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ORIGINAL ARTICLE

Exogenous Progesterone Produces Significant Histomorphological Changes in the Lungs of Experimental BALB/c Mice

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ABSTRACT

Background: Progesterone is used in combined oral contraceptive pills and also has its effect on the respective receptors which are responsible for sexual development. Bronchial asthma emerges to be a major health issue in Pakistan with estimated prevalence is about 5% of the total population. Epidemiological studies highlight that one of the considerable risk factor for morbidity and mortality in inflammatory lung diseases is female gender. This suggests that sex-related hormones might play an imperative role in asthma disease progression. Understanding the biological role of sex hormones in regulating airway inflammation is crucial given the rising prevalence of chronic diseases among women worldwide.

Aim: To determine the effect of progesterone on lungs of adult male mice by assessing and comparing the histological parameters e.g. bronchiolar smooth muscle size and peri-bronchial lymphocytic infiltration.

Study Design: Randomized control trial

Place and Duration of Study: Department of Physiology, Islamabad Medical & Dental College Islamabad in collaboration with National Institute of Health Islamabad from 1st October 2018 to 31st July 2019.

Methodology: Sixty BALB/c mice were divided into 2 groups and each group comprised 30 mice. Group I (control group) received only distilled water and group 2 (norethisterone BP group) received pills mixed in distal water according to body weight of the mice respectively for 60 days.

Results: In response to progesterone, 30% of the total mice had nil, 40% had mild, 26.7% of the total who showed moderate while severe PBLI was observed in 3.3% of the mice.

Different frequencies of Bronchiolar Smooth Muscle Hyperplasia were observed when compared to Control group. Mild to moderate hyperplasia was produced by progesterone (norethisterone BP) group.

Conclusion: Progesterone is the sexual hormone which modulates inflammatory processes in the lungs producing pulmonary inflammatory responses leading to asthma and also causes hyperplasia of the bronchiolar smooth muscles.

Keywords: Progesterone, Asthma, Bronchiolar smooth muscle, Peri-bronchial lymphocytic Infiltration, BALB/c mice

INTRODUCTION

The combined oral contraceptive pill is the most widely used hormonal contraception method globaly.[1] Over the past 50 years, research on contraception has made steady progress. Modern contraceptives, both hormonal and non-hormonal, have improved women's lives by lowering several health issues that caused high mortality.[2]

Through a demographic shift comprising lower birth rates and longer survival rates during the past 50 years, the accessibility of effective contraception has contributed to a dramatic change in the structure of the global population.[3] Oral contraception is a highly effective type of contraception, with additional health advantages beyond preventing pregnancy.[4,5]

Progesterone is a synthetic preparation and its form used in combined oral contraceptive pills (COCPs) is levonorgesterol. Progesterone has the ability to prevent pregnancy on its own in a number of ways; it inhibits luteinizing hormone (LH), and maintains a strong barrier keeping the cervical mucus thick and sticky which prevent sperm from entering the uterus.[6] It decreases the motility of the fallopian tubes, preventing sperm transport. Additionally, it alters the lining of the uterus, making it more challenging for the fertilised egg to implant. Hypothalamus reduce the production gonadotropin releasing hormone (GnRH) due to Negative feedback from progesterone, which in turn decreases the release of follicle stimulating hormone (FSH) and greatly reduces the release of luteinizing hormone (LH) by the anterior pituitary. Reduced FSH levels impede follicular growth, limiting the rise of estradiol levels. A mid-cycle LH surge is prevented by the negative feedback of progesterone and the absence of the positive feedback of oestrogen on mid cycle LH surge. Ovulation is prevented by the suppression of follicular development and the lack of the LH surge.[7] Two types of oral contraceptive pill are widely available: the pills containing only progesterone and the COCPs containing both estrogen and progesterone.[8]

MATERIALS AND METHODS

This study was conducted in Physiology Department, Islamabad Medical & Dental College, Islamabad, in alliance with National Institute of Health Islamabad from 1st October 2018 to 31st July 2019. Sixty BALB/c mice were taken and divided into two groups of thirty each control and interventional group, selected by Simple random sampling. The inclusion criterion was healthy adult male mice of 5-6 weeks age, post puberty, weighing 40-50 grams, bred in the animal house of NIH in compliance with the international criteria. They were provided access to water and regular feed pellets throughout study.

