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DISPOSITION KINETICS AND DOSAGE REGIMEN OF TAMOXIFEN IN ADULT HEALTHY FEMALE VOLUNTEERS

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ABSTRACT

Breast cancer is the second most pervasive cause of mortalities in the world and Tamoxifen is the hormone therapy of choice in pre-menopausal estrogen receptor positive breast cancer women and sometimes in post-menopausal women. The pharmgeonetics factors widely affect the pharmacokinetic parameters. Information regarding this anti breast cancer drug shows that bio disposition of Tamoxifen has not been widely studied in local healthy adult female subjects. Disposition kinectics and dosage regimen were investigated in eight healthy adult females of a specific age group 35-55 years. Blood samples were collected at various intervals after oral administration 20mg Tamoxifen tablet. Plasma concentrations were determined with HPLC. Plasma concentration versus time curve was analyzed with one compartment pharmacokinetic model to calculate the kinetic parameters such as Cmax and volume of distribution etc. The pharmacokinetic analysis revealed Cmax of 28.11 ± 2.11 ng/mL at mean Tmax of 7.7 hours.The mean ± SE volume of distribution was 306.6 ± 3.681 L/kg ,respectively while extrapolated zero time drug concentration of elimination phase 0.037 ± 0.005ng/mL. Mean ± SE rate constant for elimination phase was 0.12 ± 0.002 hr^{-1}. Due to decrease in Cmax attained after single oral dose, it is recommended that dosing interval of Tamoxifen should be decreased as to attain steady state–levels for pharmacotherapeutic results in breast cancer females. However, 5-10ng/mL change is due to the changes of

environment, epigenetic and genetic variation between Pakistan and drug manufacturing foreign countries.

KEYWORDS: Pharmacokinetics, Tamoxifen Dosage Regimen, Disposition Kinetics.

INTRODUCTION

Breast cancer is the second highly important cause of mortalities in the world. Unfortunately our health sector is far away from the standard treatment guidelines (Shahbaz et al., 2015) to cure such killing diseases. Metastatic breast cancer is more drastic as the main causative agent is not known. Although, multiple causes have been known to be involved in the metastatic breast cancer, but still chemotherapy is a question to absolutely treat this cancer (Shahbaz et al., 2014). 1.3 million Women suffer from breast cancer in United States. Similarly 1 out of every 8 women had breast cancer reported in 2014 (Breast Cancer, 2013). Not only humans but also animals become victim of breast cancer as shown by the research on cow's breast cancer at Harvard University in 2006. Among the chemotherapeutic anti-estrogens, tamoxifen is the most essential as being used for 40 years worldwide and for 20 years in Pakistan. Yet there is no appropriate data for this medicine to support its entire kinetics and dynamics in the environment of Pakistan. Contrary to the previous decade when cervical cancer was the major cause of death now breast cancer accounts for highest morbidity and mortality rate in females (Jemal et al, 2011). The precise number of mortality and number of cancer cases in Pakistan are unknown (Hanif et al., 2009). It is for sure to research those areas which causes highest human deaths as human life and its quality is the focus.

Tamoxifen is on the World Health Organization's List of Essential Medicines (WHO, 2014). Tamoxifen was introduced by EstraZeneca of UK for the first time and being frequently prescribed as hormonal therapy of estrogen positive breast cancer in the clinics of Pakistan. It is far better to use right medicine with right dose at the right stage of the disease to opt maximum curative results. The exact kinetic and dynamic mechanism within human is necessary for manufacturing drugs against any disease.

It binds to estrogen receptors competitively in tumor cells and other tissue targets, thus producing a nuclear complex that decreases DNA production and inhibits estrogen action. It is not a steroidal agent with potent antiestrogenic properties which compete with estrogen binding sites in breast and metabolized in liver by Cyp 2D6 (cytochrome P450, family

2,subfamily D, polypeptide 6), rendering active metabolites of tamoxifen includes N-desmethyl Tamoxifen, endoxifen and 4 hydroxy Tamoxifen (Fuchs et al., 1996). It binds competitively to estrogen receptor in such an intact lock that no space remains for estrogen, hence tumor growth decreases. Its half-life is 7-14 hours. A single oral tablet of 20 mg of Tamoxifen shows an average peak plasma concentration of 40 ng/mL (range 35 to 45 ng/mL) observed approximately five hours after dosing. The decline in plasma concentrations of Tamoxifen is biphasic with a terminal elimination half-life of about 5 to 7 hours (Furr et al., 1984).

