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EFFECT OF CYCLIC FLUCTUATING OF OVARIAN HORMONES ON SALIVARY PMN COUNT

Dental Science

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ABSTRACT

Neutrophil forms the first innate immune response of inflammation in the oral cavity. In reproductive age females, the changes in the levels of ovarian hormones during the menstrual cycle induce the anti-inflammatory response. This anti-inflammatory response in the oral cavity indicates the variation in the salivary neutrophil count according to the changing levels of ovarian hormones. The aim of this study is to determine the effect of fluctuating ovarian hormones on salivary Poly-morpho nuclear neutrophil (PMN) count. **Objectives:** To determine the salivary neutrophil count at different phases of menstrual cycle i.e., at follicular phase (7th day),ovulation (14th day),leutal phase (21st day) and menstruation (28th day).**Methodology:** Salivary Neutrophil from 30 eligible females at day 7,14, 21 and28 of the menstrual cycle were collected through oral rinse and PMN quantification was done. Results were analyzed using Friedman test and Post hoc test. **Results:** According to Friedman test there was highly statistically significance seen among the neutrophil count of the four groups.($p<0.05$).Post hoc test result showed that there was no statistically significance between the menstruation, follicular and leutal phase ($p>0.05$) and there was highly statistically significance between the follicular, ovulation and leutal phase ($p<0.05$).**Conclusion:** The study results confirm the influence of hormonal fluctuation on salivary Neutrophil (primary inflammatory mediators).

KEYWORDS

ovarian hormones ,salivary PMN,quantification, inflammatory mediators,

INTRODUCTION

The endocrine system consists of hormones, which are chemical messengers that pass the information between tissues or organs through the blood. They play an important role in signal transduction mechanisms[1].One of the important parts of the endocrine system is the steroid hormones also regarded as ovarian hormones. The ovarian hormones play an important in the reproductive aged female body by guiding the menstrual cycle every month[2].

Each menstrual cycle is a cycle of 28 days. It may be divided into two phases: follicular phase and the luteal phase during these phases the variation in the levels of steroid hormones i.e. estrogen and progesterone can be seen. The first day of menses until ovulation is the follicular phase in this phase the ovarian hormone estrogen level increases. Approximately 10-12 hours after the estrogen peak produced by the pre-ovulatory follicle the ovulation occurs. During the mid-follicular phase estrogen levels rise and then drop steeply after ovulation. This is followed by a secondary rise in estrogen levels during the mid-luteal phase with a decrease at the end of the menstrual cycle with the menstruation. The serum progesterone parallels the secondary rise in estrogen[3].

The important effector cells in the innate arm of the immune system are neutrophils.Jennifer M. Smith et al concludes in the study that there is anti-inflammatory effect of ovarian hormones on neutrophils[4].O'Brien S.M. et al also conducted the study which supported the same[5]. Bender JS et al and Nussbaum G et al showed by his work that the periodontal disease severity is best indicated by the neutrophils[6-7].

Literature shows that the previously conducted studies were on the properties and activities of peripheral blood neutrophils during the different phases of menstrual cycle.The collection of blood involves potential risks to subjects.The amount of inflammatory action due to the effect of ovarian hormones on the periodontal tissue is unknown . The present study is conducted in the anticipation of the evidence for the above by evaluating the effect of cyclic fluctuating ovarian hormones on salivary PMN count.

The objectives of the present study is to determine the neutrophil count at different levels of ovarian hormone during 7th day(follicular phase),14th day(ovulation),21st day(luteal phase),28th day(menstruation).

MATERIALAND METHODS

The observational descriptive study was conducted in SDM Dental college Dharwad over a period of 30 days from June to July 2018 which included one menstrual cycle of each study subject. 30 reproductive aged female students of SDM Dental college Dharwad who were willing to participate in the study, who met all the inclusion criterias of regular menstrual cycle of 28 days and with no systemic or oral diseases were recruited in the study. Subjects not willing to participate in the study, Pre-pubertal and menopause age females, Irregular menstrual cycles, With systemic oral diseases and other habits were excluded from the study.

The recruited subjects were asked for the date of menstruation of the last menstrual cycle. The subjects were grouped into four phases as follicular phase (7th day), ovulation (14th day), leutal phase (21st day) and during mensuration (28th day) depending on the information collected. Appointments were given on their respective 7th day,14th day,21st day and 28th day of menstrual cycle.

On the day of appointment the subjects were called to the department of Public Health Dentistry around 12:30 pm. The subjects were instructed not to eat or drink anything half n hour before giving the oral rinse. The gingival index was recorded of the subjects on the baseline visit for standardization, to avoid bias. The subjects were given 10ml of normal saline in a 50ml falcon tube to rinse thoroughly for 30 seconds covering all the parts of the oral cavity and spit back into the falcon tube. The neutrophil counting was immediately done from the collected oral rinse.

Neutrophil counting

Reagents used

Sodium chloride injection IP (0.9% W/V) NS,each 100 ml contained sodium chloride IP,water for injection IP,mol/L:Na+ 154,Cl-154,mOsmol/:308(calc.). Acridine orange [3,6-bis(dimethylamino) acridium chloride hemi(zinc chloride salt)]. A fluorescence lysosomal marker that gives a characteristic fluorescent staining pattern in neutrophils and helps differentiate them from other cells, was added to the rinse samples after rinse collection.

vertexed oral rinse.
The stained sample was kept aside for 5 minutes

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Neutrophil counting was done from all the 4 quadrants of the neubaeurs chambers(N) under 100X lense fluorescent microscope.

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The total Neutrophil count would be N×500 Cells/ml.