They were divided in two groups; group A Mice in this group served as controls and group B Mice were given progesterone (norethisterone BP) 0.406 mg/kg/day, once daily using oral gavage tube and standard laboratory diet for eight weeks.

At the end of 8 weeks, animals were anaesthetized, dissected and lungs were removed. After Tissue processing, standard procedure for Sectioning and staining was followed in histology section Pathology Laboratory of Islamabad medical & dental College. Later for the examination of qualitative and quantitative data a light microscope was used.

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 ocular micrometre at a 40X magnification, three sections per animal were examined. Images were captured using an Olympus digital camera (12-mega pixel) from each section. These images were then shifted to laptop. In image J version 1.48, each image was opened. The diameter was measured in micrometres using a scale that was set at 40X. The measurement tool "straight" was chosen, and a straight line was drawn to calculate the diameter to be measured. The measurements were subsequently evaluated and documented.

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).

Photography: The Olympus DP21 light microscope's ocular was used in conjunction with the Olympus stylus 1010 digital camera (12 mega pixel). By using Photoscape software, the photographs were rectified and adjusted for contrast, brightness, sharpness, and colour balance.

The data was entered in SPSS-24. The significant difference was determined using ANOVA and multiple comparison were analysed by using Tukey's test. 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

Inter group comparison of control, progesterone bronchiolar smooth muscle hyperplasia is shown in Table 1. In response to progesterone, 30% of the total mice had nil, 40% had mild, 26.7% of the total showed moderate while severe PBLI was observed in 3.3% of the mice (Figs. 1-2)

When the control group was compared to experimental progesterone group B, a statistically significant difference was discovered. (p=0.000). Highly significant results were observed on comparisons of control and progesterone administered group for Bronchiolar Smooth muscle hyperplasia by Applying Post Hoc Tukey Test. (Table 2)

Under a light microscope, the lung segment slides were examined for microscopic study, and observations were taken. The control group's H&E stained sections displayed normal architecture with normal alveoli and alveolar sacs (Fig. 3). Lung sections of experimental progesterone group B showed marked lymphocytic infiltration, with no infiltration in 09, mild in 12 (Fig. 4), moderate in 8 (Figs. 5-6) and severe in 1 mice (Figs. 7-8).

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

Group A	Group B	P value
.07±0.025	0.44±0.333	0.000

P<0.000 (highly significant)

Table 2: Comparisons of control and progesterone administered group was documented to observe, bronchiolar smooth muscle hyperplasia

Dependent variable	Group (I)	Group (J)	Mean±SD	P value
Bronchiolar Smooth muscle hyperplasia	Control	Estrogen	.36±.07	.000
	Estrogen	Control	.46±.07	.000

P<0.001(Highly significant)

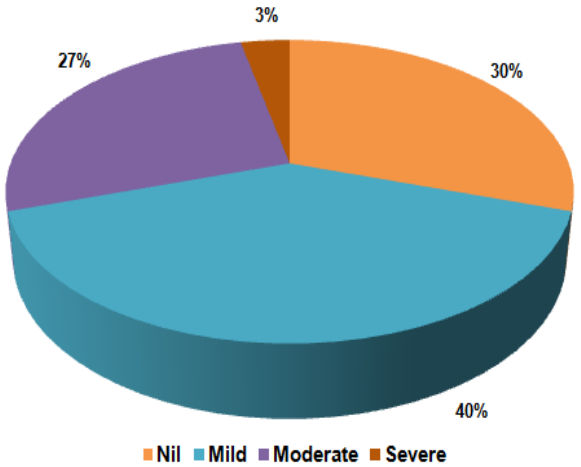

Fig. 1: Percentage of mice showing nil, mild, moderate and severe progesterone induced Peri-bronchial lymphocytic infiltration

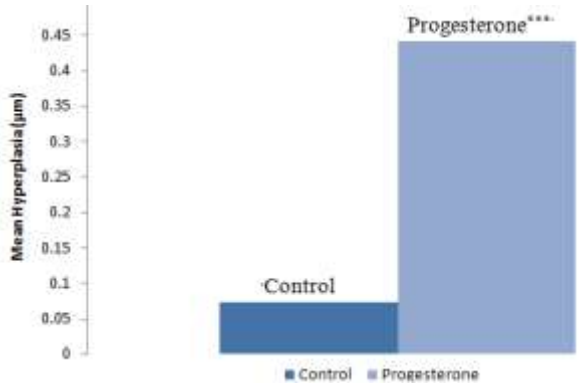

Fig 2: Comparison of mean values of control and Progesterone Induced Bronchiolar Smooth Muscle Hyperplasia (P<*** highly significant)

