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Acute Effect of Water Pipe Smoke on Sensitized Animals

Majid Mirsadraee¹, Mohammad Reza Khakzad², Maryam Ahmadzadeh¹, Vajiheh Shayesteh¹, Mohammad Vejdananparast¹, Ali Tabatabaee¹, Kamran Ghafarzadegan³

¹ Internal Medicine, Division of Pulmonary Medicine, ² Department of Immunology, Medical School of Islamic Azad University, Mashhad Branch, ³ Dr. Moayed Pathobiology Laboratory, MASHHAD- IRAN.

ABSTRACT

Background: This study aimed to compare the inflammatory effects of water pipe smoke with cigarette smoke on inducing exacerbation in asthmatic murine model, under similar conditions of exposure.

Materials and Methods: Thirty-six BALB-C mice in six different groups (one control group, one asthmatic group and four groups of asthmatic exposed to smoke) were entered the study. Animals were exposed to cigarette and water pipe smokes and samples were obtained after 6 and 24 hours. Bronchoalveolar lavage fluid (BALF) was collected and analyzed for neutrophils, eosinophils, nitric oxide, Interferon- γ (IFN- γ) and Interleukin-4 (IL-4). Serum Interferon- γ and IL-4 were also evaluated. Both lungs were sent for histopathological examination.

Results: In all sensitized animals, BALF cytology showed a significant decrease in neutrophils and lymphocyte percentage and significant increase of eosinophils.

The level of nitric oxide in all smoke exposed animals was significantly higher than sensitized controls. Water pipe smoke did not affect the IL-4 level, but cigarette smoke decreased IL-4 after 6 hours. The level of IFN- γ in BALF decreased after 6 hours of exposure in both exposed groups and returned to baseline after 24 hours.

Conclusion: Exacerbation of asthma by water pipe smoke is comparable to that of cigarette smoke. The mechanism of action may be through the suppression of the type 1 T helper cells. (*Tanaffos*2010; 9(4): 39-47)

Key words: Water pipe, Cigarette, Nitric oxide, IL-4, INF- γ , Asthma, Tobacco smoke

INTRODUCTION

Asthmatic patients are vulnerable to harmful effects of air pollution, especially different types of smokes (1,2). The inflammatory effect of cigarette smoke as a leading cause of lung disease was

studied in asthmatic patients (3). Main toxic and carcinogenic compounds in cigarette smoke were previously described, but the most noxious material in the cigarette smoke is nicotine (4). The effect of this material on asthma is well understood and includes increasing symptoms of airway irritation, cough and exacerbation of disease (3). During exacerbation of asthma due to cigarette smoke, inflammatory mediators, such as nitric oxide and

Correspondence to: Mirsadraee M

Address: No 80, 15th Kosar, Kosar Ave., Vakilabad Blv. Mashhad, Iran.

Email address: mirsadra@mshdiau.ac.ir

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acetylcholine may increase (4,5).

Hookah smoking (also called water pipe, narghile, qelyan, and hubble bubble) is a traditional way of smoking that is especially prevalent in our region (6). The popularity of water pipe smoking has increased around the world, especially amongst the young population (7). The effect of water pipe smoke on the lungs has been studied (8). The pattern of water pipe smoking is different from that of cigarette (the smoke in water pipe passes through the instrument and water and therefore it is cooler than cigarette smoke, and the duration and amount of smoke is more than that of cigarette). For this reason, many users believe that it is less harmful than cigarette (9).

Recently some researchers reported reduced allergic activation of mast cells and IgE production by cigarette smoke (10). The triggering effect of cigarette smoke is well described (3) and usually asthmatic patients avoid exposure to cigarette smoke as it induces cough and dyspnea after sudden exposure. In our experience, exacerbation of asthma by acute inhalation of water pipe smoke was observed in asthmatic subjects as well. According to these experiences and the controversies mentioned above, a study about the acute effect of these smokes on asthma and related allergic inflammation is required. Also, considering the different nature of tobacco consumption in the water pipe, a research stimulating similar conditions in amount and time of exposure to cigarette and water pipe smoke was needed to compare the effect of these two types of smoke on asthma. The objective of this study was to evaluate the acute inflammatory effect of cigarette and water pipe smoke on asthma and compare cigarette smoke to water pipe smoke with pure tobacco and without any additives on sensitized animals for inducing asthma exacerbation, under similar conditions of exposure.

