Original Research Article

Exploring Lung Histopathology in White Rats After Cigarette Smoke Exposure

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ABSTRACT

Smoking-induced respiratory epithelium changes, driven by nicotine and smoke components, can lead to severe lung alterations with prolonged exposure. However, until now, no one has examined the histopathological appearance of bronchioles and alveoli in Wistar rats following exposure to cigarette smoke. This study aimed to assessed lung histopathological changes in white rats (Rattus norvegicus) exposed to cigarette smoke. A descriptive study used a post-test group control design, exposing Rattus norvegicus to cigarette smoke and dividing them into control, 1-week, 2-week, and 4-week groups. Histopathological findings revealed that the control group had intact bronchioles; the one-week group showed lymphocyte infiltration and typical alveolar structure; the two-week group had increased respiratory epithelial cells; the four-week group displayed dominant bronchiole changes; the one-week group had varying alveolar septal thickness; the two-week group had narrowed alveolar lumens; four-week group showed thickened septa and pronounced pigmented macrophages. Cigarette smoke exposure affects changes to Rattus norvegicus lung histology. Prolonged exposure to cigarette smoke induces damage to the structure of the bronchioles and alveoli.

Keywords: Cigarette smoking, histopathology, lung damage, rattus norvegicus

INTRODUCTION

According to WHO 2020, 1.3 billion tobacco users live in low-and middle-income countries. In Indonesia, the average proportion of smokers aged ten years and over is 29.3%.[1] Cigarette contains additives such as ammonia, butane, cadmium, stearic acid, acetic acid, arsenic compounds, carbon monoxide, methane, and methanol. Cigarette smoke is the result of incomplete combustion of cigarettes. Cigarette smoke contains carbon monoxide, which is colorless but is toxic to the human body. Nicotine in tobacco ranges from 0.6 to 3% by weight tobacco. In nature, nicotine in plants repels insects, or nicotine is a natural insecticide. Tar is an aromatic hydrocarbon polynuclear compound that is carcinogenic.[2]

The effect of cigarette smoke on morphological changes in the respiratory epithelium, in general, is desquamation, loss of cilia, and an increase in goblet cells. This morphological change is correlated with the amount or level of active substances in the form of nicotine, acetaldehyde, and acrolein in cigarette smoke.[3,4] It can be seen that the more prolonged exposure to cigarette smoke in the alveolus, the more changes to the lung structure, starting from the erosion of the epithelium, the number of inflammatory cells, the loss of cilia, and the appearance of pigmented macrophages (smoker macrophages).[5]

Cigarette smoke contains more than 4000 chemicals generated from the combustion of tobacco plant leaves and is known to cause several respiratory ailments, including chronic bronchitis, emphysema, and lung cancer. Cigarette smoking is also considered a principal causative factor responsible for the development of certain diffuse interstitial andbronchiolar lung diseases, namely respiratory bronchiolitis-interstitial lung disease

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(RB-ILD), desquamative interstitial pneumonia (DIP), and adult pulmonary Langerhans' cell histiocytosis (PLCH). Histopathological changes of respiratory bronchiolitis, DIP, and PLCH (with or without co-existent emphysema) may be found on lung biopsy in the same individual, implicating smoking as a common inciting agent of these diverse lesions. Cigarette smoke can also cause both apoptosis and necrosis, and these changes contribute to irreversible pathological changes in lung structure and function.[2,3]

Cigarette smoking induces various histopathological changes in the lungs. Exposure to cigarette smoke has been found to lead to an increase in the number of macrophages in the pulmonary tissue, especially in the group that inhaled smoke for long periods. Additionally, statistically significant increases were observed in malonaldehyde levels of pulmonary tissue and plasma, as well as in the catalase activity levels of erythrocytes in the experimental groups. Furthermore, chronic whole-body inhalation exposure to mainstream cigarette smoke in rats and mice has been shown to produce a statistically significant increase in cigarette smoke-induced lung cancer. Alveolar macrophages, which play a crucial role in the defense against inhaled particulates and pathogens, have been found to undergo dysregulation following exposure to cigarette smoke, contributing to the understanding of cigarette smoking-induced lung disease. These findings highlight the detrimental effects of cigarette smoking on lung histopathology and the associated health risks.[2,5]

In several previous studies, the same research objective was to see changes in lung histopathology but in different animal strains.[6] Until now, no one has tested exposure to cigarette smoke in Wistar rats by looking at the histopathological appearance of the bronchioles and alveoli. Although there are a large number of studies on the effects of cigarette smoke exposure on lung histopathology in animal models, including white rats, there are potential research gaps in understanding the specific mechanisms of lung pathology, the impact of different types of cigarettes, and novel exposure methods in these studies. This study assessed lung histopathological changes in white rats (Rattus norvegicus) exposed to cigarette smoke.

