



Study of the immunological changes associated with *Aspergillus* infection among ceramic workers

Nadia Y. S. Morcos · Amal Saad-Hussein · Khadiga S. Ibrahim ·
Sanaa R. Abou-ElMakarem · Naglaa Abd El-Zaher · Gehan Moubarz

Received: 19 December 2018 / Accepted: 9 July 2019
© Springer Nature B.V. 2019

Abstract Ceramic workers are exposed to high levels of respirable dust that contains high microbial levels such as *Aspergillus*. It is recommended to consider fungal exposure in ceramic industry as a hazardous problem. This work aimed to study the impact of occupational exposure to high environmental concentrations of *Aspergillus* on the immune parameters related to the respiratory system in ceramic workers. Serum levels of total IgE, intercellular adhesion molecule-1 (ICAM-1), alpha 1-antitrypsin (α_1 AT), and tumor necrosis factor alpha (TNF- α) were assessed in 40 ceramic workers and 56 matched controls. Statistical analysis revealed that the levels of total IgE and ICAM-1 were higher in both non-smoker and smoker ceramic workers compared to their controls. While TNF- α was significantly lower in non-smoker ceramic workers compared to non-smoker controls. In addition, ceramic workers with sputum negative PCR for *Aspergillus* had lower TNF- α than those with positive PCR. α_1 AT levels did not differ significantly among the examined groups. These

changes were not correlated to the duration of exposure. In conclusion, occupational exposure to *Aspergillus* may cause different effects on immunological parameters related to respiratory problems in ceramic workers.

Keywords *Aspergillus* · Total IgE · Intercellular adhesion molecule-1 · Alpha 1-antitrypsin · Tumor necrosis factor alpha

1 Introduction

Aspergillus (A.) is a saprophytic filamentous fungus that is distributed environmentally worldwide, and its spores are regularly inhaled, but fungal diseases are uncommon (Park and Mehrad 2009). Over two-hundred species of *Aspergillus* are present; however, approximately ten percent are pathogenic to humans (Kauffman 2003). Although *Aspergillus* can affect many organs, respiratory tract is involved in more than 90% of the affected patients (Segal 2009; Saad-Hussein et al. 2016). It was proved that pulmonary manifestations due to environmental exposure to *Aspergillus* have extended from hypersensitivity response to invasive cavitations secondary to spore germination and hyphal infiltration (Cramer et al. 2011; Varga et al. 2011; Nath et al. 2017). Depending on the underlying state of the human immune system, the most severe infections occur in those who are

N. Y. S. Morcos · N. Abd El-Zaher
Biochemistry Department, Faculty of Science, Ain Shams
University, Cairo, Egypt

A. Saad-Hussein · K. S. Ibrahim (✉) ·
S. R. Abou-ElMakarem · G. Moubarz
Department of Environmental and Occupational
Medicine, National Research Centre, El-Behoos St.
(Tahrir St. Prev.), Dokki, Cairo 12311, Egypt
e-mail: khadigasalah@yahoo.com

immunocompromised, where invasive pulmonary aspergillosis results in high mortality rates (Chotirmall et al. 2013).

Atmospheric conditions have a great impact on mold growth, and the most important variable is the humidity (Gibson et al. 1994). Previously, Saad-Hussein et al. (2006) found that the average airborne fungi in different departments of a ceramic factory were 7×10^4 CFU/g with high concentrations of *Aspergillus* due to the higher environmental humidity and the reasonable temperature of the workplace. They also found the prevalence of the manifestations of chronic respiratory disease, the decline of the respiratory functions, and the positive prick skin test to *Aspergillus* were significantly higher among the ceramic workers compared to their controls. Therefore, they concluded that ceramic workers were at risk of developing respiratory problems not only due to the dusty environment, but also due to their occupational exposure to airborne fungi. Moreover, in a recent study among the same ceramic workers, Saad-Hussein et al. (2012) revealed that the positive sputum to *Aspergillus* by PCR was about 32.5% of the workers, and the prevalence was significantly dependent on the duration of exposure. For *Aspergillus* positive samples, it was found that 69.2% were positive for *A. flavus*, 15.4% for *A. niger*, 7.7% for *A. fumigatus*, and 7.7% for combined *A. flavus* and *A. fumigatus*. Additionally, many reports from Japan have shown that exposure to high concentrations of *Aspergillus* spores during the production of soybean products can lead to allergic bronchopulmonary aspergillosis (ABPA) (Akiyama et al. 1987; Kurosawa et al. 1990; Ashok and Chandramani 2016). Therefore, ABPA can be considered as an occupational disease.