Like other chronic ailment's treatment, the treatment of breast cancer can be at a better stance if there is more patient compliance and less side effects. The Most common side effects caused by Tamoxifen are nausea, hot flashes, vaginal dryness loss of sexual desire. It is notable that Tamoxifen is not an antagonist all tissues like in breast tissue. It, therefore, has agonist effect in bones and ovaries. In addition, it does not has a cardioprotective effect on heart and causes thromboembolism and fatty liver. It shows a reduction in libido and evidences of decreased cognition have been reported.

Its use comprises of ER positive breast cancer, bipolar disorder (Yildiz et al., 2008), infertility (Steiner et al., 2005) and gynaecomastia. It is approved by the FDA for preventing breast cancer in woman at highest risk (FDA 2007). Similarly, it is effective in both pre and postmenopausal women. In addition to its use as prophylaxis of breast cancer risks, it reduces the recurrence after mastectomy.

Genetic variations in Cyp-2D6 enzyme among various people have shown 20% of decrease in the pharmacological action of Tamoxifen (schroth et al. 2009). It is a prodrug and its active metabolites may only support an antiestrogen therapy as they change by Cyp-3A4 and Cyp-2D6 (Desta et al., 2004). Genotype, therefore, has an essential implication on the outcomes of patients taking Tamoxifen as breast cancer therapy. On Oct 18, 2006 the Subcommittee for Clinical Pharmacology recommended relabeling of Tamoxifen to include information about this gene in the package insert (DNA Direct, Inc. 2012-2013). There is a huge impact of genetic polymorphism on the pharmacokinetics in various people due to the change in various enzymes and proteins (Mizuno et al., 2012).

Pakistan imports raw and finished drugs for human and veterinary use. It has been reported that genetic make-up in local human & animals and environmental conditions are different

than those of their foreign counterparts (Javed et al., 2006). These differences are manifested through the variation in pharmacokinetic and bioavailability parameters suggesting that pharmacokinetic and bioavailability of these drugs should be investigated in the species and environment in which drug is going to be utilized ultimately (Javed et al., 2009a). From these studies it may be concluded that biochemical milieu interieur and physiological parameters are influenced by environmental and genetic conditions, which ultimately affect the disposition kinetic and are likely to affect response to the drugs. Several investigations in animal models have shown that biodisposition of certain drugs i.e. sulfonamides and antibiotics under indigenous conditions is different from the disposition recorded elsewhere (Nawaz et al., 1989; Javed et al., 2009b; Hussain et al., 2014). Therefore, it is necessary that an optimal dosage regimen should be based on the pharmacokinetics data determined in the local species and environment in which a drug is to be used clinically.

Information regarding this anti breast cancer drug shows that biodisposition of Tamoxifen has not been widely studied in local healthy adult female subjects. In view of the stated facts, the present project has been planned to investigate pharmacokinetics of Tamoxifen in local healthy adult female subjects. Also the eight female volunteers were observed for 4 weeks to check any sign or symptom i-e., nausea, vomiting, hot flashes etc. after single oral dose. However, this is the first study about Tamoxifen pharmacokinetics in Pakistan to benefit Breast cancer patients in our environment.

MATERIAL AND METHOD

For the study of Pharmacokinetics of Tamoxifen eight female volunteers were considered. These volunteers were selected from University or nearby residential area of Faisalabad.

Selection Criteria

Volunteers of age group 35-65 years were selected after physical examination and clinical history to be declared as healthy**.** All volunteers were informed about the objective of study, frequency of sampling and possible side effects of drug and written consent with each volunteer 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 volunteer 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). Pharmacokinetic calculations were done with the computer programme MW/PHARM version 3.02 by F. Rombout, in cooperation with University Centre for Pharmacy, Department of Pharmacology and Therapeutics, University of Gronigen & Medi/Ware, copy right 1987-1991. Based on kinetic parameters; optimal dosage regimens of Tamoxifen was calculated in healthy adult female subjects (Baggot, 1977).

Statistical analysis

The mean value and standard error of mean ±SE for each concentration and parameter was calculated. Plasma concentration versus time data was subjected to regression analysis following one compartment open model for calculations of disposition kinetic parameters. The mean ±SE was computed according to the standard statistical method and data was analyzed by least square regression/correlation analysis using Microsoft Excel version.

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 70/30 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. The Tamoxifen plasma concentrations were determined by regression equation from the standard curve of Tamoxifen, Figure 2.

