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PHARMGEONETIC EFFECT ON PLASMA CONCENTRATION OF TAMOXIFEN THROUGH HPLC IN FEMALE SUBJECTS OF PAKISTAN

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ABSTRACT

The pharmgeonetic and pharmenzymonetic factors alter plasma concentration of a drug achieved after the same dosage regimen in various individuals. Breast cancer being second most killing disease holds tamoxifen as a treatment of choice for years for woman. Its plasma concentration determines therapeutic output for that eight healthy female volunteers were selected from Pakistan region and single dose 20mg of Tamoxifen was given. After collection of blood samples at known intervals, plasma concentration was determined by high performance liquid chromatography. The maximum plasma concentration was 32.54±0.44 ng/mL at time 6 hours after administration. Mean ± SE initial tamoxifen concentration was 4.8 ± 0.41 ng/mL and at 24 hours it was recorded as 7.4 ± 2.06 ng/mL respectively. The concentration obtained was lesser than literature values proving geographical region dependent decrease altering therapeutic outcome and therapeutic drug monitoring is essential for breast cancer patients in short and for every ailment in long term.

KEYWORDS: Tamoxifen Plasma Concentration, HPLC for Tamoxifen, Breast Cancer Medicine, Pharmenzymonetics, Pharmgeonetics.

INTRODUCTION

Plasma concentration of Tamoxifen is studied widely in other countries except Pakistan. After the understanding of the concept of pharmenzymonetics and pharmgeonetics (Shahbaz, 2016) the need of inculcating effect of enzymes and environment on the medicine emerged. Such changes in the concentration of drug achieved among the individuals of separate origins determine success of therapy. Our health system is negligent of the advanced treatment guidelines proposed by authorized bodies like WHO (Shahbaz *et al*., 2015), due to which an individual patient suffers. That is the reason chemotherapy is a question mark for the best treatment of breast cancer (Shahbaz *et al.*, 2014) where one out of every 8 woman has this mortality causing ailment (Breast Cancer, 2013). Tamoxifen is the anti-estrogen chemotherapy of first line in post and pre menopausal breast cancer woman used for more than ten years after diagnosis of the disease. Its plasma concentration is not determined before in Pakistan hence data from foreign countries was used as standard which is unsatisfactory. Highest level of deaths has been reported due to breast cancer (Jemal *et al*, 2011). It is without a doubt to research those regions which causes most noteworthy human passings as human life and its quality is the core interest (Shahbaz *et al*., 2016).

It tamoxifen to estrogen receptors aggressively in tumor cells and other tissue targets, in this way creating an nuclear complex that abatements DNA generation and hinders estrogen activity. It is not a steroidal element with intense antiestrogenic properties which rival estrogen binding destinations in bosom and metabolized in liver by Cyp 2D6, rendering dynamic metabolites of tamoxifen incorporates N-desmethyl Tamoxifen, endoxifen and 4 hydroxy Tamoxifen (Fuchs et al., 1996). It ties aggressively to estrogen receptor in such an in place lock, to the point that no space stays for estrogen, thus tumor development diminishes. Tamoxifen use comprises of ER positive breast cancer, bipolar disorder (Yildiz *et al*., 2008), infertility (Steiner *et al*., 2005) and gynaecomastia therefore the data about its concentration in the plasma of healthy Pakistani woman helps for the treatment output of all diseses for which this medicine is used. It is FDA approved medicine for breast cancer and recurrence after the removal of memory glands (FDA 2007).

Pakistan imports drugs from foreign countries where environment varies considerably and the effect of medicine changes in pakistani individuals from leaflet values (Javed *et al*., 2006). Such effect is also explained by the concept of pharmageonetics discussing mainly the epigenetic factors (Shahbaz. 2016) in its consideration thus altering therapeutic results. The

present study was designed on eight healthy subjects namely KA, KB, KC, KD, KE, KF, KG and KH respectively. The plasma concentration achieved after single oral dose was determined through HPLC method.

MATERIAL AND METHOD

For the study of Pharmgeonetic effect on plasma concentration of Tamoxifen eight female subjects were considered. These subjects were selected from nearby residential area of Faisalabad, Pakistan.