MATERIALS AND METHODS

Thirty-six BALB-C mice used in this study were

divided into six groups of six. Five groups of animals were sensitized to ovalbumin and one group was considered as control. Sensitization of animals was performed using the standardized method (11). Sensitization started by intraperitoneal injection of ovalbumin (as the antigen) and aluminum hydroxide (as the adjuvant) once a week for three weeks. Then animals were exposed to ovalbumin aerosol 0.1% for 20 minutes in a close chamber, once a day for 8 days for a total of 8 times (up to day 75). Ovalbumin aerosol was generated using an Omron CX3 nebulizer (nebulized particle size was 3-5mm with an output of 5 L/min). At the end of this period, animals were sensitized to ovalbumin and all of them began to cough, a symptom that showed successful disease induction. One group was kept as the sensitized control group and four groups were exposed to smoke (two groups to cigarette smoke and two groups to water pipe smoke). One of these groups was tested after 6 hours and the other after 24 hours (representative of early and late phases of asthma). Smoke exposure was done using an instillator that suctioned smoke from a cigarette or water pipe outlet (Figure 1) into the mouse chamber. In the water pipe group, smoke was produced by the traditional water pipe (hookah) and was heated by charcoal. The amount of tobacco used each time was 10 gr of 100% pure moistened shredded tobacco leaves, with a nicotine content estimated to be 2.5% and carbon monoxide concentration of 0.44%. The cigarette company reported that the nicotine content of its cigarettes was 2% and CO concentration was 0.39%. In both groups, five doses of smoke were instilled for 10 minutes. Then sensitized controls received fresh air. After 6 or 24 hours, depending on the understudy group, the animals were anesthetized using 44 mg/kg intraperitoneal ketamine. A blood sample was taken from each animal and tracheostomy was then performed at the cricothyroid membrane region. Bronchoalveolar lavage fluid was collected from the tracheostomy using a 0.9mm catheter and 0.4ml

sterile PBS. Lungs were lavaged four times. Bronchoalveolar lavage fluid (BALF) was evaluated for the cellularity and asthma markers.

