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RESEARCH ARTICLE



Effect of prenatal waterpipe tobacco smoke on airway inflammation in murine model of asthma of adult offspring mice

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ABSTRACT

Objective: Worldwide popularity of waterpipe tobacco smoking has increased, including in pregnant women. This study investigates the effect of prenatal waterpipe tobacco smoke (WTS) exposure on airway inflammation in a murine model of asthma of adult offspring mice.

Materials and methods: Pregnant BALB/c mice were exposed to fresh air or WTS, using a whole-body exposure system that mimics human use during WTS. Adult male offspring mice were divided into; (1) control (prenatal fresh air, postnatal ovalbumin sensitization and saline challenge), (2) postnatal Ova S/C (prenatal fresh air, postnatal ovalbumin sensitization and challenge (Ova S/C)), (3) prenatal WTS (prenatal WTS, postnatal ovalbumin sensitization and saline challenge) and (4) prenatal WTS + postnatal Ova S/C. Cells from the bronchoalveolar lavage fluid, cytokines, and oxidative stress markers (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARS)) from lung homogenates were evaluated.

Results: Prenatal WTS increased recruitment of cells in lungs and levels of SOD and catalase when compared to unexposed offspring's. The levels of cytokines, GPx and TBARS were not affected by prenatal WTS. Prenatal WTS exposure and postnatal Ova S/C increased airway inflammation and activity of SOD compared to control and Ova S/C mice and reduced IL-18 levels compared to Ova S/C mice.

Discussion and conclusions: Prenatal exposure to WTS induced airway inflammation, further enhanced by a murine model of asthma in adult offspring. Prenatal exposure to WTS adversely affects the lung function of the offspring and careful strategies for increasing public awareness regarding the harmful effects of WTS during pregnancy is important.

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KEYWORDS

Waterpipe tobacco smoke; murine model of asthma; inflammatory cells; oxidative stress; cytokines

Introduction

Tobacco smoking accounts for 6.1 million deaths worldwide and was the second leading cause of deaths in 2013 (GBDRF Collaborators et al., 2015). Maternal cigarette tobacco smoking is the leading cause of premature morbidity and mortality in the United States (Ramo et al., 2015) and it increases the risk of childhood asthma in the first seven years of life (Jaakkola & Gissler, 2004). In addition, prenatal exposure to nicotine, the major constituent of tobacco, resulted in reduced lung size and volume (Hodges, 1996) and abnormal collagen expression (Sekhon et al., 2002). Prenatal exposure to cigarette tobacco smoke altered lung development during infancy (DiFranza et al., 2004; Herrmann et al., 2008; Stocks & Dezateux, 2003) leading to negative effects on lung function and respiratory health in adulthood (Goksor et al., 2007).

The prevalence of waterpipe tobacco smoking, also known as hookah, shisha, narghile and arghile, has increased dramatically in recent years especially among youth (Palamar et al., 2014; Park et al., 2016; Rahman et al., 2014). According to World Health Organization (WHO) and other authorities, waterpipe tobacco smoking has been stated as a

public health problem. The prevalence of waterpipe smoking among pregnant women is around 6% in Lebanon (Akl et al., 2011) and 9% in Jordan (Azab et al., 2013). In a recent human study, maternal waterpipe tobacco smoking was shown to be associated with reduced newborn's anthropometric measurements that include birth weight, newborn length and head circumference (Al-Sheyab et al., 2016; Zhou et al., 2014). Our previous study showed that chronic waterpipe tobacco smoke (WTS) exposure increased airway inflammation in an allergen-induced murine model of asthma (Al-Sawalha et al., 2017b). However, the effect of prenatal WTS on airway inflammation of offspring has not been investigated. The objective of this study was to examine the effect of prenatal exposure to WTS on airway inflammation, cytokine levels and oxidative stress markers in offspring mice using a whole-body exposure system.

Materials and methods

Animals

Twenty male and 20 female BALB/c mice (10-weeks old) were purchased from the Animal Care Unit at Jordan

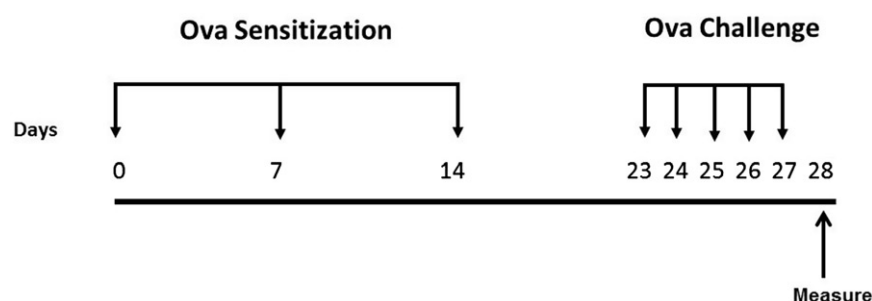


Figure 1. Treatment protocol. Mice received intraperitoneal (i.p.) ovalbumin (Ova) (2 mg/kg/day in 2 mg of alum) on days 0, 7 and 14; followed by nebulized Ova (1%) for 50 min on days 23, 24, 25, 26, 27. The mice were killed 24 h after the last nebulized Ova.