Pulmonary disease associated with *Aspergillus* involves a complex interplay between the respiratory epithelium and the host response in the presence of inhaled spores, which triggers a cascade of consequences determined by the immunological status of those affected. Accordingly, the role of inflammatory and immune responses is both critical to the progress of the disease and in eliminating sequelae (Chotirmall et al. 2013; Sales-Campos et al. 2013). The major components of the innate immune system involved in the recognition and removal of the fungi include phagocytosis, antimicrobial peptide production, and recognition by pattern recognition receptors. The cytokine response is also critical for facilitating cell-

to-cell communication and promoting the initiation, maintenance, and resolution of the host response by a mechanism that remains poorly understood (Vijverberg et al. 2013).

A key concept in studying the immune responses to *Aspergillus* is that, fungal spores in the working environment may be responsible for allergic symptoms and respiratory impairments; as well as high total IgE levels among the exposed workers (Chattopadhyay et al. 2007). Alteration in the total IgE response was reported in several respiratory diseases, including extrinsic and intrinsic asthma, as well as allergic ABPA (Denning et al. 2006; Jat et al. 2018). Although the importance of the innate response has been well described both in vitro and in vivo, Cenci et al. (1998) and (1999) mentioned that the murine model of invasive aspergillosis showed resistance to infection that was associated with the production of gamma interferon (IFN- γ), tumor necrosis factor- α (TNF- α) and interleukins. The phagocytosis of *Aspergillus* resulted in a strong and rapid release of TNF- α , besides its key contribution to the anti-*Aspergillus* innate response (Roilides et al. 1998). But, in other studies, patients infected with *Aspergillus* appeared to be lower producers of TNF- α (Sambatakou et al. 2006; Chiang et al. 2008).

The objective of this study was an estimation of the effect of occupational exposure to *Aspergillus* among ceramic workers on the immune parameters related to respiratory problems such as intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor alpha (TNF- α) and alpha 1-antitrypsin activity (α_1 AT). The role of smoking habit was also studied as a confounding factor.

2 Subjects and methods

2.1 Study design and study setting

This study was cross-sectional comparative study as a part of the project “General Environmental Management in the New Industrial District in Sadat City-Egypt”. The current work is a continuation of the study of Saad-Hussein et al. (2012). The study included two comparative male workers’ groups. Forty of ceramic workers were chosen from a factory in the industrial Sadat city after exclusion of workers with silicosis and workers employed for less than

5 years, and matched with 56 control workers from the National Research Centre of the same socioeconomic status.

Prior to the interview, explanation of the purpose of the study was done and verbal consents were obtained from all participants. Contribution was on a voluntary basis.

2.2 Questionnaire

The questionnaire was completed by the ceramic workers and their controls during the personal interview about: personal characteristics, smoking habits, detailed current and previous occupational history, and medical history to exclude confounding factors such as silicosis or other immune disturbing health problems.

2.3 Laboratory investigations

About 5-ml blood samples were collected in sterile tubes and were left to clot for 30 min at 37 °C and then centrifuged at 3000 rpm for 10 min. The separated sera were divided into several aliquots and kept frozen at − 80 °C, to be thawed once on demand.

