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INFLUENCE OF FOLLICULAR AND LUTEAL PHASE ON LEVOFLOXACIN PHARMACOKINETICS IN FEMALE VOLUNTEERS

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ABSTRACT

The cyclic changes of menstrual cycle alter the pharmacokinetics of levofloxacin. The effect of follicular and luteal phase on levofloxacin pharmacokinetics was studied in 15 healthy female volunteers taking a single dose of 500mg tablet on day 10th (follicular phase) and day 21 (luteal phase). Levofloxacin levels in saliva samples at different time points were estimated by HPLC. Follicular phase has high levels of estrogen which has an inhibitory effect on drug metabolism by inhibiting liver microsomal enzymes, where as luteal phase has high levels of progesterone which induces drug metabolism. All results were expressed as Mean±SD, data was analysed using paired t-test followed by Newman Keuls multiple comparison test. A value less than $p<0.0001$ was considered as statistically significant. The mean salivary concentrations of levofloxacin were decreased in luteal phase compared to follicular due to which bacteria may develop resistance in several infections and diseases may not be cured easily during this phase and may require dosage adjustments.

KEY WORDS : Levofloxacin Pharmacokinetics, Menstrual Cycle, Indian Females

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INTRODUCTION

Levofloxacin (LVFX) is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive, Gram-negative bacteria and atypical respiratory pathogens [1]. It is more active against penicillin-susceptible, penicillin-resistant, streptococcus pneumoniae compared to ofloxacin. Monitoring levofloxacin concentrations in body fluids is valuable to adjust the drug dosage to achieve minimum inhibitory concentration and protect from side effects [2]. The menstrual cycle is often divided into two phases, the follicular phase and the luteal phase. The follicular phase spans from the first day of menstrual cycle until the day of ovulation, while the luteal phase starts at ovulation and ends the day before the next menstrual cycle. Ovarian hormones alter physiological functions and there by modifies the pharmacokinetics of drugs which inturn modulate pharmacodynamics [3]. The level of female hormones is phase specific and the pharmacokinetic parameters of drugs are altered by the cyclic changes in menstrual cycle i.e., luteal phase has high levels of progesterone which relaxes gastrointestinal smooth muscle, alters gastrointestinal transit time and drug absorption. It also has enzyme induction capacity. Follicular phase has higher levels of estrogen which inhibit drug metabolism by inhibiting liver microsomal enzymes [4]. Antibiotics are second most commonly prescribed drugs indicating the incidence of microbial infection. Drug concentration modulation below or above minimum effective concentration and maximum tolerable concentration leads to resistance to antibiotics, therapeutic failure and antibiotic induced adverse effects [5]

Antibiotics can affect estrogen and progesterone metabolism in 2 ways. Most antibiotics are metabolized in the liver and their presence can affect the rate of metabolism of estrogen and progesterone [6]. This can change the supply of estrogen and progesterone availability in blood this can cause irregularity of the menstrual cycle. Thus by changing the levels of estrogen and progesterone in the blood the cycle will get disturbed because the pituitary gland secretes it's hormones depending on the level of estrogen in the blood. These changes in menstrual cycle alter the major pharmacokinetics of various drugs [7]. Saliva is used for the monitoring of systemic levels of drugs, as it offers distinctive advantages over serum. It is a readily available specimen, which is collected by non-invasive procedures [8] , helpful when multiple serial samples are needed, the concentration of most drugs in saliva corresponds to the free or unbound plasma drug concentrations [9]

MATERIALS AND METHODS

Chemicals and Instrumental conditions

Levofloxacin (standard) and paracetamol (internal standard) of HPLC grade were purchased from NEQ pharmacopeia, Hyderabad. Methanol, Water, Acetonitrile of HPLC grade were purchased from E-MERCK, Mumbai. LEON tablets were purchased from local chemist. The HPLC system consists of WATERS2487Dual absorbance detector, The mobile phase for levofloxacin estimation in saliva consists of 75:10:15 of water, acetonitrile, methanol and 50µl of acetonitrile which is mixed thoroughly and sonificated for 30 minutes and used for HPLC analysis. Flow rate was set to 1.0 ml/min, the eluent was monitored using a UV-VIS detector set at 285nm. Volume of 10µl was injected into kromasil C_{18} column.