METHOD

The descriptive method in this study uses a research design with the posttest-only control group design method which aims to determine the description of rat lung histopathology after exposure to nicotine smoke from cigarettes. The experimental method in this study uses a research design with the posttest-only control group design method, which aims to determine the description of rat lung histopathology after exposure to nicotine smoke from vapor. We chose this design because this study will serve as a follow-up evaluation of histopathologic changes in expression in the lungs, which is relatively inexpensive and efficient. This study was conducted in the Research Laboratory Faculty of Medicine, Indonesian Muslim University. White rats (Rattus norvegicus) as animal models were exposed to cigarette smoke with 2 cigarettes once a day. The inclusion criteria in this study were healthy white mice who had never been exposed to cigarette smoke and weighed 150-250g. Animal models were excluded if mice were found to be sick or died during the study period. The cigarettes used brand filter cigarettes with suction plugs. These cigarettes contain 1.10-2.17% nicotine and 0.05-0.175% tar.

In this study, we divided 24 white rats aged 2-3 months into four groups equally, each 6; Group A as control (not exposed to cigarette smoke for 4 weeks), Group B (exposed to cigarette smoke for 1 week), Group C (exposed to cigarette smoke for 2 weeks), and group D (exposed to cigarette smoke for 4 weeks). After the 4th week of intervention, the animal models were terminated and dissected to obtain lung tissue. Lung tissue specimens were stained using Hematoxylin and Eosin (H&E), and we performed a histopathological examination. Data obtained from observations under a microscope were reported descriptively. Ethical clearance was approved by the Ethics Committee of the Faculty of Medicine, Universitas Muslim Indonesia (No: 756/B06/KTI FK UMI/VII/2019).

RESULT AND DISCUSSION

Effects of cigarette smoke on the bronchioles

In our study, the control group, at 40x enlargement of the bronchioles, showed ciliated and non-ciliated parts that were still clearly visible. The columnar epithelium was intact; the lamina propria layer contained capillaries and blood vessels. Dogan et al. revealed that the control group showed no epithelial proliferation, and the cilia appeared intact.[6]

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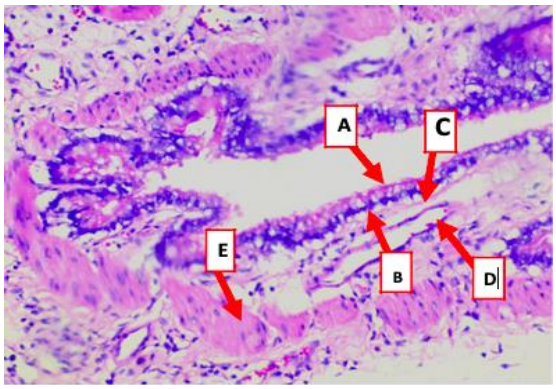

Figure 1. Histopathological appearance of bronchioles in the control group; x20; H&E (A) cilia, (B) columnar epithelium, (C) basement membrane, (D) lamina propria layer containing capillaries and blood vessels, (E) fragments of smooth muscle cells

In the study that we did in the bronchioles in the one-week group, the exposure given was still very short, so there was little change. Lymphocyte inflammatory cells were seen in the peribronchial (peribronchial inflammation) and perivascular inflammation. The cuboidal epithelium also looked intact, but in some parts, erosion began to appear so that, in general, the cilia seemed slightly incomplete. At 40x magnification in the microscope, there was a lot of extravasation of erythrocytes in the first week of exposure. The goblet cells had no significant changes, and the numbers were relatively normal.