University of Science and Technology (Irbid, Jordan). The protocol of this study was approved by the Animal Care and Use Committee (ACUC) of Jordan University of Science and Technology. Mice were kept at a 12:12 light/dark cycle at standard room temperature and acclimated for at least 10 days before mating. Food and water were provided *ad libitum*. Female mice were randomly assigned into two groups: control group and WTS group. One pair of male and female mice were kept in the same cage for mating. The pregnancy was detected by vaginal plug method (Al-Sawalha et al., 2017a). If the female mouse was not pregnant, mating was repeated the next day. After birth, the male offspring mice were kept in fresh air and monitored until the age of 8 weeks. Only male offspring were used in this study to prevent any confounding differences due to sex (Card & Zeldin, 2009).

Adult male offspring mice were divided into; (1) control (exposed to prenatal fresh air, postnatal ovalbumin sensitization and saline challenge), (2) postnatal Ova S/C (exposed to prenatal fresh air and postnatal ovalbumin sensitization and challenge (Ova S/C)), (3) prenatal WTS (exposed to prenatal WTS, postnatal ovalbumin sensitization and saline challenge), (4) prenatal WTS + postnatal Ova S/C.

Waterpipe tobacco smoke exposure

Female mice in the WTS group were exposed to WTS for two hours one day prior to mating and the exposure was continued for one hour twice daily until delivery. WTS exposure was done using whole-body exposure system as described previously (Khabour et al., 2012). The WTS exposure system is composed of a standard waterpipe that was obtained from the local market, smoke generated apparatus (diaphragm pump and puff topography control device) and an exposure chamber. About 10 g of two Apples flavor ma'assel (Nakhlbrand, Egypt) was loaded in the waterpipe head. Quick-light charcoal briquettes were used as the heat source to burn the ma'assel (Shaban brand, Egypt). Waterpipe smoke is generated through the action of a diaphragm pump. The smoke bubbles was generated in the waterpipe water and was transferred to the waterpipe exposure system through the waterpipe hose, which was about 1 m long. The pump was controlled according to the Beirut method to give 171 puffs (per hour) of 2.6 seconds duration and 17 seconds interval between each puff (Katurji et al., 2010). The puffs were monitored by puff topography device to provide 530 mL mean puff volume that was similar to the

measured human puff topography during waterpipe smoking session (Shihadeh et al., 2004). Mice were not allowed access to food or water during the exposure. The CO concentration was maintained at similar levels (340 ± 50 ppm, mean $\pm$ SD) during exposure sessions (Singh & Scott, 1984; Venditti et al., 2011) via controlling the amount of pumped fresh air into the chamber (Al-Sawalha et al., 2017b). Mice in the control group were exposed to fresh air only in an exposure chamber similar to the one used for waterpipe groups. The exposure was done simultaneously with that of the waterpipe groups but in a separate room.

Ovalbumin sensitization and challenge

Eight-week-old male offspring mice ($n = 10$ in each group, one pup/litter) were sensitized with three intraperitoneal injections of 2 mg/kg/d of ovalbumin (Ova) (Sigma-Aldrich, St. Louis, MO) and 2 mg of Alum (Imject Alum; Thermo Scientific, Pierce, Rockford, IL) as described previously (Al-Sawalha et al., 2017b). The challenge phase was accomplished by exposing mice to either nebulized 1% Ova for 50 minutes (Ova S/C) (Al-Sawalha et al., 2017b) or saline (control) as illustrated in Figure 1.

Bronchoalveolar lavage fluid (BALF) collection

The mice were killed 24 h after the last ovalbumin or saline challenge by administering 0.1 mL of ketamine and xylazine combination (containing 87.5 mg/kg ketamine and 12.5 mg/kg xylazine). After cannulating the trachea, BALF was collected by perfusing both lungs, *in situ*, with 1 mL of cold phosphate-buffered saline and drawn back through the cannula and the procedure was repeated once. The collected BALF was centrifuged at $200 \times g$ for 15 minutes and the pellet was suspended in 1 mL phosphate buffered saline. Total cells were counted by Hemacytometer (Hausser Scientific, Horsham, PA). The cell pellet was cytocentrifuged using a Cytospin 4 (Thermo Electron Corporation, Waltham, MA) at 2000 rpm for 5 min. The number of differential cells was determined by Wright-Giemsa stain (Sigma-Aldrich, St. Louis, MO).