Commercial kits were used throughout the study. Total IgE by EIAgen total IgE ELISA kit (ADALTIS Kit) according to the method used by Geha (1984). Intercellular adhesion molecule-1(ICAM-1) by an ICAM-1 ELISA kit (DIACLONE Kit) by the method described by Rothlein et al. (1991), Alpha 1-antitrypsin (α_1 AT) by diffusion plate kit (Bioscientific Kit) according to Berne (1974), and Tumor necrosis factor alpha (TNF- α) by human TNF- α ELISA kit (DIACLONE Kit) according to the method described by Aderka et al. (1992). From the previous, the sputum of ceramic workers and the controls was done for *Aspergillus* by PCR techniques (Saad-Hussein et al. 2012).

2.4 Statistical analysis

Statistical analysis was performed using the Statistical Package of Social Science (SPSS) version 18. The qualitative results were expressed as number and percent, and then compared using Chi-square test. Due to the wide range of the variances, the quantitative results were expressed in the form of median with the minimum and maximum values and compared by

Mann–Whitney U and Kruskal–Wallis H tests. The comparisons of normal distributed results were done using an independent t test and ANOVA test. The level of statistical significance was set at $P < 0.05$.

3 Results

There were no significant differences between the workers and their controls in their ages (35.8 ± 5.4 and 37.3 ± 9.8 , respectively), and their smoking habits (40% smoking workers and 35.7% smoking controls). Total IgE and ICAM-1 were significantly increased in the ceramic workers compared to their controls (Table 1).

Table 2 shows that total IgE levels were significantly higher in smokers and non-smoker ceramic workers compared to their corresponding controls. While TNF- α was significantly lower in the smoker ceramic workers compared to their controls. There was no significant difference between the two groups in the ICAM-1 levels and α_1 AT activity.

There was no significant difference in the levels of total IgE, ICAM-1, and α_1 AT between the ceramic workers with negative and positive results for *Aspergillus*, while TNF- α was significantly higher in workers with positive results for *Aspergillus* compared to those with negative results (Table 3).

Table 4 shows no significant relationship between the duration of occupational exposure and the levels of total IgE, ICAM-1, α_1 AT, and TNF- α .

4 Discussion

Many studies mentioned that fungal spores in working atmosphere may be responsible for allergic symptoms and respiratory impairments among exposed workers (Chattopadhyay et al. 2007; Saad-Hussein et al. 2012, 2016). Alteration in the immune response was proved to be associated with the progress of fungal infections. According to Pelegrino et al. (2013), the markers that characterize local and systemic inflammation in pulmonary disease remain unclear, as do their correlation with smoking status and presence of disease. The present study estimates the impact of occupational exposure to *Aspergillus* among ceramic workers on these their immune parameters: total IgE, ICAM-1, TNF- α , and α_1 AT.

Table 1 Comparison of some bio-parameters between the control and the worker groups

		Controls (56) Range (Median)	Workers (40) Range (Median)	Nonparametric test	
				Mann–Whitney <i>U</i> test	<i>P</i> value
	Total IgE (KIU/L)	15–1200 (100)	25–1250 (525)	490	<i>P</i> < 0.001
	ICAM-1 (ng/ml)	4–350 (180)	4–950 (247.5)	540	<i>P</i> = 0.01
^a Mean ± SD	α ₁ AT (mg/dl)	(163.1 ± 47.2) ^a	(160.1 ± 45.7) ^a	0.293 ^b	NS
^b Independent <i>t</i> test	TNF-α (pg/ml)	5–19	3.75–90	365	NS

Table 2 Comparison of some bio-parameters between the control and worker groups (smokers and non-smokers)