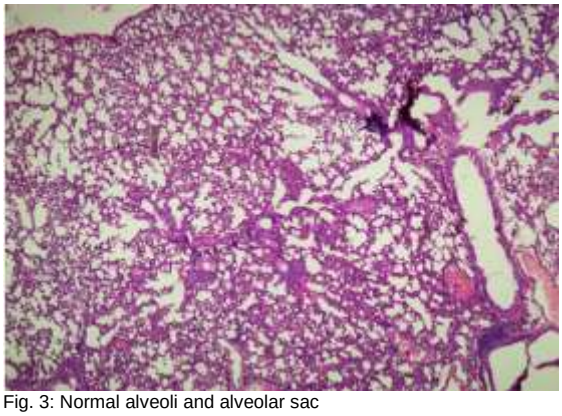

Fig. 3: Normal alveoli and alveolar sac

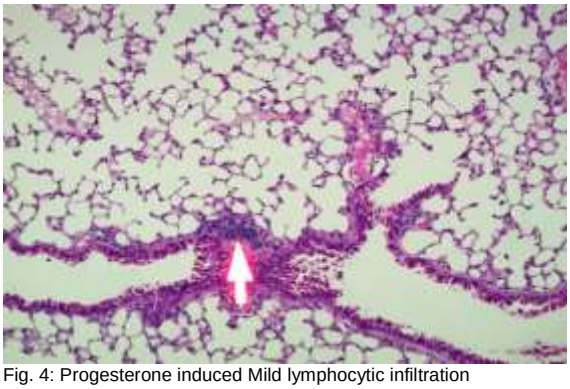

Fig. 4: Progesterone induced Mild lymphocytic infiltration

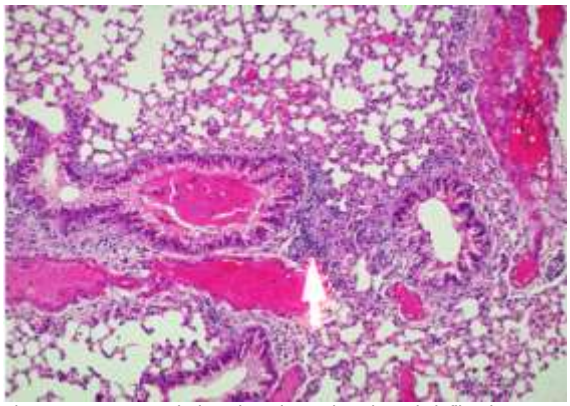

Fig. 5: Progesterone induced Moderate lymphocytic infiltration

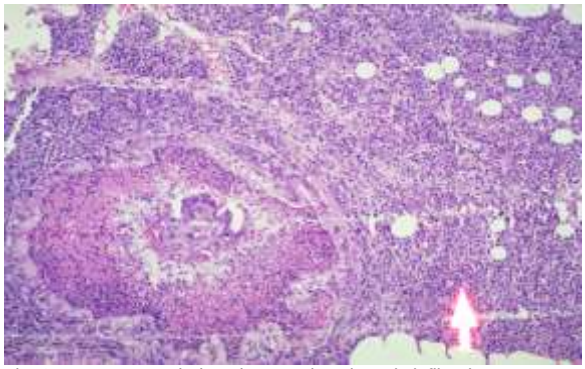

Fig. 6: Progesterone induced severe lymphocytic infiltration

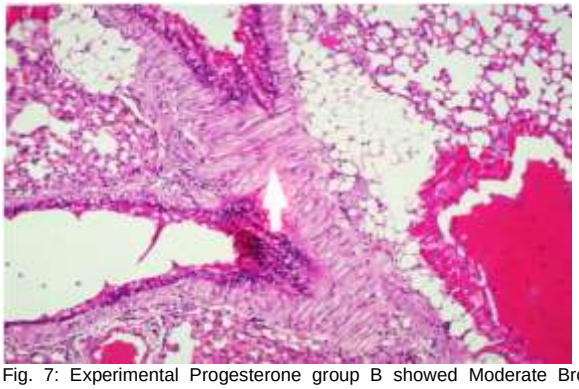

Fig. 7: Experimental Progesterone group B showed Moderate Bronchial smooth muscle hyperplasia

