimens for *Aspergillus*. (b) Percentage of ceramics workers according to the results of the second amplification of PCR in the sputum specimens for the different *Aspergillus* species.

compared to the control subjects were variable, but all were positive. The percentage change in the sIgE for *A. fumigatus* in the ceramic workers was over 100% of that in the control subjects, 50% for *A. niger* and 21% for *A. flavus* (Figure 4).

The serum levels of the sIgE for the three *Aspergillus* species were not significantly higher in the

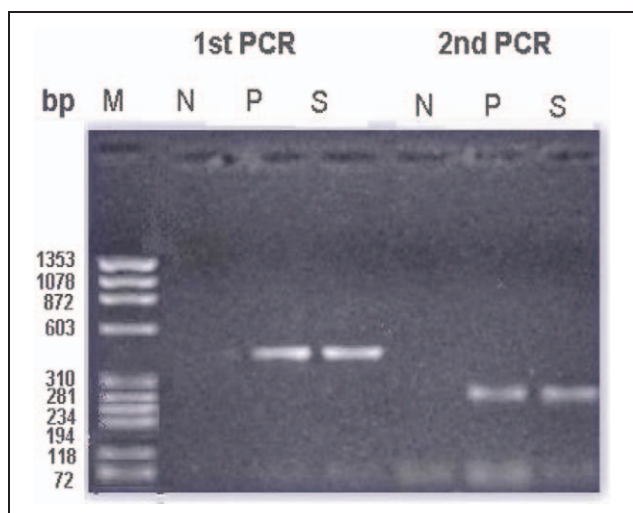


Figure 2. Polymerase chain reaction (PCR) product using *Aspergillus* specific primer ASAP (0.5bp) and species-specific primers sets (first amplification) in the sputum. (a) Positive PCR sample for *A. flavus*, (b) positive PCR sample for *A. niger*, (c) positive PCR sample for *A. fumigatus*, (d) positive PCR sample for mixed *A. fumigatus* and *A. flavus*. M: DNA Marker IX, 72–1393 bp, N: negative control amplification product, P: positive control amplification product, S: sample amplification product.

workers with positive PCR compared to the workers with negative PCR (Table 3).

The duration of exposure of the ceramic workers as not significantly correlated with the levels of sIgE for *A. flavus*, *A. niger* and *A. fumigatus* ($r = -0.1$; $p > 0.05$, $r = -0.2$; $p > 0.05$ and $r = 0.1$; $p > 0.05$, respectively; Figure 5).

Discussion

In ceramic industry, the workers are exposed to complex mixture of particles, usually very fine in size; upto 1 mm in diameter. It may contain metals, such as lead, respirable fibrogenic dusts such as silica (Dorevitch and Babin, 2001) and microorganisms such as bacteria, fungi and actinomycetes (Saad-Hussein et al., 2006). It was found that inhalation of respirable silica dust during ceramic manufacture may lead to immunological abnormalities (Otsuki et al., 2007; Polatli et al., 2008), which may increase susceptibility to fungal diseases. Chronic exposure to high levels of silica dust may not necessarily cause disabling silicosis but can lead to chronic bronchitis, emphysema and/or small airways disease that can end up with airflow obstruction causing COPD, even in the absence of radiological silicosis (Hnizdo and Vallyathan, 2003; Rushton, 2007).

The present study was considered a continuation of the study done by Saad-Hussein et al. (2006). Saad-Hussein et al. (2006) found that *Aspergillus* was one of the major environmental pollutants in the selected ceramic factory. These high concentrations of *Aspergillus* could explain the high percentage of *Aspergillus*-positive sputum among the examined ceramic workers (32.5%).

Climate and geographical factors are important determinants of the local prevalence of *Aspergillus* infections. In countries like Saudi Arabia and Sudan, with semiarid and arid dry weather conditions, *A. flavus* is the main etiological agent of invasive aspergillosis (Khairallah et al., 1992). *A. flavus* is also one of the main pathogens responsible for pulmonary aspergillosis in Africa (Hedayati et al., 2007).

The results of PCR in the present study revealed that the most prevalent *Aspergillus* species in the ceramic workers was *A. flavus*, followed by *A. niger*. Whereas only one worker was PCR positive for *A. fumigatus* and another worker was positive for mixed *A. flavus* and *A. fumigatus*.