	Controls (56)		Workers (40)		Nonparametric	
	Non-smokers (36) Range (Median)	Smokers (20) Range (Median)	Non-smokers (24) Range (Median)	Smokers (16) Range (Median)	Kruskal–Wallis test	<i>P</i> value
Total IgE (KIU/L)	15–1200 (190)	15–1200 (75)	25–1160 (525)	80–1250 (700)	17.381	<i>P</i> < 0.001
Mann–Whitney	(wnsm, wsm)	(wnsm, wsm)	(cnsm, csm)	(cnsm, csm)		
ICAM-1 (ng/ml)	4–350 (120)	100–350 (200)	95–950 (262.5)	4–300 (200)	11.245	<i>P</i> < 0.01
Mann–Whitney	(wnsm)	(–)	(cnsm)	(–)		
α ₁ AT (mg/dl)	156.7 ± 44.8 ^a	178.2 ± 51.2 ^a	169.9 ± 47.8 ^a	145.4 ± 39.1 ^a	1.566 ^b	NS
TNF-α (pg/ml)	5–19	5–19	3.75–15	5–90	10.223	<i>P</i> < 0.05
Mann–Whitney	(8)	(8)	(5.6)	(8.4)		
	(wnsm)	(wnsm)	(wsm, cnsm, csm)	(wnsm)		

^aMean ± SD^bANOVA: Analysis of variance

N.B.: wnsm: non-smoker workers, wsm: smoker workers, cnsm: non-smoker controls, csm: smoker controls

According to Vijverberg et al. (2013), re-exposure of allergen-sensitized patients to an aeroallergen will lead to an IgE-mediated inflammatory cascade in the airways. In allergic inflammation, there seems to be a disturbed balance in T helper (Th) 1-type and Th2-type cytokines with dominance toward Th2 cytokines, which induces a class switch in B cells to the production of IgE. This disturbed balance could explain the significant elevation in total IgE among the ceramic workers compared to the controls in the present study as they are continuously exposed to the aeroallergens in their working environment, although the increased IgE levels in the ceramic workers did not significantly differ among workers with positive PCR

results for *Aspergillus* compared to those with negative PCR results for *Aspergillus*.

Previous studies have explained that total IgE responses were increased among cigarette smoker asthmatic patients (Mlinaric et al. 2011; Golpe et al. 2017). Although the mechanism is unknown, some believe that smoking promoted the development of airway inflammation in association with Th-type immune responses (Nakamura et al. 2008). But, the present study revealed that the increase in IgE levels in the workers was not related to smoking habits, as there was no significant difference in total IgE according to their smoking habits. Likewise, Janson et al. (2001) found that there was no significant association

Table 3 Comparison of bio-parameters between the workers with negative and positive PCR for *Aspergillus*

	Workers with negative PCR (27) Range (Median)	Workers with positive PCR (13) Range (Median)	Nonparametric test	
			Mann–Whitney <i>U</i> test	<i>P</i> value
Total IgE (KIU/L)	25–1200 (400)	80–1160 (1000)	122.5	NS
ICAM-1 (ng/ml)	4–950 (212)	110–800 (270)	121	NS
α_1 AT (mg/dl)	(163.1 $\pm$ 49.4) ^a	(153.9 $\pm$ 37.9) ^a	0.59 ^b	NS
TNF- α (pg/ml)	3.75–75 (5.8)	4.75–90 (10.5)	63.5	<i>P</i> < 0.05

^aMean $\pm$ SD^bIndependent *t* test**Table 4** Relationships between the duration of occupational exposure and the levels of immunological parameters

	Duration of exposure (Years)	
	<i>r</i>	<i>P</i> value
Total IgE (KIU/L)	– 0.2	NS
ICAM-1 (ng/ml)	0.4	NS
α_1 AT	0.01	NS
TNF- α (pg/ml)	0.3	NS

between smoking exposure and the levels of total serum IgE.

Epithelial cells in the respiratory system are not passive bystanders during the assault of the epithelial barrier, but participate actively in the inflammatory response to defend the airway. Because epithelial cells are located at sites of contact with the external environment, they are often the first cells to interact with potential microbial pathogens. Through this interaction, epithelial cells may have the opportunity to detect and respond to pathogens by increased cell surface expression of intercellular adhesion molecule-1 (ICAM-1) in response to the inflammatory stimuli (Frick et al. 2000).