Interleukins level and oxidative stress markers measurement in the lungs

The extracted lung tissues were homogenized with lysis buffer and protease inhibitor cocktail (Sigma-Aldrich

Corp., St. Louis, MO) using tissue homogenizer (Tissue Master-125, Omni International, Kennesaw, GA) as described previously (Al-Sawalha, et al., 2017b). Homogenates were centrifuged at $14,000 \times g$ for 5 min at 4°C to remove insoluble debris. The levels of interleukin (IL)-2, 10 and 18 (R&D Systems, McKinley Pl NE, MN) were measured by ELISA following manufacturer's instructions. The absorbance was read at 450 nm (Epoch Biotek microplate reader, BioTek, Winooski, VT). The cytokine level was normalized to total protein in each sample. Total protein concentration was estimated by BioRad kit (Hercules, CA).

The activity of anti-oxidative enzymes superoxide dismutase (SOD) (Sigma-Aldrich Corp., St. Louis, MO), catalase (Cayman Chemical, Ann Arbor, MI) and glutathione peroxidase (GPx) (Sigma-Aldrich Corp., St. Louis, MO) and the amount of thiobarbituric acid reactive substances (TBARS) (Cayman Chemical, Ann Arbor, MI) were measured in lung tissue homogenates by calorimetric enzymatic assays following manufacturer's instruction. The enzyme activity was normalized to total protein in each sample that was measured using BioRad assay using bovine serum albumin as the standard.

Statistical analysis

Comparisons of the number of inflammatory cells, oxidative stress markers and cytokine levels were made using one-way ANOVA followed by Bonferroni's *post-hoc* test. Graph Pad Prism (version 5.0) computer program was used to carry out the statistical tests. All values were represented as mean $\pm$ standard error of the mean (SEM). $p < .05$ was considered statistically significant.

Results

Effect of prenatal waterpipe smoke exposure on airway inflammation in antigen-driven murine model of asthma in adult offspring mice

To determine the effect of prenatal WTS exposure on airway inflammation in adult male offspring mice using a murine model of asthma, pregnant mice were exposed to WTS for 2 h per day during the whole pregnancy period and BALF was evaluated for inflammatory cells in male offspring mice after exposure to Ova (antigen). Control mice were exposed to prenatal fresh air and postnatal ovalbumin sensitization and saline challenge. Mice that were exposed to prenatal WTS had higher number of the total number of cells as compared to control ($36.45 \pm 2.48 \times 10^5$ versus $0.67 \pm 0.13 \times 10^5$, $p < .05$). The recruitment of cells was also increased in Ova S/C mice ($21.49 \pm 7.32 \times 10^5$) compared to control mice ($0.67 \pm 0.13 \times 10^5$, $p < .05$). In addition, prenatal exposure to WTS and postnatal Ova S/C further increased the recruited cells ($47.05 \pm 2.81 \times 10^5$) compared to control and Ova S/C mice ($p < .05$). However, postnatal Ova S/C did not significantly increase the level of recruited cells to the airways in mice that were exposed to prenatal WTS (Figure 2(A)).

Asthma is characterized by eosinophilic type of airway inflammation. The airway eosinophils level was induced by Ova S/C ($2.13 \pm 0.63 \times 10^5$) and prenatal WTS exposure ($3.65 \pm 0.63 \times 10^5$) compared to control mice ($0.34 \pm 0.14 \times 10^3$, $p < .05$). Prenatal WTS and postnatal Ova S/C further potentiate the airway influx of eosinophils to the airways ($6.93 \pm 0.50 \times 10^5$) as compared to control, Ova S/C mice as well as prenatal WTS exposed mice ($p < .05$) (Figure 2(B)).

The influx of other types of inflammatory cells was also affected by prenatal WTS exposure and postnatal Ova S/C. Mice that were exposed to Ova S/C had higher levels of neutrophils ($3.60 \pm 1.12 \times 10^5$), macrophages ($7.52 \pm 2.65 \times 10^5$) and lymphocytes ($5.06 \pm 1.77 \times 10^5$) as compared to control mice ($0.02 \pm 0.006 \times 10^5$, $0.31 \pm 0.09 \times 10^5$ and $0.23 \pm 0.08 \times 10^5$, respectively, $p < .05$).

