A STUDY ON THE INFLUENCE OF PLASMA CONCENTRATIONS OF ADRIAMYCIN, CYCLOPHOSPHAMIDE AND PACLITAXEL ON THE CLINICAL OUTCOME OF PATIENTS WITH BREAST CANCER IN A TERTIARY CARE HOSPITAL

DISSERTATION

SUBMITTED FOR

M.D PHARMACOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE -641004

TAMILNADU

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PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that the dissertation titled, **"A STUDY ON THE INFLUENCE OF PLASMA CONCENTRATIONS OF ADRIAMYCIN, CYCLOPHOSPHAMIDE AND PACLITAXEL ON THE CLINICAL OUTCOME OF PATIENTS WITH BREAST CANCER IN A TERTIARY CARE HOSPITAL"** submitted by **Dr.M.S.YAMUNA DEVI.,** is an original work done by her during the study period of June 2015 to May 2018, under the guidance of **Dr.K.BHUVANESWARI. M.D.,** Professor and Head of the Department of Pharmacology, PSG Institute of Medical Sciences and Research, Coimbatore.

Dr.K.Bhuvaneswari M.D.,
Professor & Head,
Department of Pharmacology,
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(Guide)

Dr. S. Ramalingam MD.,
Dean,
PSG IMS&R.

DECLARATION

I solemnly declare that this dissertation titled, **"A STUDY ON THE INFLUENCE OF PLASMA CONCENTRATIONS OF ADRIAMYCIN, CYCLOPHOSPHAMIDE AND PACLITAXEL ON CLINICAL OUTCOME OF PATIENTS WITH BREAST CANCER IN A TERTIARY CARE HOSPITAL"** was done by me in the Department of Pharmacology, PSG Institute of Medical Sciences and Research, Coimbatore under the guidance of **Dr.K.BHUVANESWARI M.D.,** Professor & Head, Department of Pharmacology, PSG Institute of Medical Sciences and Research.

This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the university regulations for the degree of M.D. Pharmacology – Branch VI examinations to be held in May 2018.

Place: **DR.M.S.YAMUNA DEVI**

Date:

ACKNOWLEDGMENT

I express my sincere thanks to ***Dr.S.Ramalingam M.D.,*** *Dean, PSG Institute of Medical Sciences and Research for granting me permission to conduct this study and utilize the facilities required for the study.*

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I also wish to convey my sincere gratitude to our institution for funding my project through ***PSG PRIME category E grant.***

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*With a deep sense of gratitude I acknowledge the help rendered by **Ms.Shanmuga Priya, Ms.Dency, Ms.Kaleeswari, Ms.Vanitha, Ms.Kavitha and Ms.Chithra** Staff nurses, Medical Oncology ward, PSG IMS&R.*

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*I am extremely thankful to all the **patients** who consented to be a part of my study without whom the whole study would have been impossible. I wish them all good health and long life!*

DR.M.S.YAMUNA DEVI

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To
Dr M S Yamuna Devi
Postgraduate
Department of Pharmacology
Guides: Dr K Bhuvaneswari / Dr Vignesh Kanda Kumar
PSG IMS & R
Coimbatore

Ref: Project No.15/380

Date: December 29, 2015

Dear Dr Yamuna Devi,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 16.12.2015 to conduct the research study entitled *"A study on the influence of plasma concentrations of Adriamycin, Cyclophospamide and Paclitaxel on clinical outcome of patients with breast cancer in a tertiary care hospital"* during the IHEC meeting held on 24.12.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 16.12.2015)
3. Informed consent forms (Version 1 dated 16.12.2015)
4. Data collection tool (Version 1 dated 16.12.2015)
5. Current CVs of Principal investigator, Co-investigators
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 24.12.2015 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr. R. Nandakumar	BA., BL	Legal Expert, Chairperson	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

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Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

PSG Institute of Medical Sciences & Research
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November 29, 2016

To
Dr M S Yamuna Devi
Postgraduate
Department of Pharmacology
Guide/s: Dr K Bhuvaneswari / Dr Vignesh Kanda Kumar
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore - 4, has reviewed your proposal on 25th November 2016 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request to renew the approval for the study entitled:

"A study on the influence of plasma concentrations of Adriamycin, Cyclophospamide and Paclitaxel on clinical outcome of patients with breast cancer in a tertiary care hospital"

The following documents were received for review:

1. Request for renewal dated 24.11.2016
2. Status report

After due consideration, the Committee has decided to renew the approval for the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The approval is valid for one year (29.12.2016 to 28.12.2017).

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
COIMBATORE-641004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member – Secretary
Institutional Human Ethics Committee

Proposal No. 15/380

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Analysed Document: Influence of plasma conc of ACT on Ca Breast.docx (D31283989)
Submitted: 10/13/2017 11:29:00 AM
Submitted By: dr.yamuna14@gmail.com
Significance: 1 %

Sources included in the report:

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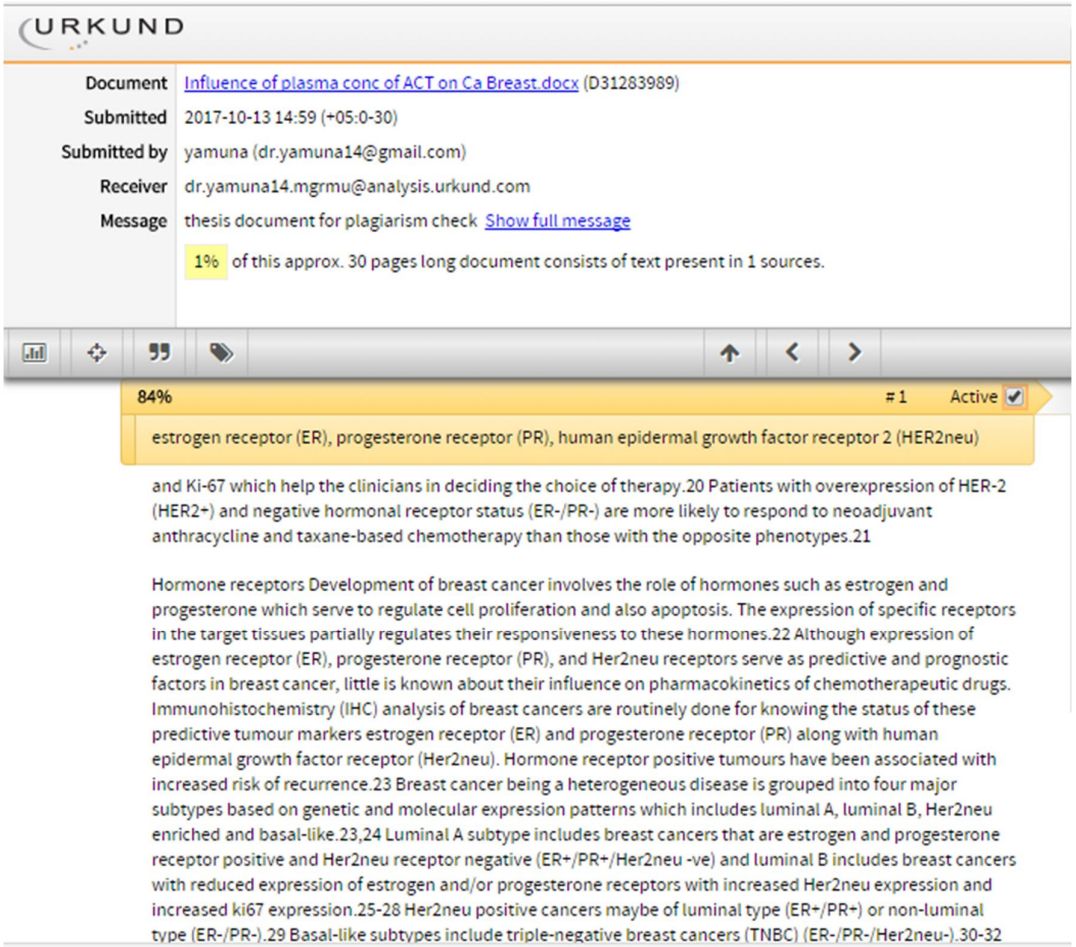
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Active
estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2neu)
and Ki-67 which help the clinicians in deciding the choice of therapy.20 Patients with overexpression of HER-2 (HER2+) and negative hormonal receptor status (ER-/PR-) are more likely to respond to neoadjuvant anthracycline and taxane-based chemotherapy than those with the opposite phenotypes.21
Hormone receptors Development of breast cancer involves the role of hormones such as estrogen and progesterone which serve to regulate cell proliferation and also apoptosis. The expression of specific receptors in the target tissues partially regulates their responsiveness to these hormones.22 Although expression of estrogen receptor (ER), progesterone receptor (PR), and Her2neu receptors serve as predictive and prognostic factors in breast cancer, little is known about their influence on pharmacokinetics of chemotherapeutic drugs. Immunohistochemistry (IHC) analysis of breast cancers are routinely done for knowing the status of these predictive tumour markers estrogen receptor (ER) and progesterone receptor (PR) along with human epidermal growth factor receptor (Her2neu). Hormone receptor positive tumours have been associated with increased risk of recurrence.23 Breast cancer being a heterogeneous disease is grouped into four major subtypes based on genetic and molecular expression patterns which includes luminal A, luminal B, Her2neu enriched and basal-like.23,24 Luminal A subtype includes breast cancers that are estrogen and progesterone receptor positive and Her2neu receptor negative (ER+/PR+/Her2neu -ve) and luminal B includes breast cancers with reduced expression of estrogen and/or progesterone receptors with increased Her2neu expression and increased ki67 expression.25-28 Her2neu positive cancers maybe of luminal type (ER+/PR+) or non-luminal type (ER-/PR-).29 Basal-like subtypes include triple-negative breast cancers (TNBC) (ER-/PR-/Her2neu-).30-32