In the present study, ICAM-1 was significantly elevated among the ceramic workers compared to their controls, and smoking plays a significant role in that elevation, as ICAM-1 was significantly higher in the ceramic non-smoker workers compared to the non-

smoker controls, while this significance was disappeared between the smokers of the two groups.

Similarly, Backé et al. (2002) reported elevated ICAM-1 levels in persons exposed to ceramic dust. Furthermore, Lain et al. (2006), Ueno et al. (2006) and Halvorsen et al. (2007) reported consistent elevated ICAM-1 levels in smokers, suggesting inflammatory processes following strong irritants of tobacco smoke. Conversely, the present results revealed that there was no significant difference in ICAM-1 between the workers with positive and negative PCR for *Aspergillus*. This is consistent with Chiang et al. (2008) who found that *A. fumigatus* infection caused suppression in the expression of ICAM-1.

Alpha 1-antitrypsin (α_1 AT) is an important anti-protease in the lung, known to decrease the destructive effects of major proteases such as neutrophil elastase (Hunt and Tuder 2012). Recent studies suggested that α_1 AT plays an important role in modulating immunity, inflammation, proteostasis, apoptosis, and possibly cellular senescence programs. These processes contribute to the lung maintenance program that preserves it despite constant bombardment by damage initiated by cigarette smoke, pollutants, or infections (Lee et al. 2013).

The results of the present study found that there was no significant difference in the levels of α_1 AT between the studied groups. Foreman et al. (2012) explicated the conflicting results concerning the effect of smoking on α_1 AT; by pointing to that the development of chronic airflow obstruction in response to cigarette smoking is highly variable in the general population,

suggesting that individuals vary in their genetic susceptibility to the detrimental effects of smoking on lung functions.

In the current study, an important marker, tumor necrosis factor- α (TNF- α) has been analyzed. TNF- α was originally described as a circulating factor that can cause necrosis of tumors, but has been identified as a key regulator of the inflammatory response (Bradley 2008). Vascular endothelial cells respond to TNF- α by undergoing a number of pro-inflammatory changes, which increase leukocyte adhesion, transendothelial migration, and vascular leak and promote thrombosis. The increased incidence of infection in patients receiving anti-TNF treatment has highlighted the physiological role of TNF- α in infectious diseases (Bradley 2008; Fei et al. 2011). In the present study, TNF- α varied largely among the studied groups, it was decreased in ceramic non-smoker workers compared to the smoker workers and the controls (smokers and non-smokers). While TNF- α was significantly elevated in ceramics workers with positive PCR results for *Aspergillus* than those with negative results.

Attik et al. (2008) found that dust in the ceramics industry induced a significant expression of TNF- α . Churg et al. (2007) found that smoke-conditioned medium caused alveolar macrophages to increase secretion of TNF- α , while, Halvorsen et al. (2007) found that the concentration of TNF- α was unchanged in the smokers compared to the non-smokers. Some studies reported an increase in TNF- α in patients infected with *Aspergillus* (Sambatakou et al. 2006); others reported a decrease (Warris et al. 2003). Fei et al. (2011) studied the mechanisms of fungus-induced airway neutrophilia by persistent exposure of mice to *A. fumigatus*. They found that specific immunoregulatory mechanisms came into play only during the repeated challenges.

According to Serapinas et al. (2012), the possible explanation for this elevation in TNF- α could be attributed to that primary pro-inflammatory cytokines mainly act in peripheral lung tissues and differences in their levels could be detected in induced sputum but not always in systemic circulation.

5 Conclusion

Occupational exposure to *Aspergillus* in the ceramic industry proved to be significantly associated with the

elevation of the levels of total IgE. Levels of TNF- α varied according to type of work, smoking habits, and presence of *Aspergillus*. It is recommended to consider fungal exposure in ceramic industry as a chest problem risk and study its immunological sequel.

Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interest.

