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**ORIGINAL ARTICLE**

# Effect of Estrogen on Bronchiolar Smooth Muscle and Peri-Bronchial Lymphocytic Infiltration in Adult Male Mice

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## ABSTRACT

**Objective:** To study the effect of estrogen on lungs of adult male mice by assessing and comparing the following histological parameters including bronchiolar smooth muscle size and peri-bronchial lymphocytic Infiltration.
**Study Design:** Randomized control trial
**Place and Duration of Study:** National institute of Health sciences, Islamabad from 1st October 2018 to 31st July 2019.
**Methodology:** Ninety BALB/c mice divided into 2 groups (n=30 per group) were enrolled. The control (male) group received only distilled water, while the interventional groups received pills (estradiol valerate) mixed in distal water according to body weight of the mice for (60) day.
**Results**: 10% of the total mice had nil, 60% had mild, 30% of the total showed moderate while no severe peri-bronchiolar lymphocytic infiltration in response to estrogen. The results also showed that estrogen produced marked hyperplasia of bronchial smooth muscle cells.
**Conclusion:** Estrogen is the sexual hormones which modulate inflammatory processes in the lungs producing pulmonary inflammatory responses leading to asthma and causes hyperplasia of the bronchiolar smooth muscles.
**Keywords:** Estrogen, Asthma, Bronchiolar smooth muscle, Peri-bronchial lymphocytic infiltration, BALB/c mice

## INTRODUCTION

Birth control methods have been used around the world for many thousands of years.The combined oral contraceptive pill (COCPs) and the minipill are the two most used hormonal contraceptive methods.[1]With the advent of oral contraceptive pills the contraceptive methods have advanced significantly since then.Oral contraception is a highly efficient method of contraception with additional health advantages beyond pregnancy prevention.[2,3]

All COCPsare not same, and brands are different with the amount of estrogen they contain. Estradiol is the estrogen compound used in most oral contraceptives and its derivative form calledethinylestradiol[4]inlower doses (20 microgram and below) which may also reduce side effects.[5]

The beneficial effects of COCP on the reproductive system are most convincing, symptomatic menorrhagia or endometriosis[6], 50% reduction in risk of endometrial cancer[7]and benign breast disease.[8] In the long term, the risk of cancer of the ovary, uterus, colon and rectum is also reduced.[9] Thus the benefits of oral contraceptives in young healthy women outweigh the side effects which have been more widely publicized.

For many years, estrogen is known to play a role in sexual development,[10,11] however it is now becoming increasingly understood that they have effects beyond the reproductive system. There are two estrogen receptors (ER) types exist: ER-α and ER-β, which are expressed in rats[12], mouse[13] and humans.[14] In addition to the mammalian female and male reproductive systems, ER-α and ER-β expression has also been found in the mammary glands, bone, cardiovascular tissues, lung, and brain of female.[10,15] The ER-β protein is expressed more in the lungs than ER-α.[16]ER-α and ER-β are members of a super family of nuclear hormone receptors, many of which are ligand-activated transcription factors that bind to the promoter region of genes to regulate gene expression.[10]

Both forms of ER, ER-α and ER-β are necessary for the development of complete alveolar units in female mice. ER-α promotes normal alveolar density per unit of surface area by ensuringadequate lung differentiation during development. ER-β on the other hand, controls extracellular matrix growth, resulting in normal elastic tissue recoil pressure in the lungs.[17] Compared to female and male mice's ERs have lesser impact on the alveolar dimension.[18-20]

Asthma is a chronic airway disease.It hasheterogeneous process and exhibit significant phenotypic variation and affects 300 million people worldwide.[21,22] Variable bronchoconstriction, airway hyper-responsiveness (AHR), airway inflammation, and airway remodelling are the characteristics of asthma.

Epidemiological research on adults has shown that women have a higher prevalence of asthma than men do.[21] Asthma prevalence, morbidity, and mortality are rising in women.[17] Female gender is a significant risk factor for morbidity and mortality in inflammatory lung diseases. It suggests that sex-related hormones might play a key role in asthma disease progression. Additionally, it also reported that estrogen level variations are also responsible foraggravation in asthma in women.[22]

Due to the increasing prevalence of chronic diseases among women globally, understanding the physiologic functions of sex hormones in controlling airway inflammation and other processes associated with asthma is crucial. So therefore, it was planned to measure smooth muscle changes in small bronchioles after sacrificing the mice which have been administered estrogen. The reason for selecting male mice was to rule out the effect of endogenous hormones which are produced in female mice in their normal monthly hormonal fluctuations.

