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TANAFFOS

Three Consecutive Episodes of Thunderstorm Asthma in Ahvaz, Iran: the Possible Role of Conocarpus Pollen

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Background: The aim of this study is to evaluate the clinical pattern of thunderstorm asthma (TA) and the possible environmental hypotheses involved in the escalation of these epidemics.

Materials and Methods: This retrospective descriptive study examined patients with respiratory problems referred to the clinics and emergency departments in Ahvaz, Iran during 2013-2016 periods following the first episodes of rainfall in autumn. The seasonal profile of airborne pollens and fungal spores in Ahvaz were characterized and clinical and spirometry findings of 443 patients were evaluated.

Results: Of 56,000 people referred to the emergency department due to respiratory problems associated with the first rainfall in Khuzestan province, 91.4% displayed asthma-like symptoms and 71.3% had a history of allergic rhinitis. According to the results of spirometry test 38%, 52.9%, and 9.1% of patients had normal, obstructive, and restrictive patterns, respectively. Our results highlight the importance of allergic rhinitis as risk factors of TA epidemics. In terms of pollen, seasonal pollen integral was much higher in autumn than in winter.

Conclusion: Our results highlight the importance of seasonal allergy and rhinitis as risk factors for thunderstorm asthma epidemics.

Key words: Epidemics; Asthma; Pollen; Emergency Service Hospital

INTRODUCTION

Thunderstorm asthma (TA) refers to marked increase in acute bronchospasm cases following thunderstorms in the local vicinity. According to various evidences, this phenomenon occurs in the pollen seasons. It has been shown that thunderstorms are a risk factor for the incidence of asthma attacks in allergic patients, causing severe asthma epidemics during thunderstorms (1-4). Several types of TAs have been reported around the world. The largest outbreak ever recorded occurred on 24 June 1994 in London (5). However, the first report about the relationship between thunderstorm and asthma symptom outbreaks was released at Birmingham Hospital on 6-7 July 1983 (6). Other types of asthma outbreaks during thunderstorms have also been reported in Australia, Italy, Canada, and the USA (7-10). In Australia, major epidemic asthma coincided with thunderstorms in Melbourne in 2016 (11-13). The nature of relationship between asthma attacks and thunderstorms should be explored. However, there is convincing evidence highlighting a pollen etiology, as TA epidemics mainly occur in seasons with a high atmospheric concentration of airborne allergic pollen (3, 14-17). Besides, most people affected by this phenomenon have allergies (18, 19) and if these people stay indoor with

windows closed during thunderstorms, they can prevent possible consequences. Under thunderstorm conditions, pollen grains may be concentrated on the ground releasing allergic particles into the atmosphere after rupturing particles by the osmotic shock. One characteristic of these allergic particles is their respirable size. During a thunderstorm lasting for 20-30 minutes, allergic patients inhale a high dose of concentrated allergic materials. This is followed by an unprecedented surge in the number of people seeking emergency medical care for respiratory distress within the first hours of the thunderstorm and continues in the following days (1, 20, 21). Most people affected by asthma-like symptoms during thunderstorm events do not use asthma medications to control their condition. The proper use of asthma medications, as well as good control of asthma and allergic rhinosinusitis may protect asthmatic patients against symptoms of TA.

As the capital of Khuzestan province, Ahvaz is the seventh most populous city in Iran. According to the World Health Organization's annual report in 2013, Ahvaz was ranked as the most polluted city in the world with an annual mean value of 372 $\mu g/m^3$ for PM_{10} (22). Numerous assumptions were made about the respiratory autumn events from 2013 to 2015, which directed a heavy treatment load towards Ahvaz Jundishapur University of Medical Sciences (23). Perhaps the rise in pollen concentration and air pollutants in the autumn, especially dust storms from Arab countries, as well as the synergistic effects of particles and pollens together with industrial pollutants could be cited as the main causes of recent events. Thus, this study was conducted to evaluate the clinical pattern of patients referring to health centers along with the possible environmental hypotheses regarding the causes of these epidemics.

MATERIALS AND METHODS

This descriptive-analytical cross-sectional study was conducted in Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran from 2013 to 2016. Patients with respiratory symptoms following the first episodes of rainfall during these three years who had referred to pulmonary clinics were evaluated by a questionnaire containing demographics data, past medical history of respiratory diseases such as asthma, family history asthma and allergy, and history of smoking. Spirometry was performed for patients.