The plasma concentration versus time data was plotted on semilogrithmic graph paper and analysed by one compartment open model. The pharmacokinetic parameters calculations were computed with the computer programme MW/PHRAM version 3.02 by F. Rombout, a MEDI WARE product APO pharmacological analysis in the cooperation with University Centre for Pharmacy, Department of Pharmacology and Therapeutics, University of Gronigen and Medi/Ware, copy right 1987-1991.

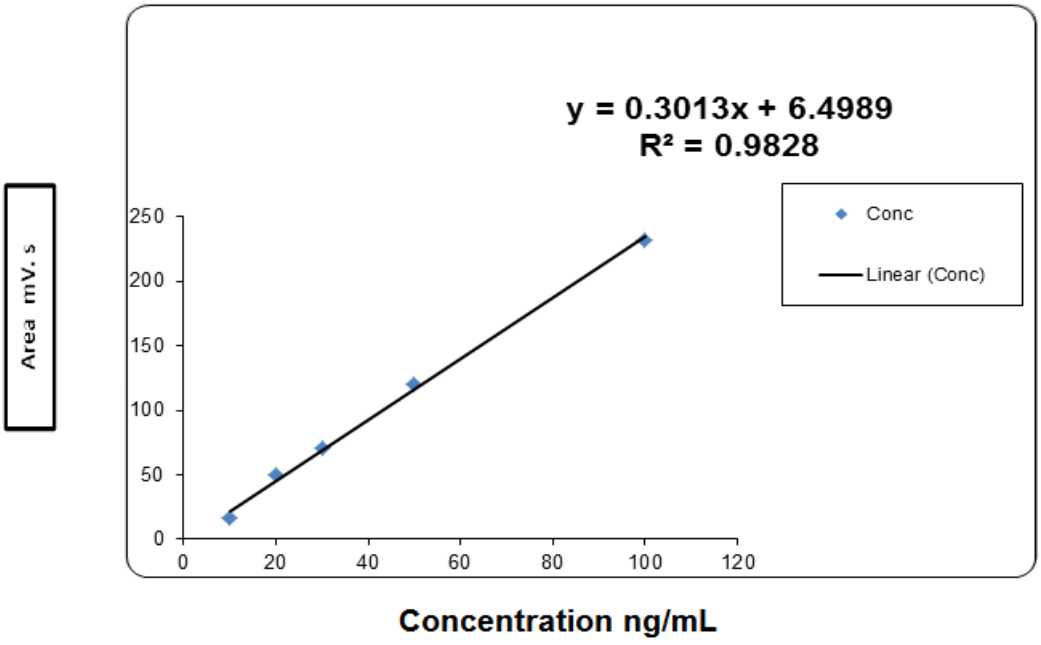

Figure 2: Standard Curve of Tamoxifen

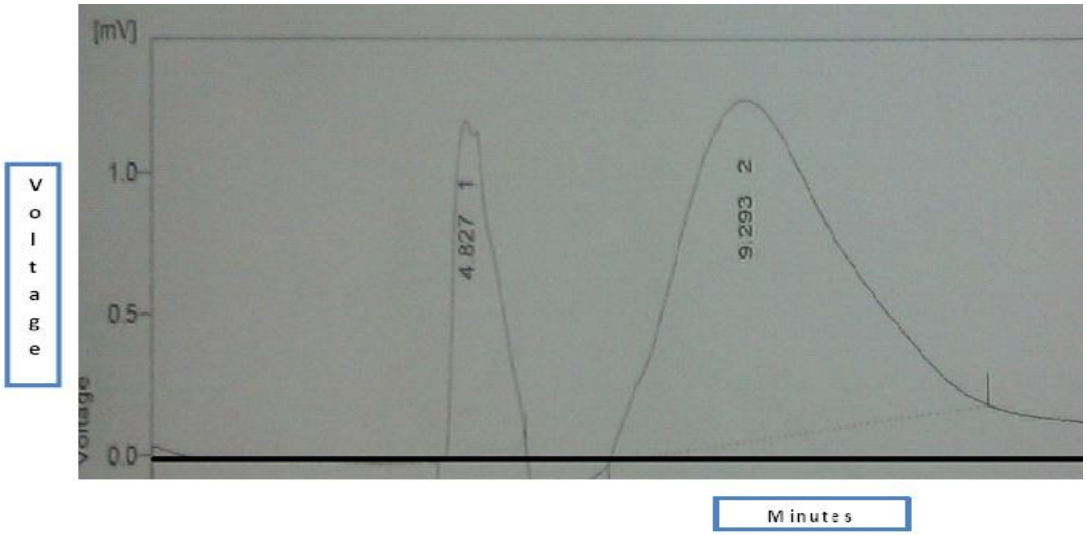

Figure 3 Chromatogram of 100 ng/ml of Tamoxifen Standard peak at right side.