Selection Criteria

Subjects of age group 35-65 years were selected after physical examination and clinical history to be declared as healthy**.** All subjects were informed about the objective of study, frequency of sampling and possible side effects of drug and written consent with each subject was made.

Drug/ Chemicals

Tamoxifen, 20 mg tablet from ICI Pvt. Ltd., Lahore, Pakistan was taken.

The following chemicals used in the entire study were of HPLC grade:

- Ammonium acetate(Merck, Germany)
- Acetonitrile (Fischer Scientific Limited, UK)
- Methanol (Fischer Scientific Limited, UK)
- Deionized water

A single dose of Tamoxifen 20 mg tablet of Nolvadex brand was given orally to each subject after breakfast. In all experiments, a blood blank sample was collected before drug administration. Blood sample of 5ml each was collected from the cubital vein of each volunteer either directly with the help of a disposable syringe or through I.V cannula of 20 gauge needle at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24 hours after oral dose. The pH of fresh sample of blood was noted in each experiment by a pH meter (Beckman HS, Germany) with a glass electrode at 37^0C. Collected blood was centrifuged and plasma was separated and stored at -$20C^0$.

Drug Analysis

Tamoxifen concentration in plasma samples was determined using HPLC (Sykam, S-3210) analytical method using UV/Vis detector (Sykam, S-3210) (Kashtiaray *et al. 2011*).

Statistical analysis

The mean value and standard error of mean ±SE for each concentration was calculated. Plasma concentration versus time data was subjected.

Stock solution was prepared by dissolving 1 mg of the reference standard in 1.0 ml of HPLC-grade methanol and mixture was diluted to 10 ml with distilled water. This stock solution was refrigerated at $3C^0$ for up to one week. Further 1.0mg per ml of the stock solution was diluted with distilled water to prepare an additional standard that was 100 nanogram per ml.

Calibration standard for the plasma assay was prepared by adding 100 microliter of the 100 nanogram per ml Tamoxifenstock solution to appropriate volume of drug-free plasma. Plasma Tamoxifen calibration standard curve was prepared at concentration of 0.5, 1, 2 and 4 Nanogram per ml.

The mobile phase was prepared fresh on the day of analysis by combining 75/25 v/v of acetonitrile and ammonium acetate (0.05M). For the adjustment of pH ammonium acetate was used and was filtered and degased by vacuum before use. A 200µl aliquot of the plasma standard was filtered to a propylene 1.5 ml snap-Cap centrifuge tube & 20µl of the working acetonitrile was added. The tube was vortexed at high speed for 15 second and centrifuged at 12000G for 10 min. The clear supernatant was taken and 100 µl was injected for each analysis.