The spirometry assessment was performed using a spirometer (Ganshorn medizin electronic, Germany) in accordance with the standards of lung function testing of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) guidelines (24).

Hourly data about the pollutants were obtained from Khuzestan Environmental Protection Department, which included ozone (O_3), sulfur dioxide (SO_2), nitrogen oxides (NO_x), carbon monoxide (CO), and inhalable particulars with a diameter less than 10 microns (PM_{10}) during the epidemics.

Since the first TA occurred in Iran in 2013 and there was no experience in this field and pollens were not measured routinely, the sampling of aeroallergens including pollen grains and fungal spores was conducted using a Hirst-type volumetric sampler (Hirst, 1952, UK) placed on the rooftop of the sampling site in 2016. The detailed illustration of the method and sampling time have been described by Rad in 2019 (25).

Data analysis was conducted using SPSS 22. Qualitative variables were presented as frequency and frequency percentage, and quantitative variables were expressed as the mean and standard deviation.

RESULTS

In the evening of November 10, 2013, the cities of Khuzestan province witnessed the first rainfall. The number of patients with asthma-like symptom gradually rose to 2,200 within 10 hours after the rainfall. In the period of 1 to 20 November, 2013, the number of patients referring to emergency departments with respiratory symptoms in Ahvaz was 15,506 and this number from all other cities of Khuzestan was 2,274 cases. While 93% of patients were discharged after receiving urgent clinical care on an outpatient basis with mitigated symptoms, 7% of patients

were hospitalized due to the severity of the illness. The same event was repeated in 2014 and 2015, when during the period from October 17 to November 2, 2014, a total of 7,744 patients referred to hospitals in Ahvaz, and 8,336 patients in the entire province. This figure reached a new peak in 2015 when from October 28 to November 9, a total of 20,235 and 22,610 patients referred to hospitals in Ahvaz and other cities of Khuzestan province, respectively. In terms of geographic distribution, more than three-quarters of the cases with asthma-like symptoms during these three outbreaks were reported in Ahvaz. However, after massive pruning in 2016 following the first autumn rainfall, the number of referrals dramatically dropped in Ahvaz and all other cities in the province (Figure 1). Demographics with clinical and spirometry data are presented in Table 1.

The levels of airborne contaminants, including PM_{10}, CO, SO_2, O_3, and NO_X in the periods before and during the respiratory epidemics (except for ozone contamination from 28 October to 10 November 2016, which was higher than the limit) were lower than the values stipulated by the National Ambient Air Quality Standard (NAAQS) (Table 2).

Table 1. Frequency and mean distribution of demographic, clinical and spirometric data of referrals during the epidemic periods

Variable		N	%
	Male	214	48.3
Sex	Female	229	51.7
	Total	443	100.0
	Yes	61	15.6
Smoking	No	331	84.4
	Total	392	100.0
	Yes	246	58.0
History of attack in the last year	No	178	42.0
	Total	424	100.0
	Yes	57	14.8
Family history of asthma	No	329	85.2
	Total	386	100.0
	Yes	316	71.3
Allergic Rhinitis	No	127	28.7
	Total	443	100.0
	Yes	405	91.4
Asthma-like symptoms	No	38	8.6
	Total	443	100.0
	Normal	112	38.0
	Obstructive	156	52.9
Spirometry results	Restrictive	27	9.1
	Total	295	100.0
Variable	Mean ± SD	Min	Max
Age (yr)	38.2±13.2	20	88.0
FEV1 (%)	66.9±19.9	17.0	106.0
FVC (%)	78.1±19.3	30.0	135.0
FEV1/FVC	70.0±10.2	13.0	91.0
FEF25-75 (%)	47.7±45.2	6.0	79.0

Table 2. Concentrations of airborne contaminants in the timeframe before and during the respiratory epidemics

