Histopathological Changes in the Lung of Rat Following Long -Term Exposure to Biomass Smoke

Uzun Süre Biyomas Dumanına Maruz Bırakılan Sıçan Akciğerindeki Histopatolojik Değişiklikler

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ABSTRACT Objective: Experimentally to show the histopathologic alterations in the trachea and lungs due to long term exposure to biomass smoke in rats. **Material and Methods:** 30 female Wistar-Albino adult rats were used in the experiment. The rats were exposed to biomass smoke for one hour a day for 3, 6, and 9 months. At the end of the experiment, the rats were decapitated under deep anesthesia. The tissue samples were taken and were processed for histopathological examinations. **Results:** Gross findings were focal small anthracotic accumulations, multiple abscesses, cysts with mucinous fluid and emphysematic changes in the lungs. Histopathologically, lymphocytic, eosinophilic and macrophage infiltrations in varying densities were observed in the interstitium of the lungs. Based on the exposure time, small papillary structures were observed in the airway mucosa. As most apparent in pulmonary veins, the intimae and media of the pulmonary vessels were thickened and their lumens were narrowed. In one case, we noticed papillary adenomatous proliferation of the mucosa. In tracheal mucosa, the anthracotic spots were encountered. We also observed ulceration, erosion areas and squamouse metaplasia in the tracheal mucosae. **Conclusion:** Long-term exposure of rats to biomass smoke was associated with chronic inflammatory and premalignant alterations in different regions of the respiratory trac such as trachea, bronchia, bronchioles, and parenchyma including subpleural areas.

Key Words: Lung; smoke; biomass; pathology

ÖZET Amaç: Önemli halk sağlığı sorunlarına yol açan biyomas yakıtları gelişmekte olan ülkelerde sıklıkla kullanılmakta ve önemli halk sağlığı sorunlarına yol açmaktadır. Bundan en çok etkilenen organ akciğerler olmakla birlikte, değişen derecelerde diğer organlar da etkilenmektedir. Bu çalışmanın amacı, bölgemizde yaygın olarak kullanılan tezeğin akciğerlere olan etkisini deneysel olarak göstermektir. **Gereç ve Yöntemler:** Çalışmada her biri yaklaşık 300 gr ağırlığında 30 dişi Wistar Albino sıçan kullanıldı. Sıçanlar günde bir saat olmak üzere 3, 6, ve 9 aylık sürelerle tezek dumanına maruz bırakıldı. Süreç sonunda sıçanlar derin anestezi altında dekapite edildiler. Dokular parafin bloklarda mikroskobik inceleme için hazır hale getirildi. **Bulgular:** Makroskobik incelemede değişen sürelerle tezek dumanına maruz bırakılan sıçanlarda fokal küçük antrakotik lezyonlar, ve pnömoniye bağlı, müsinöz sıvıyla dolu birden çok apse görüldü. Mikroskobik incelemede hemen hemen tüm sıçanlarda perivasküler, peribronşiyolar ve interalveoler septalarda lenfosit infiltrasyonları, değişen yoğunlukta makrofaj ve eozinofil infiltrasyonları görüldü. Maruz kalma süresiyle orantılı olarak bronş ve bronşiyollerin submukozalarında hiyalinizasyon ve kollajen artışı, ayrıca mukozada küçük papiller oluşumlar gözlendi. Bazı sıçanlarda alveoler septalarda belirgin incelme, bazıları perfore geniş amfizematöz bölgeler görüldü. Pulmoner venalarda belirgin olmak üzere pulmoner damarların intima ve media tabakalarında kalınlaşma, lümende daralma (sklerozis) vardı. Bir olguda mukozanın papiller adenomatöz proliferasyonu fark edildi. Hem trakea hem de interstisyumda granüler ve siyah renkli lekeler görüldü. Trakea mukozasında skuamöz metaplazi, geniş ülserasyon ve erozyon alanları da vardı. **Sonuç:** Çalışmada, tezek dumanına maruz kalmanın trakea, bronş, bronşiyol, akciğer interstisyumu ve damarları gibi solunum yollarının farklı bölgelerinde kronik yangısal ve premalin değişikliklere yol açtığı gösterilmiştir.