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1. INTRODUCTION

Cancers are one of the most common causes of morbidity and mortality worldwide and the incidence has been in the raising trend in the recent years. The Global Burden of Disease (GBD) study estimates that cancer of all types resulted in 8,235,700 deaths in the year 2013.[1] Cancer has now become the second leading cause of death worldwide next only to cardiovascular diseases.[2]

Breast cancer is one among the three most common cancers in the world, which includes lung & colon cancer. It is the most common cancer in women.[3] According to the recent global statistics, it was estimated that about 1.7 million people suffered from breast cancer in 2012 and about 0.5 million deaths were reported.[4]

In Asia, the incidence of breast cancer is found to be on rise, which might be attributed to the life-style modifications, increase in screening programmes and increase in awareness among the public about the disease. However the mortality from breast cancer is also on the rising trend due to various reasons like lack of accessibility to diagnosis and treatment.[5]

In India, there has been an increase in the incidence of breast cancer in the recent years. It has become the most common cancer in women pushing cervical cancer behind which was once the most common cancer.[6] According to Globocan 2012, India along with China and United States accounts for about a third of the world breast cancer burden. There was an increase in incidence of

about 11.54% and increase in mortality of about 13.82% due to breast cancer during the year 2008 to 2012.[4,7]

Urbanisation and life-style modifications seem to have an impact on the incidence of breast cancer. Statistics from various cancer registries have shown that the incidence rates are higher in cities like Mumbai, Chennai, Bangalore, etc with crude incidence rates of 33.6% in Mumbai and 40.6% in Chennai.[8]

Recent studies have shown that the incidence of breast cancer disease peaks between the age group of 40 to 50 years in Indian women.[9] A vast majority of patients in India, present late with either a locally advanced Breast cancer disease or with metastasis which increases the mortality risk and reduces the survival rate in such cases.[8] According to various studies, about 45.7% cases present late with advanced disease.[10,11]

Obesity, increasing age, low parity, marital status, early menarche, late menopause, alcohol, smoking, tobacco chewing are strongly associated with breast cancer.[8] About 5% of breast cancers are influenced by genetic factors. Genetic mutations in BRCA 1 and BRCA 2 genes are associated with increased risk.[12] Breast feeding and increased physical activity seems to be protective against breast cancer.[13]

Breast cancer is considered curable if detected at an early stage before the evidence of distant metastasis.[3] Surgical treatment in the form of breast conservation surgery is the most important modality of treatment in early breast

cancer.[14] In situations where breast conservation is not possible because of the bulk of the tumour, neoadjuvant chemotherapy becomes an effective treatment option.[15] Based on the tumour type, both cytotoxic chemotherapy and hormonal therapy are given.[16]

In improving survival rates, both pre-operative and post-operative chemotherapy are equally effective.[17] Neoadjuvant chemotherapy offers the advantage of better operability & is mainly recommended for patients with triple negative and Her2neu positive disease. Adjuvant chemotherapy must be started within few weeks following surgery to improve the patient outcome.

Despite adverse effects, numerous chemotherapeutic regimens are being followed to modify the clinical outcome in these patients. The current treatment options for early breast cancer are anthracyclines and taxanes given either as a combination therapy or in sequence over a period of 18 to 24 weeks.[3]

There are numerous studies on individual pharmacokinetics of Adriamycin, Cyclophosphamide and Paclitaxel. Yet no single study has been done on the AC/T regimen as a whole. Since the pharmacokinetics of majority of anticancer drugs show a large inter-individual variations, this study by exploring the influence of plasma concentration of these drugs on the clinical outcome of breast cancer patients, helps in optimizing the therapy by allowing individualization of dosage, thereby reducing the adverse outcomes.

2. AIM & OBJECTIVES

2.1. Aim

- To study the influence of plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel on clinical outcome of patients with breast cancer.

2.2. Objectives

- To study the influence of plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel on the liver and renal functions of patients with breast cancer.

- To study the relationship between the plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel and the clinical reduction in tumour size of patients with breast cancer.

- To study the role of estrogen and progesterone receptors on plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel and the related adverse drug reactions in patients with breast cancer.

3. REVIEW OF LITERATURE

Breast cancer

Breast cancer being the most frequently diagnosed cancer in women has become a major cause of morbidity and mortality among those in the age group of 35 to 60 years.[18] It has also become the most important health care problem contributing substantially to the health care burden, especially in a developing country like India.

Before the advent of chemotherapy, the treatment of breast cancer depended solely on surgical intervention, which resulted in increased rate of recurrence and inoperable cases were given only palliative care. In the era of modern medicine, chemotherapy is considered as the major breakthrough.

Chemotherapy is a well-established therapeutic modality known for ages for the treatment of breast cancer. In spite of the diagnostic and therapeutic developments that have been made over the last few decades in the field of breast cancer management, it still remains a deadly disease associated with social stigma. This may be attributed to the inter-individual differences in drug responsiveness. There is an increased incidence of recurrence or metastasis seen in patients who are unresponsive to chemotherapy.[19]

Both adjuvant and neoadjuvant chemotherapy has significantly reduced the morbidity and mortality associated with breast cancer. However, chemotherapy has its own advantages and disadvantages. It is associated with an increased

incidence of adverse effects attributed to the drug as well as patient characteristics.

Inspite of the adverse effects, several different chemotherapeutic regimens have been followed universally depending on the clinic-pathological characteristics of tumour.

Biomarkers in breast cancer

Biomarkers help in predicting the responses to therapy - systemic or hormonal or radiation therapy. They are biomolecules which derived from disease related processes and are also associated with specific clinical outcomes and are amenable to detection by various methods. Several biomarkers are available for the detection of a variety of tumours and they also serve as prognostic indicators.

The role of these biomarkers in breast cancer is that, they play an important role in allowing individualized therapy for each patient. Some of the most widely used biomarkers in breast cancer management includes the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2neu) and Ki-67 which help the clinicians in deciding the choice of therapy.[20]

Patients with overexpression of HER-2 (HER2+) and negative hormonal receptor status (ER-/PR-) are more likely to respond to neoadjuvant anthracycline and taxane-based chemotherapy than those with the opposite phenotypes.[21]

Hormone receptors

Development of breast cancer involves the role of hormones such as estrogen and progesterone which serve to regulate cell proliferation and also apoptosis. The expression of specific receptors in the target tissues partially regulates their responsiveness to these hormones.[22] Although expression of estrogen receptor (ER), progesterone receptor (PR), and Her2neu receptors serve as predictive and prognostic factors in breast cancer, little is known about their influence on pharmacokinetics of chemotherapeutic drugs.

Immunohistochemistry (IHC) analysis of breast cancers are routinely done for knowing the status of these predictive tumour markers estrogen receptor (ER) and progesterone receptor (PR) along with human epidermal growth factor receptor (Her2neu). Hormone receptor positive tumours have been associated with increased risk of recurrence.[23]

Breast cancer being a heterogeneous disease is grouped into four major subtypes based on genetic and molecular expression patterns which includes luminal A, luminal B, Her2neu enriched and basal-like.[23,24] Luminal A subtype includes breast cancers that are estrogen and progesterone receptor positive and

Her2neu receptor negative (ER+/PR+/Her2neu -ve) and luminal B includes breast cancers with reduced expression of estrogen and/or progesterone receptors with increased Her2neu expression and increased ki67 expression.[25-28] Her2neu positive cancers maybe of luminal type (ER+/PR+) or non-luminal type (ER-/PR-).[29] Basal-like subtypes include triple-negative breast cancers (TNBC) (ER-/PR-/Her2neu-).[30-32]

The tumour marker of interest in the recent years is Ki67. It is used as a marker of cell proliferation as it is present only during active phases of the cell cycle.[22]

The estrogen receptor (ER) and progesterone receptor (PR) are steroid hormone receptors which is in turn a part of the larger superfamily of nuclear hormone receptors. They function as ligand gated transcription factors which modulate the gene expression. The steroid receptors that are unbound are located in the cytosol. Binding of a ligand induces receptor dimerization and brings about conformational changes which in turn cause translocation into the nucleus. After entering the nucleus, the receptor dimer recognizes and also binds specific DNA sequences which in turn results in transcription of specific genes that are regulated by the receptor.[23]

Estrogen receptor

The estrogen receptors were identified in 1950s by Dr. Elwood V. Jensen.[33,34] Later, it was found that there are three isoforms of estrogen receptors- ERα, ERβ, and GPR 30.[35,36] The three different ERs are encoded by three different

genes located on different chromosomes. The ESR1 gene which is located on chromosome 6 encodes ERα; the ESR2 gene located on chromosome 14 encodes ERβ; and the GPER gene located on chromosome 7 encodes GPR30. Only the ERα and ERβ are nuclear hormone receptors while GPR30 is a G-protein coupled receptor which binds and responds to estrogen.[37-39]

Both ERα and ERβ are expressed in the breast tissue and they bind to estrogen with equal affinities.[40] However, studies have found that only the ERα has an important role in the development of normal mammary gland.[41,42] Most importantly, the fact that ERβ receptor expression represses ERα receptor expression as well as function was also evident from large number of studies.[43,44] Both these nuclear receptors are also expressed in numerous other tissues in the human body, like the ovary, endometrium, cerebral cortex, testes, thyroid and myocardium. Although they are present in various tissues, their pattern of expression differs in different tissues.[23]

George T. Beatson in 1896 found that bilateral oophorectomy in patients with advanced breast cancer disease resulted in significant regression in tumour size, which later proved the stimulating effect of estrogen on breast cancer. Hence, removal of ovaries which are the major source of estrogen and/or the administration of drugs that target the (ER) estrogen receptor have become standard care therapies for treating breast cancers that are estrogen-responsive. The relationship between estrogen receptor (ER) and breast cancer primarily depends on one receptor in particular – the ERα. In fact, the classification of

breast cancer as estrogen receptor positive (ER+) or negative (ER-), is determined mainly based on the presence or absence of ER-α. ER-α negative breast cancer may express other hormone receptors like PR, AR, and even ER-β, however they are not estrogen-responsive. ER-α positive breast cancer disease is the most prevalent among all the breast cancer types, and it accounts for approximately about 75% of all breast cancers.[45]

Several studies have shown that ER-α positive breast tumours are of lower grade and are less aggressive and hence patients with this type of breast cancer tend have a better prognosis when compared to those who are negative for ER-α. The same can be applied to patients with metastatic tumours expressing ER-α who had significantly better survival outcomes when compared to patients with ER-α negative tumour, and this is because ER-α positive tumours responds better to the standard endocrine therapies.[46] In spite of their better clinical response to hormonal therapy they may eventually become resistant as the breast cancer disease progresses. ER-α plays an important role in the development as well as progression of the breast cancer disease and its presence is used to determine whether the breast cancer is likely to respond to hormonal treatment hence it is considered as a good prognostic marker for breast cancer.