Standard solutions

Primary stock solutions of levofloxacin 1mg/ml and paracetamol 1mg/ml were prepared in methanol. levofloxacin stock solution was further diluted with methanol to get a working standard solutions ranging from 0.5,1,2,5,10,20 and 50µg/ml.

Saliva Sample extraction

To 0.2 ml human saliva containing Levofloxacin in a glass screw-capped tube 20µl of paracetamol(1mg/ml) is added. To this 0.4ml of methanol is added to precipitate proteins. The tubes were vertexed for 30 seconds and centrifuged for 10minutes at 8000rpm, later the supernatant is collected and evaporated. The dried eppendroff tube is

reconstituted with 0.2ml of mobile phase and vortex for 30seconds from this 10µl of volume is injected in to column.

Standard graph preparation

To 20µl of each of 0.5,1,2,5,10,20 and 50µg/ml of working standard concentrations add 0.2ml of blank saliva, 20µl of 1mg/ml of internal standard(paracetamol) and 0.4ml of methanol.Then vortex for 1minute and centrifuge at 8000 rpm for 10minutes later collect supernatant and evaporate to dryness and reconstitute with 0.2ml of mobile phase. Inject 10µl of this sample in to column. The retention time of levofloxacin and paracetamol were 5.3 and 4.4 respectively. The peak area ratios obtained at different concentrations of the drug were plotted against the concentrations of the drug [17]. The slope of this plot was determined by least squares regression analysis and was used to calculate levofloxacin concentrations in unknown saliva samples. Then various pharmacokinetic parameters of levofloxacin were obtained using KINETICA software.

Table 1

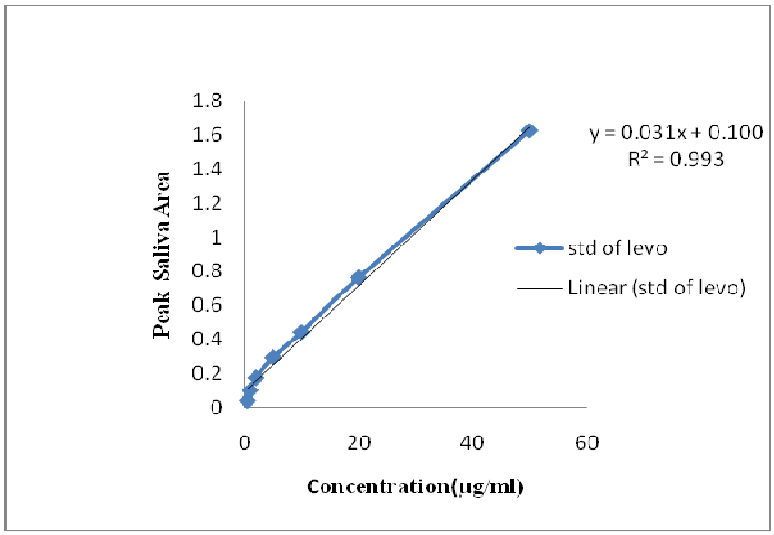
Table showing drug concentrations and peak areas

Conc of levofloxacin(µg/ml)	Conc of IS*(µg/ml)	Peak Area
0.5	1000	0.042
1	1000	0.1021
2	1000	0.1764
5	1000	0.296
10	1000	0.443
20	1000	0.761
50	1000	1.623