Pasqualotto (2008) mentioned that the bigger size of *A. flavus* spores (25 μm in diameter), in comparison to that of *A. fumigatus* spores (23 μm in diameter), may favor their deposit in the upper respiratory tract and thus become the common etiological agent of fungal sinusitis and cutaneous infections but not invasive fungal pneumonia. Additionally, Hedayati et al. (2007) found that *A. flavus* is the second leading cause of invasive aspergillosis after *A. fumigatus* and it is the most common cause of superficial infection. This may explain the high percentage of PCR-positive sputum for *A. flavus* in the current results and the minimum percentage for *A. fumigatus* and for the mixed (*A. fumigatus* and *A. flavus*), as the sputum of the examined ceramic workers could be contaminated from their postnasal discharge. Moreover, this high percentage of positive *A. flavus* could also be attributed to the high vitality of *A. flavus* compared to *A. fumigatus*. Since 1967, Ford and Friedman proved that *A. flavus* grows well at 37°C (the body temperature), while *A. fumigatus* grows at temperatures of 40°C or above.

Although, reports of invasive pulmonary aspergillosis caused by *A. niger* are uncommon in the literature, in some studies exposure to *A. niger* colonization has been suggested as a potential risk factor for invasive disease (Husain et al., 2006), and *A. niger* has been detected in bronchial lavage fluids (Isobe et al., 2007).