Progesterone receptor

The expression of progesterone receptor (PR) is regulated primarily by the estrogen receptor (ERα) at the transcriptional level. Similar to estrogen receptor

there are two different isoforms of PR, the PR-A and PR-B. The two receptor proteins are transcribed from two promoters that are different but are located within the same gene on chromosome number 11[47,48] and both can form homo- or hetero-dimers. The functional importance of both the isoforms were confirmed by gene knock-out studies in mice.[49,50] Although PR-A knock-out mice did not show any significant developmental effects in the mammary glands, they displayed severe dysfunctions in their uterus and ovaries which resulted in infertility, which is suggestive of its primary function of maintaining normal uterine and ovarian functions. Conversely, mice lacking PR-B retained their normal uterine and ovarian functions but displayed a significant reduction in mammary gland ductal morphogenesis[23], suggesting that PR-B receptor has a major role in maintaining the proliferative effects in the mammary gland.

The two PR isoforms, PR-A and PR-B bind to progesterone and activate transcription differentially which is suggested by their structural and functional analyses as evidenced in various studies. Richer et al. found that, approximately about 27% of PR regulated genes are controlled by both PR-A and PR-B isoforms. This study has also shown that the expression majority of the PR-regulated genes were controlled by PR-B alone in comparison to PR-A alone (69% vs 4%).[51] This may be partly due to the fact that, PR-B isoform is an intrinsically stronger activator of transcription than PR-A isoform. In addition to that, PR-A isoform has been found to function as a repressor of transcriptional activity under certain conditions.[52] However, the isoform which

is more often over-expressed in breast cancer is PR-A and not PR-B.[53] Few studies have even showed that the ratio of the two isoforms is more important in the development of breast cancer rather than their relative amount of expression. For instance, a high ratio of PR-A/PR-B has been correlated with poorer prognosis and poor clinical response to hormone therapy in patients with breast cancer.[54]

Of all the ER-α+ breast tumours, about 50-60% are PR+.While the significance of ER status has been well known as a predictor for the prognosis and treatment of breast cancer, little has been known about the role and significance of PR in the presence of ER-α. However, tumours that express both estrogen and progesterone receptors were found to be less aggressive and are less likely to metastasize according to several studies. These kind of tumours expressing both the receptors also have a better prognosis. A study by Dunnwald et al. in breast cancer patients found that patients with ERα+/PR+ tumours had significantly lower mortality rates when compared to women with ERα-/PR+, ERα+/PR- and ERα-/PR-. The highest mortality rate was seen in patients who were negative for both the receptors. This has also been confirmed by another study done by Salmen et al. In addition to that, it has also been found that the tumours that are PR- have a more aggressive course and negative prognoses, suggesting that PR is also an important prognostic and predictive indicator of the progression of breast cancer disease.[23]

Her2 receptor

The HER2 (Human epidermal growth factor receptor 2) is an oncogene that encodes the transmembrane tyrosine kinase receptor which belongs to the epidermal growth factor receptor family. It is involved in activation of various signal transduction pathways involving cell adhesion, motility and proliferation.[55] Approximately about 18 –22% of breast cancers over-express HER2 and are associated with highly aggressive clinical behaviour that includes transition to high-grade tumors, increased growth and increased rates of tumour recurrence and death. HER2 overexpression also has a predictive value for HER2 targeted therapy in both adjuvant and metastatic settings.[20]

Overexpression of this oncogene HER2 in most of the invasive breast tumour cases has been found associated with extra copies of the HER2 gene on chromosome number 17. The cause of HER2 gene amplification that is found only in the breast cancer cells of patients is still unknown. Yet its implications in the tumour biology is clear—high density of HER2 gene on the surface of malignant tumour cells leads to the formation of homodimers and HER2 heterodimers and this results in conformational change in the transmembrane receptor, thereby activating the tyrosine kinase moiety which in turn triggers a cascade of intracellular signaling pathways which enhances cell proliferation and growth, resists apoptosis, and promotes metastasis. Anti- HER2 directed therapy prevents the activation of this pathway and thus increases the sensitivity of cells to other anti-tumour treatment.[56]

Retrospective analyses of various adjuvant chemotherapy trials identified that patients with early HER2+ cancers benefitted from regimens that contained both anthracycline and taxane. However, there was an increased risk of recurrence in HER+ patients than patients with HER2 – cancers.

The role of Her-2 in predicting clinical response to anthracycline versus non-anthracycline regimens has been assessed in various trials. However, those studies yielded inconsistent results. While some studies say that Her-2 overexpression is associated with improved efficacy of anthracycline-based adjuvant chemotherapy, the other studies have not. This has been confirmed in two recent meta-analyses which showed that patients with Her-2 overexpression had greater benefit from the anthracycline-based therapy in terms of increased disease-free survival and overall survival. But the underlying mechanism of the interaction between anthracycline-based chemotherapy and Her-2 is still unclear and Her-2 may serve as a surrogate marker.[58]

Three trials in early breast cancer in adjuvant setting and one trial in neoadjuvant setting have retrospectively assessed Her-2 as a predictive marker for clinical response to taxane-based chemotherapy, one of these in the neoadjuvant setting.[59-62] The CALGB 9344 trial which compared anthracycline-based chemotherapy alone with the addition of paclitaxel to anthracycline-based chemotherapy showed that there was a statistically significant improvement in DFS (disease free survival) and OS (overall

survival) with the addition of paclitaxel only in breast cancers overexpressing Her-2.[59] There was no benefit with addition of taxane seen in the Her-2-negative patients. Another meta-analysis of the three trials on adjuvant chemotherapy suggested that both Her-2 positive and Her-2 negative patients benefit from addition of taxane, however greater benefit was associated with Her-2 amplified patients.[63]

Hormone receptors and chemotherapy

For patients with locally advanced breast cancer neoadjuvant chemotherapy has become a standard therapy. Major advantages of neoadjuvant chemotherapy include 1) converting inoperable breast cancer to operable one by reducing the tumour bulk, 2) increasing the chances of amenability to breast conserving surgery, and 3) serving as an *in vivo* chemo-sensitivity test of the tumour. But, the loss of prognostic value indicated by the tumour size and lymph nodal status following neoadjuvant therapy is an important disadvantage associated with it.[64]

A large number of studies have investigated the role of prognostic factors in the setting of neoadjuvant chemotherapy. Currently, pathologic complete response (pCR) is a widely used independent prognostic factor and a better survival rate was associated with the breast cancer patients who achieved pCR than those with the residual tumour.[65-68] However, only a small proportion of patients achieved pCR, and a significant proportion of patients with pCR had recurrent breast cancer disease [9]. Biomarkers like estrogen receptor (ER), progesterone

receptor (PR), p53 and Ki-67 are considered as predictive or prognostic indicators in the neoadjuvant setting. However, these molecular markers are often contradictory and inconclusive because of heterogeneous patient populations and various chemotherapeutic regimens. Because of the above said reasons it is often difficult to accurately define risk profiles and choose optimal post-operative treatment.[64]

Developing methods for predicting the response of tumour to chemotherapy has been an interesting area of research and several studies have been undertaken so far. Some in vitro chemo-sensitivity tests like the human tumour clonogenic assay (HTCA), the succinic dehydrogenase inhibition test (SDI test), the thymidine incorporation assay (TIA), etc have been available. However, these tests are not used routinely in clinical practice in the neoadjuvant setting and they are not of much value in a metastatic setting.[69]

In vitro studies like ATP-based chemotherapy response assay ATP-CRA has shown that a few biological and histological factors in breast cancers have importance

as predictive factors of chemo-responsiveness to specific chemotherapeutic agents. Tumours with high histologic and nuclear grade have higher rates of response to Adriamycin. ER-α negative tumour responds well to Adriamycin and PR negative tumour responds well to all chemotherapeutic drugs. The HER2+/ ER- breast cancers have a higher response rate to Adriamycin.[19]

Breast cancer and chemotherapy

Though the primary modality of treatment of local and regional breast cancer is surgical intervention, anti-tumour therapy with cancer chemotherapeutic agents also plays an equally important role in the medical management of breast cancer disease.

Several different groups of anti-neoplastic drugs are available for treating breast cancer. It can be managed by systemic chemotherapy or endocrine / hormonal therapy or targeted therapy (Her2neu receptor directed therapy). The choice of therapy is determined mainly based on the disease severity and characteristics of the tumour. For early stages of breast cancer (stage I, stage II and stage III), the nature of chemotherapy is determined by ER, PR and Her2neu status, involvement of lymph nodes and the size of the tumour.[70]

In patients with early breast cancer, pre-operative chemotherapy is almost equally effective as post-operative chemotherapy with respect to disease-free survival rate and overall survival rate. However, only if the patient has an indication for adjuvant chemotherapy, neoadjuvant chemotherapy should be performed. Here, loco-regional tumour spread, molecular type and as well as the risk of relapse has to be considered because low absolute risk suggests low absolute benefit. Though nodal involvement is associated with high risk of relapse, their effect on outcome is considered less and hence can be ignored while making a clinical decision.[3]

In addition to the advantage of better operability after neoadjuvant chemotherapy, this method is recommended particularly in patients who are negative for all three tumour markers (triple-negative breast cancer) and in those with HER2neu-positive disease. These subtypes have a pathological complete response which is correlated well with the patient outcome. [71] This association will help surgeons to decide on their prognosis after surgery which can be communicated to the patients. All chemotherapy should have to be administered before surgery to maintain the intensity of the dose in the setting of neoadjuvant chemotherapy.