References

- Aderka, D., Engelmann, H., Maor, Y., Brakebusch, C., & Wallach, D. (1992). Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. *Journal of Experimental Medicine*, 175, 323–329.
- Akiyama, K., Takizawa, H., Suzuki, M., Miyachi, S., Ichinohe, M., & Yanagihara, Y. (1987). Allergic bronchopulmonary aspergillosis due to *Aspergillus oryzae*. *Chest*, 91, 285–286.
- Ashok, S., & Chandramani, P. (2016). Allergic bronchopulmonary aspergillosis: A perplexing clinical entity. *Allergy Asthma & Immunology Research*, 8(4), 282–297.
- Attik, G., Brown, R., Jackson, P., Creutzenberg, O., Aboukhamis, I., & Rihn, B. H. (2008). Internalization, cytotoxicity, apoptosis, and tumor necrosis factor-alpha expression in rat alveolar macrophages exposed to various dusts occurring in the ceramics industry. *Inhalation Toxicology*, 19, 1–8.
- Backé, E. M., Lotz, G., Tittelbach, U., Bolm-Audorff, U., Wallenstein, G., & Schneider, W. D. (2002). Soluble intercellular adhesion molecules in the serum of subjects exposed to dust at different workplaces—correlation to airway symptoms, lung function, tobacco and dust exposure. *International Journal of Hygiene Environmental Health*, 204(5–6), 377–379.
- Berne, G. H. (1974). Differing methodology and equations used in quantitating immunoglobulins by radial immunodiffusion—a comparative evaluation of reported and commercial techniques. *Clinical Chemistry*, 20(1), 61–89.
- Bradley, J. R. (2008). TNF-mediated inflammatory disease. *The Journal of Pathology*, 214(2), 149–160.
- Cenci, E., Mencacci, A., Del Sero, G., Bacci, A., Montagnoli, C., d'Ostiani, C. F., et al. (1999). Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through suppression of protective type I responses. *The Journal of Infectious Diseases*, 180(6), 1957–1968.
- Cenci, E., Mencacci, A., Fe d'Ostiani, C., Montagnoli, C., Bacci, A., Del Sero, G., et al. (1998). Cytokine and T-helper-dependent immunity in murine aspergillosis. *Research in Immunology*, 149, 445–454.
- Chattopadhyay, B. P., Das, S., Adhikari, A., & Alam, J. (2007). Exposure to varying concentration of fungal spores in grain storage godowns and its effect on the respiratory function status among the workers. *Industrial Health*, 45(3), 449–461.