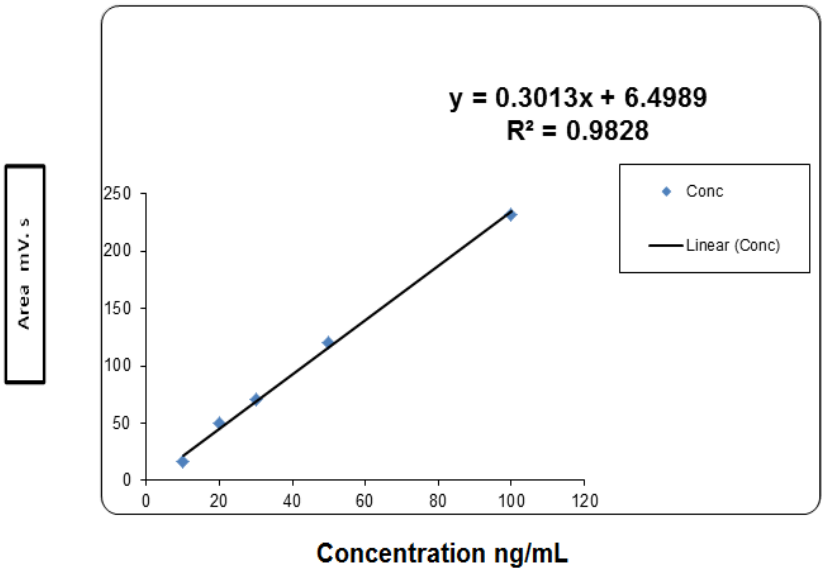

Figure 1: Standard Curve of Tamoxifen

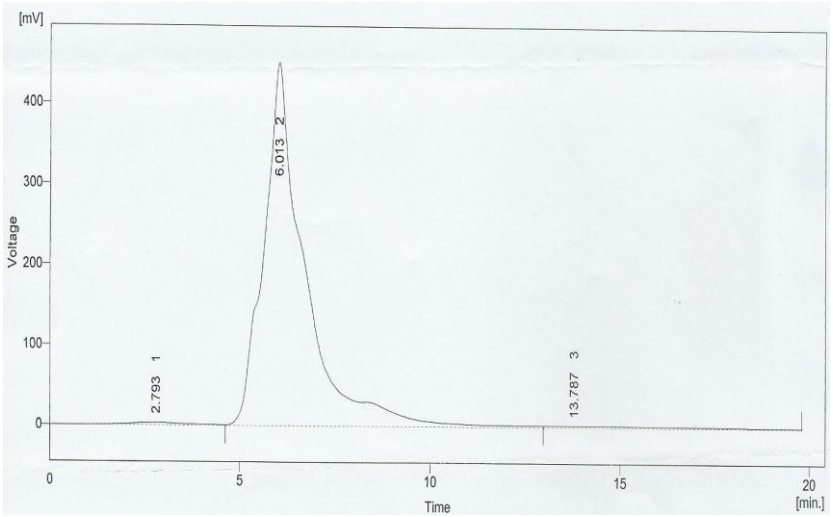

Figure 2: Chromatogram of 50 ng/ml of Tamoxifen Standard peak at right side.

RESULTS

The values of plasma concentrations of Tamoxifen in healthy adult females are determined which are far from plasma concentration of tamoxifen in other countries due to pharmgeonetic effects.

Plasma concentration of the drug

After oral administration the concentration of Tamoxifen at various time intervals for subjects has been presented in respective figure 4. Mean ± SE (ng/mL) values for these results are given in table 1. The chromatogram for various Tamoxifen concentrations at different times is also shown as figures 4 and 5 respectively.

Table 1: Mean ± SE Plasma Concentration (ng/ml) of Tamoxifen Following a Single Oral Administration 20mg In 8 Healthy Adult Female subjects

Subject No.	Time after administration (hours)										
	0.5	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**16**	**24**
1	5	7	7.4	13	21	30	31.5	26	21	17.1	7.4
2	5.1	7.1	7.6	13.5	21.6	30.5	35	26.1	20.8	9	3
3	4.9	7	7.4	13	21.1	30	31.5	26	20.8	17.8	7.5
4	5	7	7.4	13.1	21	30	31.7	26.9	20	17.9	7.7
5	3	8	7.8	13.5	21.5	30.6	32.5	26	20	17.9	8
6	5.1	7.1	7.6	13.6	21	30.9	33.2	26.1	21	17.4	9
7	5	7	7.4	13	21	30	31.5	26	21	17.5	9.2
8	5.9	7.6	7.6	13.1	21	30	33.4	26	21.84	17.4	9
Mean	4.87	7.22	7.52	13.22	21.15	30.25	32.54	26.13	20.80	16.2	6.47
±SE	0.410	0.186	0.130	0.125	0.181	0.181	0.442	0.155	0.297	0.307	0.705

The figure 3 dpicts graphical representation of plasma tamoxifen concentration in eight healthy subjects. KB reached highest level of tamoxifen in plasma and at 24 hrs concentration of tamoxifen decreased rapidly in KB. On the other hand overal plasma concentrationn in eight individuals is schematic that is lower at initial hours and reaches maximum at 5 to 6 hours and then decreases after 8 Hrs.