Anahtar Kelimeler: Akciğer; duman; biyomas; histopatoloji

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Biomass smoke in our region widely results from burning of biomass, which is made of animal manure/dried dung. Biomass is widely used under primitive and inefficient conditions as the major source of domestic energy in developing countries. Exposure to biomass, during cooking, baking and heating has been suggested to be a potential risk factor for obstructive airway disease (OAD) and bronchopulmonary disease.[1-3] Two reports have suggested that exposure to biomass smoke increases the prevalence of chronic bronchitis and produces lung damage.[4,5] Biomass smoke resulted from burning up dried dung includes NO2 (oxides of nitrogen), NH3 (ammonia), hydrogen cyanide aldehyde, ketotie acrolein etc. and particles.[6] In the study of Barış et al, chronic obstructive pulmonary diseases (COPD) was significantly more common in women who cooked in a traditional underground oven, which is called "tandır" for they were more exposed to biomass smoke than others were.[7] Additionally, they observed that COPD prevalence rose as exposure time increased.[7] In previous studies performed in women highly exposed to biomass smoke examining functional and radiological effects of smoke on lung, severe obstruction and increased lung volumes were reported. In addition, radiological examination showed diffuse emphysema, interlobular septal thickenings, focal emphysematous areas, increased cardiothoracic index, and bronchovascular arborisations. Biomass smoke was suggested te lead to obstructive and restrictive pathologies and hazardous effects on pulmonary function and structure.[8,9] Fidan et al examined the acute effects of environmental tobacco smoke and dried dung smoke on lung histopathology in rabbits, and they stated that although less than the effects of cigarette smoke, dried dung smoke had severe histopathological effects on rabbit lungs.[10] However, the amount of biomass smoke related to animal dung affecting the lung was not studied. Therefore, in this study we aimed to investigate the effect of long–term exposure to biomass smoke on rat lung.

MATERIAL AND METHODS

ANIMALS

30 adult inbred female Wistar Albino rats weighing about 300 g were obtained from the Laboratory of Animal Science, Medical School of University. The animals were given standard rat pellets and tap water ad libitium. The rats were housed in individual cages (360 mm x 200 mm x 90 mm) 1 month before the start of the experiments. Rats were exposed to biomass smoke obtained by burning up to 500 g animal dung one huor daily for 3, 6 and 9 months via inhalation chambers equipped with a trap and designed to sustain dynamic and adjustable airflow using a modified smoking cabin.[6] CO and NO concentrations ranged between 40-270 ppm and 2-5 ppm, respectively.[11] Neither food nor water was given to animals during the exposure, which was made in a 2 m closed area. All animals were housed in stainless cages under standard laboratory conditions (light period 7.00 a.m to 8.00 p.m, 21 ± 2°C, relative humidity 55%), and received humane care according to the criteria outlined in the "Guide for the care and use of laboratory animals" prepared by the National Institutes of Health.

HISTOLOGICAL PROCEDURE

At the end of the experiment, rats were sacrificed by decapitation, under chloralhydrate anesthesia (6 ml of 7% chloralhydrate/kg). Lung and tracheal tissue samples were taken from rats in each group for histological examination at previously mentioned time points. Lungs were inflated with fixative via the trachea. The lungs were maintained in inflation for 24 hours and were sectioned in a sagittal plane. The midsagittal slices of the lungs were embedded in paraffin wax. Replicate sections were cut at 4-5 μm thickness and were stained with haematoxylin and eosin (HE). Additional sections were stained with van Gieson and Mason trichrome.

RESULTS

MACROSCOPIC FINDINGS

There was no clear alteration macroscopically in the group of three months. Small blackish spots were detected in the lungs of rats of six months. These spots became more evident at nine months. Additionally, in one case, many gray and yellowish colored focal abscesses in a nodular manner were observed in the lungs. In another case, there were large pneumonic areas in the diaphragmatic lobes. In two cases, many small sacs filled with grayish

colored mucinous fluid were particularly seen in the diaphragmatic lobes of the lungs (Figure 1A).