RESULTS

The values of plasma concentrations, disposition kinetic parameters of Tamoxifen in healthy adult females are determined which are far from pharmacokinetic parameters of tamoxifen in other countries due to epigenetics.

Plasma concentration of the drug

After oral administration the concentration of Tamoxifen at various time intervals for volunteer has been presented in respective figure 4. Mean ± SE (ng/mL) values for these results are given in table 1.

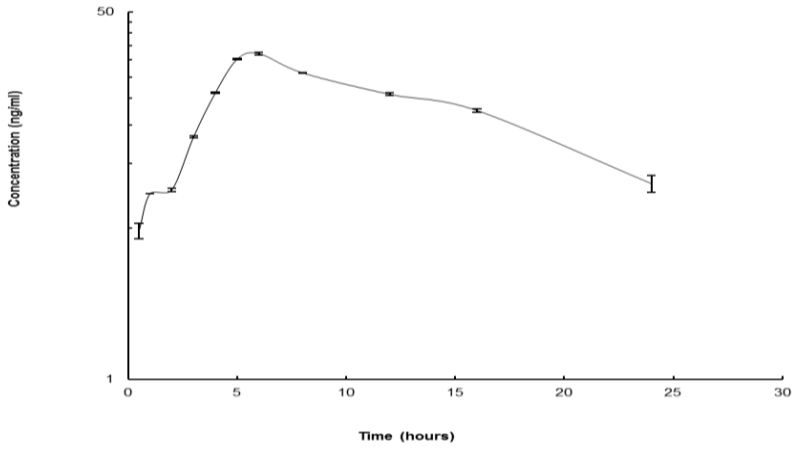

Figure: 4 Plasma concentration of Tamoxifen on a semilogarithmic scale versus time after single oral administration of Tamoxifen 20mg tablet in 8 healthy females.

Optimal dose

The optimal dosage regimens of Tamoxifen based upon the pharmacokinetic parameters were calculated in female volunteers. The calculations of priming and maintenance doses of Tamoxifen after oral administration were based upon the minimum effective concentration (MEC) of Tamoxifen in blood. The minimum effective concentrations of Tamoxifen CP (min) **20, 30, 40** ng/ml have been used in the calculations for each time interval.

The oral doses of Tamoxifen in mg/kg body weight for 8, 12 and 24 hour intervals in females have been presented in Table 2. Graphically, dose and dosing intervals of Tamoxifen in the adult female volunteers have been presented as "dosogram" in Fig 4. The doses of Tamoxifen in females at CP (min) 30ng/ml were 10.4, 15.6 and 21mg/kg for 12, 18 and 24 hour dosing intervals, respectively. The calculated doses for females at CP (min) 20ng/ml, were 4, 6 and 8 mg/kg for 12, 18and 24 hour dosing intervals.

Table 1. Oral Dosage Regimen of Tamoxifen (mg) to Achive the MEC (ng/ml) in Eight Healthy Females.

Volunteer		Dosing Interval (hours)								
		12			18			24		
		MEC (ng/ml)								
		20	30	40	20	30	40	20	30	40
Healthy Females	Dose	4	10.4	20.9	6	15.6	31.38	8	21	41

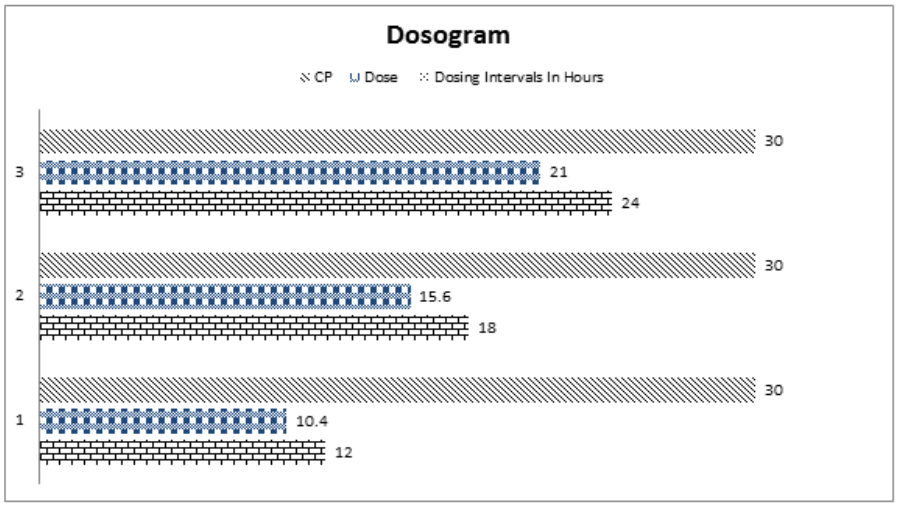

Figure 5 Dosage Regimen For Therapeutic CP^{o} Levels At Particular Dosage Intervals Determined After Single Dose of Tamoxifen 20mg tablet.