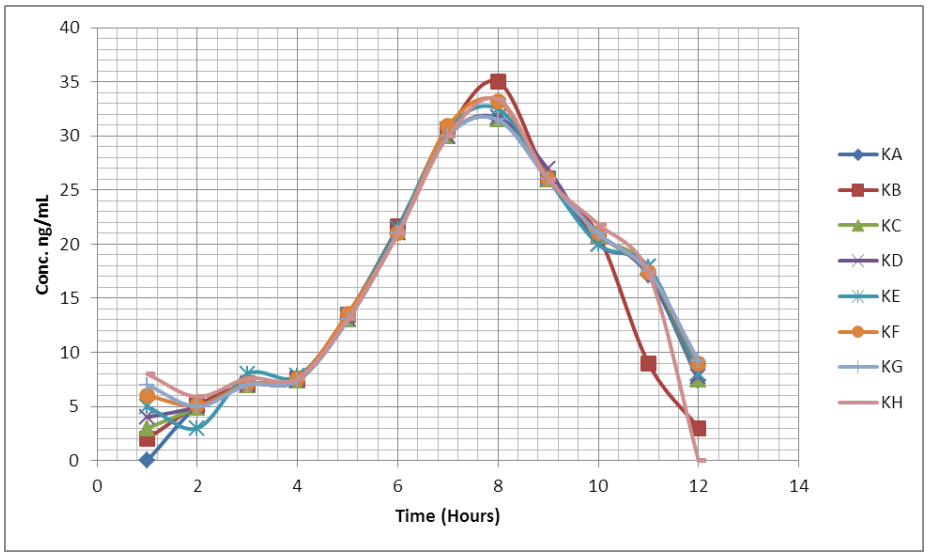

Figure 3: Plasma concentration of Tamoxifen versus time after single oral administration of Tamoxifen 20mg tablet in 8 healthy females.

