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Original Research Article

Nasal Mucociliary Clearance in Various Phases of Menstrual Cycles

Sneh Lata Garg[1], Jyoti Yadav[2]

[1]Resident, [2]Professor,
Department of Physiology, Pt. B.D.S PGIMS Rohtak, Haryana.

Corresponding Author: Sneh Lata Garg

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ABSTRACT

Introduction: Various phases of menstrual cycle not only affect reproductive system but other systems also. So this study was performed to see effect of various phases of menstrual cycle on nasal mucosa.
Materials and methods: Study was performed on 30 healthy female medical students of 18-24 years having regular menstrual cycles. Nasal mucociliary clearance (NMC) time was recorded during menstrual (2^{nd} to 4^{th} day), proliferative (9^{th} to 12^{th} day) and luteal phases (19^{th} to 21^{st} day) of menstrual cycles. NMC time was assessed by Andersen's saccharin technique.
Results: The mean values of NMC of two menstrual cycles were 10.81± 2.143, 8.233± 1.942 and 11.12 ± 2.118 in menstrual, proliferative and luteal phases respectively. On comparing proliferative phase with menstrual and luteal phases, NMC time difference was highly significant ($p<.001$) and when luteal and menstrual phases were compared results were not significant ($p>.05$).
Conclusion: Nasal mucociliary clearance time was significantly less in proliferative phase when compared with other two phases of menstrual cycles. Thus various phases of menstrual cycle do have effect on nasal mucosa. This may be related to change in hormonal levels in different phases of menstrual cycle.

Key words: menstrual cycle, menstrual, proliferative, luteal phase, NMC.

INTRODUCTION

Various phases of menstrual cycle not only affect reproductive system but other systems also as the sex hormones play a major role in virtually all physiological processes and have potential effect on cardiovascular system, sleep, nasal mucosa, breathing, upper respiratory muscle, etc. [1] So this study was performed to see effect of various phases of menstrual cycle on nasal mucosa. Nasal mucociliary clearance (NMC) is generally determined to obtain an in vivo measurement of the effectiveness of interaction between cilia and mucus. The mucociliary clearance occurs in trachea and main bronchi at a similar rate as in the nose. The nasal and tracheobronchial clearance in normal subjects revealed that saccharin test is a useful screening technique. The results obtained using saccharin test is similar to those obtained using radioactively labeled particles. It has been postulated that the nasal mucosa, like other human tissues, is affected by a complex interactive network of neuropeptides, allergic and inflammatory mediators and hormones. Thus, associations between hormonal changes and nasal conditions have been described in the literature based on clinical trials, thus showing connections between symptoms such as nasal stuffiness and coryza and hormonal variations in pregnancy, use of contraceptives and menstrual cycle phases. [2] It has been observed that there is

increased nasal obstruction in women at times of high blood estrogen levels, compared with a control group, through using acoustic rhinometry and anterior rhinomanometry. Nasal congestion therefore occurs in conjunction with the rise in serum estrogens that occur at ovulation in the normal menstrual cycle. [3] Haeggström et al. (2000), found a connection between high blood estrogen levels and nasal mucosal reactivity. Another study also suggested that the nasal mucosa became hyper-reactive to histamine in connection with ovulation, when the blood level of estrogen reached its peak suggesting some role of estrogen on nasal mucosa. [4] Several factors and diseases like smoking, lower respiratory tract diseases, bronchial asthma and perennial allergic rhinitis affect NMC. [5-8]

MATERIALS AND METHODS

The present study was conducted in the department of Physiology, Pt. B.D. Sharma, PGIMS, Rohtak. Thirty healthy, unmarried female volunteers of 18-24 years age with history of normal regular menstrual cycle were included in the study. Nasal mucociliary clearance time on both sides was recorded during various phases of menstrual cycle i.e. menstruation (2^{nd} to 4^{th} day), follicular (9th to 12^{th} day) and luteal phase (19^{th} to 21^{st} day) in the same 30 girls during two menstrual cycles. The mean of both menstrual cycles was taken as mean for calculations. Persons having anaemia, common cold, nasal polyps, deviated nasal septum, chronic sinusitis, allergic rhinitis, atrophic rhinitis, chronic smokers and patients with recent nasal packings/surgery, cardiovascular and respiratory system diseases and on hormone therapy were excluded from study.