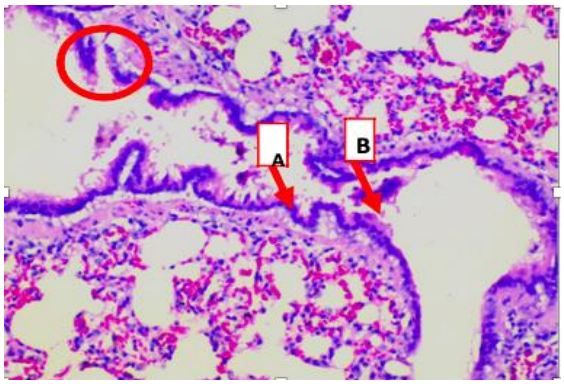

Figure 2. Histopathological appearance of bronchioles in the cigarette smoke-exposed 1-week group; x20; H&E. (A) cuboidal epithelium appears to be intact, but in some parts (circled) erosion appears to occur, (B) cilia appear incomplete like falling

In the two-week group, inflammatory cells were accumulated in the peribronchial area (peribronchial inflammation), and the area started to show damage. At 40x magnification, it was apparent that the respiratory epithelium cells were more proliferative, and the smooth muscle layers were visible.

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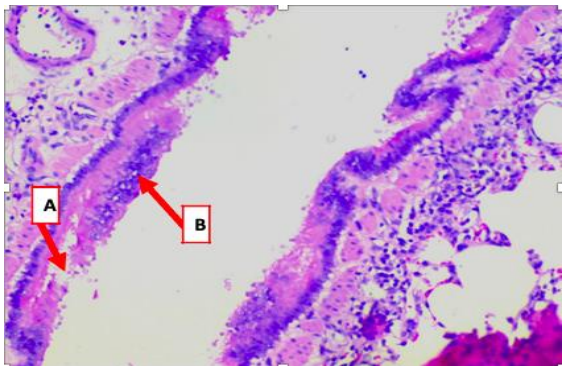

Figure 3. Histopathological appearance of bronchioles in the cigarette smoke-exposed 2 weeks group; x20; H&E. (A) Erosion in the alveoli, (B) Inflammatory cells infiltrate

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The four-week group showed changes in the structure of the dominant bronchioles. There was an erosion of the respiratory epithelium with an extensive infiltration of inflammatory cells. The respiratory epithelium was still visible in some areas with a standard ciliary structure. The lymphocyte cells looked dense, which is an indication that chronic inflammation has occurred. [7]

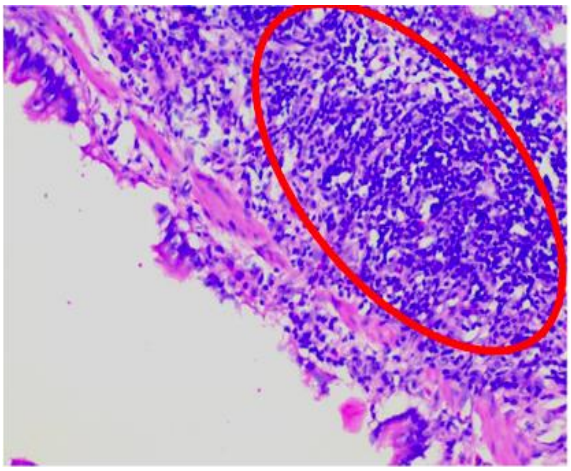

Figure 4. Histopathological appearance of bronchioles in the cigarette smoke-exposed 4 weeks group; x20; H&E. Dense lymphocyte cells (chronic inflammation marker) (circled)

The control group can be seen as a comparison to see the different histopathological changes seen in the group exposed to cigarette smoke for 1, 2, and 4 weeks. Several significant changes we found in bronchioles before (Figure 1), and after cigarette smoke exposure (Figures 2, 3, and 4), it is believed inflammatory effects were induced by nicotine from cigarette smoke. Previous studies have reported that the group exposed to cigarette smoke showed histopathological changes in the respiratory epithelial structure in the form of epithelial cell hyperplasia and loss of missing cilia. The effects of cigarette smoke on morphological changes in the respiratory epithelium are desquamation, loss of cilia, and an increase in goblet cells. These morphological changes correlate with the amount or level of active substances such as nicotine, acetaldehyde, and acrolein in cigarette smoke.[3]