## MATERIAL AND METHODS

The study was carried out in the Physiology Department, Islamabad Medical & Dental College (IMDC), Islamabad. The animal house was in National Institute of Health (NIH), Islamabad and later histological studies were conducted at Pathology Laboratory (IMDC). The Ethical Committee of IMDC approved the experiment. It was laboratory based randomized control trial with the duration of study having 10 months from 1st October 2018 to 31st July 2019. Sixty BALB/cmice bred in NIH were taken and divided into two groups of thirty each control and interventional group. The controls were given standard laboratory diet and water ad libitum and experimental mice were givenstandard laboratory diet and estrogen (estradiol valerate) 0.16mg/kg/day once daily by an oral gavage tube for eight weeks. The inclusion criterion was healthy adult male mice of 5-6 weeks age, post puberty, weighing 40-50 grams.

At the end of 8 weeks, animals were anaesthetized by chloroform. Dissection was carried out for removal of lung. Both lungs were removed and placed into 10% formalin. Tissue processing, sectioning and staining procedures were carried out in Histopathology Laboratory of Islamabad Medical &Dental College.

Qualitative and quantitative parameters were observed on light microscope.

**Quantitative parameters of lungs: Hyperplasia of Bronchiolar smooth muscle:** Increase in smooth muscle in the wall of pulmonary blood vessels were measured and estimated for each animal. Using an Olympus digital camera, images were taken from each section (12-mega pixel). To determine the diameter in micrometers, a scale set at 40X was used.Measurements were analyzed and recorded. Results were calculated as average means.

**Qualitative parameters of lungs peri-bronchiolar lymphocytic infiltration:** Peri-bronchiolar lymphocytic infiltration was seen under the microscope and on the basis of the presence of lymphocytes was marked as mild, moderate and severe according to their appearance. Considering less than 25% as mild (low infiltrate <4 cells thick), 25-50% as moderate (medium infiltrate 5-10 cells thick) and more than 50% as Severe (high infiltrate >50% visualized lumen with increased cellularity/thickening).

The data base used was SPSS-24. Chi square test was applied. One way analysis of variance (ANOVA) was used to determine significant difference & Tukey's test for multiple comparisons. It was used to determine significance of changes in lung histology following medication in different groups. p value less than 0.05 was considered statistically significant.

## RESULTS

In response to estrogen 10% of the total mice had nil, 60% had mild, 30% of the total showed moderate while no severe PBLI was observed in this group (Fig. 1). When the control was compared with estrogens group, highly significant difference was noted (Table 1). Percentage of mice showing nil, mild, moderate estrogen induced peri-bronchial lymphocytic infiltration (Fig. 2).

Different frequencies of BSMH observed in control are shown in Fig. 1. The means of control group A and experimental group's estrogen (B) respectively are given in (Table 2, Fig. 3). Statistically highly significant difference was observed on intergroup comparison of control group with experimental estrogen group (p=0.000). Multiple comparisons of control and estrogen bronchiolar smooth muscle hyperplasia were done by applying post hoc Tukey's Test. It showed highly significant results of control with experimental groups (Table 4).

Table 1: Inter group comparison of control and Estrogen, Bronchiolar Smooth Muscle Hyperplasia

| Group A | Group B | P value |
|---|---|---|
| .07±0.025 | 0.54±0.331 | 0.000 |

P<0.000 (highly significant)

Table 2: Multiple comparisons of control, estrogen administered groups were documented to observe, bronchiolar smooth muscle hyperplasia

| Dependent variable | Group (I) | Group (J) | Mean±SD | P value |
|---|---|---|---|---|
| Bronchiolar Smooth muscle hyperplasia | Control | Estrogen | .47±.07 | .000*** |
| | Estrogen | Control | .47±.07 | .000*** |

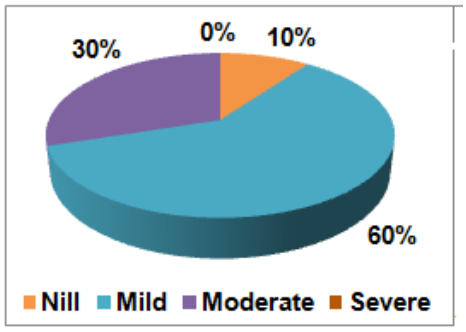


Fig. 1: Percentage of mice showing nil, mild, moderate estrogen induced peri-bronchial lymphocytic infiltration

Microscopic study was carried out and by examining the lung section slides under the light microscope,observations were made. H&E stained sections of control group revealed normal structure with normal alveoli & alveolar sac (Fig. 4). Estrogen produced marked hyperplasia of bronchial smooth muscle cells, to orally administered ethinyl estradiol (Fig. 5).Lung sections of experimental estrogen group B showed marked transitions. The lungs not only showed hyperplasia (Fig. 6) but also mild to moderate peri-bronchial lymphocytic infiltration lacking its normal architecture (Fig. 7)