IS* expressed as Internal Standard

Graph 1

Standard Graph of Levofloxacin



RESULTS

The results are summerised in table 2 and table 3. Significant differences were observed during day 10 and day 21. The mean peak salivary concentrations of levofloxacin during follicular phase and luteal phase were 128.1±14.1 and 76.8±7.8 µg/ml shown in graph 2. Mean AUC total values of levofloxacin during follicular and luteal phase were 1239±315.14 and 255.1±78.47. Mean t ½ during follicular and luteal phase were

9.93±3.5 and 4.59±2.1. Mean MRT values of levofloxacin during follicular and luteal phase were 14.4±4.5 and 5.6±1.7. Mean CL values were 6.83±1.6 and 15.7±3.71 respectively during follicular and luteal phase. Mean Vd values during follicular and luteal phase were 104±33.8 and 150.2±29.13.

Table 2
Statistical significance of various pharmacokinetic Parameters during follicular and luteal phase.

Pharmacokinetic parameters	Follicular phase (Mean±SD)	Luteal phase (Mean±SD)	Statistical significance	p-value
C_{max}(µg/ml)	128.4±14.1 Reference	76.8±7.6 ↓59.8%	L vs F<0.0001***	
T_{max}(hrs)	1±0	1±0	NS	
$AUC_{0-\infty}$(µg/ml/hr²	1239±315 Reference	255.1±78.4 ↓20.58%	L vs F<0.0001***	
$t_{½(hrs)}$	9.93±3.5 Reference	4.59±2.1 ↓46.22%	L vs F<0.01**	0.0025
V_d(ml/kg)	104±33.8 Reference	150±29.13 ↑144.2%	F vs L<0.01**	0.0092
MRT(hr)	14.4±4.5 Reference	5.64±1.76 ↓39.16%	Lvs F<0.0001***	0.0002
CL(ml/hr/kg)	6.8±1.6 Reference	18.7±3.7 ↑220.12%	F vs L<0.0001***	

F expressed as follicular phase, L expressed as luteal phase

Table 3
shows percentage variations in pharmacokinetic parameters L expressed as luteal phase, F expressed as follicular phase.

Pharmacokinetic parameters	Follicular phase (Mean±SD)	Luteal phase (Mean±SD)	Statistical significance	p-value
C_{max}(µg/ml)	128.4±14.1 Reference	76.8±7.6 ↓59.8%	L vs F<0.0001***	
T_{max}(hrs)	1±0	1±0	NS	
$AUC_{0-\infty}$(µg/ml/hr²	1239±315 Reference	255.1±78.4 ↓20.58%	L vs F<0.0001***	
$t_{½(hrs)}$	9.93±3.5 Reference	4.59±2.1 ↓46.22%	L vs F<0.01**	0.0025
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CL(ml/hr/kg)	6.8±1.6 Reference	18.7±3.7 ↑220.12%	F vs L<0.0001***	

Values are expressed as Mean ± SD,**P<0.01 and ***P<0.0001 is considered as statistically significant Abbr: AUC - Area under the curve; Vd/f - Apparent volume of distribution for fraction of drug absorbed; MRT – Mean residence time ; CL- clearance; Tmax time of peak concentration; Cmax-peak drug concentration; T half-biological half life for elimination.

Statistical analysis

All the results were expressed as mean±S.D, data was analysed using paired t-test, followed by Newman keuls test. T half of levofloxacin on day 21 was significantly smaller than T half of levofloxacin on day 10 (p<0.01), volume of distribution of levofloxacin on day 10 was significantly smaller than day 21 levofloxacin Vd(p<0.01). Peak plasma concentration, Area under curve total (0-∞), Mean Residence time of levofloxacin on day 21 were significantly smaller than day 10

levofloxacin parameters(p<0.0001). Clearance of levofloxacin on day 10 is significantly smaller than day 21 levofloxacin Clearance (p<0.0001). T max has no significant differences between day 10 and day 21. Mean Cmax value, AUC total, t half, MRT was decreased by 59.8%, 20.5%, 46.22% and 39.16% in luteal phase. Volume of distribution and clearance was increased by 144.2% and 220% respectively in luteal phase.