Variable	NAAQS	Thunderstorm-Year	Date	Mean	Status
PM_{10} (µg/m³)	150µg/m³(Daily)	The first-2013	From Oct. 29 to Nov. 21, 2013	144.7	Lower than NAAQS
		The second -2014	From Oct. 17to Nov. 2, 2014	128.8	Lower than NAAQS
		The third -2015	From Oct. 28 to Nov. 10, 2016	81.9	Lower than NAAQS
CO (ppm)	9 ppm(Average 8 hours)	The first-2013	From Oct. 29 to Nov. 21, 2013	1.7	Lower than NAAQS
		The second -2014	From Oct. 17to Nov. 2, 2014	0.9	Lower than NAAQS
		The third -2015	From Oct. 28 to Nov. 10, 2016	5.6	Lower than NAAQS
SO_2(ppm)	0.14ppm(Daily)	The first-2013	From Oct. 29 to Nov. 21, 2013	0.02	Lower than NAAQS
		The second -2014	From Oct. 17to Nov. 2, 2014	0.02	Lower than NAAQS
		The third -2015	From Oct. 28 to Nov. 10, 2016	0.02	Lower than NAAQS
O_3(ppm)	0. 08 ppm(Average 1 hours)	The first-2013	From Oct. 29 to Nov. 21, 2013	0.07	Lower than NAAQS
		The second -2014	From Oct. 17to Nov. 2, 2014	0.04	Lower than NAAQS
		The third -2015	From Oct. 28 to Nov. 10, 2016	0.12	Higher than NAAQS
NO_x(ppm)	0.24ppm(Daily)	The first-2013	From Oct. 29 to Nov. 21, 2013	0.02	Lower than NAAQS
		The second -2014	From Oct. 17to Nov. 2, 2014	0.03	Lower than NAAQS
		The third -2015	From Oct. 28 to Nov. 10, 2016	0.04	Lower than NAAQS

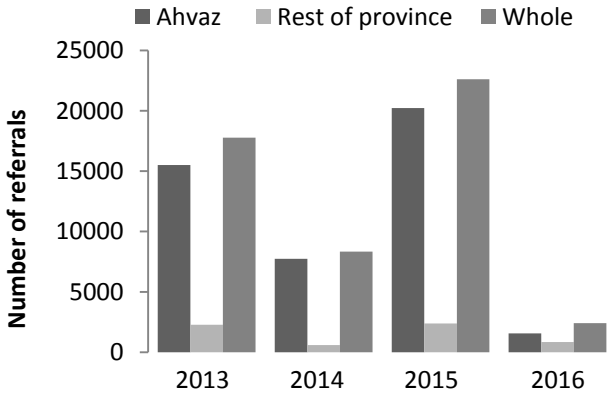

Figure 1. Frequency distribution of referrals with respiratory problems during the respiratory epidemics from 2013 to 2016

DISCUSSION

It is not clear why TA occurs only in some specific years and not annually. However, the importance of thunderstorm coexistence with the large amounts of airborne allergens has been underlined.

In our study, out of a total of 56,000 individuals referred to the emergency department due to respiratory problems associated with the first rainfall in Khuzestan province from 2014-2016, 91.4% displayed asthma-like symptoms and 71.3% had a history of allergic rhinitis. Seemingly, it was one of the greatest thunderstorms in the world. Although several thunderstorm episodes in Melbourne occurred in the aftermath of humid winters, they did not represent all events. All allergic people at risk of asthma during thunderstorm-induced asthma epidemics may also suffer from asthma even in the absence of any thunderstorms. Other environmental factors, such as a sudden decline in temperature, also play a role in this process. The 2016 Melbourne event saw a significant drop in temperature and a relative increase in humidity as the storm front passed (26). Another similar pattern was observed in London in 1994 (5).

In the present study, allergic rhinitis, family history of asthma, and similar symptoms in last year were seen in 71.3%, 14.8%, and 58% of cases, respectively. The most important clinical feature observed in our patients was allergic rhinitis, which was in agreement with the study conducted by Hew et al. (26), reporting that allergic rhinitis was a major finding in 87% of patients and a history of asthma accounted for 28% of patients. In this study, the mean age of patient was 38 years old which was similar to the mean age of incidence allergic rhinitis in middle age (27). For the first time, in this study, pulmonary function test was evaluated in patients with TA and results were found to have an obstructive, restrictive, and normal pattern in 52%, 9%, and 38% of cases, respectively. The