Pharmacokinetics

The log plasma drug concentration versus time profile of Tamoxifen showed a monophasic curve, so the kinetic parameters were best fitted by one compartment model method for Tamoxifen. However, a decision about two or three compartment model seems to depend on the frequency of blood sampling during the initial phase of experiments. For the current experiment data one compartment model best fits to the situation. The kinetics parameters for Tamoxifen were calculated according to the method (Baggot, 1977).

Volume of Distribution (Vd)

Mean value of volume of distribution (Vd) of Tamoxifen was 306.6 L/kg in the local female population after single oral administration of 20mg tablet which was greater than 50-60 L/kg after oral dose of 20 mg in females of breast cancer (Martindale 1989).

Species Difference

A comparison of different pharmacokinetics parameter of various drugs in different species of animals verified considerable deviation when compared with the literature values (Nawaz and Khan , 1979; Nawaz, 1980; Khan et al., 1980; Nawaz, 1982; Shah and Nawaz, 1986; Javed et al., 1984; Ghaffar et al., 1996; Muhammad, 1997 and Javed et al., 2009).Not only species but we may observe this change in various age groups. As the value obtained of various parameters in current studyare differentas compared to other species as mice, rat and even dogs.

Dosage Regimen

The most common cause of ineffectiveness of Tamoxifen treatment is the inadequate tissue concentration (Kiss et al., 1976). The inhibition of estrogen by the Tamoxifen varies not only among the species but also among the population within the same species resulting in a very wide range of CP^{o} (min) or minimum effective concentration (MEC) against the hormone.

Table: 2 Mean ± SE pharmacokinetic parameters of Tamoxifen Following Oral Administration of 20mg in 8 Adult Healthy Female Subjects By One Compartment Model

Subject No.	Cmax (ng/mL)	β (hr^{-1})	V_d (l/kg)	B -
1	39.74	0.13	311.0	0.03
2	29.55	0.13	306.6	0.03
3	23.71	0.13	311.0	0.06
4	23.83	0.13	307.9	0.03
5	23.39	0.12	314.8	0.03

6	30.23	0.13	306.3	0.03
7	24.22	0.13	311.1	0.03
8	30.22	0.13	302.4	0.03
Mean	28.11	0.12	306.6	0.037
±SE	2.811	0.002	3.681	0.005

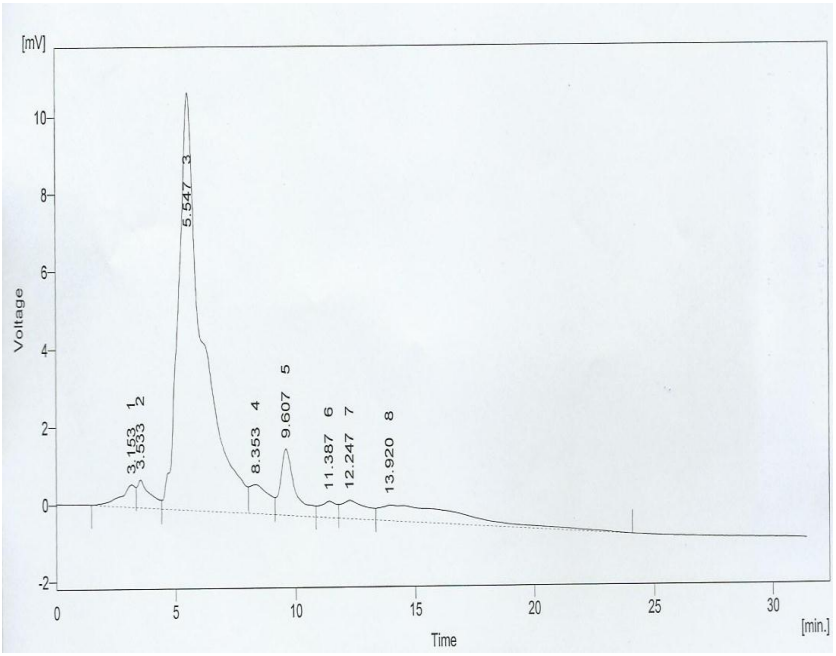

Figure: 7 Chromatogram of 30.5 ng/ml Tamoxifen in the Plasma of Female Volunteers.