Smoking exposure can cause histopathological changes in the bronchioles. Cigarette smoke can cause chronic inflammation, enlargement of the mucous glands, and remodeling of the walls of both large and small bronchi, which reflects a deregulated healing process in tissue persistently damaged by the inhalation of tobacco smoke. An increase in the number of macrophages was observed in the pulmonary tissue of rats exposed to cigarette smoke, especially in the group that inhaled the smoke for long periods.[8]

Cilia damage may be related to the high concentration of nicotine administered to experimental animals. The effect can damage microtubule structures and alter the polymerization and depolymerization. Other components known to be contained in cigarettes in the form of acetaldehyde and acrolein compounds are thought to have a dominant role in causing ciliary damage. Acetaldehyde can damage mucociliary function by inhibiting the activity of the ciliary dynein ATPase enzyme and binding to ciliary proteins that are important in dynein and tubulin function. Acrolein compounds, also found in cigarettes, can damage cilia by reducing the frequency of cilia movement in the respiratory tract. Damage to ciliary cells in the bronchioles will result in mucus hypersecretion, causing hyperplasia of goblet cells.[9] Cigarette smoke interferes with the metabolism of human airway basal stem/progenitor cells, affects the replenishment of mucociliary epithelium, and impairs the integrity of the airway epithelium, mainly by disruption of intercellular contacts.[10]

The observed inflammatory cells infiltrated the chronic inflammatory process in the bronchioles, strengthening the indication that tissue damage had occurred, which was mediated by free radicals and the secretion of proteolytic enzymes.[4] The experimental model resulted in immunohistochemical changes caused mainly by exposure to cigarette smoke. The positive tracheal immunoexpression for surviving in animals exposed to tobacco smoke with a negative immunostaining for P53 may represent early detection of future carcinogenesis development.[11]

Cigarette smoking induces various

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histopathological changes in bronchioles, which can lead to the development of lung diseases.

Some of the key changes include Basal-cell hyperplasia: This is a common change observed in the bronchial epithelium of cigarette smokers, which can be associated with the loss of cilia and the occurrence of cells with atypical nuclei. Mucous hyperplasia: Smoking can induce structural changes in the airways, such as mucous hyperplasia in the airways of COPD patients. This is associated with increased numbers of mucus-producing cells and increased mucosal permeability. Epithelial remodeling and aberrant repair responses: Cigarette smoke can cause damage to human bronchial epithelial cells, leading to disturbances in mucosal barrier function in COPD. This can be attributed to the activation of the epidermal growth factor receptor (EGFR)/extracellular signal-related kinase (ERK) pathway and the subsequent delocalization of tight junction (TJ) proteins. Increased permeability to allergens: Cigarette smoking has been shown to increase the permeability of the airways to allergens in vitro, which can contribute to the development of allergic lung diseases. Smoking-related interstitial fibrosis (SRIF): This is a morphologically distinct finding in the lung tissue of cigarette smokers and can be associated with various smoking-related lung diseases.[12]

These histopathological changes can lead to the development of lung diseases, such as lung cancer, chronic obstructive pulmonary disease (COPD), and interstitial lung diseases. The severity of these changes can vary depending on the intensity and duration of cigarette smoking.[12]

Effects of cigarette smoke on the alveolus

In our study, in the control group, it was observed that the structure of the alveoli was typical without any particular damage, which consisted of connective tissue septa that lined the air spaces. These septa were composed of collagenous connective tissue, and within which there were capillaries containing erythrocytes, type I pneumocyte cells, and type II pneumocyte cells. It can also be observed macrophage cells (dust cells) and the presence of a bit of extravasation of erythrocytes in the lumen of the alveoli. Previous studies have reported on the histopathological features of the alveolus in the control group that the structure of collagen and elastin fibers was intact, and the lumen of the alveolus appeared normal. There was no damage and the presence of alveolar macrophages in the alveoli.[13]