A written informed consent for nasal mucociliary clearance was taken after explaining the method of study. This study was approved by institutional ethical committee. Subjects were instructed to come to the laboratory on 2^{nd}-4^{th} day, 9^{th}-12^{th} day and 19^{th}-21^{st} day of menstrual cycles. NMC assessment was carried out during two menstrual cycles. If NMC assessment in any phase of a cycle was missed due to holiday or some other reason, then all the phases of next regular menstrual cycle were considered.

NMC was assessed by Andersen's saccharin method in the present study. Saccharin test can be used for serial measurements during treatment, although it should be repeated only after the sweet taste has completely disappeared. [9] A 1 mm particle of saccharin was placed on the floor of the nose approximately 1 cm behind the anterior end of inferior turbinate under direct vision with the subject in sitting position. They were asked to swallow at about every 30 seconds and to report the first change in their sensation of taste. The test was carried out in both the nostrils and the mean of the two was taken as mucociliary clearance time. This was done to obviate the effect, if any, of nasal cycle on the mucociliary clearance time. The time taken by the subjects from placement of particle to perception of sweet taste was recorded as the nasal mucociliary clearance time in minutes. All subjects were tested in similar environmental conditions and were instructed not to inhale or exhale forcefully, sniff, eat or drink and avoid coughing and sneezing during that time. Whenever there was coughing or sneezing, then the test was repeated.

Statistical analysis

Data was analyzed by repetitive ANOVAs for comparison between all three phases of menstrual cycle and results were expressed in mean ± SD.

OBSERVATIONS AND RESULTS

The mean values of NMC time in minutes in cycle 1 were 10.63 ± 2.23 , 8.258 ± 2.116 and 10.94 ± 2.208 in menstrual , proliferative and luteal phases respectively while the mean values of NMC time in cycle 2 were 10.98 ± 2.107, 8.209 ± 1.795 and 11.3 ± 2.101 in menstrual , proliferative and luteal phases respectively. When both cycles were considered, the mean values of

NMC time were 10.81± 2.143; 8.233± 1.942 and 11.12 ± 2.118 in menstrual, proliferative and luteal phases respectively (Table 1, Fig 1). It was found that there was significant decrease in NMC time in proliferative phase as compared to luteal and menstrual phases.

Table 1: NMC time values (minutes) in different phases of menstrual cycles

Cycles	Menstrual phase (2^{nd} - 4^{th} day)	Proliferative phase (9^{th} -12^{th} day)	Luteal phase (19^{th} -21^{st} day)
Cycle1	10.63±2.23	8.258±2.116	10.94±2.208
Cycle 2	10.98±2.107	8.209±1.795	11.3±2.101
Both cycles	10.81± 2.143	8.233± 1.942	11.12 ± 2.118

Mean ± SD

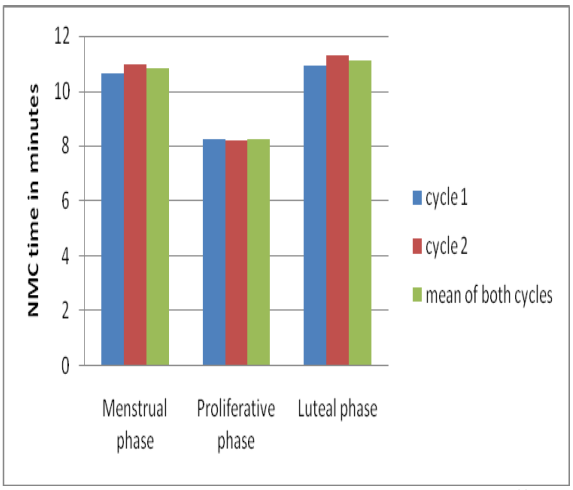

Fig 1: Graph showing NMC values (minutes) in different phases of menstrual cycles

On comparing menstrual and proliferative phases, the NMC time differences were found highly significant (p<.001). Similarly on comparing luteal and proliferative phases, the NMC time differences were found highly significant (p<.001). But on comparing menstrual and luteal phases, the NMC time differences were not found significant (p>.05) as shown in Table 2.