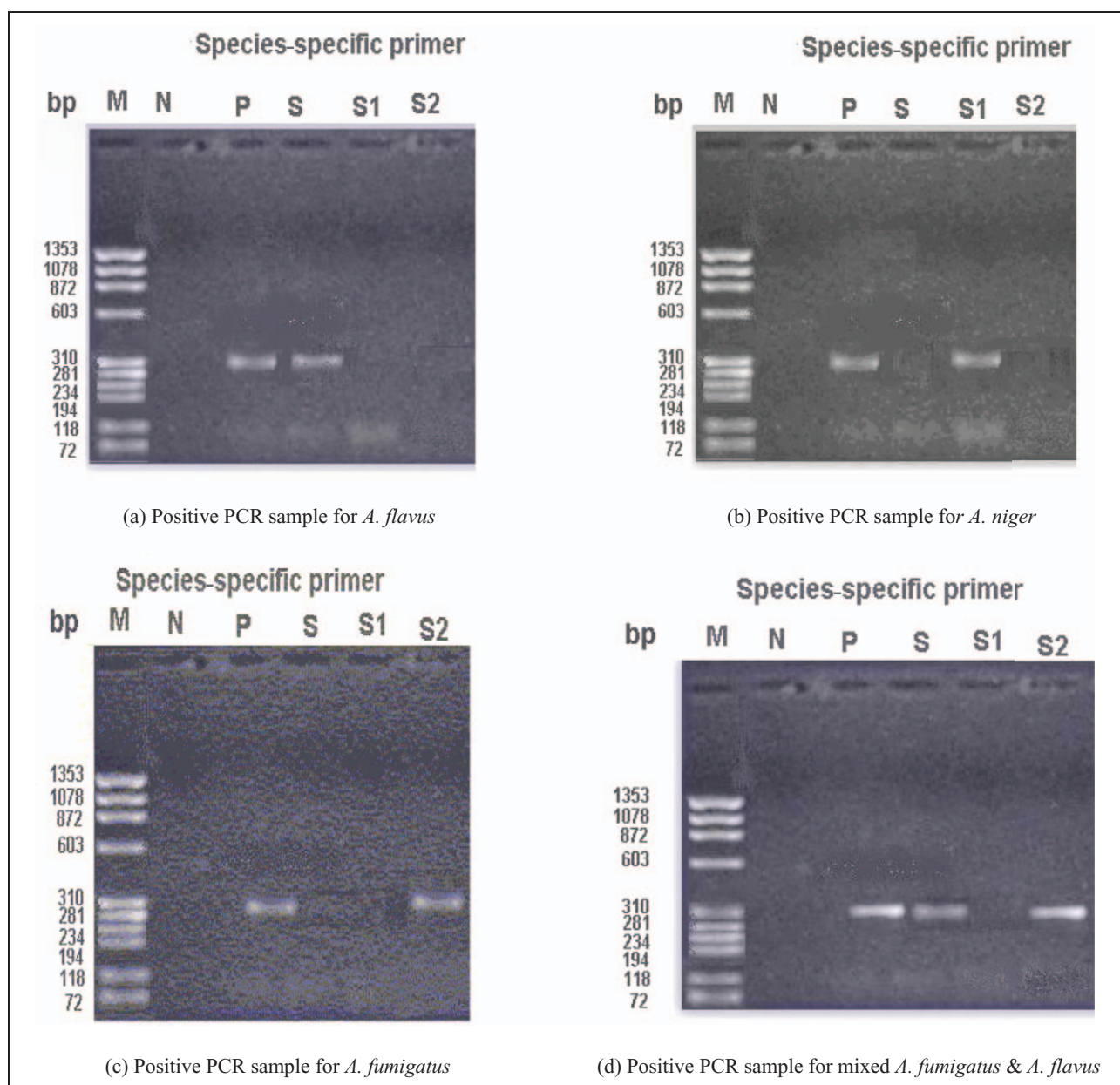


Figure 3. The positive polymerase chain reaction (PCR) sample for the different *Aspergillus* species using species-specific primer sets comparing to the positive control in the sputum. M: DNA Marker IX, 72–1393 bp. N: negative control amplification, P: positive control amplification, S: sample second amplification product using *A. flavus* primer sets, S1: sample second amplification product using *A. niger* primer sets, S2: sample second amplification product using *A. fumigatus* primer sets.

Table 2. Comparison of serum-specific IgE for the different *Aspergillus* species between the workers and their controls

sIgE (IU/ml)	Controls (56) Range (Median)	Workers (40) Range (Median)	Nonparametric	
			Mann-Whitney <i>U</i> test	<i>P</i> value
<i>A. flavus</i>	0.07–0.75 (0.28)	0.04–3.28 (0.29)	791	NS
<i>A. niger</i>	0.06–0.39 (0.24)	0.04–3.50 (0.24)	755	NS
<i>A. fumigatus</i>	0.06–0.68 (0.24)	0.03–3.90 (0.27)	322	NS

A.: *Aspergillus*.

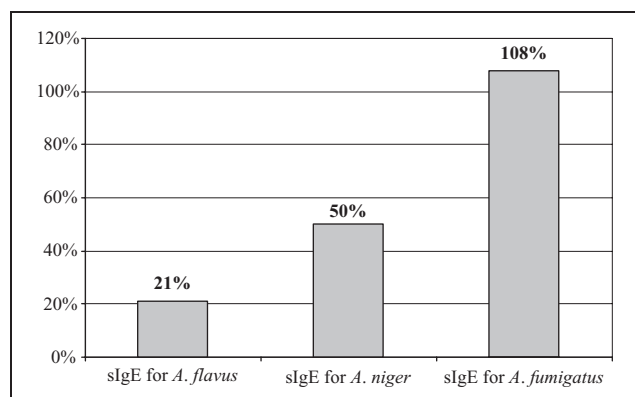


Figure 4. Percentage change in serum-specific immunoglobulin E (IgE) for the three *Aspergillus* species in the workers compared to the controls.

Few case reports on invasive pulmonary aspergillosis due to *A. niger* have been linked with some physiological, structural and acidophilic characteristics (Abdel-Rahim and Arbab, 1985; Severo et al., 1997). First, the small size of *A. niger* conidia (6–7 µm) and the presence of strong interspore bridges impair *A. niger* penetration into the lower respiratory tract. Therefore, after inhalation, the spores are easily captured and eliminated by the host mucociliary system. Second, *A. niger* is less thermotolerant than *A. fumigatus* and *A. flavus*, and its ideal temperature for fungal growth is around 30°C. This makes germination difficult in the presence of the human body temperature (~37°C). This may explain the low percentage of the ceramic workers with PCR positive for *A. niger* in the current study.

The present study revealed that the percentage of the sputum samples of the workers with PCR positive for *A. niger* was much low than for *A. flavus* but higher than for *A. fumigatus*. This seemed to be due to the virulence of *A. niger* that requires the presence of a severe immunosuppressed host to cause invasive disease or severely affected lung tissues. Xavier et al. (2008) found that *A. niger* may cause tracheobronchitis and invasive pulmonary aspergillosis in a lung transplant recipient.