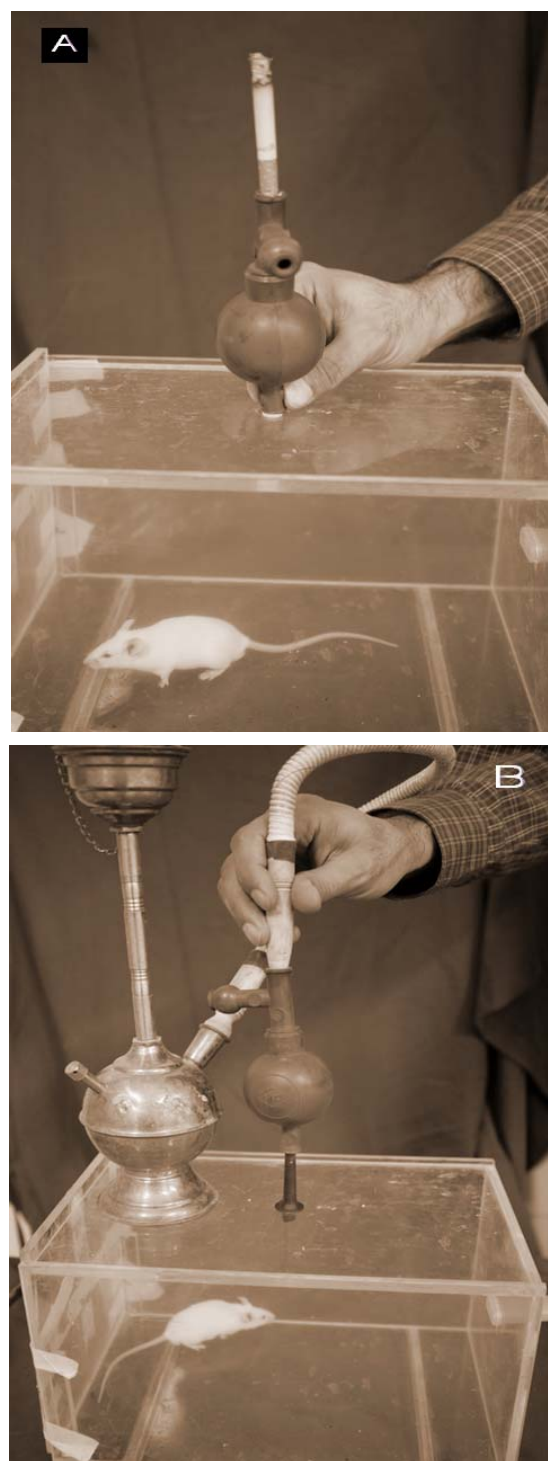


Figure 1. Environment and method of instillation of cigarette (a) and water pipe smoke (b).

Total white blood cell (WBC) count was measured using a torch stain and by Neubauer slide. Differential cell count was made using the criteria to classify cells as eosinophils, neutrophils, lymphocytes and basophiles and the results were expressed in cell percentage. Measurement of nitric oxide (chlorimetry method, Roche Company), interleukin-4 (IL-4) (U-CYTech, cytokine ELISA kit) and interferon- γ (U-CYTech, cytokine ELISA kit) in BALF was also done. Then autopsies were performed and both lungs were extracted and inflated with 0.5ml formalin and sent for a histopathology examination which certain criteria such as basement membrane thickening, smooth muscle hyperplasia and eosinophilic infiltration were evaluated to confirm asthma induction. Hematoxylin 8-Eosin staining was used for this evaluation.

This study was approved by the Ethical Committee of the Islamic Azad University of Mashhad.

Statistical analysis: Normal distribution of the data was checked using the Bartlett's test. Non-parametric analysis was used when normal distribution was not observed. Statistical analysis was done using the chi square test for qualitative histopathologic findings and unpaired "t-test for quantitative measurements of inflammatory cells and mediators. EPI INFO 2007 (v3.4) and SPSS ver.14 software were used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

Thirty mice successfully finished the course of asthma induction. Smoke exposure was performed in 24 mice, but one of them died (in the second group of water pipe smoke exposure). Histopathological study showed mucosal and smooth muscle changes consistent with asthma and eosinophilic infiltration in all asthmatic cases without any significant difference in severity ($X^2=0.44$, $p=0.58$) (Figure 2). In the control group, bronchial mucosa and smooth muscle were normal, but eosinophilic infiltration was seen in

one subject although still significantly less than the asthmatic group ($X^2=5.5$, $p=0.01$).