Table 2: Comparison of NMC time among different phases of menstrual cycles

Cycles	Menstrual vs. Proliferative	Proliferative vs. Luteal	Luteal vs. Menstrual
Cycle1	p< .001^{s}	p < .001^{s}	p >.05 ^{ns}
Cycle 2	p < .001^{s}	p < .001^{s}	p >.05 ^{ns}
Both cycles	p < .001^{s}	p < .001^{s}	p >.05^{ns}

p<.001 = highly significant(s), p >.05 = not significant (ns)

DISCUSSION

Menstrual cycle is an integral part of female reproductive system which reflects a complex interplay between brain, pituitary gland and ovary. Menstrual cycle occurs in three phases i.e. menstrual, follicular and luteal. The mean menstrual cycle is 28 days long. Levels of hormones in the three phases of menstrual cycle are fluctuating. This fluctuation in sex hormones plays a major role in virtually all physiological processes and hence affects various systems of human body. [1] In our study, we studied the effect of different phases of menstrual cycle on nasal mucociliary clearance. There is connection between symptoms such as nasal stuffiness and coryza and hormonal variations in pregnancy, use of contraceptives and menstrual cycle phases. Anderson et al (1974) described saccharin test, which is simple, inexpensive and reproducible clinical test for determining abnormal nasal mucociliary clearance. [9] Deborah et al (2014) also emphasized that saccharin test is a simple, inexpensive and non-invasive method while methods using radiolabeled particles are time consuming, cumbersome and expensive. [2]

Mechanism of action of sex steroid hormones is via their own unique receptors: estrogen receptor (ER-α or ER-β), progesterone receptor (PR-A or PR-B), and an androgen receptor (AR). Estradiol binds with a higher affinity to ER than its metabolic products such as estrone and estriol. It is known that estrogen and progesterone receptors are responsible for sexual development but their effect beyond the reproductive system is becoming increasingly recognized. There are two types of estrogen receptors (ER-α or ER-β), two types of progesterone receptors (PR): PR-A and PR-B, all of which are expressed in rats, mice and humans. While androgen receptors (AR) are expressed primarily in

mammalian reproductive tissues, ER-α, ER-β, PR-A and PR-B receptors expression have been found not only in the mammalian female and male reproductive tracts, but also in the female mammary glands, bone, cardiovascular tissues, lung, brain and nasal mucosa. [10] Among the factors influencing NMC, the most important is acetylcholine (Ach), a vasodilator neurohumoral transmitter secreted at somatic and autonomic sites under normal and physiological conditions. Most of the Ach is present in the ionic solution within the synaptic vessels but some is also found in free form in the cytoplasm of cholinergic nerve endings. It is destroyed by enzyme acetylcholinesterase. Muscarinic receptors are believed to play an important role in modulation of ciliary action in respiratory system's activity. The cryostimulation by methacholine in human upper airway mucosa involves M_1- and M_3- muscarinic receptor subtypes, but not the M_2-receptor subtype. Aerosolized methacholine stimulated the ciliary beat frequency (CBF) from the base line of 5.8 +/- 0.7 to 9.4 +/- 3.0 Hz. [11]

Ovarian hormones may have roles in maintaining the normal balance and functional interactions between different neurotransmitter systems. The combination of hormone manipulation with qualitative and quantitative analysis of immunocytochemistry for dopamine beta-hydroxylase, choline acetyltransferase and serotonin in the primate prefrontal cortex revealed quantitative responses in both cholinergic and monoaminergic axons to changing ovarian hormone levels. [12]

The hypothalamic stimulus that leads to release of female hormones also releases Ach in nasal mucosa. The hormones are selectively concentrated in nasal mucosa almost one thousand fold and inhibit acetylcholinesterase, hence increases local concentration of acetylcholine that lead to increase in vasomotor reaction and hence mucociliary clearance. Acetylcholine itself increases ciliary beat frequency while atropine causes the reduction in the secretion of the nose and hence depresses the ciliary beat frequency. [13,14]

A study was done on dogs to find stimulation of ciliary beat frequency (CBF) by autonomic agonists in vivo. It was assumed that increase in autonomic activity would result in increase in CBF in vivo. It was found that CBF in the lower respiratory tract is regulated by autonomic agonists. This was perhaps due to effect of estrogen on autonomic system. [15]