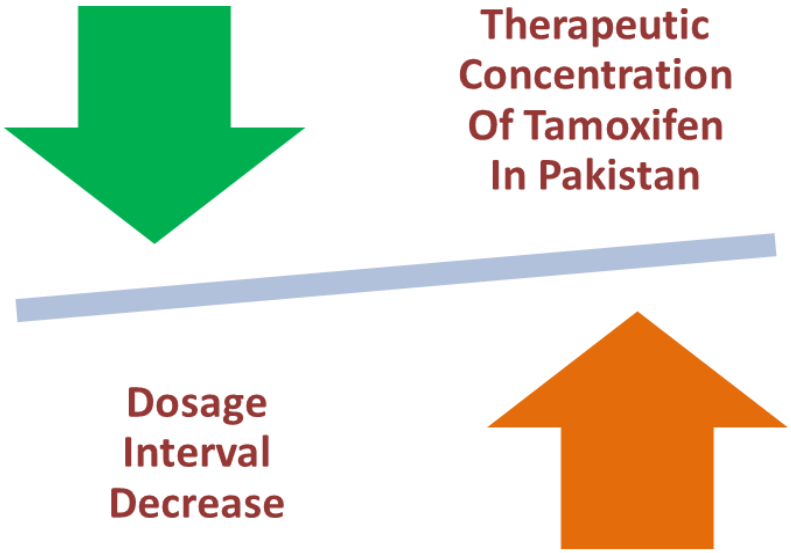

Figure: 8. Diagrammatic representation of dosage scheme for the breast cancer females patients of Pakistan under same environmental conditions to achieve therapeutic levels of Tamoxifen.

Figure: 9 Healthy female volunteers after administration of single dose of tamoxifen 20mg and providing blood samples at regular designed intervals.

DISCUSSION

Pharmacokinetics of Tamoxifen (Nolvadex) of Tamoxifen was investigated following an oral dose of 20 mg tablet in eight healthy female volunteers. Plasma samples were collected at different time intervals following drug administration, and analyzed for Tamoxifen concentration by HPLC method. The minimum effective concentration of Tamoxifen is 30 ng/ml, that is 40ng/mL in another study (Santana et al., 2008). The minimum effective concentration of the Tamoxifen in plasma was persistent during the eight hours (0.05-8.0 hours) that will assure its pharmacotherapeutic action.

C_{max} is defined as the maximum concentration of drug achieved in the plasma. The peak plasma concentration is the factor which depends on both the rate of drug absorption and extent of drug absorption in the plasma. If less time is required for the drug absorption then maximum drug concentration achieved very rapidly. The maximum concentration of single dose of Tamoxifen 20 mg after oral administration in the female volunteers with mean ± S.E was 28.11 ± 2.11ng/ml. C_{max} was achieved at 2.07 hours under the range of 31-33.4 ng/ml. However following singe oral dose of tamoxifen 20mg tablet the maximum plasma concentration achieved was 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) and 17.8ng/mL(Kashtiaray et al.,2011). T_{max} of the present study calculated with ranged 6.05- 7.7

hours with mean ± S.E 7.717 ± 0.22 hours and this time increases as its 99% protein boind (Ferner et al., 1990).

The absorption rate constant was under the ranged from 0.132-0.148 of with mean ± S.E was 0.134 ± 0.17hr^{-1}in the present study. The value of the distribution phase constant of Tamoxifen 20 mg after oral administration with mean ± S.E was 0.120 ± 0.09 hr^{-1} under the ranged of 0.04- 0.125 hr^{-1}. The extrapolated zero time drug concentration of distribution phase of present study ranged from 0.07-0.19 µg/ml with mean ± S.E was 0.14 ± 0.02 µg/ml and distribution half-life of 5.26 ± 0.05Hrs.

The overall elimination rate constant ranged from 0.132-0.135 $hr^{-1}$of with mean ± S.E was 0.129 ± 0.002hr^{-1} and Extrapolated zero time drug concentration of elimination phase (B, µg/ml) is 0.129 ± 0.002hr^{-1}. Apparent volume of distribution was (mean ± S.E) 306.6 ± 3.68 L/Kg. This Vd parameter is very important for many other kinetics parameters; if volume of distribution is altered it may responsible for the change of other parameters. Volume of distribution depends on many biological factors. Vd range is vast 201-1071 L (Mahra 2005), 50-60L/Kg in another study (Ernst et al., 1991) thus if Vd increases the half-life of a drug also increases and vice versa.