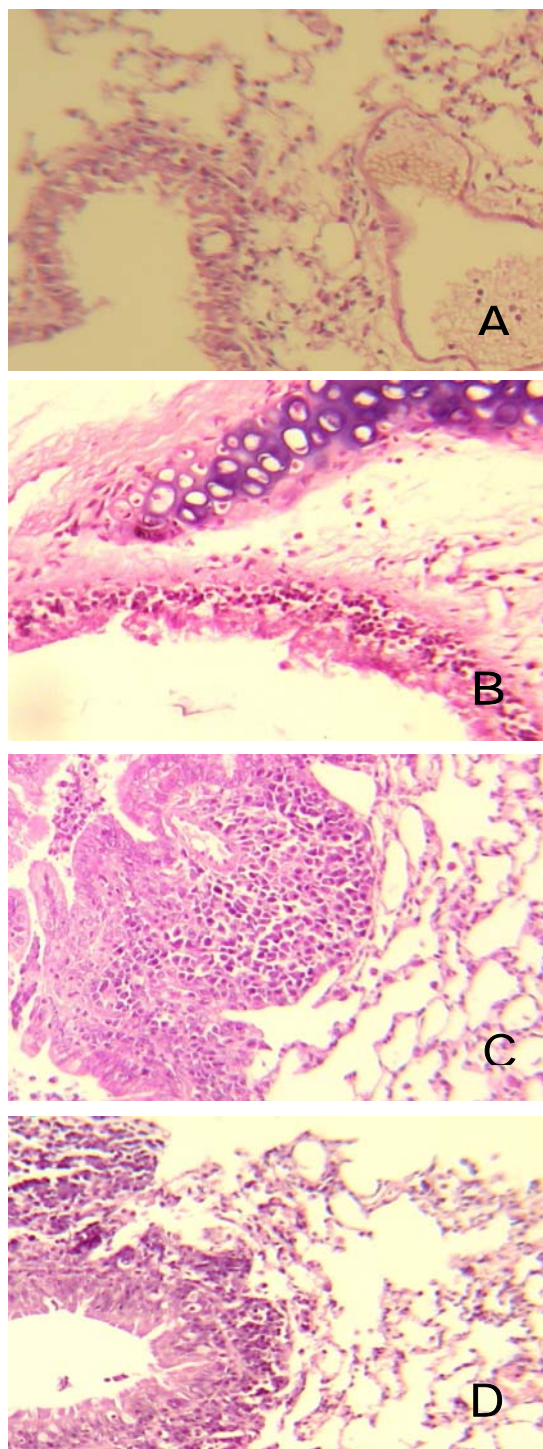


Figure 2. Histopathological evaluation of lung specimen from A: Non sensitized non smoke exposed mouse, B: Sensitized non exposed mouse, C: Sensitized that exposed to cigarette smoke and D: Sensitized that exposed to water pipe smoke.

Inflammatory cells in BALF

Total number of inflammatory cells increased in all groups of sensitized animals (Figure 3), and was significantly higher than that of the control group ($t=13.1$, $p=0.0001$). Mean \pm SD of cells in sensitized controls, cigarette and water pipe groups were not significantly different after 6 hours (1895 ± 312 , 1838 ± 467 and 1850 ± 242 cells per ml, respectively), but after 24 hours of exposure the total cell count decreased significantly (1453 ± 143 and 1454 ± 234 cells per ml respectively) ($p < 0.02$).

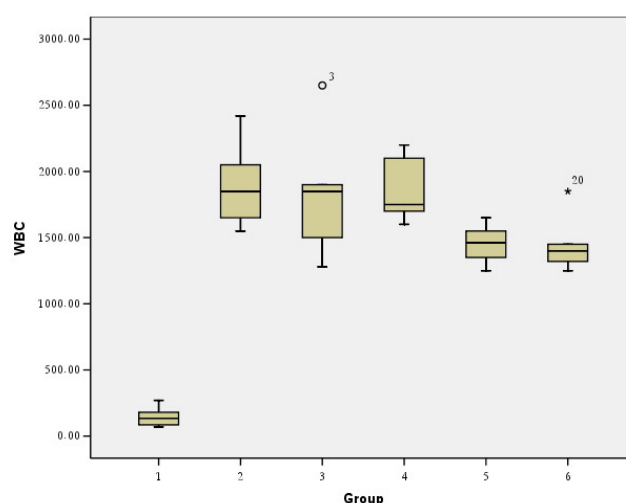


Figure 3. Box plot demonstration of total inflammatory cells in BALF of samples exposed to smoke (1- normal control group, 2- control asthmatic group, 3- cigarette after 6 hours, 4- water pipe after 6 hours, 5- cigarette after 24 hours, 6- water pipe after 24 hours)

Mean \pm SD of the four major groups of inflammatory cells is shown in Table 1. In comparison to the normal control group, in all sensitized groups, percentage of neutrophils decreased significantly (t test from 4.06 to 11.12 and p -value less than 0.004); whereas, eosinophils' percentage increased significantly (t test from 10.5 to 35.2 and p -value less than 0.001) (Figure 4). In addition to these changes, the lymphocyte percentage decreased significantly in both smoke groups after 24 hours ($t=6.17$, $P=0.001$ in the cigarette group and $t=4.81$, $p=0.001$ in the water pipe group).