MICROSCOPIC FINDINGS (TABLE 1)

Histopathologic Findings at three months

Lung

Interalveolar septae were thickened due to little connective tissue increase associated with lymphocyte and macrophage infiltration in the perivascular, peribronchiolar and interalveolar septae. In addition, eosinophilic infiltrations of various densities were also seen in these areas, especially in the perivascular areas. Small black dusts were encountered in the cytoplasms of macrophages and freely in the intercellular regions. Similar linear accumulations were detected in the apical surfaces of the bronchial and bronchiolor epithelial cells. There was degeneration and desquamation in the mucosal epithelium of the bronchia and bronchioles, as well. Further, focal emphysematous alterations and rupture of the alveolar septae had taken place. Minimal thickening was detected in the intimae and media layers of pulmonary veins. In two cases, mucosal small papillary proliferations were seen toward the lumen of the bronchia, and hyalinized tissue had increased in the bronchial and bronchiolar

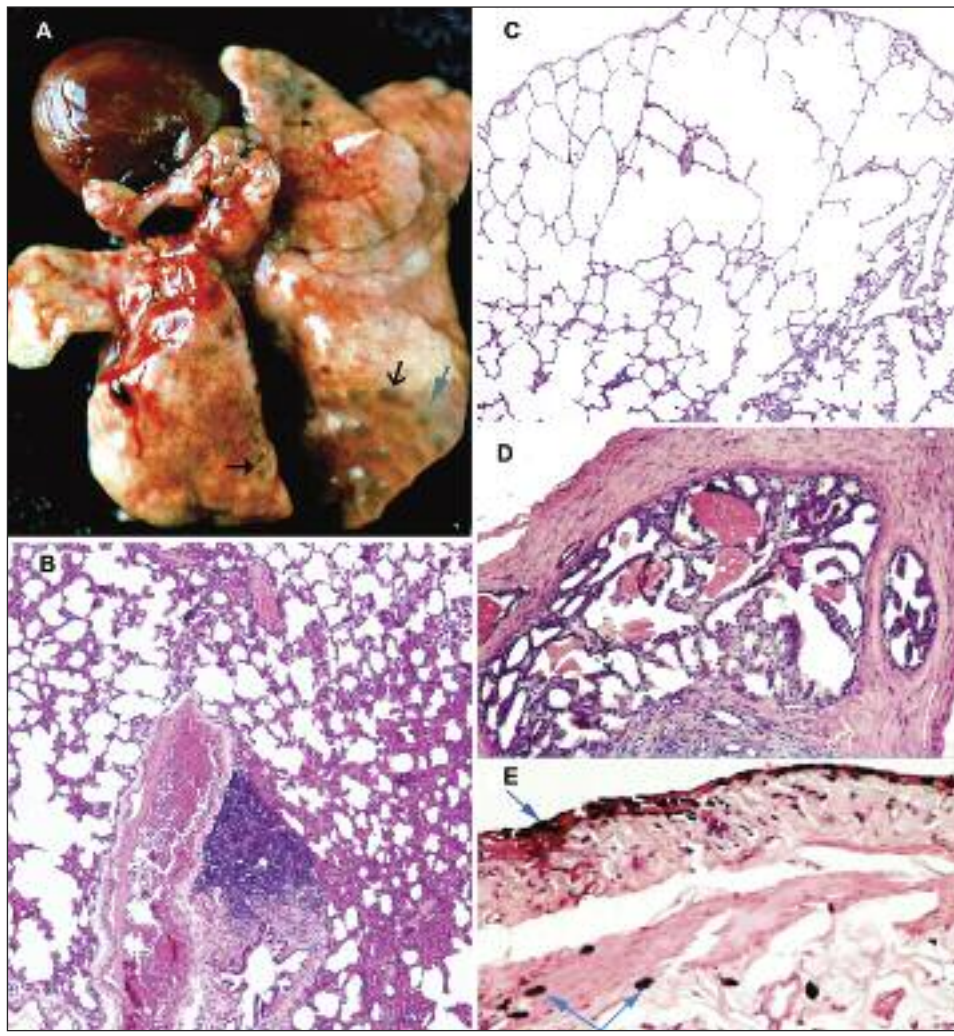

FIGURE 1: (A) Showing many small grayish sacs (arrows) filled with mucinous fluid, particularly seen in the diaphragmatic lobes of the lung.
(B) Alveolar septae thickening due to mononuclear cell infiltrations and emphysematic alveoli. HE, x100.
(C) Note alveolar septae had rather thinned, some destructed, and large alveolar emphysematous areas had occurred. HE, x60.
(D) Note the papillary adenomatous proliferation of bronchiolar mucosa and peribronchiolar fibromatous developing. HE, x100.
(E) Black pigment depositions (arrows) in the epithelial surfaces and submucosa of the trachea. HE, x140.

TABLE 1: Microscopic findings and severity of lesions in time course.

Lesions	At 3 months		At 6 months		At 9 months	
Intraparenchymal infiltration	+	8/10*	++	10/10	+++	10/10
Intraparenchymal fibrosis	+	5/10	++	10/10	+++	10/10
Alveolar destruction	+	4/10	+	10/10	+++	10/10
Emphysema	+	10	++	10/10	+++	10/10
Perivascular infiltration	+	4	++	7/10	+++	10/10
Peribronchial-bronchiolar infiltration	+	3	++	10/10	+++	10/10
Intraparenchymal haemorrhage	+	3/10	++	6/10	+++	810
Intraparenchymal vascular congestion and thrombosis	+	2/10	++	5/10	+++	8/10
Sclerosis	+	3/10	++	4/10	+++	8/10
Bronchial-bronchiolar papillary formations	0	10/10	++	10/10	+++	10/10
Bronchiolitis obliterans	+	3/10	++	7/10	+++	8/10
Abscesses	0	0/10	0	0/10	+++	1/10
Necrotizing pneumonia	0	0/10	0	0/10	+++	1/10
Adenoma	0	0/10	0	0/10	+	2/10
Tracheitis	+	5/10	++	7/10	+++	10/10