The entire procedure was carried out in a dark room.The preparation of

orange acridine dye used for staining of neutrophils to be viewed under fluorescent microscope was done by adding orange acridine powder to 10ml of distilled water or normal saline. The solution was refrigerated for one hour, the solution prepared is stock solution and care was taken to not to expose the orange acridine stock solution to light.0.1ml (100µL)of stock solution was added to 50 ml of distilled water or normal saline to get the working solution. The working solution was prepared every day freshly in the morning. The subjects was recalled again after 7 days and the same procedure was repeated in the subsequence visits.

Statistical Analysis

The collected data was entered in Microsoft Office Excel Worksheet. The data analysis was done using SPSS 20 version software.The statistical analysis included Friedman test to test the significant difference in the neutrophil counts at different intervals. Wilcoxon signed-rank test was done to see among which two intervals the significance difference was found.

Sl.no.	Different age groups (years)	Number of subjects (n)
1	22-23	13
2	24-25	14
3	26-27	3

Table 1 shows the age-wise distribution of 30 reproductive aged female subjects with the mean age of 23.83 years. 13 subjects were between the age group of 22-23 years, 14 subjects were between the age group of 24-25 years and 3 subjects were between the age group of 26-27 years. The mean value of gingival index scores of the study subjects was 0.515±0.233.

Table 2: Comparison of the mean neutrophil count during various phases of menstrual cycle using Friedman test.

Different phases of menstrual cycle	Mean salivary PMN count	Standard deviation
Follicular phase	41.55	28.07
Ovulation	51.83	33.56
Luteal phase	41.86	27.00
Menstruation	41.05	27.76
Chi-square value – 52.20 P value – 0.000*		

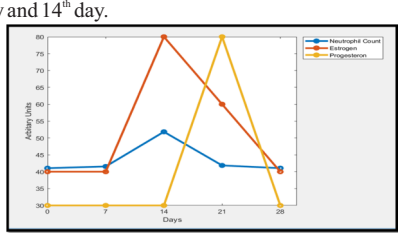
Table 2 shows the comparison of mean salivary PMN count during various phase of menstrual cycle.The mean±SD salivary PMN count was 41.55±28.07 during follicular phase, the mean±SD salivary PMN count was 51.83±33.56 during ovulation, , the mean±SD salivary PMN count was 41.86±27.00 during luteal phase and the mean±SD salivary PMN count was 41.05±27.76 during menstruation. Friedman test showed that there was statistical significant difference among salivary PMN count of various phases of menstrual cycle P value= <0.001.

Table 3:Significant pair differentiation of salivary PMN count during various phases of menstrual cycle using Post-hoc Wilcoxon signed ranks test.

	7th day vs 28 day	14th day vs 28th day	21st day vs 28th day	14th day vs 7th day	21st day vs 7th day	21st day vs 14th day
Asymp.sign(2-tailed)	1.56	0.000*	.107	.000*	.980	.000*

P value <0.001 is significant*

Table 3 shows that significant pair differentiation of salivary PMN count during different phases of menstrual cycle using Post-hoc Wilcoxon signed ranks test. The statistical significance of salivary PMN was found between 14th days and 28th day,14th day and 7the day and 21st day and 14th day.



Graph 1:Comparison between fluctuating salivary PMN count and ovarian hormones.

Graph 1 shows the comparison between fluctuating salivary PMN and ovarian hormones.As the level of estrogen hormone increases in-between 7th day (follicular phase) and 14th days (during ovulation) the salivary PMN count also increases. During the luteal phase(21st day) estrogen starts decreasing and reaches the baseline level during menstruation(28th day), salivary PMN count follows the estrogen hormone.

DISCUSSION

In this observational study the effect of fluctuating ovarian hormones on salivary neutrophils was studied. Friedman and Post-hoc Wilcoxon Signed Ranks tests showed that there was significant difference of the neutrophil count between 14th, 28th day and 14th ,7th day.

This test was conducted in 30 voluntary reproductive aged females who met the inclusion criterias and voluntarily participated in the study.The selected subjects were the students from the same college to avoid the confounding factors in the study. The study duration was 28 days which constituted one menstrual cycle which was divided into four phases by assuming that the ovulation would be on 14th day of the cycle, with 7 days apart.

On the day of saliva sample collection the gingival index was recorded for each subjects for standardization and to avoid the bias .The subjects were instructed to not to eat or drink anything half an hour before giving the sample. The instructions were given to allow the neutrophils to pool in the oral cavity.30 seconds of through rinsing ensured to cover all the surfaces of the oral cavity. Acridine orange reagent was used in the neutrophil counting procedure because it is a fluorescence lysosomal marker that gives a characteristic fluorescent staining pattern in neutrophils and helps differentiate them from other cells[6].In recent years, the quantification of Pmns has been proposed as a screening tool for various conditions, ranging from gingivitis and periodontitis to systemic inflammation. This study showed that the dental treatment should be planned one week before or after the menstruation during the reproductive age in females ,anti-inflammatory effect will be less and the prognosis of the dental treatment will be better.

The present study is in accordance with the study conducted by Aruna Bhatia, Harmandeep Kaur Sekhon, and Gurpreet Kaur which showed that ovarian hormones of reproductive system are regulate the immune system due to the presence of hormone receptors on immune cells[9].The studies conducted by Barbara J. Bain, J. M. England et al[10] and **S.** M. COCKLE et al[11] has showen that neutrophil phenotype varies minimally during the menstrual cycle. In the present study increase in the neutrophil count was observed during the ovulation. The limitation of the present study is that the follow up was only for one cycle,the follow up of more cycles would give more clarity. *Conclusion* : From the present study we can conclude that the neutrophil count tend to be at the peak level in the ovulation phase than on other phases of the menstrual cycle, similar to the fluctuating ovarian hormones during the various phases of menstrual cycle.

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