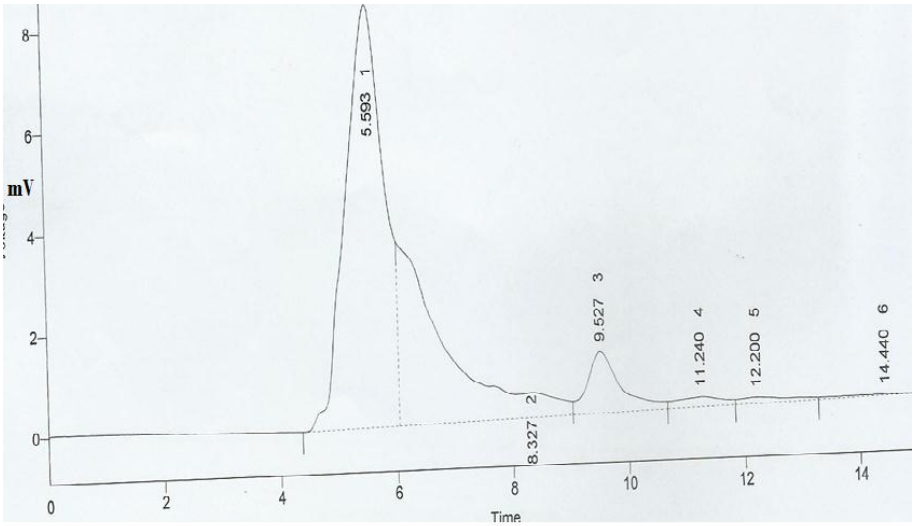

Figure 4. Chromatogram of 21 ng/ml Tamoxifen in the Plasma of Female Subjects

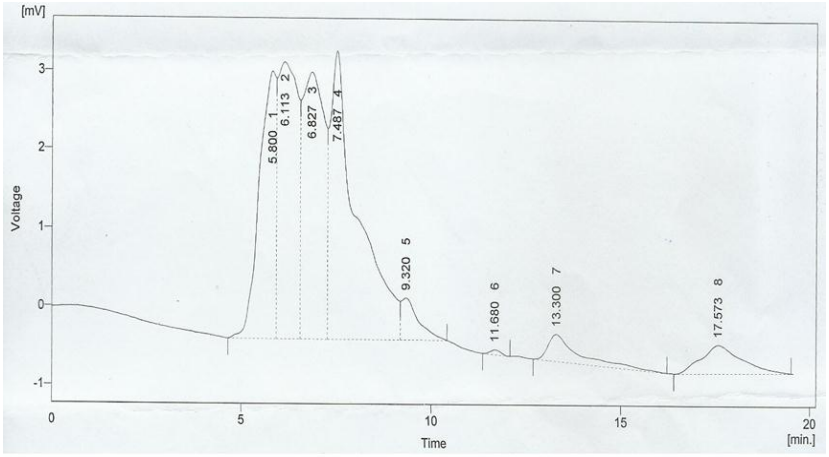

Figure 5 Chromatogram of 17.1 ng/mL tamoxifen in plasma of female subjects

DISCUSSION

Plasma concentration of Tamoxifen (Nolvadex ICI) was investigated following an oral dose of 20 mg tablet in eight healthy female subjects KA, KB,KC,KD,KE,KF,KG and KH respectively. Plasma samples were collected at different time intervals following drug administration, and analyzed for Tamoxifen concentration by HPLC method using acetonitrile and ammonium acetate 75/25 v/v as mobile phase. The mean ±SE for highest level of Tamoxifen in plasma was 32.54 ± 0.44 while in another study of different geographical region is 40ng/mL(Santana et al., 2008), 141ng/L in adults and 209ng/L in pediatrics (Mahra, 2005), 147ng/mL (Aman *et al*., 1994), 42 ng/mL (Adam *et al*., 1980), 17.8ng/mL (Kashtiaray *et al*.,2011) and 28 ng/mL (Shahbaz *et al*., 2016). The possible variation is due to environmental effects and changed physiology of individuals belonging to various regions. Also various other studies were done after many doses while in current study single dose used for one time administration, as in another study 78ng/mL of Tamoxifen found in serum after 7 days of treatment (Lien *et al.*, 1991) and serum concentration of 83.6ng/mL after 28 days 20mg Tamoxifen treatment (Kisanga *et al.*, 2004).

Thus mean ± S.E maximum concentration of given medicine in the female subjects was Cmax = 32.54 ± 0.44 ng/mL achieved at 6 hours i-e. Tmax. T_{max} in the present study found to be 6 hours may increase more than that because drug is 99% bound to proteins thus have a slower release rate (Ferner *et al*.,1990).

One of the most interesting finding of this research is lower age and lower body weight (Cheymol. 2000) shows higher level of Tamoxifen in the plasma at 6 hours shown by KB shown in table 2. Also the KB was premenopausal and may have better water intake because

the drug concentration decreased rapidly to 3ng/mL, trend not observed in other subjects. However our study do not focus on the effect of subject characteristics on Tamoxifen plasma concentration and study has a short coming that by only one subject results we cannot assume exact impact of age and body weight on the Tamoxifen metabolism. Similarly in a trial of 105 healthy women the Tamoxifen blood concentration was 230.6 ng/Ml after 20mg/day dose in two months (Decensi *et al.*, 1999) which shows that long term use of Tamoxifen exceeds the plasma drug concentration in the body thus concentration obtained after single dose may not be therapeutic. In other words concentration gathered in months of Tamoxifen use may be toxic as patients are using this medicine for up to 5 years in Pakistan. Serious individual concerned studies regarding geographical dependent changes in the effective concentration of drug should be performed.

Table 2: Subject Characteristics And Tamoxifen Maximum Concentration

Subject	Age (Years)	Dose mg	Weight (Kg)	Menopausal Status	Concomitant Disease	TAM Cmax (ng/mL)	TAM Tmax (Hrs)
KA	53	20	72	Post	Nil	31.5	6
KB	43	20	67	Pre	Nil	35	6
KC	50	20	70	Post	Nil	31.5	6
KD	51	20	71	Post	Nil	31.7	6
KE	47	20	72	Post	Nil	32.5	6
KF	52	20	69	Post	Nil	33.5	6
KG	49	20	72	Post	Nil	31.5	6
KH	54	20	68	Post	Nil	33.4	6

Cyp2D6 varies in various individuals, (Jorde *et al.*, 2004, Abraham *et al.*, 2010) is metabolizer of tamoxifen to other active metabolites and its test before start of therapy is necessary for pharmenzymonetics study. Possible change in its concentration the body due to environmental change is also under consideration that favors pharmgeonetics.

CONCLUSION

Effect of geographical region and environment on the plasma concentration of Tamoxifen is proved with lower level of Tamoxifen found in the healthy individuals of Pakistan however it suggests epigenetic effects are also one of the reason when pharmgeonetics is considered. Importantly HPLC method was slightly modified which gave exact results in the environment of Pakistan for Tamoxifen on the selected chromatogram. Thusly for required level of Tamoxifen in blood the levels of drug dose ought to be expanded. Likewise different

metabolites concentration ought to be determined as Tamoxifen is a prodrug and metabolized to dynamic components for instance endoxifen is another research territory.

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