Toppozada et al (1981) through their studies on humans demonstrated that the morphological and histochemical changes occurring in the nasal mucosa were associated with estrogen in healthy fertile women during the menstrual cycle. [16] Navarrete et al (2003) did cytological analysis in different phases of the menstrual cycle which revealed that both nasal and vaginal smear showed the same characteristics, suggesting that cell turnover in the nasal epithelium is influenced by hormonal state during the menstrual cycle. [17] Serra et al. in 2004 studied 88 women with ovulatory menstrual cycle, who underwent nasal sampling with a cytobrush of the middle and inferior nasal turbinates under direct vision during the follicular, periovular and luteal phases of the menstrual cycle. Hematoxylin-eosin staining revealed the cytological characteristics of the nasal respiratory epithelium and of vaginal smears co-related according to the three different phases of the menstrual cycle, suggesting that the vaginal cells as well as the nasal respiratory epithelium is an ovarian steroid target. [18] Millas et al (2010, 2011) evaluated the presence of specific estrogen receptors (alpha and beta) in the inferior turbinate of asymptomatic patients, in order to characterize the influence of hormones on physiology and pathological nasal processes and showed the presence of alpha and beta receptors, with higher beta expression and higher intensity in the anterior portion of the inferior turbinate. [19,20] Shirasaki et al (2004) studied nasal mucosa by using immunohistochemistry and observed antibodies to glucocorticoid

receptor (GR) that showed the presence of GR within all cells of nasal mucosa, with the highest quantities of GR being localized in epithelial cells, submucosal glands and inflammatory leukocytes. Immunohisto-chemical analysis of sex steroid receptor revealed anti-estrogen receptor ERα antibody labeled mast cells and anti-ERβ antibody labeled submucosal glands showed the presence of ERα and ERβ but no progesterone receptor (PR) or androgen receptors (AR). [21]

Armnostone et al studied nasal mucociliary transport time using the vegetable charcoal powder technique. Three measurements were made at different points of the cycle i.e. during the early follicular phase, periovulatory phase and luteal phase. Transit was significantly accelerated during the periovulatory phase ($p < 0.01$), when the serum estrogens are at their highest level. [22] In our study we found almost same results by using saccharin method. Transit was significantly accelerated during the proliferative phase ($p < 0.01$).

Littlejohn et al studied nasal mucociliary clearance in subject in both the congested and decongested phases of the cycle. The results were statistically significant and highly suggestive of a difference in nasal mucociliary clearance between the two phases of cycle, with the congested phase having the more rapid clearance. [23] Stubner et al reported that, for influencing the neurogenic nasal symptoms, higher hormone concentrations are apparently necessary than those achieved after administration of oral contraceptives. [24] Haeggstrom et al (2000) found a connection between high blood estrogen levels and nasal mucosal reactivity. They found that the nasal mucosa became hyperreactive to histamine during ovulation, when the blood level of estrogen reached its peak suggesting some role of estrogen on nasal mucosa. [4]

Philpott et al (2004) also found some association between nasal symptoms and blood estrogen levels. It was observed that there was increased nasal obstruction in women at times of high blood estrogen levels when compared with a control group by using acoustic rhinometry, anterior rhinomanometry and measurements of peak inspiratory nasal flow. It was due to nasal congestion at the periovulatory stage of the cycle, of which anterior rhinomanometry and mucociliary time were decreased significantly ($p < 0.05$). Nasal congestion therefore occurs in conjunction with the rise in serum estrogens that occur at ovulation in the normal menstrual cycle. So it was suggested that pharmacological antagonism of estrogens may therefore relieve nasal congestion and should be further researched. [3] Nappi et al (2004) found that both intranasal and transdermal hormonal therapy (HT) with 17beta-estradiol improve nasal symptomatology and nasal mucosa appearance and reduced mean mucociliary transport time. [25] Soylu et al (2015) studied in premenopausal and postmenopausal women the mean NMC time and found that in postmenopausal women NMC time was significantly longer than in premenopausal women ($p < 0.0001$). There was positive correlation between menopause duration and nasal mucociliary clearance time in postmenopausal women ($p < 0.0001$). [26] These studies are at par with our study which showed that there was significant decrease in NMC time in proliferative phase as compared to luteal and menstrual phases. On comparing menstrual and proliferative phases, the NMC time difference was found highly significant ($p<.001$). Similarly on comparing luteal and proliferative phases, the NMC time difference was found highly significant ($p<.001$). But on comparing menstrual and luteal phases, the NMC time difference was not found significant ($p>.05$).

CONCLUSION

It can be concluded that various phases of menstrual cycle in young healthy females not only affect reproductive system but also affect nasal mucosa by significant decrease ($p<.001$) in NMC time in

proliferative phase as compared with other two phases.

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