Prenatal WTS exposure induced higher level of airway recruitment of neutrophils ($5.86 \pm 0.77 \times 10^5$), macrophages ($17.22 \pm 1.69 \times 10^5$) and lymphocytes ($10.95 \pm 0.88 \times 10^5$) as compared to control mice ($p < .05$). Further, prenatal WTS and postnatal Ova S/C increased the airway influx of neutrophils ($9.73 \pm 0.80 \times 10^5$), macrophages ($18.49 \pm 1.15 \times 10^5$) and lymphocytes ($14.53 \pm 0.97 \times 10^5$) as compared to control mice and Ova S/C mice ($p < .05$) (Figure 2(C-E), respectively).

Effect of prenatal waterpipe smoke exposure on cytokine level in antigen-driven murine model of asthma in adult offspring mice

The levels of IL-2 and IL-10 in lung homogenate were not affected by Ova S/C or prenatal WTS and postnatal Ova S/C (Figure 3(A,B)). Lung level of IL-18 was increased in Ova S/C mice (14.71 ± 1.77 pg/mg) as compared to control (9.34 ± 0.76 pg/mg) and prenatal WTS exposed mice (9.61 ± 0.60 pg/mg, $p < .05$; Figure 3(C)).

Effect of prenatal waterpipe smoke exposure on oxidative stress biomarkers in antigen-driven murine model of asthma in adult offspring mice

Prenatal exposure to WTS significantly increased catalase and SOD enzyme activities in lung tissue homogenates of offspring mice (44.00 ± 4.09 and 51.36 ± 3.10 units/mg protein, respectively) compared to control (29.76 ± 2.11 and 38.44 ± 2.19 units/mg protein, respectively, $p < .05$) and Ova S/C mice (30.47 ± 2.61 and 35.22 ± 3.90 units/mg protein, respectively, $p < .05$) (Figure 4(A,B)). The catalase activity in prenatal WTS and postnatal Ova S/C exposed mice was higher as compared to control (41.48 ± 3.14 versus 29.76 ± 2.11 units/mg protein, $p < .05$). In addition, the SOD activity in prenatal WTS and postnatal Ova S/C exposed mice (50.09 ± 3.01 units/mg protein) was higher as compared to control (38.44 ± 2.19 units/mg protein) as well as Ova S/C mice (35.22 ± 3.90 units/mg protein) (Figure 4(A,B)). However, exposure to prenatal WTS or prenatal WTS and postnatal Ova S/C did not affect the lung level of GPx and TBARS in adult offspring mice (Figure 4(C,D)).