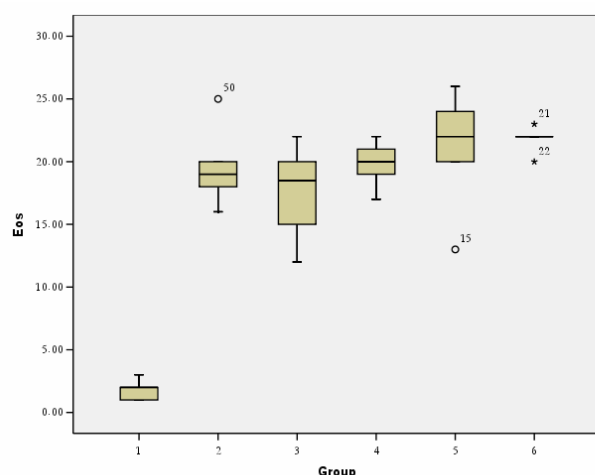


Figure 4. Box plot demonstration of eosinophils in BALF of mice exposed to smoke (1- normal control group, 2- asthmatic control group, 3- cigarette after 6 hours, 4- water pipe after 6 hours, 5- cigarette after 24 hours, 6- water pipe after 24 hours)

Comparisons between cigarette smoke and water pipe showed that after 6 hours, neutrophils percentage was significantly higher in the cigarette group compared to the water pipe group. However, after 24 hours no significant changes were observed between the two groups.

Nitric oxide

Nitric oxide was significantly higher in the sensitized control and smoke groups than the normal control group (Table 2) (t test ranged from 2.67 to 11.61, $p < 0.029$).

Nitric oxide in all smoke groups was also significantly higher than the sensitized control group (t test ranged from 6.09 to 13.61, $P < 0.001$). Nitric oxide significantly decreased in both cigarette and water pipe groups after 24 hours in comparison to 6 hours ($t = 4.12$, $P = 0.002$ and $t = 3.9$, $p = 0.004$ in cigarette and water pipe groups respectively). The difference in the level of nitric oxide in cigarette and water pipe groups after 6 hours was not significant; however, after 24 hours the level of nitric oxide in the water pipe group was significantly higher than in the cigarette group ($t = 3.36$, $P = 0.008$).

Interleukin-4

BALF IL-4 increased significantly in all groups (except for the cigarette group after 6 hours) in comparison to the non-sensitized group ($p < 0.01$), (Table 2). Compared to the sensitized controls, IL-4 showed two contradictory changes in the cigarette groups that decreased significantly in the cigarette group after 6 hours and then increased after 24 hours. No significant difference was detected in IL-4 in the water pipe groups compared with that of the sensitized controls. After 6 hours, IL-4 in cigarette groups was significantly lower than in the water pipe groups ($t = 3.42$, $p = 0.006$), but it increased significantly after 24 hours ($t = 8.85$, $P = 0.001$), making the difference between the cigarette and water pipe groups insignificant ($t = 0.31$, $P = 0.76$).

Serum IL-4 alterations are shown in Table 2. Differences between groups were not statistically significant except for the water pipe after 24 hours, which showed a significant increase in comparison to the non- sensitized and sensitized control groups ($t = 0.98$, $p = 0.0001$ and $t = 5.02$, $p = 0.0002$, respectively).

Interferon γ

BALF IFN- γ in the sensitized controls showed a mild increase (Table 2) ($t = 3.72$, $P = 0.004$). Six hours after exposure to cigarette and water pipe smoke, BAL IFN- γ decreased to levels below the non-sensitized and sensitized control groups (Table 2) ($t = 4.82$, $t = 12.4$ and $t = 4.27$, $t = 10.2$ respectively for non-sensitized and sensitized controls in the two smoke groups, $P < 0.002$ in all groups) and after 24 hours it returned to levels similar to the baseline level of non-sensitized cases, but still lower than the sensitized control group ($t = 3.96$, $P = 0.003$ and $t = 2.39$, $P = 0.04$). Differences between the two smoke groups were not significant.

Serum IFN- γ did not change after becoming sensitized or when exposed to cigarette or water pipe smoke (Table 2).