Adjuvant chemotherapy must be started within the first few weeks following surgery and delaying chemotherapy beyond 3–4 weeks of surgery could impair the clinical outcome.[72] However, all studies do not give such a narrow time period. A population based analysis in 2016, showed that delaying the initiation of adjuvant chemotherapy beyond 91 days following is associated with an impaired clinical outcome, particularly in case of triple-negative breast cancers.[73]

The current standard of chemotherapy in early breast cancer includes taxanes and anthracyclines. These chemotherapeutic drugs are given either as a combination or in sequence over a time period of 18–24 weeks. Neoadjuvant and adjuvant settings generally do not differ in their chemotherapeutic regimens and the standard chemotherapeutic agents do not show considerable differences in the clinical response. The EBCTCG meta-analysis[74] showed that

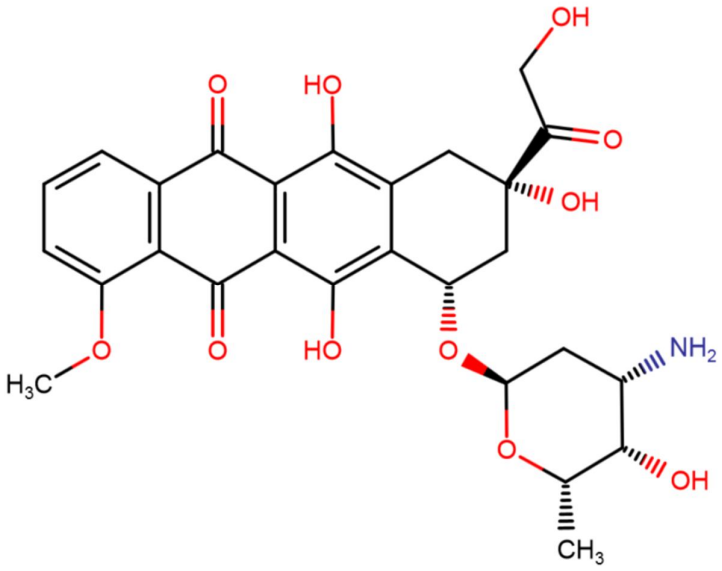
there was a one-third reduction in the 10-year breast cancer mortality in patients who received anthracycline-containing and taxane-containing chemotherapy. An anthracycline with taxane sequence is equally effective as their combination.[75,76]

Systemic chemotherapy in adjuvant setting has been found to reduce the risk of recurrence and mortaliy in breast cancer. Adjuvant regimens containing taxanes have been proved to be more effective than the regimens containing anthracyclines for node-positive breast cancer. Adriamycin (Adriamycin) / Cyclophosphamide followed by Paclitaxel (AC/T) is well established as a standard regimen for patients with node-positive breast cancer.[77] The dose of Adriamycin is usually between 50 to 60 mg/m^2, Cyclophosphamide 600 mg/m^2 and paclitaxel 175 mg/m^2.[78]

Clinical data from various trials on chemotherapy exist for early breast cancer patients up to the age group of 70 years, and biological age plays an important role than the chronological age with respect to chemotherapy in geriatric patients. Standard chemotherapy regimens are preferred for elderly patients who are fit with no major comorbidities. The International Society of Geriatric Oncology (SIOG) recommends tailoring of dosage and schedule of chemotherapeutic agents as per the the special requirements of older patients. For patients with ER-/PR-/Her2neu- (triple-negative) breast cancer, standard chemotherapeutic regimens containing anthracyclines and taxanes must be used, commonly as neoadjuvant therapy.[3]

Adriamycin

Figure.1 Structure of Adriamycin



Doxorubicin (Adriamycin) is an anthracycline derivative and is one of the most widely used anticancer chemotherapeutic agents. It has a very broad spectrum of activity against a number of malignancies including breast cancer. However, the routine clinical use of Adriamycin in cancer chemotherapy is limited by its cumulative, dose-dependent side effects.[79]

In its unaltered form, Adriamycin has shown great potential in the treatment of various cancers, and is regarded as one of the most potent of the Food and Drug Administration-approved chemotherapeutic agents. The ability of the drug to fight rapidly dividing cells and slow progression of disease has been well established for several decades, and is limited only by its toxic effects on non-

cancerous cells in the body. The drug is a non-selective, class I anthracycline, possessing aglyconic and sugar moieties.[80]

Several studies on the pharmacokinetics of Adriamycin have been done to assess the treatment range from single-agent to multi-agent therapy against a wide range of tumours. Most of these studies have shown that there is multiphasic drug disposition following intravenous injection. When administered intravenously, it is followed often by triphasic plasma clearance which gives the distribution half-life of 3–5 minutes, suggesting the rapid uptake of drug by cells. Adriamycin's terminal half-life of 24–36 hours suggests that the drug takes a longer time to get eliminated from the tissue than its uptake.[81]

Steady-state distribution of the drug is important to reduce the risk of toxicity. The range of steady distribution varies from 500 to 800 litre/m^2, and this allows the body tissues to take up a considerable amount of drug.[81]Adriamycin and its major metabolite Adriamycinol bind to plasma proteins. The free intracellular drug left (2%) is equally distributed among the other cell components.[82]

Adriamycin is found to accumulate mostly in the liver, which is mostly due to the role of hepatic cells in metabolism. Furthermore, the concentration of Adriamycin in white blood cells and bone marrow is 200 to 500 times higher than in the plasma. As the tissue distribution is very rapid, it is reflected by the rapid fall in the drug level in the plasma.

Adriamycin has a greater ability to penetrate tissues effectively. In spite of its high penetrating power, it does not have the ability to pass through the blood–brain barrier. Plasma clearance of Adriamycin normally ranges from 324 to 809 ml/min/m^2 and the elimination is by the hepatobiliary pathway. About 50% of the drug is eliminated through bile, usually within 5 to 7 days of drug administration, while only 5–12% of it appears in the urine during the same time period and 3% of the drug found in urine is in the form of its metabolite, Adriamycinol.[80]

Adriamycin acts by binding to DNA associated enzymes and intercalates with the base pairs of the DNA double helix. By binding to several molecular targets like topoisomerases, it brings about cytotoxic effects along with anti-proliferation, which in turn results in DNA damage.[83]

As fore-mentioned, the adverse effects associated with anthracyclines are the multi-directional cytotoxic effects, of which cardiotoxicity is the most prominent. Early phase II and III trials showed common side effects of nausea, vomiting, gastro-intestinal disturbances, alopecia, and neuro-psychiatric disturbances like hallucinations and light-headedness.[84,85]

Adriamycin exerts its effects not only on target tumour cells but also affects the growth of other non-neoplastic cells in the human body. This results in the depression of the immune system which in turn increase the patients' susceptibility to various microbial infections, fatigue and also increases the time to healing. The severity of these toxic effects and their occurrence often

depends on the dose and duration of Adriamycin therapy. Continuous intravenous administration of the drug may cause local reactions like phlebosclerosis, cellulitis, thrombophlebitis, etc.[84]

Adriamycin is responsible for various structural alterations in the heart, leading to enlargement of cardiac myocytes. Adriamycin can trigger the production of cytokines and also stimulate cytotoxic T lymphocyte responses while simultaneously increasing the activity of natural killer (NK) cells simultaneously. The stimulation of innate and adaptive immune responses will eventually result in aggressive cardiac damage.[80]

Yet another common site of Adriamycin-induced cell death and tissue damage is the liver, with approximately about 40% of patients ending up with some form of hepatotoxicity. When a patient is being treated with the drug it gets accumulated in the liver following which it plays an important role of metabolising it. In the event of metabolising high concentrations of Adriamycin, an increased number of reactive oxygen species (ROS) are produced which then results in oxidative stress culminating in cellular damage. The final major effect brought about by Adriamycin-mediated toxicity is that it is responsible for reduced levels of inorganic phosphate, both in the form of ADP and ATP as well as AMP which in turn is responsible for major hepatocyte pathology. The ATP-binding cassette is needed for mediating the efflux drug from the cells which requires a constant supply of ATP. Since Adriamycin reduces ATP levels through production of ROS, the ability of cells

to perform these energy-dependent tasks also decrease resulting in muscular and mental fatigue.[80]

Adriamycin is found to cause nephropathy and proteinuria by injuring glomerular podocytes.[86] Adriamycin-induced nephropathy occurs due to the drugs interference with the normal functioning of mitochondria which thereby results in increased levels of triglycerides, superoxides and citrate synthase, whilst decreasing the levels of vitamin E and other antioxidants due to lipid peroxidation. There is an alteration in the structure of nephrons when the proteins which leak through the local passages come in contact with renal tissue, leading to glomerulosclerosis which may cause hypertension, steroid resistance and proteinuria, eventually culminating in renal failure.[84] Unlike the liver, the regenerating ability of the kidney is poor, reducing its ability to heal by itself when the glomeruli are damaged.