FIGURE 1
standard chromatogram of levofloxacin

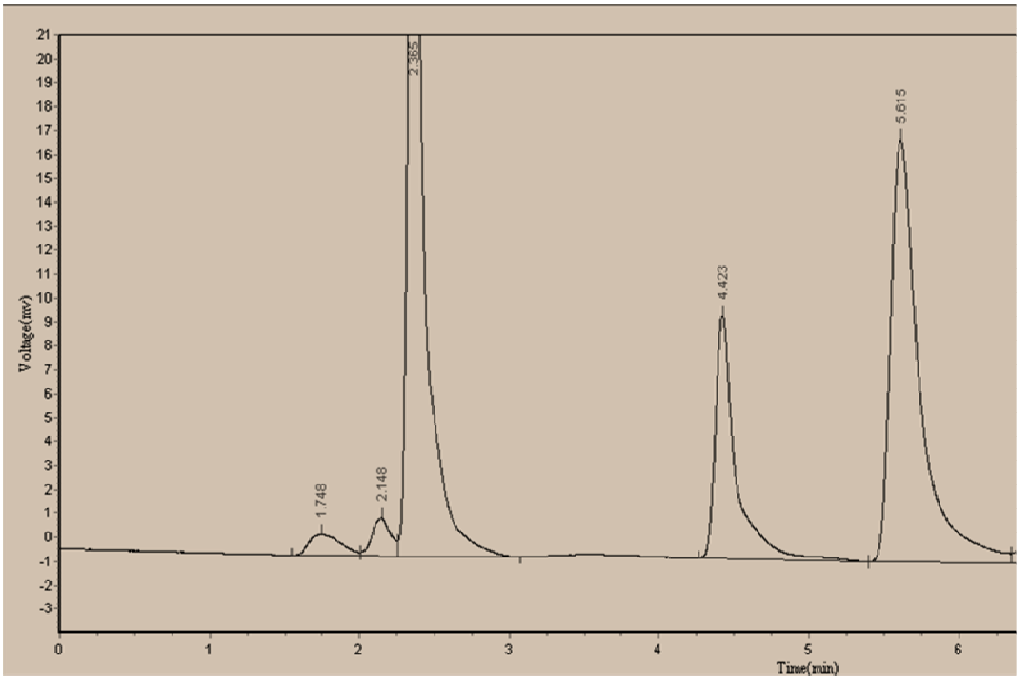


FIGURE 2
Test chromatogram of levofloxacin during follicular phase at 1st hour showing paracetamol RT 4.3 and levofloxacin RT 5.1