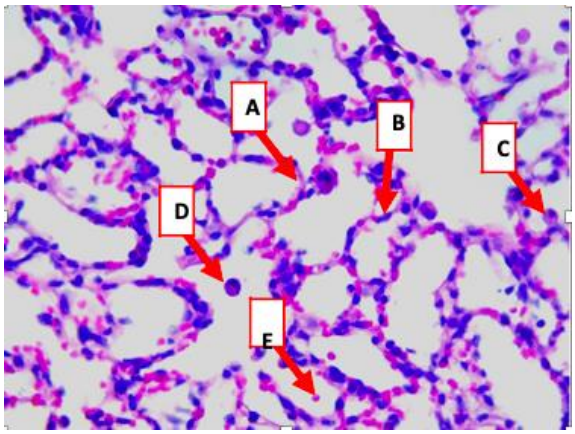

Figure 5. Histopathological appearance of alveolus in the control group; x20; H&E (A) collagen depositions within alveolar septum and capillaries containing erythrocytes, (B) type I pneumocyte cells are flat and attached directly to the basement membrane, (C) type II pneumocyte cells (cuboidal form) and protrude into the lumen, (D) Macrophage cells in the lumen, (E) normal appearance of erythrocyte extravasation

The study that we did in the alveolus in the one-week group showed changes in the thickness of the alveolar septa in several areas. Generally, the cause of the thickening of the alveolar septum is infiltrating inflammatory cells (interstitial pneumonitis), which causes impaired diffusion of oxygen and carbon dioxide as a respiration process. At 40x magnification, the erythrocyte extravasation was clear, and the lymphocyte infiltrate was dominant.

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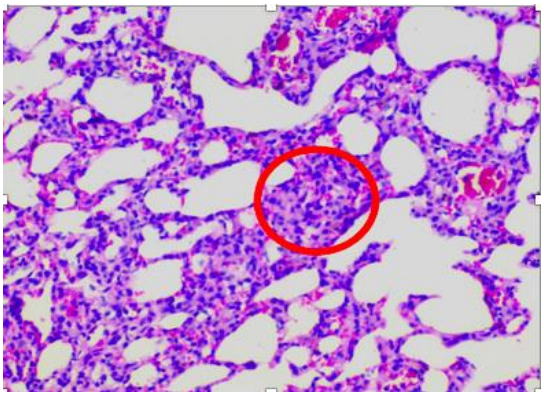

Figure 6. Histopathological appearance of an alveolus in the cigarette smoke-exposed 1-week group; x20; H&E. The thickening of the septum (circled) due to a large number of inflammatory cells (interstitial pneumonitis) makes it difficult for oxygen diffusion

In the two-week group, we could observe a collection of inflammatory cells and a narrowing of the alveolar lumen. At 20x magnification, a lot of extravasation of blood vessels and infiltration of inflammatory cells were seen. In this group, it could be observed the presence of pigmented macrophages. These cells' presence was a typical sign of a history of chronic cigarette smoke exposure.

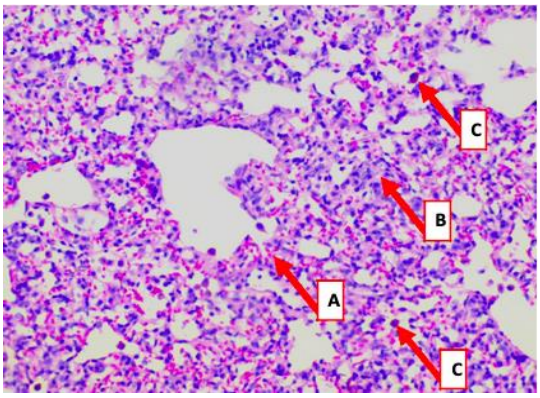

Figure 7. Histopathological appearance of alveolus in the cigarette smoke-exposed 2 weeks group; x20; H&E. (A) a lot of blood vessels extravasation, (B) inflammatory cells infiltrate, (C) pigmented macrophages

In the four-week group, apparent changes were seen, namely the thickening of the septum; the inflammatory cells also looked very abundant and clear. There were dilated blood vessels and an infiltrate of macrophage alveolar cells, very clear pigmented macrophages (Smoker macrophages)