Table 1. Frequency (percentage) of four major inflammatory cells in bronchoalveolar lavage of understudy subjects

	Lymphocyte (% in BALF)	Neutrophil (% in BALF)	Macrophage (% in BALF)	Eosinophil (% in BALF)
Non-sensitized	53.16±2.16	40.04±2.16	4.33±2.33	1.8±0.15
Sensitized control	51.33±2.65	26.5±2.81†	2.66±0.81	19.5±3.08†
Cigarette after 6 hours	48.66±6.62	31.5±3.72*†	2.66±0.81	17.66±3.61†
Water pipe after 6 hours	50±3.54	27.1±2.04†	2.83±0.4	19.8±1.83†
Cigarette after 24 hours	43.3±2.9†	33.1±3.97*†	2±0.63†	21.1±4.53†
Water pipe after 24 hours	42.6±4.61*†	33.4±3.62*†	3±1	21.8±1†

* Significant difference between groups and sensitized control group

† Significant difference between groups and non-sensitized control group

Table 2. Mean and standard deviation of nitric oxide, interferon gamma (IFN-γ) and IL-4 in bronchoalveolar lavage of subjects exposed to smoke.

	Nitric Oxide (μM/L)	IL-4 BALF (Pg/ml)	IFN-γ BALF (Pg/ml)	IL-4 Serum (Pg/ml)	IFN-γ Serum (Pg/ml)
Non-sensitized	22.55±2.34	3.16±0.33	3.15±0.53	7.16±2.19	19.7±3.3
Sensitized control	28.63±1.64†	5.28±0.3†	4.08±0.3†	11.18±3.82	15.3±4.3
Cigarette after 6 hours	55.8±13.05*†	3.36±0.61*	1.96±0.28*†	50.47±66.2	21.6±13.3
Water pipe after 6 hours	50.76±4.77*†	4.81±0.8†	2±0.38*†	113±142	20.4±5.7
Cigarette after 24 hours	33.3±3.05*†	6.08±0.42*†	3.21±0.44*	27±28	21.9±9.2
Water pipe after 24 hours	40.34±3.85*†	5.94±1.02†	3.52±0.47*	30±8.05*†	25.1±11.3

IL-4= Interleukin-4, IFN-γ = Interferon-γ

* Significant difference between groups and sensitized control group

† Significant difference between groups and non-sensitized control group

DISCUSSION

In this study, sensitized mice were exposed to cigarette and water pipe smokes and the effect of smoke on BALF and serum was evaluated. Results of this study showed acute increase of nitric oxide and eosinophilic infiltration in BALF after exposure to cigarette and water pipe smoke. IL-4 increased in serum and BALF INF-γ as a hallmark of the type 1 T helper cells showed significant decrease in favor of a decreasing inhibitory effect of the T helper 1 system by cigarette and water pipe smoke.

Several studies described the harmful effects of active and passive cigarette smoking on adult and childhood asthma (1-3,12). Water pipe smoke as an old way of tobacco consumption is growing in western countries of Asia and in other parts of the world (6-9). Usually smokers use 10-20g of tobacco

while smoking a water pipe (8). The smoke passes through the water in the body of the pipe, where it is diluted and cooled and soluble compounds are dissolved (8). The usual duration of smoking is 40-50 minutes. A widespread perception among smokers, and even physicians, is that the water through which the smoke bubbles, filters the toxic components, rendering the smoking considerably less harmful than cigarette smoking (13,14). Recent studies on the main stream of water pipe smoke showed that the nicotine content of the water pipe tobacco is more than cigarettes (2% to 4%, in comparison with 1% to 3% for cigarettes) (8). Level of carbon monoxide (CO) in water pipe smoke is variable according to the instrument and type of charcoal used for burning the tobacco, but the amount of carbon monoxide

produced by a small hookah is three times the amount in cigarette smoke (15). For this reason, the mean concentration of carboxyhemoglobin was higher among water pipe smokers (10.1%) than cigarette smokers (6.5%) or nonsmokers (1.6%) (16). Previous studies showed that tar in the smoke of the water pipe was less than cigarette smoke because tobacco in the water pipe is heated to 450°C, approximately half the temperature of cigarette (900°C) (17). But in a recent study, Shihadeh and Saleh (18) showed that pyrolysis-synthesized Polycyclic aromatic hydrocarbons were present in the “tar” despite the low temperature characteristic of the tobacco in the water pipe. According to this review, many lung injuries can be expected from water pipe smoke.