- Chiang, L. Y., Sheppard, D. C., Gravelat, F. N., Patterson, T. F., & Filler, S. G. (2008). *Aspergillus fumigatus* stimulates leukocyte adhesion molecules and cytokine production by endothelial cells in vitro and during invasive pulmonary disease. *Infectious Immunology*, 76(8), 3429–3438.
- Chotirmall, S. H., Al-Alawi, M., Mirkovic, B., Lavelle, G., Logan, P. M., Greene, C. M., et al. (2013). *Aspergillus* associated airway disease inflammation and the innate immune response. *BioMed Research International*, 2013, 723129.
- Churg, A., Wang, X., Wang, R. D., Meixner, S. C., Prydzial, E. L., & Wright, J. L. (2007). α 1-Antitrypsin suppresses TNF- and MMP-12 production by cigarette smoke-stimulated macrophages. *American Journal of Respiratory Cell and Molecular Biology*, 37, 144–151.
- Cramer, R. A., Rivera, A., & Hohl, T. M. (2011). Immune responses against *Aspergillus fumigatus*: What have we learned? *Current Opinion Infectious Diseases*, 24(4), 315–322.
- Denning, D. W., O'Driscoll, B. R., Hogaboam, C. M., Bowyer, P., & Niven, R. M. (2006). The link between fungi and severe asthma: A summary of the evidence. *European Respiratory Journal*, 27, 615–626.
- Fei, M., Bhatia, S., Oriss, T. B., Yarlagadda, M., Khare, A., Akira, S., et al. (2011). TNF-alpha from inflammatory dendritic cells (DCs) regulates lung IL-17A/IL-5 levels and neutrophilia versus eosinophilia during persistent fungal infection. *Proceedings of National Academy of Sciences of the United States of America*, 108(13), 5360–5365.
- Foreman, M. G., Campos, M., & Celedón, J. C. (2012). Genes and chronic obstructive pulmonary disease. *The Medical Clinics of North America*, 96(4), 699–711.
- Frick, A. J., Joseph, T. D., Pang, L., Rabe, A. M., St Geme, J. W., 3rd, & Look, D. C. (2000). *Haemophilus influenzae* stimulates ICAM-1 expression on respiratory epithelial cells. *Journal of Immunology*, 164, 4185–4196.
- Geha, R. S. (1984). Human IgE. *The Journal of Allergy and Clinical Immunology*, 74, 109–120.
- Gibson, A. M., Baranyi, J., Pitt, M. J., Eyles, M. J., & Roberts, T. A. (1994). Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *International Journal of Food Microbiology*, 23, 419–431.
- Golpe, R., Martín-Robles, I., Sanjuán-López, P., Pérez-de-Llano, L., González-Juanatey, C., López-Campos, J. L., et al. (2017). Differences in systemic inflammation between cigarette and biomass smoke-induced COPD. *International Journal of Chronic Obstructive Pulmonary Disease*, 1(12), 2639–2646.
- Halvorsen, B., Lund Sagen, E., Ueland, T., Aukrust, P., & Tonstad, S. (2007). Effect of smoking cessation on markers of inflammation and endothelial cell activation among individuals with high risk for cardiovascular disease. *Scandinavian Journal of Clinical and Laboratory Investigation*, 67(6), 604–611.
- Hunt, J. M., & Tuder, R. (2012). Alpha 1 anti-trypsin: one protein, many functions. *Current Molecular Medicine*, 12(7), 827–835.
- Janson, C., Chinn, S., Jarvis, D., Zock, J. P., Torén, K., & Burney, P. (2001). Effect of passive smoking on respiratory symptoms, bronchial responsiveness, lung function, and total serum IgE in the European Community Respiratory Health Survey: across-sectional study. *Lancet*, 358(9299), 2103–2109.
- Jat, K., Walia, D., & Khairwa, A. (2018). Anti-IgE therapy for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database of Systematic Reviews*, CD010288. <https://doi.org/10.1002/14651858>.
- Kauffman, H. F. (2003). Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. *Frontiers in Bioscience*, 8, e190–e196.
- Kurosawa, M., Kobayashi, S., Yanagihara, Y., & Shida, T. (1990). case of occupational allergic bronchopulmonary aspergillosis unique to Japan. *British Journal of Clinical Practice*, 44, 482–489.
- Lain, K. Y., Luppi, P., McGonigal, S., Roberts, J. M., & DeLoia, J. A. (2006). Intracellular adhesion molecule concentrations in women who smoke during pregnancy. *Obstetrics Gynecology*, 107(3), 588–594.
- Lee, S., Lee, Y., Hong, K., Hong, J., Bae, S., Choi, J., et al. (2013). Effect of recombinant α 1-antitrypsin Fc-fused (AAT-Fc) protein on the inhibition of inflammatory cytokine production and streptozotocin-induced diabetes. *Molecular Medicine*, 19, 65–71.
- Mlinaric, A., PopovicGrle, S., Nadalin, S., Skurla, B., Munivrana, H., & Milosevic, M. (2011). Passive smoking and respiratory allergies in adolescents. *European Review for Medical and Pharmacological Sciences*, 15(8), 973–977.
- Nakamura, Y., Miyata, M., Ohba, T., Ando, T., Hatsushika, K., Suenaga, F., et al. (2008). Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to T (H) 2-type immune responses and airway inflammation. *The Journal of Allergy and Clinical Immunology*, 122(6), 1208–1214.
- Nath, A., Khan, A., Hashim, Z., & Patra, J. K. (2017). Prevalence of *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma at a tertiary care center in North India. *Lung India*, 34, 150–154.
- Park, S. J., & Mehrad, B. (2009). Innate immunity to *Aspergillus* species. *Clinical Microbiology Reviews*, 22, 535–551.
- Pelegrino, N. R., Tanni, S. E., Amaral, R. A., Angeleli, A. Y., Correa, C., & Godoy, I. (2013). Effects of active smoking on airway and systemic inflammation profiles in patients with chronic obstructive pulmonary disease. *American Journal of the Medical Sciences*, 345(6), 440–445.
- Roilides, E., Katsifa, H., & Walsh, T. J. (1998). Pulmonary host defenses against *Aspergillus fumigatus*. *Research Immunology*, 149, 454–465.
- Rothlein, R., Czajakowski, M., & Malin, D. (1991). A form of circulating ICAM-1 in human serum. *Journal of Immunology*, 147, 3788–3793.
- Saad-Hussein, A., Awad, A. H., & Aziz, H. (2006). Assessment of respiratory health problems due to exposure to air borne fungi in ceramics industry. *Egypt Journal of Occupational Medicine*, 30(2), 193–216.
- Saad-Hussein, A., Mona, M. T., Fadl, N. N., Awad, A. H., Mahdy-Abdallah, H., Moubarz, G., et al. (2016). Effects of airborne *Aspergillus* on serum aflatoxin B1 and liver enzymes in workers handling wheat flour. *Human and Experimental Toxicology*, 35(1), 3–9.
- Saad-Hussein, A., Morcos, N. Y. S., Rizk, S. A., Ibrahim, K. S., El-Zaher, N. A., & Moubarz, G. (2012). *Aspergillus*