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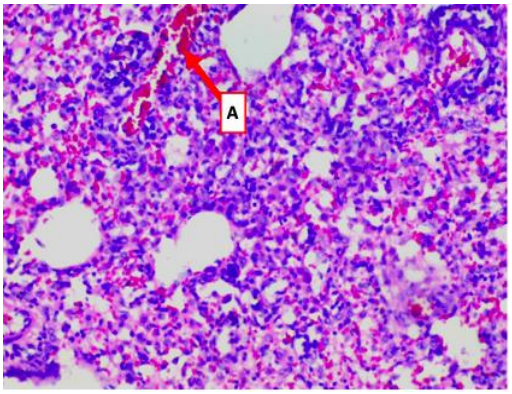

Figure 8. Histopathological appearance of alveolus in the cigarette smoke-exposed 4 weeks group; x20; H&E. (A) smoker's macrophages

The control group can be seen as a comparison to see the different histopathological changes seen in the group exposed to cigarette smoke for 1, 2, and 4 week. Several significant changes we found in alveolus before (Figure 5), and after cigarette smoke exposure (Figures 6, 7, and 8), it is believed inflammatory effects were induced by nicotine from cigarette smoke. Previous studies have shown that the group that had been exposed to cigarette smoke showed that macrophage alveolar cells were seen infiltrating the lumen of the alveoli, accompanied by a narrowing of the diameter of the alveolus and Bronchus Associated Lymphoid Tissue Hyperplasia (BALT). Ultrastructural changes of type I pneumocyte cells, type II, and Clara cells also lead to cell death. Alveolar cell hyperplasia is a feature of damaged lung tissue.[14] Alveolar macrophages are implicated in cigarette smoke-related disease. For a short time, tobacco smoke exposure can generally stimulate immunity, activating and increasing macrophages. Alveolar macrophages function by releasing free radicals such as nitric oxide (NO) and will phagocytize dust particles and particles of pathogenic microorganisms that enter the lung alveoli.[15]

As antigen-presenting cells (APCs), macrophages eliminate pathogenic microorganisms when they invade the body. Macrophages will also increase inflammatory mediators by secreting interleukin (IL)-1β, IL-8, tumor necrosis factor-α (TNFα), interferon-gamma (IFNγ), and chemokines. Interleukins and TNFα will activate the systemic immune system and then attract leukocytes to the site of inflammation. Activation of alveolar macrophages will release chemotactic factors so that neutrophils are activated to secrete proteases that will destroy parenchymal tissue and cause excess elasticity in the lung, resulting in damage to the walls of the alveoli and hypersecretion of mucus.[16] A collection of inflammatory cell infiltrates can cause thickening of the walls of the alveoli.[17] Infiltration of lymphocytes or bronchi-associated lymphoid tissue (BALT) occurs due to the accumulation of lymphoid cells where there is a grouping of B lymphocyte cells mainly in the follicles and T lymphocytes more towards the edges of the bronchial wall venule.[5]

Long-term and high-dose exposure to cigarette smoke can significantly damage the immune system and cause an imbalance in the inflammatory response.[18] Under the influence of cigarette smoke, it can be seen that the more prolonged exposure to cigarette smoke, the more changes to the lung structure occur, starting from the erosion of the epithelium, the number of inflammatory cells, the loss of cilia and the appearance of pigmented macrophages (smoker macrophages).[19,20,21]

Individual responses regarding macrophage activation, polarization, and lung compartmentalization can greatly influence chronic inflammatory processes after exposure to cigarette smoke. The difference in the pattern of polarization and distribution in the lung can lead to damage and dysfunctional tissue repair, which in some respects are similar to those that can characterize COPD phenotypes in humans.[22] The accumulation of foamy alveolar macrophages may play a vital role in developing smoking-induced emphysema. Increased surfactant protein-D (SP-D) may play a protective role in developing smoking-induced emphysema, partly by preventing alveolar cell death.[23]

CONCLUSION

Cigarette smoke exposure affects changes to Rattus norvegicus lung histology, especially damage to the structure of the bronchioles and alveoli. Further research is needed to determine other effects of exposure to cigarette smoke in white rats. Continue this research with an observational analytic design so that correlations can be found between related variables. Further research with a larger sample size is needed to see the effects of histopathological changes due to exposure to cigarette smoke.

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