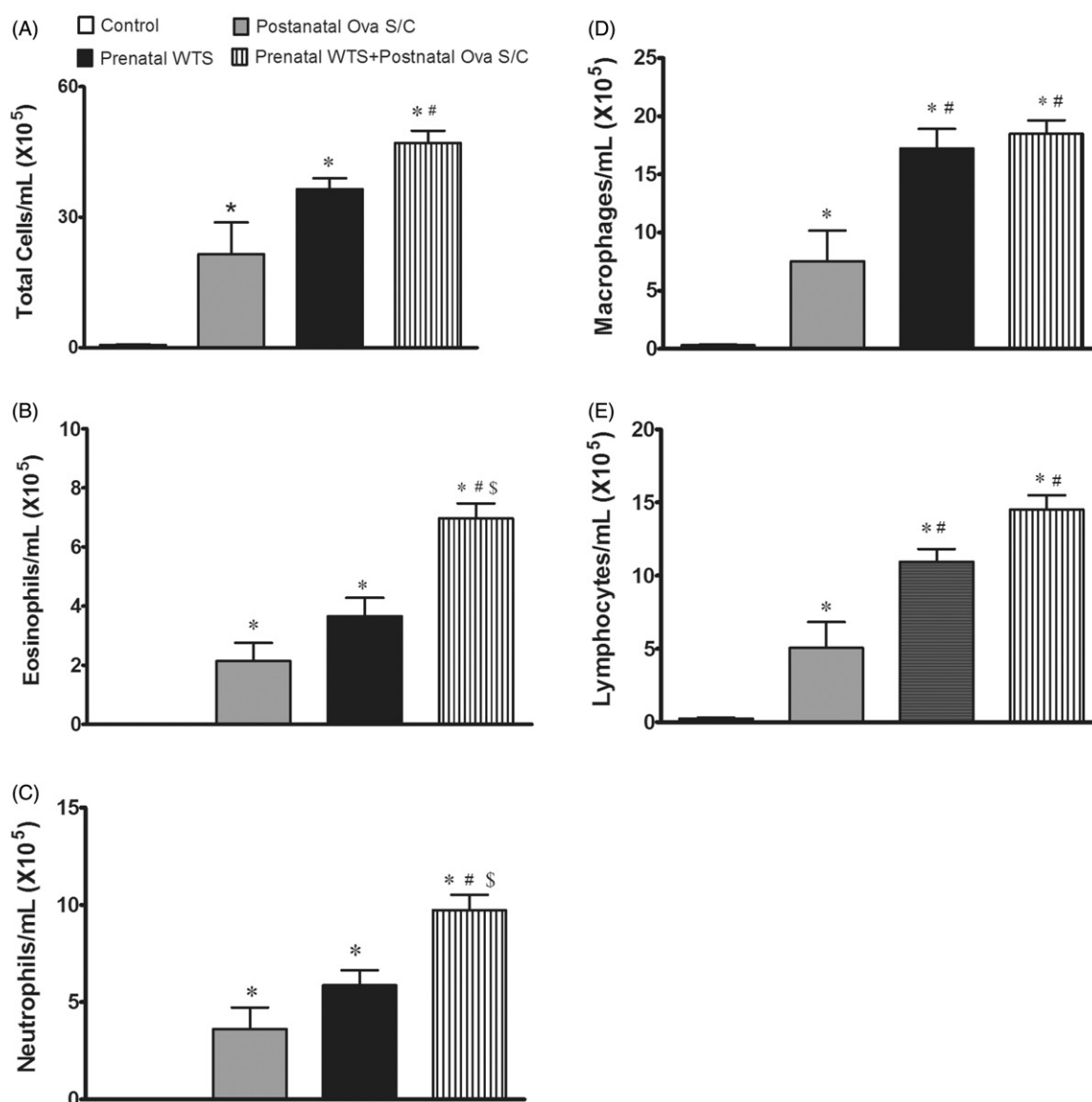


Figure 2. Effect of prenatal waterpipe smoke exposure on inflammatory cells in bronchoalveolar lavage fluid (BALF) of adult offspring mice. BALF was collected from adult offspring mice that were exposed to prenatal fresh air and postnatal ovalbumin sensitization and saline challenge (control group), prenatal fresh air and postnatal ovalbumin sensitization and challenge (postnatal Ova S/C group), prenatal WTS and postnatal saline challenge (Prenatal WTS group) or prenatal WTS and postnatal ovalbumin sensitization and challenge (Prenatal WTS + Postnatal Ova S/C group). The number of (A) total cells, (B) Eosinophils, (C) Neutrophils, (D) Macrophages and (E) Lymphocytes, were analyzed from the BALF. Values represent the mean $\pm$ SEM of data from 10 adult male offspring mice in each group. $p < .05$ was considered significant. * indicates significant difference from control group, # indicates significant difference as compared to Ova S/C group and § indicates significant difference as compared to prenatal WTS group.

Prenatal exposure to WTS did not affect GPx enzyme activity or TBARS level in lung tissue homogenates of offspring mice (0.66 ± 0.18 units/mg protein and 4.69 ± 0.32 μ M/mg protein, respectively) compared to control (0.73 ± 0.16 units/mg protein and 3.81 ± 0.33 μ M/mg protein, respectively, $p > .05$) and Ova S/C mice (0.58 ± 0.14 units/mg protein and 3.57 ± 0.35 μ M/mg protein, respectively, $p > .05$) and prenatal WTS and Ova S/C (0.89 ± 0.12 units/mg protein and 4.76 ± 0.60 μ M/mg protein, respectively, $p > .05$).

Discussion

This study evaluated, for the first time, the effect of prenatal WTS and postnatal allergen exposure on airway

inflammation and oxidative stress levels in a murine model of asthma using adult offspring mice. Mice that were prenatally exposed to WTS had higher levels of airway inflammation and increased activity of catalase and SOD in the adult offspring's lungs. Moreover, prenatal WTS exposure with postnatal allergen exposure further increased the level of airway inflammation and the activity of catalase and SOD.

In the past, the effects of cigarette tobacco smoke on airway inflammation have been studied extensively (Moerloose et al., 2005; Sato et al., 2008). However, more recently, the attention has been directed towards the effect of WTS in the development of respiratory diseases since waterpipe smoking becomes a global trend of tobacco use. Exposure to WTS has been associated with reduced lung function