obstructive pattern had the highest prevalence consistent with asthma diagnosis. In some patients, the forced expiratory volume (FEV) may be reduced due to air trapping, resulting in pseudo-restriction on spirometry in the presence of normal to high FEV1/FVC% ratio, high residual volume (RV), and normal to high total lung capacity (TLC) (28). Many patients with underlying asthma have normal lung functions, and this relationship is recognized in asthma guidelines (29). The severity of obstruction does not always correlate with symptoms. It has been shown that patients with acute obstruction are more likely to perceive the symptom of dyspnea than patients with chronic obstruction (30). The pulmonary function test (PFT) results could be low during the pollen season when the airborne pollen count was very high. It was inferred that high airborne pollens have a negative effect on PFT allergic patients (31).

Air pollution has not been proven to be involved in the development of TA. According to the Victoria Environment Protection Authority (EPA) report, at 2016 Melbourne episode, the measurement of $PM_{2.5}$ and PM_{10} air pollutants did not demonstrate any increase in $PM_{2.5,}$ but elevated PM_{10} levels (particles ≤ 10 μm) were observed after the storm. However, high PM_{10} levels are a common finding in severe wind events and their association with previous TA days has not been established (27).

Ahvaz has been at the mercy of Middle Eastern dust storms over the two decades. Given the extreme pollution of the city throughout the year and the absence of considerable respiratory syndromes in citizens during the peak pollution days in recent years, it is unlikely that these factors play a role in the outbreak, as the concentration of these particles in the air tend to quickly fall to the normal level after rainfall. People living in Khuzestan province, which is in the vicinity of oil fields, are exposed to organic matter and aromatic hydrocarbons. Studies have shown that the combination of these substances and microgranule exacerbates cellular damage and could be carcinogenic (32).

Other criteria of air pollutants, including sulfur dioxide, nitrogen dioxide, ozone, and carbon monoxide are the major causes of respiratory problems and induce significant morbidity and mortality every year in periods of intensified air pollution. According to the Environmental Protection Agency's reports, the level of pollutants during outbreaks was low, especially during and after the rain (33).

Air pollution can escalate the irritability of the airways to aeroallergens in atopic people. With the pollutants binding with the surface of pollen grains, the sensitization potential of these particles changes, leading to elevated airway inflammation, lung mucosal permeability, and IgE-mediated responses to airborne allergens. There has been an increase in the prevalence of asthma and irritability of airways due to air pollution in Ahvaz. Agricultural activities have also contributed to the TA by releasing large amounts of fungal spores and grass pollens (32).

Features such as seasonal nature, provincial scale, and repeatability in the first rainfall along with the results of previous studies on TA in different parts of the world indicate that this phenomenon originates from increased concentrations of allergen matters following the rain (1, 3-14, 17, 19-21). Over the past 15 years, the vegetation of Ahvaz and some other parts of the province has been altered by the planting of a non-indigenous plant called Conocarpus. Although this imported tree has induced beneficial changes in the urban green infrastructure of Ahvaz, it has engendered serious side effects. One characteristic of Conocarpus is that it pollinates and flowers in the spring and autumn, releasing a plethora of pollens in the air along with other species of native plants, such as pigweed (amaranthus retroflexus) and Salsola rigida (25).

To control this disaster, the concentration of allergens should be reduced by pruning Conocarpus plants. Also, prophylactic treatment for vulnerable individuals and increase of society awareness were done. The prophylaxis protocol was distributed by the Ahvaz Jundishapur

University of Medical Sciences from the beginning of October 2016 at emergency departments and clinics. Target people included asthmatic patients, allergic rhinitis, and patients with a history of attack in the past years. These people were frequently encouraged by health and social media to receive health care protocols.

As mentioned earlier, there were no pruning activities prior to 2016. These activities were launched by the green infrastructure bureau of municipality in 2016 and have continued so far. As a result, it was observed that the number of patients admitted to hospitals for asthma attacks syndrome dropped sharply after conducting prune strategies in Ahvaz. The present research is in line with previous studies on this phenomenon (23, 25).