Few patients have shown other adverse reactions from Adriamycin therapy such as cutaneous injuries. These patients may show hyperpigmentation of dermal creases and nail beds, alopecia, photosensitivity, itching and rashes. Adriamycin is also known to cause fever, urticaria and even anaphylaxis. Several studies have also shown Adriamycin affecting the gastrointestinal system frequently resulting in nausea, vomiting and mucositis during the initial stages of therapy (5–10 days). Most patients recover within a period of 10 days following treatment, however those with severe reactions often show signs of

ulceration and necrosis, causing severe infections in the lower gastrointestinal tract, which can be fatal.[80]

Adriamycin is commonly administered clinically as an intravenous bolus dose of 60 to 90 mg/m^2 every 3 weeks. The plasma pharmacokinetics of the drug following bolus administration exhibit an initial rapid decline followed by a slow decline in the plasma concentration which has been related to rapid redistribution of the drug intracellularly followed by its slow release from the tissue stores as plasma levels fall due to drug elimination.[87]

The optimal dose of an anti-neoplastic drug should produce a maximal anti-tumour effect with minimal and acceptable levels of toxicity. Although toxicity is the most important effect to be taken into consideration, the risk of under-dosing and reduced efficacy of the drug should also be considered as much as that of over-dosing.[88] Evidence of this comes from a study done by Budman et al, done in breast cancer patients treated with adjuvant chemotherapy which has shown that under-dosing of anti-tumour drugs may lead to a relative reduction in disease-free survival rate of nearly 20%.[89]

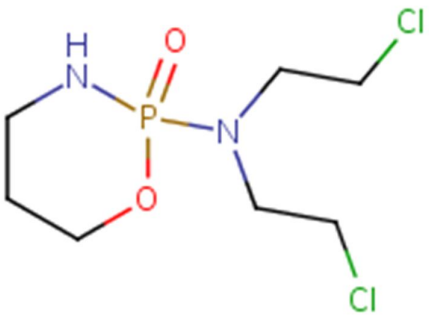
Even knowing that the measurement of the therapeutic response to cytotoxic drugs is a difficult challenge, it is reasonable to assume that in order to provide a similar response to the treatment, the pharmacokinetic profile of a specific drug in all patients must be similar, reaching the same maximum concentrations (Cmax) and producing the same area under the plasma concentration–time curves (AUC).

Several pharmacokinetic studies have been done on Adriamycin using animal models and cancer patients. Most of the pharmacokinetic data were obtained initially from the estimation of plasma total anthracycline fluorescence or from thin-layer chromatographic (TLC) analysis of the drug and its metabolites. However, numerous high-performance liquid chromatography (HPLC) methods are now available and are more reliable and sensitive than other older methods; they have the advantage of not being destructive towards the drugs and can usually be performed swiftly and also allows routine serial determinations.[90]

Cyclophosphamide

Cyclophosphamide is an alkylating chemotherapeutic agent known since decades. It belongs to the class of oxazaphosphorines. It is one of the most established anticancer agents ever synthesized and is still widely used as a chemotherapeutic agent in the management of various cancers, including breast cancer.

Figure.2 Structure of cyclophosphamide



Cyclophosphamide is an inactive prodrug which requires enzymatic and biochemical activation to release active phosphoramide mustard. Hydroxylation of the oxazaphosphorine ring by the hepatic cytochrome P450 enzyme system generates a metabolite, 4-hydroxycyclophosphamide, which exists along with its tautomer, aldophosphamide. These unstable precursors freely diffuse into the cells, where aldophosphamide is degradeded into two different compounds, phosphoramide mustard and acrolein. 6-Phosphoramide mustard produces both inter-strand and intra-strand DNA crosslinks which are responsible for the cytotoxic effects of cyclophosphamide, whereas acrolein is the one which causes hemorrhagic cystitis, one of the major adverse effects of cyclophosphamide.[91]

The metabolic process of cyclophosphamide is complex and involves formation of unstable intermediate compounds which makes the pharmacokinetic studies complicated. For instance, the incidence of both cardiac toxic effects and anti-tumour activity was found to be more in women with lower cyclophosphamide plasma levels after high dose administration of the drug for metastatic breast cancer. This observation was postulated to be due to the detoxification by tissue Aldehyde dehydrogenase. Indeed, the drug has been administered safely without any dose adjustments even to patients with renal or hepatic failure. Cyclophosphamide levels become undetectable by 12–24h after administration, even in patients with end-stage renal disease.[92]

The dosage and the toxicity profile of cyclophosphamide vary widely based on the clinical indication. The range of ‘low’ dose is between 1–3 mg/kg (40–120 mg/m 2) usually administered orally daily; ‘intermediate’ or ‘pulse’ dose being 15–40 mg/kg (600–1,500 mg/m2) usually administered i.v every 3–4 weeks; and ‘high’ dose being >120 mg/kg (>5,000 mg/m2). Low to intermediate dosages are associated with fewer acute toxic effects; But, prolonged usage for >6 months may result in significant chronic toxicity. Conversely, high doses of cyclophosphamide tend to cause more acute toxic effects, but seem to reduce the risk for chronic toxic effects.[93]

Among hematologic toxic effects, the most common toxic effect of cyclophosphamide is bone marrow suppression. Neutropenia is dependent on dose administered. Patients treated with low dose of the drug should also be monitored closely, though they rarely develop significant neutropenia. Leucopenia, thrombocytopenia and anaemia are more common after high dose cyclophosphamide therapy. However rapid recovery occurs within 2–3 weeks in patients with normal bone marrow reserve, even if high doses were administered.

Cardiotoxicity is the dose-limiting adverse effect of cyclophosphamide, and is observed only after administration of high doses. The resulting cardiac manifestations are heterogeneous and range from mild to fatal. The most severe form is haemorrhagic necrotic myocarditis, with an incidence of approximately <1–9% after the administration of high doses of cyclophosphamide.[94,95] This

clinical toxicity occurs abruptly within few days of drug infusion and is often fatal. Peri-myocarditis manifests as severe congestive heart failure accompanied by ECG findings of diffuse voltage loss, cardiomegaly and pericardial effusions. Post-mortem findings show haemorrhagic cardiac necrosis. Mild form of arrhythmias and small pleural effusions can also occur. Some patients may develop symptoms of congestive heart failure; however, if ECG voltage is maintained, these symptoms are usually reversible.[96]

Gonadal failure is another major complication of cyclophosphamide administration, especially in female patients. The age at diagnosis of the patient, the cumulative dose of drug and the dosage schedule are the major determinants for this toxic effect. The risk for sustained amenorrhea in patients receiving monthly intermediate dose cyclophosphamide is 12% for women < 25 years of age, and more than 50% for women > 30 years of age. The risk for ovarian failure following a high dose cyclophosphamide administration is less than that of intermediate dose.[97]

Haemorrhagic cystitis is the most common form of bladder toxicity associated with cyclophosphamide therapy. Other toxic syndromes include bladder fibrosis and transitional or squamous cell carcinoma. Haemorrhagic cystitis may occur early or late after cyclophosphamide therapy. Early onset cystitis, in the initial days following administration, appears to be caused by the metabolite, acrolein. Vigorous hydration, forced diuresis and MESNA, a drug which interacts with acrolein to form non-toxic adducts, can prevent this acute

condition by limiting the uro-epithelial exposure to the metabolite. Late onset haemorrhagic cystitis can develop weeks to months following treatment in approximately 25% of patients receiving high dose cyclophosphamide.[98]

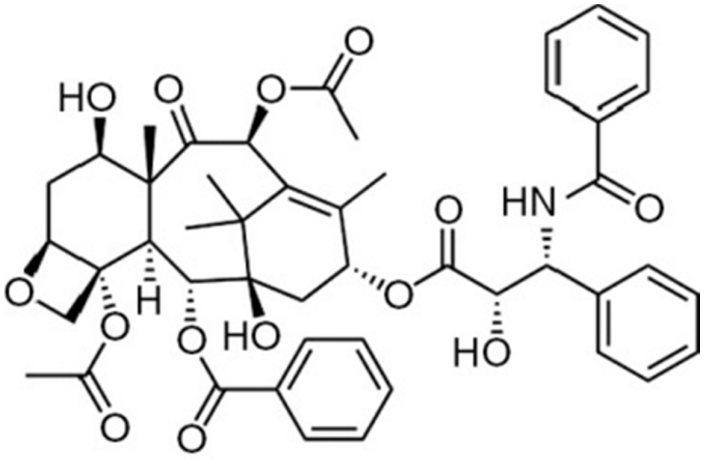
Nausea and vomiting are the most common adverse effects of cyclophosphamide administration and are most frequently associated with intermediate and high dosages of the drug. Prophylaxis with 5HT3 receptor antagonists, such as ondansetron or palanosetron, can usually prevent these adverse effects. Reversible alopecia is also commonly seen often with high dosages of cyclophosphamide. Diarrhoea may also occur following high dose therapy. Mild to moderate hyponatremia which is attributed to the syndrome of inappropriate antidiuretic hormone (SIADH) and central pontine myelinolysis have also been associated with cyclophosphamide therapy.[91]

Cyclophosphamide, in combination with other chemotherapeutic agents, has been the mainstay of adjuvant, neoadjuvant and metastatic breast cancer chemotherapy regimens such as FEC (5fluorouracil, epirubicin, cyclophosphamide) and CMF (cyclophosphamide, methotrexate, 5fluorouracil) for decades. AC (Adriamycin, cyclophosphamide) regimen was found to be equivalent to 6 months of standard CMF regimen in two separate National surgical Adjuvant Breast and Bowel Project (NSABP) studies.[99,100] The addition of a taxane to this adjuvant chemotherapy regimen further improved the outcomes and has since become the standard therapy for human epidermal

growth factor receptor 2 (HER2) negative early stage breast cancer.[101] A recent study showed that the addition of paclitaxel (T) to AC significantly reduced the hazard for disease free survival by 17% and 5 year disease free survival was 76% for patients who received AC/T compared with 72% for those who received only AC. The 5 year overall survival was found to be equal (85%) for both the groups. Toxicity associated with the AC and paclitaxel regimen was acceptable for the adjuvant setting.[102]

Paclitaxel

Figure.3 Structure of Paclitaxel



Taxol is a naturally occurring diterpene alkaloid, the source of which is the bark of the *Taxus brevifolia* tree found in western parts of the United States. Paclitaxel is a highly lipophilic, low water soluble drug, with a melting point ranging between 216℃–217℃. It has a high protein binding rate and functions by interfering with the normal structure of the inner aspect of the cell

membrane. For almost half a century the anti-cancer property of Paclitaxel has been an area of interest. It is a commonly used anti-cancer drug for treatment of a wide range of cancers from metastatic breast cancer, non-small-cell lung cancer, advanced ovarian cancer to Kaposi's sarcoma.