Tracheal ulcer	+	4/10	++	7/10	+++	10/10

Severity of lesions: no changes (0), mild (+), moderate (++), severe (+++)

* The number of lungs with lesion / The number of examined rats

submucosa. One case had fibrotic pleural thickening and nodules associated with increased hyalinized tissue and mononuclear cell infiltration in pleura. Black pigment accumulations were present in the subpleural lymphatic spaces.

Trachea

A few lymphocyte, macrophage and fibroblast infiltrations in the submucosa and small black pigment accumulations in the cytoplasms of macrophages and in areas of the interstitial space were present. Similar linear accumulations were also detected in the apical surfaces of the epithelial cells of the tracheal mucosa and as thin granules within their cytoplasms. In addition, degenerative and desquamative alterations were seen in the tracheal mucosa. In one case, the tracheal mucosa had produced small papillary proliferations toward the lumen. Fibrotic thickening and subserosal bleedings were recognized in the trachea.

Histopathologic Alterations in 6th Month

Lung

Parenchymal lesions were more apparent and widespread in the group of sik months than in the three month group. Especially, alveolar septae next to the pleura had been rather thinned and alveolar spaces enlarged, even sometimes perforated leading to emphysema areas. Some alveolar septae had thickened due to mononuclear cell infiltrations and some of the alveoli were emphysematic (Figure 1B). Increased connective tissue occurred associated with perivascular, peribronchial, and peribronchiolar dense lymphocyte, eosinophils and macrophage infiltrations, sometimes as lymphocyte follicles. Mucosa had papillary proliferations of various lengths, and the hyalinized tissue increased in the submucosa of bronchia and bronchioles. Some walls of the bronchioles were focally destructed and there were fibroblastic polypoid proliferations toward the lumen and partial obstructions (bronchiolitis obliterans). Focal nodular or diffuse fibrotic thickenings were detected in the pleura. Linear or granular black pigments (anthracosis) were encountered in the subpleural lymphatic spaces, in the cellular exudates around bronchia, bronchioles and vessels, and finally in the apical surfaces of bronchial and bronchiolar epithelial cells. These depositions were more common and intensive at six months than at three months. Degeneration, necrosis, desquamation in the epithelium of mucosa, and increased hyalinisable connective tissue were detected in the submucosae of bronchia and bronchioles.