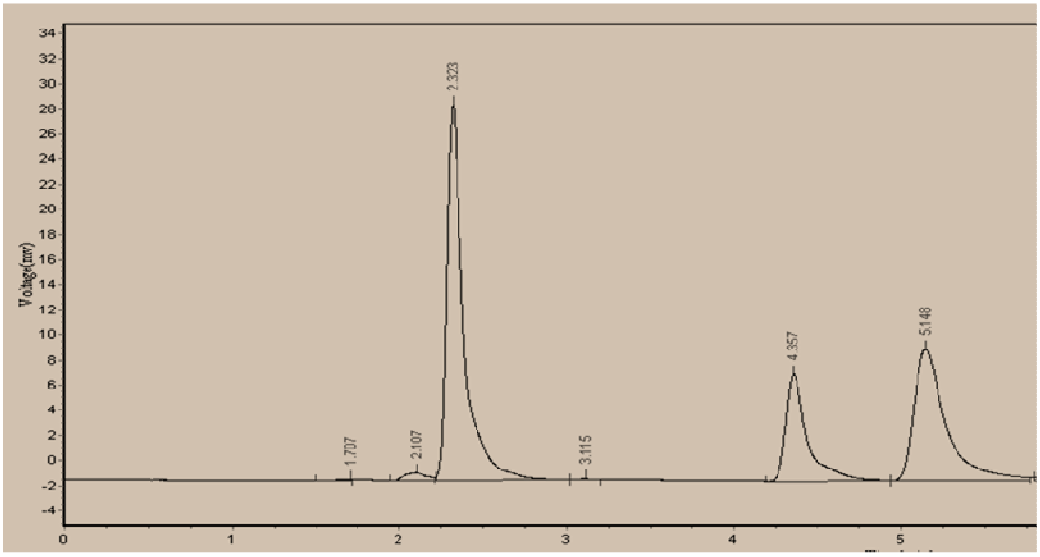
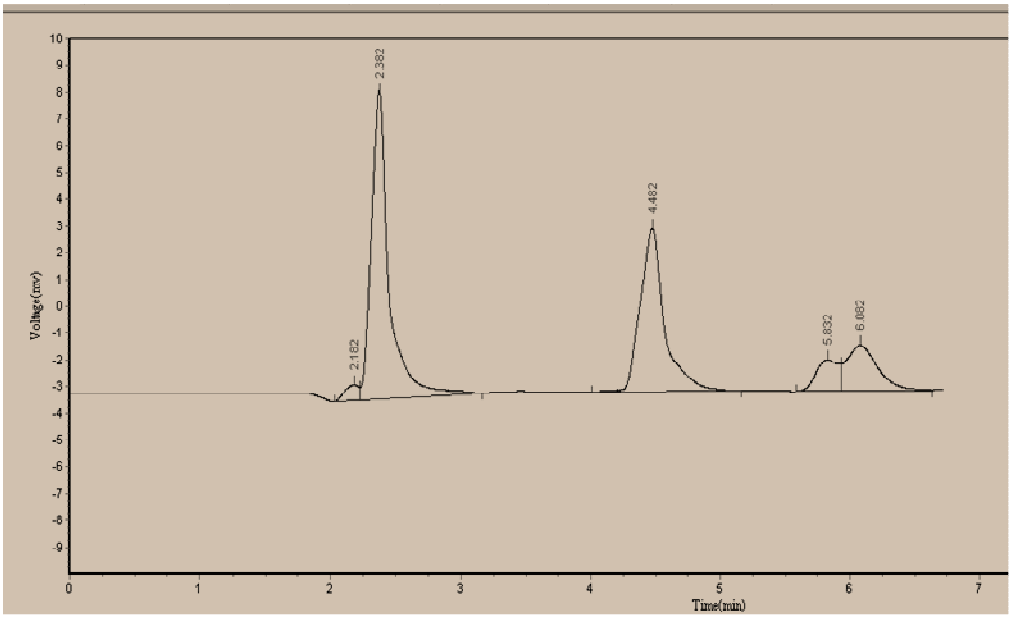
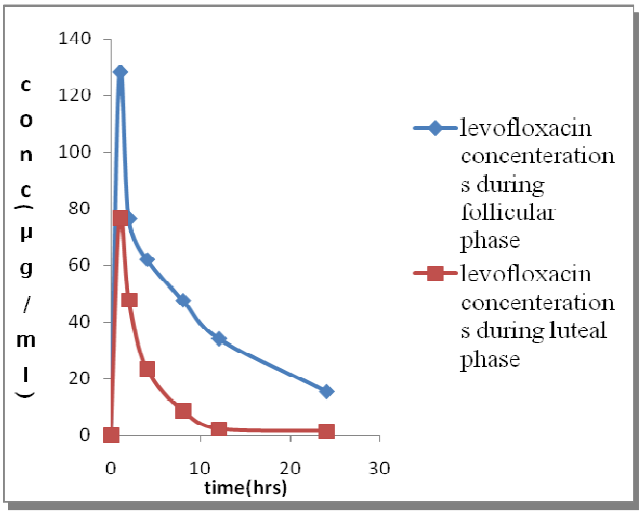


FIGURE 3
Test chromatogram of levofloxacin during luteal phase at 1st hour. Showing paracetamol RT 4.4 and levofloxacin RT 6.0



Graph 2
shows the concentrations of levofloxacin in saliva during follicular and luteal phases



DISCUSSION

Variations in Hormonal levels and consequent changes in the menstrual cycle modifies the pharmacokinetics of drugs either through difference in gastric acidity, gastro intestinal motility and plasma protein levels which alter drug absorption, drug-binding and elimination.