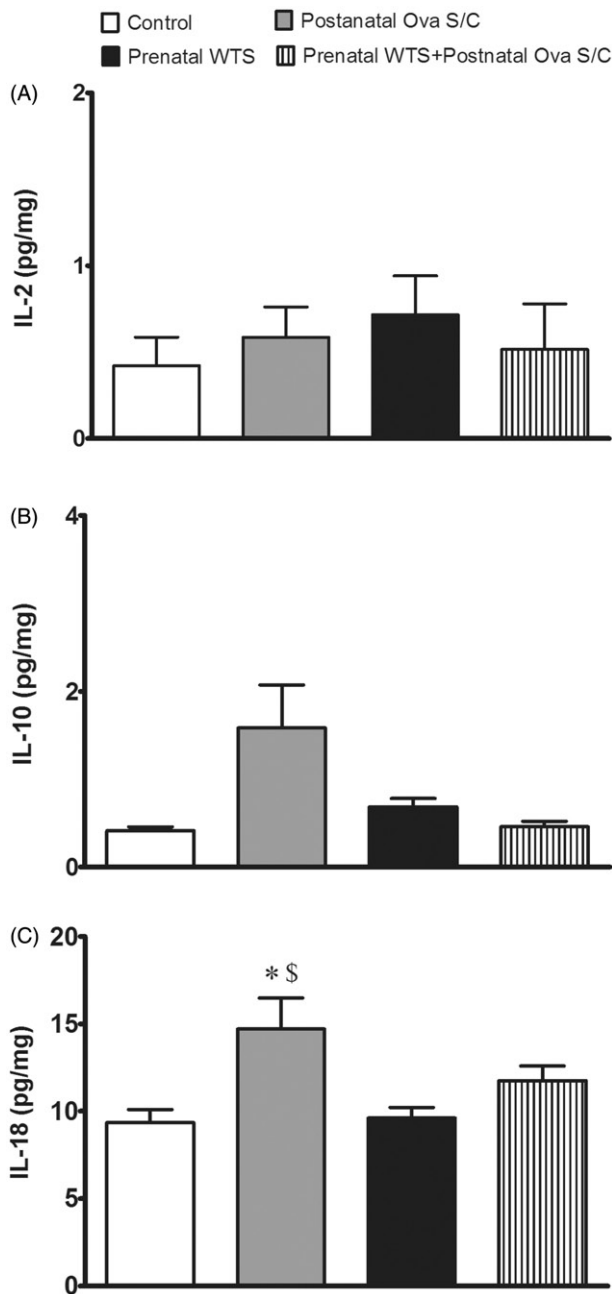


Figure 3. Effect of prenatal waterpipe smoke exposure on level of inflammatory cytokines in lungs of adult offspring mice. Lungs were collected from adult offspring mice that were exposed to prenatal fresh air and postnatal ovalbumin sensitization and saline challenge (control group), prenatal fresh air and postnatal ovalbumin sensitization and challenge (postnatal Ova S/C group), prenatal WTS and postnatal saline challenge (Prenatal WTS group) or prenatal WTS and postnatal ovalbumin sensitization and challenge (Prenatal WTS + Postnatal Ova S/C group). Lungs were analyzed for (A) interleukin-2 (IL-2), (B) interleukin-10 (IL-10) and (C) interleukin-18 (IL-18). Values represent the mean $\pm$ SEM of data from 10 adult male offspring mice in each group. $p < .05$ was considered significant. * indicates significant difference from control group, # indicates significant difference as compared to Ova S/C group and \$ indicates significant difference as compared to prenatal WTS group.

(Raad et al., 2011) and an increased respiratory rate (Bentur et al., 2014) compared to nonsmokers. Murine studies have revealed that WTS exposure increases airway inflammation and the level of oxidative stress in the lungs (Khabour et al., 2012). A recent study by Al-Sawalha et al. (2017b) revealed that chronic exposure to WTS-induced airway inflammation

in naïve mice and enhanced airway inflammation in a murine model of asthma.

Eosinophilic airway inflammation is a major characteristic of asthma (Watt et al., 2005) and the blood levels of eosinophils has been correlated with asthma severity (Griffin et al., 1991). Neutrophils accumulate in the airways in the severe stages of the disease: during acute severe exacerbation of asthma and in corticosteroid-resistant asthma (Ciepiela et al., 2015; Fahy, 2009). Macrophages and lymphocytes also contribute to airway inflammation and asthma pathogenesis (Ishmael, 2011). The results of this study showed that prenatal WTS precipitates the development of airway inflammation.

Maternal cigarette smoke exposure induced T-helper-2 (TH2) response and immunoglobulin (Ig)-E in the absence of allergen exposure in offspring (Singh et al., 2011) and therefore contributed to the subsequent increased risk of respiratory diseases. Consistent with the current results, maternal exposure to cigarette smoke increased the level of airway inflammatory cells and eosinophils as compared to unexposed mice in house dust mite (HDM) model of allergic airway disease (Eyring et al., 2015). Singh et al. (2011) found that prenatal exposure to cigarette smoke increased the level of recruited eosinophils, neutrophils, lymphocytes and macrophages in *A. fumigatus* challenged mice as compared to mice exposed to prenatal fresh air and *A. fumigatus* challenge. However, prenatal exposure to cigarette smoke did not affect the level of airway inflammatory cells as compared to mice exposed to fresh air (Singh et al., 2011), which is inconsistent with the current study. This difference could be due to the differences in the composition of cigarette and WTS. The WTS contains many toxicants such as carbon monoxide, nicotine, tar, polycyclic aromatic hydrocarbons, volatile aldehydes and a variety of heavy metals (Shihadeh et al., 2015). In addition, the charcoal that is used to heat the flavored tobacco, adds to the levels of toxicants such as carbon monoxide, metals and carcinogenic chemicals (Cobb et al., 2012).