Rate constant for elimination ranged between 0.132-0.135 hr^{-1}. K_{12} is the first order transfer rate constant for distribution between the central compartment and peripheral compartment ranged between 1.29 to 1.49 Hr^{-1} and K_{21} was 0.01 ± 0.023 hr^{-1}.

Optimal dosage regimens and hence the MEC of Tamoxifen 30ng/ml has been used in the calculations. Based on the different pharmacokinetic parameters of the present study the primary and maintenance dose 21 mg have been recommended with the dosing interval of 24 hours in local population. Following this dosage regime the population of Pakistan will get the optimum therapeutic levels of 30 ng/mL in the blood. Thus our current study supports the manufacturer's recommendation to some extent as enzymatic effects are still unknown (Shahbaz. 2016). However to get more levels of tamoxifen in blood like at 40-45 ng/mL as proposed in some studies, a tablet of more milligram should be prepared accordingly to fit the dosage in our population. It is concluded that the current dosage form of tamoxifen is not so far from the standard criterion as proposed in this research however small concentration change bring drastic changes in the course of cancer treatment. Similarly a new tablet with different dosage regimen should be designed to overcome the lower blood concentration

levels in the Pakistan's population. So, a dosage interval after 18 hours will provide best therapeutic concentration to the patients. It is because level of CYP2D6 varies in the various individuals of even same geographical region. Lower the Cyp2d6 levels lower the therapeutic metabolite of tamoxifen in the body.

CONCLUSION

On the basis of results it is concluded that epigenetic factor and level of enzyme should be checked while advising its dose. However current study proposes 21mg of tamoxifen dose every 24 hours which is very close to manufacturer's recommendations whereas when the therapeutic levels of 35-40ng/mL of tamoxifen are required according to some researches, dosage interval should be decreased. High volume of distribution is due to healthy condition of the female volunteers and shows well absorption of drug in the tissues. The value of Cmax also reveals good absorption of the drug in Pakistani woman and concept of pharmgeonetics is observed (Shahbaz. 2016).

REFERENCES

1. Abraham J, M Maranian1, K E Driver1, R Platte, B Kalmyrzaev1, C Baynes, C Luccarini, M Shah1, S Ingle, D Greenberg, HM Earl, AM Dunning1, P Pharoah, C Caldas. CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant Tamoxifen. Breast Cancer Research, 2010; 12: 64.
2. Adam HK, JS Patterson, JV Kemp. Studies in the metabolism and pharmacokinetics of Tamoxifen in normal volunteers. Cancer Treat Rep., 1980; 64: 761
3. Baggot JD. Principles of drug disposition in domestic animals: The basis of veterinary Clinical Pharmacokinetics. W.B. Saunders Co., 199 Ed, Philadelphia, 1977; 72-74.
4. Carter SJ, XF Li, JR Mackey, S Modi, J Hanson and NJ Dovichi. Biomonitoring of Urinary Tamoxifen and its metabolites from breast cancer patients using non-aqueous capillary electrophoresis with electrospray mass spectrometry. Electrophoresis, 2001; 22: 2730-2706.
5. Chen JH, CC Yeun, C Daniel, TW Yi, N Ke, FC Ruey, N Orhan, SH Chiun and YS Min. Reduction of breast density using tamoxifen treatment evaluated by 3D MRI. Magon reson imaging, 2011; 29: 91-98.