Mean saliva levofloxacin concentrations were high during follicular phase(10th day) due to decreased clearance of levofloxacin. However mean saliva levofloxacin concentrations were decreased in luteal phase due to progesterone enzyme induction on CYP3A4. Blackham and Spencer reported that estrogens inhibit and progesterone induce drug metabolism. The circulating levels of a number of hormones including estradiol, luteinizing hormone, follicle stimulating hormone and prolactin, change

dramatically around the time of ovulation and this could affect the hepatic metabolism of drugs in women [10] Volume of distribution of levofloxacin in follicular phase was decreased than luteal phase might be due to high protein binding in the present study and may be due to as progesterone retains body water due to increased synthesis of aldosterone [11] The mean AUC and elimination half life of Antipyrine was significantly smaller and mean clearance was significantly greater on 15th and 21st day when compared to 5th day of menstrual cycle [12] . In our study significant, similar results were found with levofloxacin i.e., mean $AUC_{0-\infty}$ and half-life were smaller, mean clearance was greater in luteal phases when compared to follicular phase and this might be due to progesterone inducing capacity on enzyme metabolism. [13.]

The bioequivalence studies of Williams et al., indicate an increased bioavailability reflecting an increased $AUC_{0-\infty}$ value. In fact the mid-cycle $AUC_{0-\infty}$ value of Methaqualone was smaller than that obtained at the beginning of the cycle indicated the predominant pharmacokinetic change at this stage of the menstrual cycle, increased rate of metabolite formation and hence clearance decreased [14]. Similar $AUC_{0-\infty}$ value was obtained with Levofloxacin . Bruguerolle and coworkers examined the pharmacokinetics of Theophylline in asthmatics on 0th, 10th and 20th days of the menstrual cycle and found that the maximum plasma drug concentration, minimum mean resident time and elimination half life occurred at follicular phase [15] . In our present study results were correlated positively with the earlier study results and might be due to estrogen enzyme inhibition capacity. Kellermann in a single subject with a normal menstrual cycle receiving methaquolone observed that the plasma half-life of methaquolone was high on day 10 of the menstrual cycle and declined to a minimum on day 21 . Large fluctuations in hormone concentrations throughout the menstrual cycle potentially impact hepatic enzyme activity and affect the metabolism of drugs. Progesterone has been shown to both inhibit and induce hepatic enzyme activity . In our study, the increased clearance of levofloxacin in the luteal phase is probably due to progesterone's induced hepatic enzyme activity. This value did attained significance due to large inter individual variability. Estrogen decreases oxidative drug metabolism through inhibition of certain cytochrome P450 enzymes and inhibits the hepatic clearance of Imipramine . In our study, the decreased clearance of levofloxacin significant in the follicular phase may be due to peak estrogen levels in follicular phase and its inhibition on hepatic clearance of levofloxacin. Comparison of data obtained in follicular phase and luteal phase revealed difference in most pharmacokinetic parameters, indicative of the characteristic physiological changes associated with the luteal phase that largely affect the kinetics and availability of Ranitidine [16.]

CONCLUSION

Significant mean salivary levels of Levofloxacin were lower in luteal phase compared to follicular phase, due to which bacteria may develop resistance in several infections. In these phases of menstrual cycle diseases may not be cured and may require dosage adjustments

CONFLICT OF INTERSET

Conflict of interest declared none.

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