It is possible that exposure to WTS during pregnancy could induce permanent changes in the expression of a set of genes via epigenetic mechanisms that led to the observed altered inflammatory response in the offspring mice. It has been shown by Xiao et al. (2012) that microarray analysis of genes that were affected by *in utero* exposure to secondhand cigarette smoke revealed that the expression level of *Fos* and *Fcer2* genes were downregulated in offspring mice compared to control. *Fos* is a redox-sensitive transcription factor that regulates heme oxygenase, a cytoprotective enzyme (Baglole et al., 2008). *Fcer2*, the gene that encodes CD23, is associated with an increased level of IgE-mediated hypersensitivity that is commonly seen in atopy and asthma (Chan et al., 2014). Other genes affected by maternal smoking and shown to be associated with placental methylation, are NR3C1, CYP1A1, HTR2A and HSD11B2 genes (Maccani & Maccani, 2015). Therefore, More studies are required to test the changes in the expression of different genes by prenatal WTS.

The profile of several inflammatory mediators was affected/modified by WTS exposure (Al-Sawalha et al.,

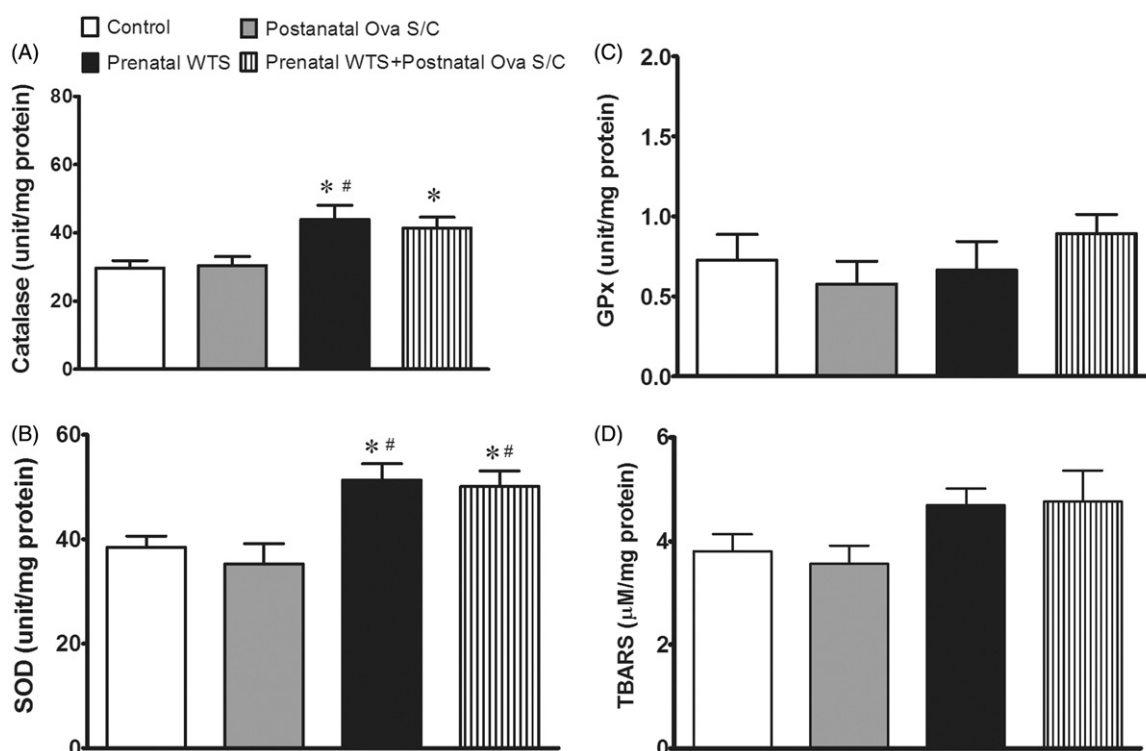


Figure 4. Effect of prenatal waterpipe smoke exposure on level of oxidative stress markers in lungs of adult offspring mice. Lungs were collected from adult offspring mice that were exposed to prenatal fresh air and postnatal ovalbumin sensitization and saline challenge (control group), prenatal fresh air and postnatal ovalbumin sensitization and challenge (postnatal Ova S/C group), prenatal WTS and postnatal saline challenge (Prenatal WTS group) or prenatal WTS and postnatal ovalbumin sensitization and challenge (Prenatal WTS + Postnatal Ova S/C group). Lungs were analyzed for (A) catalase, (B) superoxide dismutase (SOD), (C) Glutathione peroxidase (GPx) and (D) Thiobarbituric acid reactive substances (TBARS). Values represent the mean $\pm$ SEM of data from 10 adult male offspring mice in each group. $p < .05$ was considered significant. * indicates significant difference from control group, # indicates significant difference as compared to Ova S/C group and \$ indicates significant difference as compared to prenatal WTS group.

2017b; Khabour et al., 2012; Nemmar et al., 2015). However, the effect of maternal WTS exposure on airway inflammatory mediators is largely unknown. In the current study, prenatal WTS exposure or prenatal WTS exposure and postnatal Ova S/C did not affect the level of IL-10 and IL-2 in lungs of offspring rats. Our previous study reported that maternal cigarette smoke exposure did not alter the level of IL-2 as compared to unexposed offspring mice (Blacchiere et al., 2009). Further, prenatal WTS did not induce any changes in the level of IL-18 from the whole lung homogenates of offspring mice. The level of IL-18 was induced by Ova S/C, consistent with a previous report where, the level of IL-18 was higher in asthma patients as compared to healthy subjects (Wong et al., 2001). In the current study, postnatal Ova S/C significantly reduced the level of IL-18 in mice that were exposed to prenatal WTS as compared to mice exposed to Ova S/C only. The involvement of IL-18 in the development of asthma phenotype is variable. The genetic deficiency of IL-18, either enhanced (Kodama et al., 2000) or attenuated (Yamagata et al., 2008) airway inflammation in different murine models of allergic airway disease. This variation in the effect of IL-18 on airway inflammation is due to the differences in cytokine environments that either shift the immune response to T-Helper (TH)-1 or TH-2 cytokines (Ishikawa et al., 2006). However, the levels of TH2 cytokines, specifically IL-13, IL-4 and IL-5, were not measured in this study and this could be a potential target in our future studies.