The cytoskeletal tubulin is the target of Paclitaxel. It causes defective assembly of the mitotic spindle and abnormal chromosome segregation that ultimately impair cell division. Paclitaxel causes cell death by arresting the cell cycle in the G0/G1 and G2/M phases wherein it stabilizes the microtubule polymer and prevents their disassembly.[103]

Earlier studies were on prolonged infusion and found the drug to have a linear clearance, however for shorter infusions when there is a disproportionate increase in peak plasma concentration and drug exposure with increasing doses, clearance might be non-linear or saturable. In all the regimens to which patients have been assigned the peak plasma concentration was found to be in the range that would produce relevant biologic responses in vitro. Paclitaxel has a rapid plasma clearance although it is highly plasma protein bound (95 to 98 percent). It has a large volume of distribution attributed to the property of binding to cellular proteins, possibly tubulin.[104]

As only 1 to 8 percent of the total drug is eliminated by the kidneys and renal dysfunction does not necessitate dose reduction. Systemic clearance is mostly by Hepatic metabolism, biliary excretion, elimination in feces and extensive tissue binding appears. Cytochrome P450 produces a hydroxylated metabolite

of Paclitaxel. Both paclitaxel and its hydroxylated metabolite attain high biliary concentrations. For patients with hepatic dysfunction neither the optimal dose nor its potential for interacting with drugs modulating the activity of hepatic Cytochrome P450 enzymes has been established.

An increased incidence (as high as 25 to 30 percent in some studies) of major hypersensitivity reactions complicated the early development of paclitaxel. Type 1 hypersensitivity reactions encompassing dyspnea with bronchospasm, hypotension and urticaria were more commonly noted. Most of the serious reactions were observed within 2 to 3 minutes of administration of paclitaxel nevertheless, almost all the reactions were observed within the first 10 minutes. Most of the reactions were also found to occur following the first or second dose. One case fatality was reported while all of the remaining patients showed full recovering either following the discontinuation of paclitaxel alone or with occasional additional treatments with antihistamines, fluids, and vasopressors. Flushing and rashes were noted in as high as 40 percent of patients. However, minor reactions are not a warning sign for the development of major ones. Initially it was observed that the hypersensitivity reactions mediated by paclitaxel was similar to the hypersensitivity reactions to radio contrast agents by means of direct release of histamine or other vasoactive substances.[105]

The principal toxic drug reaction is neutropenia onset of which is usually on day 8 to 10 following treatment, and recovery most often complete by day 15 to 21. As neutropenia is not cumulative it is suggestive of the fact that

paclitaxel does not damage the immature hematopoietic cells irreversibly. When paclitaxel was given at doses of 200 to 250 mg per square meter over a period of 24 hours, it was found that in majority of the infusions neutropenia was mostly severe (neutrophil counts dropping to less than 500 per cubic mm) even in patients who were previously untreated.[106]

Initial studies reported a low frequency of febrile and infectious sequelae at these doses (10 percent of courses) in comparison to severe neutropenia. However, later studies showed an increase in frequency in these complications. So for trials that used Paclitaxel in that dose range, granulocyte colony-stimulating factor was given as a prophylactic measure to prevent complications of neutropenia. It was found that in most patients who had received high doses of other chemotherapeutic agents, the maximal tolerated dose of Paclitaxel in the absence of granulocyte colony-stimulating factor was 175 to 200 mg per square meter. The time duration for which the plasma concentrations are higher than biologically active concentrations (0.05 to 0.1 mmol per liter) is a critical pharmacological determinant for the severity of neutropenia — this explains the reason for longer infusions resulting in more severe neutropenia. However as the optimal dose and schedule have not been determined for most tumors shorter infusions cannot be made a choice in all patients.[104]

Due to these indifferences the extent of previous myelotoxic therapy is considered the principal clinical determinant of severity of neutropenia. In isolation Paclitaxel occasionally causes severe thrombocytopenia and anemia.

A glove-and-stocking type of peripheral neuropathy is characteristically seen with Paclitaxel that manifests clinically with sensory symptoms of numbness and paresthesia.[105] Sensations such as proprioception, vibration carried by large fibres and temperature, pinprick carried by small ones are lost distally and bilaterally .In comparison to conventional doses (135 to 250 mg/m^2), treatment with higher doses (250 mg/m^2) produces symptoms earlier. Similarly, in comparison to doses exceeding 250 mg per square meter over a period of 3 or 24, conventional doses (200 mg/m^2) severe neurotoxicity is only rarely seen, even among those previously treated with other neurotoxic agents, like cisplatin.

Higher doses exceeding 170 mg per square meter have been found to cause transient myalgia, mostly within two to five days on starting treatment, likewise myopathy has been noted when high doses of paclitaxel (250 mg per square meter) was combined with cisplatin .[107,108]

Although Cardiac rhythm abnormalities have been noted with Paclitaxel, the significance of these effects is unknown.[106,109] Transient asymptomatic bradycardia was the most common reaction noted (29 percent of patients in one trial). Isolated asymptomatic bradycardia in the absence of hemodynamic effects does is not an indication for discontinuing paclitaxel. Though clinically

significant bradyarrhythmias have been noted, the incidence as determined in a large National Cancer Institute data base was only 0.1 percent. Episodes that have been documented have mostly been asymptomatic and if symptomatic were able to be reverted. Paclitaxel was found to be a probable cause for bradyarrhythmias as related taxanes altered cardiac automaticity and conduction and it was also found that humans and animals ingesting various species of yew plants underwent similar effects.[109]

Cardiac monitoring during treatment with paclitaxel is not needed on a routine basis however it is advisable for patients who cannot tolerate the possible bradyarrhythmic effects, such as patients having atrioventricular conduction defects or ventricular dysfunction.

Gastrointestinal effects due to paclitaxel use, such as vomiting and diarrhea, are not common.[105] Leukemic patients who are prone to mucosal breakdown or in patients receiving 96-hour infusions may manifest mucositis at high doses. In patients who received high doses of paclitaxel in combination with doxorubicin or cyclophosphamide, neutropenic enterocolitis has been reported rarely.[110]

As with other chemotherapeutic agents, Paclitaxel also causes reversible scalp alopecia, and with cumulative therapy even body hair is frequently lost. Rarely injection site inflammation and inflammation along the course of the injected vein and at sites of extravasation of the drug may be noted. Similarly inflammatory skin reactions may rarely occur at previously irradiated areas.[105]

In the early 1990s, the demonstration of significant anti-tumour activity with taxanes in patients with advanced breast cancer and led to the development of new, more active adjuvant chemotherapy regimens. Although the clinical development of paclitaxel started as early as 1970s, initial progress was hampered by the occurrence of severe hypersensitivity reactions. In the late early 1990s, these hypersensitivity reactions were controlled with appropriate pre-medications and by infusing the drug over longer periods of time (24 - 96 hours). Although longer infusions demonstrated considerable clinical activity with a good safety profile, they were impractical for the adjuvant setting. Several studies then explored and eventually confirmed the safety and efficacy of a 3-hour infusion at low or high doses with 250 to 300 mg/m2 as the maximum tolerated dose without the support of colony-stimulating factor.[101]

Anthracyclines and taxanes are among the most potent and well established chemotherapeutic agents for the treatment of early breast cancer. Several different dosing regimens are commonly used which include concurrent, standard-dose sequential, and dose-dense sequential regimens. On the basis of the findings of the Cancer and Leukemia Group B 9741 trial, (Sequential Chemotherapy with Adriamycin, Paclitaxel and Cyclophosphamide verses Concurrent Adriamycin and Cyclophosphamide Followed by Paclitaxel at 14 or 21 Day Intervals in patients with Node Positive Stage II/IIIA Breast Cancer), which demonstrated significantly improved disease free survival and overall survival with dose-dense regimens over conventionally scheduled regimens.

DD AC3P is considered to be one of the most effective P-based adjuvant chemotherapy regimens.[111]

Pharmacokinetics of chemotherapeutic drugs

Pharmacokinetics is what the body does to the drug which includes absorption, distribution, metabolism and elimination. There are substantial differences between patients with respect to these physiological processes, which in turn will result in differences in plasma concentrations in these patients. Even though the dose of these chemotherapeutic agents is calculated according to the body surface area (BSA), there are considerable inter-individual variations in the concentration of the drug attained in the plasma. This pharmacokinetic variability may also be attributed to patients' non-compliance, drug-drug interactions, etc.[112] Measuring the plasma concentration routinely in the form of therapeutic drug monitoring has been done for a very few chemotherapeutic agents like 5- Fluorouracil, high dose Methotrexate, etc.

The area under plasma concentration-time curve (AUC), the maximum plasma concentration (Cmax) or the steady-state plasma concentration for continuous infusion (Css), clearance (Cl) and half-life (t1/2) are the commonly used pharmacokinetic parameters for describing drug disposition in all patients.[113] Though BSA failed to correlate with inter-individual pharmacokinetic variations of most of the cytotoxic anti-cancer drugs, previous investigations have shown that cytotoxic cancer chemotherapeutic drug induced toxicities had been correlated well with pharmacokinetic parameters such as AUC, Cmax,

Css, and Cl. Some anti-cancer efficacy had also been correlated with these pharmacokinetic parameters. These pharmacokinetic-pharmacodynamic (PK-PD) correlations have provided a background that dose optimization for individual patients could be done from these PK-PD correlations. For example, some studies have shown that docetaxel AUC was significantly correlated with the docetaxel induced hematologic toxicity and neutropenia.[113]

Several studies have identified that patients receiving standard BSA-based dosage of a cytotoxic chemotherapy drug, showed wide inter-individual variations in drug efficacy, toxicities and pharmacokinetic parameters. These variations may be attributed to non-genetic factors as well as genetic factors. Non-genetic factors include, age of the patient, liver and renal functions, drug-drug interactions, comorbidities, smoking, alcohol consumption, etc. Genetic factors are mainly due to genetic variants that are located on the genes that encode drug metabolizing enzymes, transporters and drug targets. Briefly, it is well known that inter-individual variations in efficacy and toxicity of chemotherapeutic drugs are wide when cancer patients receive a BSA standardized dose of cytotoxic anti-cancer drugs and anti-cancer drug PK-PD correlations would provide information for optimization of therapy.