Trachea

Lymphocyte and macrophage infiltration and increasedof connective tissue in the submucosa and black dust depositions in epithelial surfaces were more intensive and widespread. The tracheal epithelium was usually compressive. There was sometimes hydropic degeneration on the tracheal epithelium. In addition, focal erosion and ulceration had developed. In some cases, multifocal papillary proliferations toward the lumen in the tracheal mucosa were produced.

Histopathologic Alterations at nine months

Lung

Alveolar septae had been rather thinned, destructed, and extensive and large alveolar emphysematous areas had occurred (Figure 1C). Evident interstitial thickening had formed in the perivascular, peribronchial, and interalveolar septae with lymphocyte, macrophage, and eosinophil leukocyte infiltration, sometimes as lymphoid follicle formations. Eosinophile infiltration had intensified, especially, around bronchia, bronchioles and vessels. Clear papillary proliferations toward the lumen had formed especially in the majority of bronchial mucosa. Bronchiolitis obliterans had occurred in many bronchioles. Respiratory epithelium was transformed to monostratified squamous and/or cubic epithelium. Increased connective tissue had hyalinized as nodular in some areas. The intima and media layers of pulmonary veins were rather thickened and their lumens were narrowed (sclerosis). Black pigment accumulations were similar in localization to those at sixth months but much more intensive. In one case, pneumonia with abscesses and necrosis was also observed. In another case, papillary adenomatous proliferation in some bronchial and bronchiolar mucosa and collagenous tissue increase around them had developed (adenomatous and fibromatous premalignant alteration) (Figure 1D).

Trachea

Lymphocyte and macrophage infiltration and connective tissue increase were detected in the submucosa. Black pigment depositions in the epithelial surfaces and submucosas were more intensive and widespread than that at three and six months (Figure 1E). Tracheal epithelium was monostratified and flattened. Furthermore, focal erosion and ulceration had formed (erosive–ulcerative tracheitis). Finally, multifocal papillary proliferations had produced toward the lumen in some areas of tracheal mucosa.

DISCUSSION

We assumed that pollutants from biomass smoke obtained by burning up dried animal dung might play a role in the etiology of COPD, interstitial lung disease and pulmonary hypertension, which are common in nonsmoker female patients in living regions. Severity of chronic bronchitis and airway obstruction caused by biomass fuel was strongly correlated with duration and intensity in previous studies.[5,7-9] Thus, we wanted to show experimentally that some histopathologic alterations might develop due to smoke originated from these pollutants. We also considered that this study was the first on this subject in rats.

There are very few, articles that are related to histopathologic alterations after biomass smoke exposure in rats. Most articles include alterations and influences due to cigarette smoke exposure.[12,13] Wright et al investigated the long term influences of cigarette smoking on small airways and compared small airways alterations of new Guinea Pigs exposed to cigarette smoke for 1, 3, 6, and 12 months with not exposed ones.[11] They found that the numbers of secretory cells increased in airway epithelium of animals exposed to smoke for 3, 6, and 12 months. They could not find any alterations in the airway walls and in the dimension of peribronchial and alveolar infiltrations of both groups. In view of this observation, they concluded that airway obstruction was due to the loss of elastic recoil caused by emphysematous destruction.[11] Another study investigated the structural and functional effects of smoking 10 cigarettes daily 5 days in a week and for 1, 3, 5, and 12 months on pig lung.[12] The authors demonstrated that long-term exposure to cigarette smoke caused slow morphologic and physiologic alterations that were

characteristics of emphysema.[12] In animal models, cigarette smoke caused epithelial metaplasia and alveolar and peribronchial inflammation with accumulation of pigment-laden macrophages.[14] Wright et al observed that secretory cell metaplasia developed in Guinea Pigs exposed to smoke for more than 3 months.[12] This was attributed to a response to chronic irritation. Goblet cell metaplasia is a frequent finding in the airway epithelium of membranous bronchioles of cigarette smoking people as well.[15,16] In the present study, papillary proliferations in the bronchial-bronchiolar mucosal epithelium were seen after 6 and 9 months in rats. Papillary proliferations were also present in the tracheal mucosa.