Oxidative stress is defined as an imbalance between the production of free radicals and the antioxidant defense mechanisms, in favor of oxidation (Betteridge, 2000). WTS is known to produce large amounts of free radicals (Fahn et al., 1998) and therefore participate in tissue inflammation and damage. In the current study, prenatal WTS exposure increased the activity of catalase and SOD as compared to unexposed mice. In addition, mice that were exposed to prenatal WTS and postnatal Ova S/C had higher levels of SOD activity than mice that were only exposed to Ova S/C. The increased level of antioxidant enzymes indicates the presence of a persistent oxidative stress. However, lipid peroxidation was not altered, as levels of TBARS were not induced by prenatal WTS. Similar to previous findings, acute WTS exposure increased the level and activity of catalase and GPx (Khabour et al., 2012) while, chronic WTS exposure increased SOD, as well as reactive oxygen species (Nemmar et al., 2016).

Maternal cigarette smoke exposure increased airway resistance in adult offspring mice (Blacchiere et al., 2009). Prenatal exposure to cigarette smoke, but not adult cigarette smoking, decreased the level of cyclic adenosine monophosphate and increased the activity of phosphodiesterase in whole lung homogenates of offspring mice as compared to unexposed mice (Singh et al., 2003) resulted in attenuating bronchial smooth muscle cells relaxation. Future studies should focus on studying the effect of prenatal WTS exposure on airway resistance and lung's structural changes in murine model of asthma.

The results of this study shed some light on the negative effects of prenatal WTS exposure on lung inflammation of offspring and on potentiating airway inflammation in the Ova S/C murine asthma model. While, these studies are in murine models, the extrapolation of the data from smoking studies in mice to humans warrants detailed human studies to understand the risks of prenatal WTS exposure on children. Strategies to enhance the public awareness of the harmful consequences of waterpipe smoking during pregnancy are required. In addition, adapting waterpipe smoking cessation interventions and policies are highly needed especially among women and young smokers.

Conclusion

In conclusion, the results of this study highlight the potential harmful effect of prenatal waterpipe tobacco smoking in inducing airway inflammation in adult offspring. Mice that were prenatally exposed to WTS had higher levels of airway inflammation and increased activity of catalase and SOD in the offspring lungs. In addition, prenatal WTS exposure and postnatal allergen exposure further increased level of airway inflammation, activity of catalase and SOD.

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Disclosure statement

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