Several studies reported the effect of water pipe smoke on pulmonary function tests (19), small air way function (20) and level of tonicity in smooth muscles of bronchial trees (21). In a study conducted in Saudi Arabia on 595 water pipe smokers, decrease in vital capacity, forced expiratory volume in 1 second, and the forced vital capacity was seen (22), and a similar study performed in Turkey showed a decrease in the peak expiratory flow rate (23).

However, no study was found on the direct effect of water pipe smoke on asthma. In this study, we used a recommended experimental model (24) for evaluating the effect of cigarette and water pipe smoke on asthma. After inducing asthma on BALB-C mice, they were exposed to cigarette or water pipe smoke for a fixed short time. Total inflammatory cell count increased in all asthmatic groups in comparison to the control group and this increase continued after 6 hours (Figure 3). This result showed a strong smoke effect on asthmatic patients because total inflammatory cells decreased after 24 hours of exposure, but was still significantly higher than that of the non-exposed group. Floreani et al. reported an increase in the number and activity of macrophages, neutrophils and eosinophils after

exposure to cigarette smoke (3). In the present study, eosinophilic fraction increased and a decrement was seen in neutrophilic fraction in all groups and in lymphocytes in the smoke exposed group after 24 hours (Figure 4). It seems that this cellularity is mostly determined by pathogenesis of asthma and a single short time exposure to smoke cannot affect the previous pattern of inflammatory cells.

Measurement of exhaled nitric oxide is now considered a suitable test for diagnosis and evaluation of severity of asthma (25). Kharitonov (26) et al. reported that cigarette smoke has an inhibitory effect on the production of nitric oxide. Jang et al. (27) measured nitric oxide in asthmatic patients who smoked cigarette. Their study revealed that nitric oxide level in asthmatic smokers was not significantly different from that of nonsmokers. The level of nitric oxide in subjects who smoked hookah was not reported. In this study, nitric oxide significantly increased after a brief exposure to smoke. Nitric oxide levels in the cigarette group was the same as the water pipe group after 6 hours; therefore, inflammatory reaction with the water pipe was the same as cigarette, and in both groups, the increase was significantly greater than in the non-smoker sensitized group. It means that both cigarette and hookah smoke have severe triggering effect on asthma. After 24 hours, nitric oxide in the water pipe group dropped more than in the cigarette group. We should keep in mind that a paradox exists regarding nitric oxide in asthma. Nitric oxide is an inflammatory mediator that increases during activation of asthma, but its main role in pathogenesis of asthma is to decrease inflammation and tissue protection from excess destructions (28,29). According to our results, the water pipe can induce exacerbation in asthma as we witnessed in clinical practice. Defensive mechanisms of nitric oxide were activated with smoke, but this mechanism is more transient in water pipe compared to cigarette smoking. This nitric oxide release should be

separated from nitric oxide generated from tobacco combustion. BAL IL-4 as a marker of type 2 T helper activity decreased after exposure to cigarette smoke, but it did not change with the water pipe. In contrast, serum IL-4 increased after 24 hours of exposure to the water pipe smoke which means stimulation of the type 2 T helper system with the water pipe. IFN- γ significantly decreased in both smoke groups, meaning a decreased inhibitory effect of the type-1 T helper system on the progression of asthma. We should emphasize that this study showed some evidence of harmful effects of water pipe smoke on asthma and its exacerbation mechanism. It is not completely applicable to human beings but it is helpful enough to warn about its noxious effects on asthmatic patients.

In conclusion, these data showed that in asthmatic patients, nitric oxide was increased with cigarette and water pipe smoke in excess of the ordinary increase observed in asthma. Increased severity of asthma and its exacerbation by exposure to water pipe smoke may be related to a diminished protective effect of the type 1 T helper system.

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