The present study detected infiltrations of lymphocyte, sometimes in the shape of lymphoid follicules and macrophages and some increase in the connective tissue in perivascular and peribronchiolar areas and in interalveolar septae in rats, which were exposed to biomass smoke for 3 months. After six months of exposure, we noticed that collagen tissue increased, sometimes hyalinized, in the submucosa of bronchia and bronchioles. Hyalinization and previous alterations were highly prominent at 9 months. Parenchymal lesions seen at six months of exposure were rather clear and had become widespread. Particularly alveolar septae next to the pleura were rather thinned and were destroyed and alveolar spaces had enlarged resulting with large emphysematous areas. Biomass smoke distinctly caused emphysema in accordance with previous data on cigarette smoke and emphysema. We believe that these alterations related to traditional biomass smoke were distinctly and clearly shown in the present study for the first time in the literature. Hyalinized tissue increase and collagen collection in the interstitium seen after 6 and 9 months may be the result of the hazardous effects of biomass smoke in the etiology of both emphysema and interstitial lung diseases.

In our study, biomass exposed rats had increased hyalinized tissue in the bronchial and bronchiolar submucosa showing progression from three months to nine months correlating the duration of biomass smoke exposure with the papillary proliferations of mucosa towards the lumen. Furthermore, bronchial and bronchiolar epithelium had hyperplasia. The tracheal epithelium had sometimes transformed to monostratified squamous or cubic epithelium at nine months. Evidence of destruction in airway epithelium caused by long-term smoke was degeneration, erosion, and ulcers in bronchial mucosa, and significant small papillary proliferations, which had developed in bronchial-bronchiolar mucosa starting from 6 months of exposure. These findings were concordant with previous data of cigarette smoke exposure studies.[12,15] In 2 studies, in which pigs were exposed to smoke for 12 months, alveolar structures were enlarged.[15,16] Yamato et al observed that capillary vessels in both central and near peripheral lobules had been prominently narrowed in the study that examined the histopathology of emphysema due to cigarette smoke exposure.[17] In the same study, emphysema was related to widespread and somewhat uniform capillary narrowing and decreased density and frequency of capillaries. In the present study, rats exposed to biomass smoke showed thinning of alveolar septae near pleura and the enlargement of alveolar spaces, even some perforated alveoli constituting emphysema areas.

Wright et al also examined the effects of smoke on pulmonary vessels and reported pulmonary hypertension in pigs, however not due to capillary bed destruction.[18] They suggested that long-term biomass smoke exposure promoted the density of muscularized small arterioles and the tonus of muscular arterioles.[18] In the present study, there was increase in the media layers of the vessels and their lumens were narrowed. Hypertrophic alterations were partly seen in the adventitia. Infiltration of perivascular lymphocytes, sometimes as follicles, macrophages and eosinophiles were clearly observed. Our findings demonstrated that exposure to biomass smoke could induce pulmonary vascular changes which could lead to pulmonary hypertension with both pulmonary vascular muscle involvement and perivascular infiltration.

Some investigators suggested that acute inflammatory response due to cigarette smoke had bronchospastic and vasospastic effects. These bron-

chospastic effects may results in chronic airway obstruction and vasospastic effects in pulmonary hypertension in COPD. It is a reality that smoke increases epithelial permeability. Bronchoconstruction developing after smoke exposure may be due to inflammatory response. James et al proposed that the increased permeability could result in bronchospastic response.[19] Holtzman et al examined the effect of ozone on hypersensitivity by linking it with acute inflammatory infiltrate in the airways.[20] All findings gave rise to the idea that the morphologic and physiologic alterations of pulmonary hypertension occurring after long-term biomass smoke might possibly be originated from the recurrent episodes of pulmonary vasospasm due to inflammatory response arising after acute response to smoke.

In the final course of this experimental trial in the present study, we concluded that long-term exposure to biomass smoke was associated with chronic inflammatory and premalignant alterations in different regions of the respiratory trac such as trachea, bronchia, bronchioles, and parenchyma including subpleural areas.

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