Further research is also required to answer questions about the most powerful assumption related to pollen and fungal spores. One limitation of this study was the lack of facilities and equipment for measuring pollens during 2013 to 2015, and consequently the paucity of studies on how Conocarpus pollen concentration and other pollens are related to hospital referrals during outbreaks. There are also limited in vivo and in vitro studies for evaluating sensitivity (specific IgE) to Conocarpus and other pollens in TA patients to confirm the hypothesis regarding the effect of pollens on TA.

CONCLUSION

Our results highlight the importance of seasonal allergy and rhinitis as risk factors for epidemic TA. This catastrophic phenomenon requires authorities and people in charge of urban air quality to identify and warn sensitive individuals in a bid to minimize exposure to ambient polluted air, particularly at the time of first autumnal rainfall in upcoming years in Ahvaz.

REFERENCES

1. D'Amato G, Vitale C, D'Amato M, Cecchi L, Liccardi G, Molino A, et al. Thunderstorm-related asthma: what happens and why. ***Clin Exp Allergy*** 2016;46(3):390-6.
2. Dabrera G, Murray V, Emberlin J, Ayres JG, Collier C, Clewlow Y, et al. Thunderstorm asthma: an overview of the evidence base and implications for public health advice. ***QJM*** 2013;106(3):207-17.
3. D'Amato G, Liccardi G, Frenguelli G. Thunderstorm-asthma and pollen allergy. ***Allergy*** 2007;62(1):11-6.
4. Celenza A, Fothergill J, Kupek E, Shaw RJ. Thunderstorm associated asthma: a detailed analysis of environmental factors. ***BMJ*** 1996;312(7031):604-7.
5. Venables KM, Allitt U, Collier CG, Emberlin J, Greig JB, Hardaker PJ, et al. Thunderstorm-related asthma--the epidemic of 24/25 June 1994. ***Clin Exp Allergy*** 1997;27(7):725-36.
6. Packe GE, Ayres JG. Asthma outbreak during a thunderstorm. ***Lancet*** 1985;2(8448):199-204.
7. Newson R, Strachan D, Archibald E, Emberlin J, Hardaker P, Collier C. Acute asthma epidemics, weather and pollen in England, 1987-1994. ***Eur Respir J*** 1998;11(3):694-701.
8. Wardman AE, Stefani D, MacDonald JC. Thunderstorm-associated asthma or shortness of breath epidemic: a Canadian case report. ***Can Respir J*** 2002;9(4):267-70.
9. Girgis ST, Marks GB, Downs SH, Kolbe A, Car GN, Paton R. Thunderstorm-associated asthma in an inland town in south-eastern Australia. Who is at risk? ***Eur Respir J*** 2000;16(1):3-8.
10. D'Amato G, Cecchi L, Annesi-Maesano I. A trans-disciplinary overview of case reports of thunderstorm-related asthma outbreaks and relapse. ***Eur Respir Rev*** 2012;21(124):82-7.
11. Thien F, Beggs PJ, Csutoros D, Darvall J, Hew M, Davies JM, et al. The Melbourne epidemic thunderstorm asthma event 2016: an investigation of environmental triggers, effect on health services, and patient risk factors. ***Lancet Planet Health*** 2018;2(6):e255-e263.
12. Davidson AC, Emberlin J, Cook AD, Venables KM. A major outbreak of asthma associated with a thunderstorm: experience of accident and emergency departments and patients' characteristics. Thames Regions Accident and Emergency Trainees Association. ***BMJ*** 1996;312(7031):601-4.
13. Woodhead M. Hospitals overwhelmed with patients after "thunderstorm asthma" hits Melbourne. ***BMJ*** 2016;355:i6391.