6. CuzickJ, JF Forbes, I Sestak, S Cawthorn, H Hamed, K Holli and A Howell. Long-Term Results of Tamoxifen Prophylaxis for Breast Cancer 96-Month Follow-up of the Randomized IBIS-I Trial, JNCI J Natl Cancer Inst, 2007; 99: 272-282.
7. De Santana DP, MCB Rossana, S Ruth, MA Miracy, GB César and BL Leila. Reversed phase HPLC determination of Tamoxifen in dog plasma and its pharmacokinetic after a single oral dose administration. Quim Nova, 2008; 31: 47-52.
8. Dick DV, HTS Peter, S Derek, JB Ray. Serum elimination half-life of tamoxifen and its metabolites in patients with advanced breast cancer. Cancer Chemotherapy and Pharmacology, 1992; 31(1): 76-78.
9. Fuchs WS, WP Leary, MMJvan der, S Gay, K Witschital and AV Nieciecki. Pharmacokinetics and Bioavailability of Tamoxifen in Postmenopausal healthy women, 1996; 46: 418-422.
10. G. Milano, M. C. Etienne, M. Frenay, R. Khater, J. L. Formento, N. Renee, J. L. Moll, M. Francoual, M. Berto, and M. Namer. Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours. Br J Cancer, 1987; 55(5): 509–512.
11. Golander Y, LA Sternson. Paired-ion chromatographic analysis of Tamoxifen and two major metabolites in plasma. J Chromatogr, 1980; 181: 41.
12. Guelen PJM, D Stevenson, RJ Briggs, DD Vos. The bioavailability of Tamoplex (Tamoxifen): 2. A single dose cross-over study in healthy male volunteers. Methods Find Exp Clin Pharmacol, 1987; 9: 685.
13. Hanif M., Zaidi P, Kamal S, Hameed A. Institution-based Cancer Incidence in a Local Population in Pakistan: Nine Year Data Analysis. Asian Pacific Journal of Cancer Prevention, 2009; 10: 227-230.
14. Hussain T, I Javed, M Faqir and Z U Rahman. Disposition kinetics of enrofloxacine following intramuscular administration in goats. Pak Vet J., 2014; 34: 279-282.
15. Javed I, Z Iqbal, Z Rahman, MZ Khan, M Faqir, B Aslam, M A Sandhu and JI Sultan. Disposition kinetics and optimal dosage of ciprofloxacin healthy domestic ruminant species. Actavet Benro, 2009; 78: 155-162.
16. Javed I, ZU Rahman, FH Khan, F Muhammad, Z Iqbal and B Aslam. Renal clearance and urinary excretion of kanamycin in domestic ruminant species. Pak Vet J., 2006; 26: 1-8.
17. Jemal A., F Bray, E Melissa. Global Cancer Statistics. CA CANCER J CLIN, 2011; 61: 69– 90.

18. Kashtiaray K, H Farahani, Farhadi1, B Rochat and HR Sobhi. Trace Determination of Tamoxifen in Biological Fluids Using Hollow Fiber Liquid-Phase Microextraction Followed by High-Performance Liquid Chromatography-Ultraviolet Detection. Am J of Anal Chem, 2011; 2: 429-436.
19. Lien EA, Anker G, Lønning PE, Solheim E, Ueland PM. Decreased serum concentrations of Tamoxifen and its metabolites induced by aminoglutethimide. Cancer Res., 1990; 50: 5851.
20. Lien EA, E. Solheim, S. Kvinnsland, PM Ueland, Identification of 4-hydroxy-N-desmethylTamoxifen as a metabolite of Tamoxifen in human bile. Cancer Res., 1988; 48: 2304.
21. Lien EA, E Solheim, OA Lea, S Lundgren, S Kvinnsland, PM Ueland, Distribution of 4-hydroxy-N-desmethylTamoxifen and other Tamoxifen metabolites in human biological fluids during Tamoxifen treatment. Cancer Res., 1989; 49: 2175.
22. Lien EA, K Wester, PE Lønning, E Solheim, PM Ueland, Distribution of Tamoxifen and metabolites into brain tissue and brain metastases in breast cancer patients. Br J Cancer., 1991; 63: 641.
23. Nawaz M, and BH Shah. Renal clearance of endogenous creatinine and urea in sheep during summer and winter. Res Vet Sci., 1984; 36: 220-224.
24. Shahbaz K, A Mehfooz, W Khadam, MU Din, K Shahbaz, U Shahbaz. Breast Cancer Vaccination- An Envisioned Future, IAJPR, 2014; 3: 4.
25. Shahbaz, K. Comparison Between Standard Treatment Guidelines Of Preeclampsia Proposed By Who And Current Practice In Tertiary Care Centers. J. PP. Sci., 2015; 4(8): 1566-1593.
26. Shahbaz, K. Pharmenzymonetics And Pharmgeonetics: A New Door In Pharmacology. J. PP. Sci., 2016; 6(2).
27. Sirisabya N, Y Li, A Jaishuen, HG Zheng, DM Gershenson and JJ Kavanagh. Tamoxifen is safe and effective in gynecological cancer patients with renal dysfunction, Int J Gynecol Cancer., 2008; 18: 648-651.
28. http://www.breastcancer.org/symptoms/understand_bc/risk,2013.
29. Zhu YB, Q Zhang, JJ Zou, CX Yu, DW Xiao, Optimizing high-performance liquid chromatography method with fluorescence detection for quantification of tamoxifen and two metabolites in human plasma: application to a clinical study. J Pharm Biomed Anal, 2008; 46: 349-55.