Banerjee et al. (1998) demonstrated that patients with allergic bronchopulmonary aspergillosis (ABPA) exhibit significantly higher levels of IgE specific to the *Aspergillus* antigen, when compared with controls. This antigen appears to be involved with the adherence of the fungus to the extracellular matrix. The native and recombinant forms of this antigen were assessed and considered immunologically comparable and useful for specific diagnosis of

ABPA. It is believed that detecting sIgE to these intracellular antigens of *Aspergillus* indicates the presence of the disease. In ABPA, fungus grows in the airways and the immune reaction that follows results in the destruction of these microorganisms, with exposure of intracellular antigens.

Rydjord et al. (2007) found that high spore counts up to 107 spores/m³ air exposure were associated with a somewhat reduced level of mould-sIgE antibodies. Moreover, Greenberger (2002) mentioned that IgE was proved to be sharply elevated in the first stage of *Aspergillus* infection and returned to normal in the end stage of lung damage if untreated.

In the present study, there was no significant difference in the sIgE of the three *Aspergillus* species in ceramic workers compared to their controls, as well as in the workers with positive PCR compared to those with negative PCR. This means that we could not diagnose any case of APBA among the ceramic workers and could not exclude the positive cases. Thus, further investigations were recommended, especially with the elevation in percentage changes in the sIgE of different *Aspergillus* species in the ceramic workers compared to their controls.

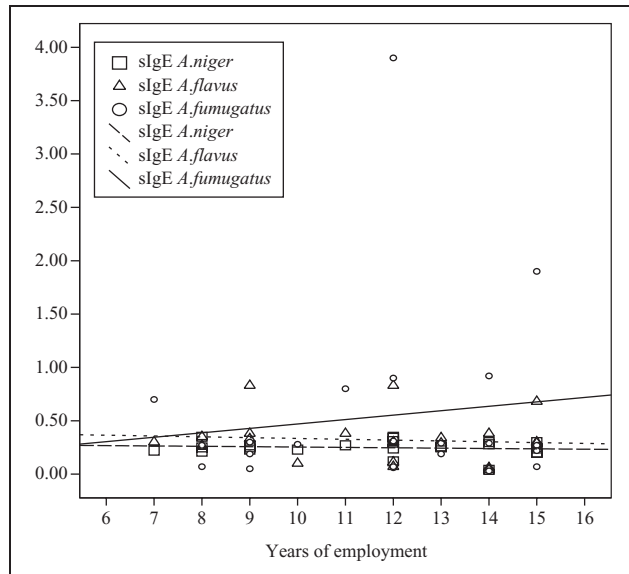
Duration of exposure in almost all the occupational diseases proved to have significant effect on the development of the health problem and in its progress. In the current study, there was no significant correlation between the duration of exposure and the levels of sIgE in the three *Aspergillus* species. Meanwhile, the inverse relation between the duration of exposure and the serum levels of sIgE for *A. flavus* and *A. niger* in the present study, though not significant, must not be neglected, as it may be considered a bad sign and needs special health care for those workers.

Conclusion

Fungal exposure in ceramic industry could be considered a potential health problem especially with the liability of low immunity secondary to chronic exposure to silica dust, as nearly one-third of the sputum samples of the ceramic workers were PCR positive for *Aspergillus*. *A. flavus* was the most prevalent species among the positive workers. The nonsignificant increase in sIgE for the three *Aspergillus* species in the ceramic workers with positive PCR compared to those with negative PCR must not be neglected and considered a dangerous marker put forward for further investigations. It is recommended to consider fungal

Table 3. Comparison of serum-specific IgE for the different *Aspergillus* species between the workers with negative and positive PCR (second amplification) in sputum

sIgE (IU/ml)	Workers with negative PCR (No.) Range (Median)	Workers with positive PCR (No.) Range (Median)	Nonparametric test	
			Mann-Whitney U test	P value
<i>A. flavus</i>	(30) 0.04–3.28 (0.29)	(10) 0.08–0.38 (0.30)	139	NS
<i>A. niger</i>	(38) 0.04–3.50 (0.24)	(2) 0.24–0.30 (0.26)	36.5	NS
<i>A. fumigatus</i>	(38) 0.05–1.90 (0.24)	(2) 0.03–3.90 (0.26)	48.5	NS

A.: *Aspergillus*.**Figure 5.** Correlation between the duration of exposure of the workers and the serum levels of sIgE for the three *Aspergillus* species.

exposure in ceramic industry as hazardous problem. Follow-up study is also recommended.

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