14. Anderson W, Prescott GJ, Packham S, Mullins J, Brookes M, Seaton A. Asthma admissions and thunderstorms: a study of pollen, fungal spores, rainfall, and ozone. ***QJM*** 2001;94(8):429-33.
15. Dales RE, Cakmak S, Judek S, Dann T, Coates F, Brook JR, Burnett RT. The role of fungal spores in thunderstorm asthma. ***Chest*** 2003;123(3):745-50.
16. D'Amato G, Baena-Cagnani CE, Cecchi L, Annesi-Maesano I, Nunes C, Ansotegui I, et al. Climate change, air pollution and extreme events leading to increasing prevalence of allergic respiratory diseases. ***Multidiscip Respir Med*** 2013;8(1):12.
17. Wallis DN, Davidson AC, Weilch J. Clinical and immunological characteristics of patients with thunderstorm asthma. ***Eur Respir J*** 1995;8(Suppl. 19):500s.
18. Rangamuwa K, Young AC, Cao R, Wong C, Khaleque A, Hanegbi N, et al. Persistence of symptoms, prevalence of asthma diagnosis, asthma control, preventer use and asthma action plan implementation in those presenting with thunderstorm asthma. ***Respirology*** 2017; 22: 118.
19. Rangamuwa K, Young AC, Nithianandan J, Demase K, Saerian N, McDonald-Wedding L, et al. Prior history of asthma, undiagnosed asthma and prevalence of rhinitis and its severity in those presenting with thunderstorm asthma. ***Respirology*** 2017; 22: 118.
20. Taylor PE, Flagan RC, Valenta R, Glovsky MM. Release of allergens as respirable aerosols: A link between grass pollen and asthma. ***J Allergy Clin Immunol*** 2002;109(1):51-6.
21. D'Amato G, Holgate ST, Pawankar R, Ledford DK, Cecchi L, Al-Ahmad M, et al. Meteorological conditions, climate change, new emerging factors, and asthma and related allergic disorders. A statement of the World Allergy Organization. ***World Allergy Organ J*** 2015;8(1):25.
22. Dastoorpoor M, Goudarzi G, Khanjani N, Idani E, Aghababaeian H, Bahrampour A. Lag time structure of cardiovascular deaths attributed to ambient air pollutants in Ahvaz, Iran, 2008-2015. ***Int J Occup Med Environ Health*** 2018;31(4):459-473.
23. Idani E, Dastoorpoor M, Goudarzi G, Khanjani N. Severe Outbreaks of Respiratory Syndromes Following Autumn Rainfall in Khuzestan, Iran. ***Arch Iran Med*** 2016;19(12):884-885.
24. van den Berge M, Ten Hacken NHT, Cohen J, Douma WR, Postma DS. Small airway disease in asthma and COPD: clinical implications. ***Chest*** 2011;139(2):412-423.
25. Rad HD, Assarehzadegan MA, Goudarzi G, Sorooshian A, Birgani YT, Maleki H, et al. Do Conocarpus erectus airborne pollen grains exacerbate autumnal thunderstorm asthma attacks in Ahvaz, Iran?. ***Atmospheric environment*** 2019;213:311-25.
26. Hew M, Lee J, Susanto NH, Prasad S, Bardin PG, Barnes S, et al. The 2016 Melbourne thunderstorm asthma epidemic: Risk factors for severe attacks requiring hospital admission. ***Allergy*** 2019;74(1):122-130.
27. Cazzoletti L, Ferrari M, Olivieri M, Verlato G, Antonicelli L, Bono R, et al. The gender, age and risk factor distribution differs in self-reported allergic and non-allergic rhinitis: a cross-sectional population-based study. ***Allergy Asthma Clin Immunol*** 2015;11:36.
28. Miller A, Palecki A. Restrictive impairment in patients with asthma. ***Respir Med*** 2007;101(2):272-6.
29. Busse WW. What is the best pulmonary diagnostic approach for wheezing patients with normal spirometry? ***Respir Care*** 2012;57(1):39-46; discussion 47-9.
30. Rietveld S, Everaerd W. Perceptions of asthma by adolescents at home. ***Chest*** 2000;117(2):434-9.
31. Harun NS, Lachapelle P, Douglass J. Thunderstorm-triggered asthma: what we know so far. ***J Asthma Allergy*** 2019;12:101-108.
32. Goudarzi G, Geravandi S, Alavi N, Idani E, Salmanzadeh S, Yari AR, et al. Association between cancer risk and polycyclic aromatic hydrocarbons' exposure in the ambient air of Ahvaz, southwest of Iran. ***Int J Biometeorol*** 2018;62(8):1461-1470.
33. Yari AR, Goudarzi G, Geravandi S, Dobaradaran S, Yousefi F, Idani E, et al. Study of ground-level ozone and its health risk assessment in residents in Ahvaz City, Iran during 2013. ***Toxin reviews*** 2016;35(3-4):201-6.