Therapeutic drug monitoring of chemotherapeutic agents

In current clinical practice, therapeutic drug monitoring (TDM), also known in the recent times as therapeutic concentration intervention (TCI) has been

widely used for optimizing drug treatment.[114] It is commonly done for drugs with narrow therapeutic index like antimicrobials, antiepileptics, etc.[112]

The application of therapeutic concentration intervention in Oncology is very much complicated and also limited due to two important reasons. Firstly, the therapeutic range of most of the chemotherapeutic drugs is not known. Secondly, almost all type of cancers are treated with multiple drugs combined together, which fails to explain which drug causes which effect. For example, both Adriamycin and Cyclophosphamide causes mucositis and myelosuppression. Hence getting a clear dose-response or concentration-effect relationship with chemotherapeutic agents is difficult. Number of studies addressing the relationship between plasma concentration of these drugs and their effects are also limited.

Another major area of concern with the use of chemotherapeutic agents is the increased incidence of adverse effects which could be prevented by optimizing the dosage of drugs based on their plasma concentration.[112] As mentioned earlier, there are considerable differences in response to chemotherapeutic agents observed between patients due to large inter-individual variations in pharmacokinetics.[114]

In majority of patients, the dose which produces the therapeutic effect may also be responsible for toxic effects. Therefore, optimizing the drug dosage to reduce the exposure may have the therapeutic advantage of maximizing the efficacy of the drug while reducing the adverse effects.

4. MATERIALS AND METHODS

4.1. Study settings

This study was done in the Department of Pharmacology, PSG Institute of Medical Sciences and Research (PSG IMS&R), Coimbatore in collaboration with the Department of Medical Oncology, PSG IMS&R. The study was done in patients with breast cancer who were on AC/T regimen (Adriamycin and Cyclophosphamide 4 cycles followed by Paclitaxel 4 cycles) during the period of November 2016 to October 2017.

4.2. Study design

The study was designed as an observational study on breast cancer patients who were on AC/T regimen both as adjuvant and neoadjuvant chemotherapy.

4.2.1 Sample size

This study was planned as an in-hospital study; hence to study the influence of plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel on the clinical outcome of patients with breast cancer we took a convenient sample size of 30 with 10 patients for each drug.

4.2.2 Study approval

The study protocol was approved by the Institutional Human Ethics Committee (IHEC) – Proposal No. 15/380 dated 29.12.2016 before the commencement of

the study. It was also registered in the Clinical trial registry of India – CTRI/2017/06/008766.

4.2.3 Inclusion criteria

- All Ca breast patients, both male and female on AC/T regimen – diagnosed based on AJCC staging criteria, belonging to Stage I, Stage II and Stage III in the age group of 30 – 65 years
- All Ca breast patients on AC/T regimen with laboratory parameters between normal limit to upper limit of contraindicated values

4.2.4 Exclusion criteria

- Patients with tumor beyond stage IV
- Patients in the age group of less than 30 years and more than 65 years
- Patients who were on other chemotherapy regimens previously
- Patients with co-morbid conditions like renal failure and liver failure

4.2.5 Study subjects

Breast cancer patients satisfying the inclusion and exclusion criteria were explained in detail about the nature of the study. A written informed consent was obtained from all patients who were willing to enroll in the study. The demographic details and data pertaining to the treatment of breast cancer such as tumour size, stage of the disease, drug and dosage, Estrogen / Progesterone

receptor (ER/PR) status, laboratory parameters including liver and renal function test and complete blood count (CBC), nature of chemotherapy (adjuvant / neoadjuvant) and number of cycles completed were obtained from the medical records of the patient. Patients were also asked about the occurrence of adverse effects like nausea, vomiting, etc.

4.2.6 Study groups

We had three independent groups of patients with a sample size of 10 patients in each group. Patients in Adriamycin group were receiving Inj.adriamycin 60 mg/m^2 given as an intravenous infusion over 15 minutes. Patients in Cyclophosphamide group were receiving Inj.cyclophosphamide (following Adriamycin) 600 mg/m^2 given as an intravenous infusion over 60 minutes. Patients in Paclitaxel group were receiving Inj.paclitaxel 175 mg/m^2 given as an intravenous infusion over 3 hours.

Table.1 Dose, route and duration of infusion of the drugs.

Drug	Dose	Route of administration	Duration of infusion
Adriamycin	60 mg/m^2	i.v	15 min
Cyclophosphamide	600 mg/m^2	i.v	60 min
Paclitaxel	175 mg/m^2	i.v	180 min

4.2.7 Sample collection and processing

About two milliliters of venous blood was collected from the study participants at the end of infusion of the drug in polypropylene tubes containing 3.6 mg of ethylene diamine tetra acetic acid (EDTA). The sample was then centrifuged at 2000 rpm for 15 minutes to separate plasma which was stored at -20□ until further processing.

4.3 HPLC analysis

4.3.1 Instrumentation and Chromatography conditions for Cyclophospamide

The UPLC instrument consisted of a Waters Acquity-H class UPLC system equipped with a quaternary pump and 96-vial autosampler coupled with a diode array UV detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC BEH C18 column from Waters (2.1 mm × 100mm; 1.7µm). The column temperature was set at 30ºC and the autosampler was kept at 15ºC. The mobile phase composed of a mixture of acetonitrile (50%, v/v) and water (50%, v/v) at a flow rate of 0.1ml/min. Before analysis the mobile phase was filtered through a 0.22µm membrane filter and degassed by ultra-sonication. A 3µl injection of each sample was loaded on to the system and total analysis time was 8 min. DAD was set at 200 nm. Data acquisition was done using Empower 3 software (Waters).

4.3.2 Preparation of stock and working standard solution for Cyclophospamide

Cyclophospamide stock solutions of 1mg/ml were prepared by dissolving suitable amount drug in methanol. Stock solutions cyclophospamide (100µg/ml) were prepared in methanol. The IS stock solution of 200µg/ml was prepared in methanol. Further dilution was made in methanol:water (50:50, v/v) to produce working stock solution for the calibration standards and quality control (QC) standards. The IS working solution (5µg/ml) was prepared in methanol-water (50:50 v/v). Calibration curve samples were prepared in drug-free human plasma with the appropriate mixture of working solution with range of 20-5500 ng/mL of cyclophospamide on the day of analysis. All the samples were stored together at -80 ± 10℃ until analysis.

4.3.3 Sample Preparation for Cyclophospamide

Sample preparation was carried out by the protein precipitation procedure. To a 250µl of aliquot of plasma, 10µl of IS working solution were mixed for 30 s on a spinix vortex shaker (Tarsons, India). Added 1.0 ml of methanol and vortex mixed for 5 min. Centrifuged at 10,000 rpm for 5 min at 4ºC on an eppendrof 5810R centrifuge (Eppendrof AG, Hamburg, Germany). The clear supernatant organic layer (0.850 mL) was transferred into 2ml glass tubes and evaporated to dryness at 35ºC using nitrogen evaporator (Turebovap®, Biotage, USA). The residue was reconstituted in 100 µl of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000rpm for 5 min. Finally, 90 µl of the clear

supernatant were transferred into glass micro-vials and 5µl were injected onto the UPLC system for analysis.

4.3.4 Instrumentation and Chromatography conditions for Adriamycin and Paclitaxel

The HPLC instrument consisted of a Waters 515 system equipped with an isocratic pump coupled with a 2489-UV and 2475 Fluorescence detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Zorbox SB-CN C18 column from Agilent (2.1 mm × 100mm; 3.5µm). The mobile phase composed of a mixture of acetonitrile (50%, v/v) and 0.1mM EDTA (50%, v/v) at a flow rate of 0.2ml/min. Before analysis the mobile phase was filtered through a 0.40µm membrane filter and degassed by ultra-sonication. A 10µl injection of each sample was loaded on to the system and total analysis time was 12 min. UV detector was set at 230 nm and Fluorescence detector set at excitation 475, emission 555. Data acquisition was done using Empower 2 software (Waters).

4.3.5 Preparation of stock and working standard solution for Adriamycin and Paclitaxel

Adriamycin and Paclitaxel stock solutions of 1mg/ml were prepared by dissolving suitable amount single drug in methanol. Mixture of stock solutions Adriamycin and Paclitaxel (100µg/ml) were prepared in methanol. The IS stock solution of 200µg/ml was prepared in methanol. Further dilution was

made in acetonitrile-water (50:50, v/v) to produce working stock solution for the calibration standards and quality control (QC) standards. The IS working solution (8µg/ml) was prepared in acetonitrile-water (50:50 v/v). Calibration curve samples were prepared in drug-free mouse plasma with the appropriate mixture of working solution with range of 20-5500 ng/mL of Adriamycin and Paclitaxel on the day of analysis. All the samples were stored together at -80 ± 10°C until analysis.