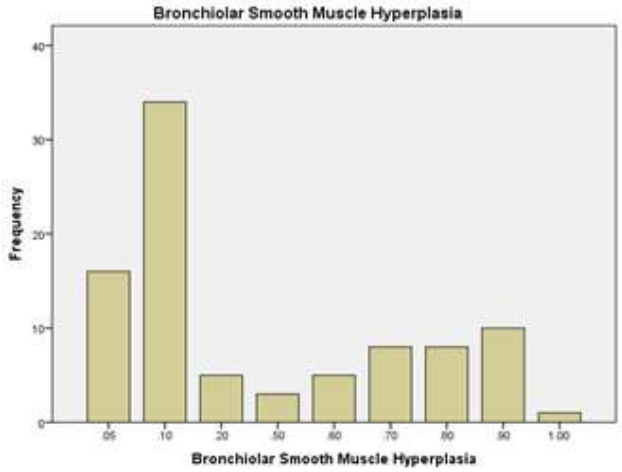


Fig. 2: Different frequencies of smooth muscle hyperplasia observed in control group

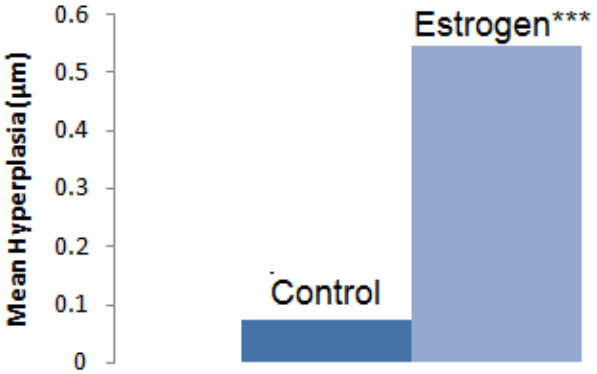


Fig. 3: Comparison of mean values of control and estrogen induced bronchiolar smooth muscle hyperplasia (***highly significant)

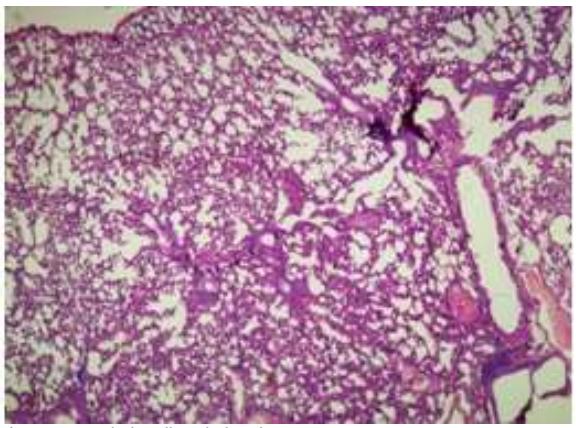

Fig. 4: Normal alveoli and alveolar sac

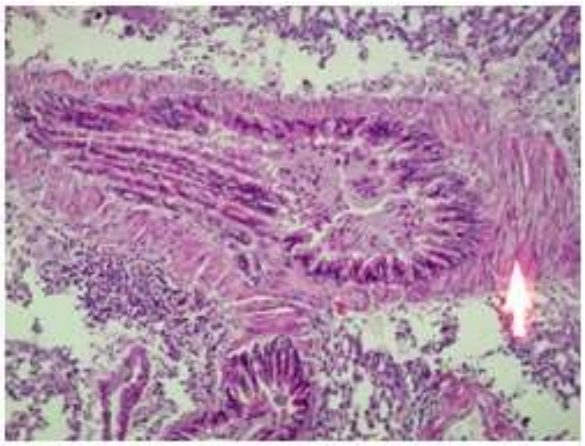

Fig. 5: Estrogen induced hyperplasia of 0.6 µm, observed with the help of ocular micrometer at 40X magnification