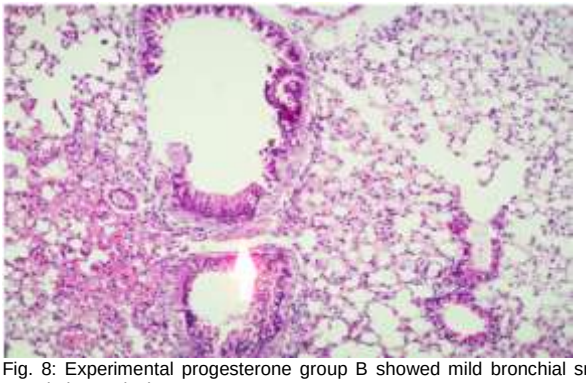

Fig. 8: Experimental progesterone group B showed mild bronchial smooth muscle hyperplasia

DISCUSSION

There are sex-related differences in the risk, incidence and pathogenesis of numerous lung disease in humans.[27] Sex steroid hormones are important for maintaining respiratory health.[28] According to the few evidences, during various phases of a female hormonal cycle, sex hormones may affect inflammatory processes in the lungs and smooth muscle tissue.[29] The exact mechanism behind this connection is still obscure. Therefore, it is crucial to evaluate the key findings relating sex hormone interactions and comprehend the pathophysiological mechanisms underlying this association.

As these changes lead to asthma, similar clinical evidence has been suggested by Macsali et al[30] that usage of contraceptives is linked to deteriorated lung functions. They proposed that using contraceptives increases the chance of developing or exacerbating asthma attacks.

In the present study, the group B showed progesterone induced peribronchial lymphocytic infiltration which has statistically highly significant result as compared to control group. This group also showed marked lymphocytic infiltration. A study by Dratva et al. [31] contradicts our findings by showing that oral contraceptives have a protective effect by reducing bronchial hyperreactivity. Another study by Nwaru and Sheikh [32,] hypothesised that hormonal contraceptives decreased asthma exacerbations and the number of episodes necessitating medical attention, which is again in conflict with our findings. In another study conducted by Lange et al, where there is no relation between using oral contraceptives and asthma.[33]

In the present study, group B showed mild hyperplasia of bronchial smooth muscle cells, to orally administered progesterone, given that an abnormality of the smooth muscle lining the airways is believed to be the underlying cause of the hyperresponsiveness of the airways that characterizes asthma and being the second factor taken into account in our research. The pathogenesis of asthma includes the remodelling of the airways. Increased airway smooth muscle mass is a significant structural alteration associated with airway remodelling. There is increasing evidence to suggest that the migration of airway smooth muscle cells may contribute to cellular hyperplasia, and therefore increased airway smooth muscle mass.[34]

There is evidence that individuals with more muscle mass have more airways smooth muscle cells overall due to both hypertrophy and hyperplasia. Two forms of airway smooth muscle hypertrophy and hyperplasia have been described by Ebina and colleagues.[35] Airway smooth muscle mass was only enhanced in the middle bronchi, where hyperplasia predominated, in patients with type 1 asthma. The tracheobronchial tree of subjects with type 2 asthma displayed increased muscle, with this increased muscle being characterised by both hyperplasia and hypertrophy, especially in peripheral airways.. This favors the findings of our study.

Our findings are in agreement with that of de Oliveira et al.[36] The authors assessed progesterone's role to allergic lung inflammation. The release of IL-10, IL-1, and TNF by BAL cells was raised by progesterone, while the synthesis of IL-4 by BM cells was also increased. The existence of such dual hormonal effects suggests that hormone therapy in premenstrual and postmenopausal asthmatic women should consider the risk that these treatments may aggravate pulmonary problems.

Mitchell et al[37] reported that in the absence of oestrogen, progesterone increased the harmful environmental tobacco smoke (ETS) induced airway remodelling and inflammation. This is also in agreement with our findings.

CONCLUSION

Progesterone is the sexual hormones which modulate inflammatory processes in the lungs producing pulmonary inflammatory responses which leads to Asthma and Leads to hyperplasia of the bronchial smooth muscles.

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