- hazardous problem in ceramics workers. *Toxicology and Industrial Health*, 28(10), 886–893.
- Sambatakou, H., Pravica, V., Hutchinson, I. V., & Denning, D. W. (2006). Cytokine profiling of pulmonary aspergillosis. *International Journal of Immunogenetics*, 33(4), 297–302.
- Segal, B. H. (2009). Aspergillosis. *The New England Journal of Medicine*, 360, 1870–1884.
- Serapinas, D., Sitkauskienė, B., & Sakalauskas, R. (2012). Inflammatory markers in chronic obstructive pulmonary disease patients with different $\alpha 1$ antitrypsin genotypes. *Archives of Medical Science*, 8(6), 1053–1058.
- Sales-Campos, H., Tonani, L., Cardoso, C. R. B., & Kress, M. R. V. Z. (2013). The immune interplay between the host and the pathogen in *Aspergillus fumigatus* Lung Infection. *BioMed Research International*. Article ID 693023. <http://dx.doi.org/10.1155/2013/693023>.
- Ueno, H., Pradhan, S., Schlessel, D., Hirasawa, H., & Sumpio, B. E. (2006). Nicotine enhances human vascular endothelial cell expression of ICAM-1 and VCAM-1 via protein kinase C, p38 mitogen-activated protein kinase, NF- κ B, and AP-1. *Cardiovascular Toxicology*, 6(1), 39–50.
- Varga, J., Frisvad, J. C., & Samson, R. A. (2011). Two new aflatoxin producing species, and an overview of *Aspergillus* section Flavi. *Studies in Mycology*, 69, 57–80.
- Vijverberg, S. J., Hilvering, B., Raaijmakers, J. A., Lammers, J. W., Maitland-van der Zee, A. H., & Koenderman, L. (2013). Clinical utility of asthma biomarkers: from bench to bedside. *Biologics*, 7, 199–210.
- Warris, A., Netea, M. G., & Wang, J. E. (2003). Cytokine release in healthy donors and patients with chronic granulomatous disease upon stimulation with *Aspergillus fumigatus*. *Scandinavian Journal of Infectious Diseases*, 35, 482–487.