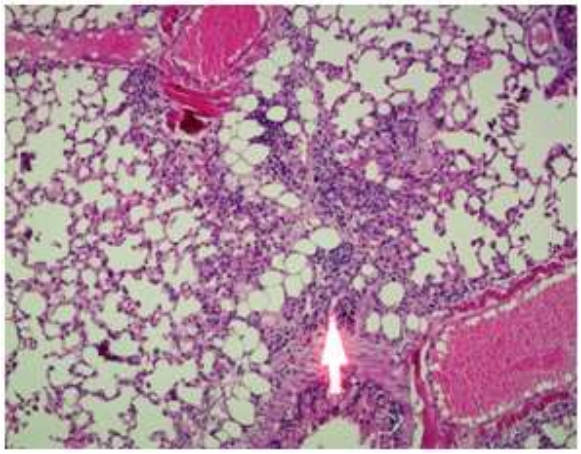

Fig. 6: Estrogen induced moderate peri-bronchial lymphocytic infiltration

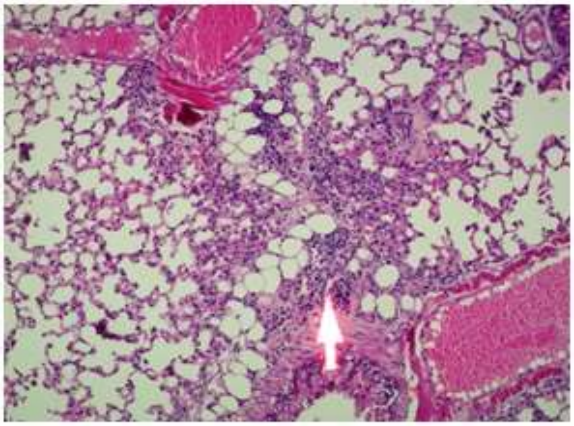

Fig. 7: Estrogen induced moderate peri-bronchial lymphocytic infiltration

## DISCUSSION

Numerous researches have investigated the relation between sex hormones and asthma.[23-26] Sex hormones are vital for maintaining respiratory health and changes in hormone levels may be accountable for aggravation of asthma in women. Hormonal changes in asthmatic women must be monitored carefully.[27] Evidence suggests that at various phases of the female hormonal cycle, both endogenous and exogenous sex hormones affect inflammatory progressions in the lungs and in smooth muscle tissue.[28] Various studies have found an association between sex hormones and inflammatory responses in the lower airway system, specifically in relation to asthma.[24,26] However, the mechanism remains unclear. Therefore, it is crucial to evaluate the key findings relating sex hormone interactions and comprehend the pathophysiological mechanismof this consortium.

In the present study, group B showed estrogen induced peribronchial lymphocytic infiltration which was statistically highly significant as compared to control (group A). Sections of experimental group B (ethinylestradiol exposed mice) showed marked transitions. The lungs not only showed hyperplasia but also mild to moderate peri-bronchial lymphocytic infiltration lacking its normal architecture. As these changes lead to asthma, similar clinical evidence has been suggested by Macsali et al[29] that use of contraceptives is linked with deteriorated lung functions.It is also suggested that using oral contraceptives has a risk factor for asthma development or aggravation.

In a study conducted by Dratvaetal[30], oral contraceptives depicted a shielding outcome, through reducing bronchial hyper-reactivity which goes against our findings. Another study by Nwaru and Sheikh[31], speculated that hormonal contraceptives decreased asthma exacerbation and the number of episodes need care which is also contradictory to our results.In another study conducted by Lange et al[32], no relationship was noticed between use of oral contraceptives and asthma.

In the present study, the bronchiolar smooth muscle hyperplasia was observed in both groups. Group B showed marked hyperplasia, to orally administered ethinylestradiol.

Considering that an abnormality of airway smooth muscle is assumed to be the basis of the airway hyper-responsiveness that characterizes asthma and being the second factor under consideration in our research. The pathogenesis of asthma includes the remodelling of the airways. Increased airway smooth muscle mass is a major structural alteration related with airway remodelling. There is emerging evidence that suggests airway smooth muscle cell migration may lead to cellular hyperplasia and, consequently, increased airway smooth muscle mass.[33]

The exact source of these cells remains unidentified. Neighboring airway smooth muscle cells in the bundle, airway myofibroblasts infiltration or circulating hemopoietic progenitor cells contribute to increased airway smooth muscle mass.[33]

Another large epidemiologic study conducted by Tam et al[17] suggested the fact that lung function changes during the menstrual cycle in female airways disease patients but not in healthy women, raises the possibility that circulating estradiol and progesterone are responsible for this phenomenon.

The ability of oestrogen to directly influence the lungs has been proven by Mitchell et al[34] using animal models.

This hormone act through a number of receptors, such as the estrogen receptors ER$\alpha$ and ER$\beta$. These are also expressed in human lungsleading to the conclusion that sex hormones play a role in lungs development. The mesenchymal and epithelial cells of the lungs also express androgen receptors.

## CONCLUSIONS

Estrogen, modulate inflammatory processes in the lungs and produce pulmonary inflammatory responses which leads to Asthma. It also leads to hyperplasia of the bronchial smooth muscles. So we must be careful prescribing women COCPs or Other oral contraceptives.

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