4.3.6 Sample Preparation for Adriamycin and Paclitaxel

Sample preparation was carried out by the protein precipitation procedure. To a 250µl of aliquot of plasma, 10µl of IS working solution were mixed for 30 s on a spinix vortex shaker (Tarsons, India). Added 1.0 ml of methanol and vortex mixed for 5 min. Centrifuged at 10,000 rpm for 5 min at 4ºC on an eppendrof 5810R centrifuge (Eppendrof AG, Hamburg, Germany). The clear supernatant organic layer (0.850 mL) was transferred into 2ml glass tubes and evaporated to dryness at 35ºC using nitrogen evaporator (Turebovap®, Biotage, USA). The residue was reconstituted in 100 µl of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000rpm for 5 min. Finally, 10 µl of the clear supernatant was injected onto the HPLC system for analysis.

4.4 Statistical analysis

All the 30 patients were included in the statistical analysis. The outcome variables were expressed as mean values. The association between the variables was analysed using Pearson correlation with a p value of 0.05 and a confidence interval of 95%. We used SPSS statistical software version 24 for the data analysis.

5. RESULTS

5.1 Study participants

A total of 30 patients with breast cancer satisfying the eligibility criteria were enrolled in the study, after obtaining an informed consent. All the patients were clinically stable at the time of enrolment and were undergoing 3rd cycle of their respective chemotherapeutic drugs [AC/T (Adriamycin, Cyclophosphamide and Paclitaxel)] and were grouped into 3, with 10 patients for each drug under study.

5.2 Baseline demographic profile

Adriamycin

The mean age at diagnosis of patients enrolled in this group was 53.8 years, with more number of patients in the age group of >50yrs (n=7) and only about 3 patients < 50 years. The drugs were administered based on the BSA. The mean BSA was found to be 1.60 $m^{2.}$ Half of the patients had co-morbid medical conditions like diabetes, hypertension, etc for which they were under relevant medications. Only 2 of 10 patients had a family history of breast cancer. Regarding the menstrual status, 4 patients were pre-menopausal, 4 were menopausal and 2 had undergone hysterectomy.

Among the 10 patients, all except 1 patient had breast fed their children and that 1 patient was a nullipara. The mean duration of disease was 3 months and we had equal number of patients having an initial tumour size of < 5 cm (5 patients) and > 5 cm (5 patients). Out of 10 patients, 7 patients were on adjuvant chemotherapy and 3 were on neoadjuvant chemotherapy. Regarding the hormone receptor status, 4 were ER+ and 6 ER- and 3 were PR+ and 7 PR-. (table 2)

Cyclophosphamide

The mean age at diagnosis of patients in this group was 47.5 years with equal number of patients in the age group of >50 years and < 50 years. The mean BSA was found to be 1.63 m^2. 3 patients had co-morbid medical conditions, 2 patients with hypertension and 1 with hypothyroidism for which they were under relevant medications. Only 1 patient had a family history of breast cancer in her second degree relative. Regarding the menstrual status, 4 patients were pre-menopausal and 6 were menopausal.

Among the 10 patients only 1 patient was a nullipara, and all others had a history of breast feeding. The mean duration of disease was 9.2 months and we had equal number of patients having an initial tumour size of < 5 cm (5 patients) and > 5 cm (5 patients). Out of 10 patients, 6 patients were on adjuvant chemotherapy and 6 were on neoadjuvant chemotherapy. Regarding the hormone receptor status, 7 were ER+ and 3 ER- and 5 were PR+ and 5 PR-. (table 2)

Paclitaxel

The mean age at diagnosis of patients enrolled in this group was 51.2 years with 7 patients in the age group of >50yrs and only about 3 patients < 50 years of age. The mean BSA was found to be 1.59 m^2. 4 patients had co-morbid medical conditions like diabetes, hypertension, etc for which they were under relevant medications. None of the patients had a family history of breast cancer. Regarding the menstrual status, 3 patients were pre-menopausal, 7 were menopausal.

Among the 10 patients, all except 1 patient had breast fed their children. The mean duration of disease was 8.2 months and we had 8 patients with an initial tumour size of < 5 cm and only 2 patients with > 5 cm tumour. All 10 patients were on adjuvant chemotherapy. Regarding the hormone receptor status, 7 were ER+ and 3 ER- and 6 were PR+ and 4 PR-.(table 2)

Table.2 Baseline demographic characteristics

	Adriamycin (n=10)	Cyclophosphamide (n=10)	Paclitaxel (n=10)
Age at diagnosis (years)			
Mean	53.8	47.5	51.2
<50	3	5	3
>50	7	5	7
BSA			
Mean	1.60	1.63	1.59
Medical history			
Yes	5	3	4
No	5	7	6
Family history			
Yes	2	1	0
No	8	9	10
Menstrual status			
Premenopausal	4	4	3
Postmenopausal	4	6	7
Hysterectomised	2	0	0
Breastfeeding H/o			
Yes	9	9	9
No	1	1	1

Baseline demographic characteristics continued

	Adriamycin (n=10)	Cyclophosphamide (n=10)	Paclitaxel (n=10)
Duration of disease (months)			
Mean	3	9.2	8.2
< 6	9	4	4
> 6	1	6	6
Initial tumour size			
< 5 cm	5	5	8
> 5 cm	5	5	2
Chemotherapy			
Adjuvant	7	6	10
Neoadjuvant	3	4	0
ER status			
Positive	4	7	7
Negative	6	3	3
PR status			
Positive	3	5	6
Negative	7	5	4

5.3 HPLC analysis

The plasma concentrations of the three drugs were analyzed using High performance liquid chromatography as mentioned above and the calibration graph plotted. The calibration curves exhibited excellent linearity with regression correlation coefficient ($r^2 > 0.99$) over the concentration range of

20.0 – 5500 ng/mL for all the drugs in human plasma. The standard calibration curve had a consistent reproducibility over the standard concentrations across the calibration range.

A typical regression equation was prepared by determining the best fit of peak-area ratio (peak area analyte / peak area IS) vs concentration, and fitted to the y = mx + c using a weighting factor ($1/x^2$). The lowest concentration with the RSD < 20% was taken as the LLOQ. The percentage accuracy observed for the mean of back-calculated concentration for four calibration curves for the entire drugs were within 97.10-103.50%.

Figure.4 Representative Chromatogram of Adriamycin in patient plasma

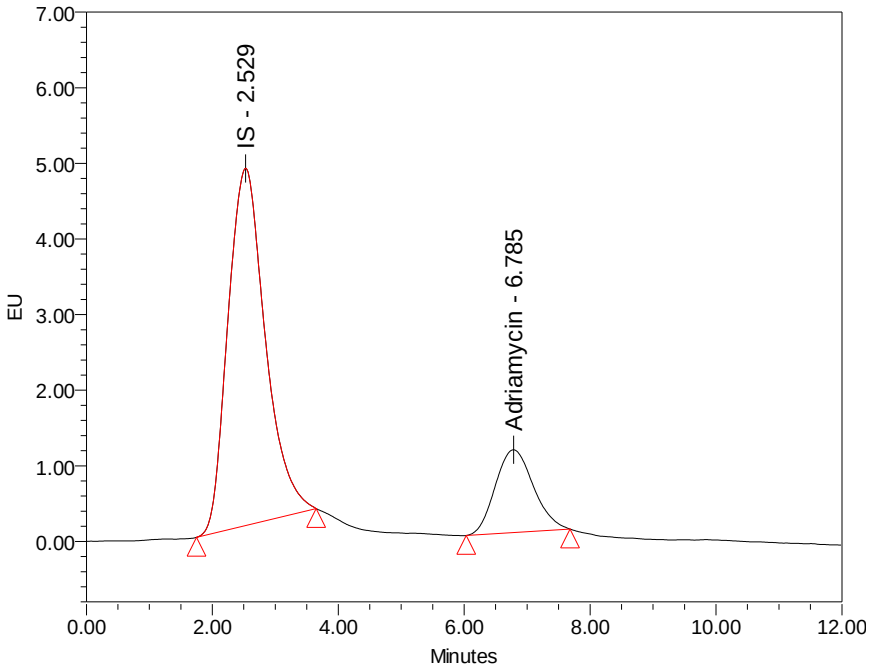


Figure.5 Representative Chromatogram of Cyclophosphamide in patient plasma

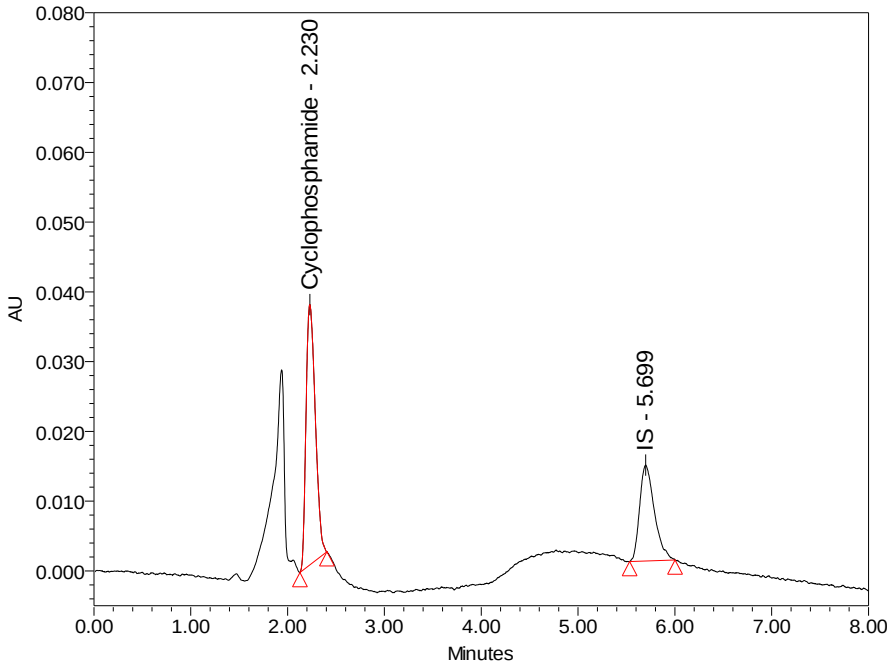
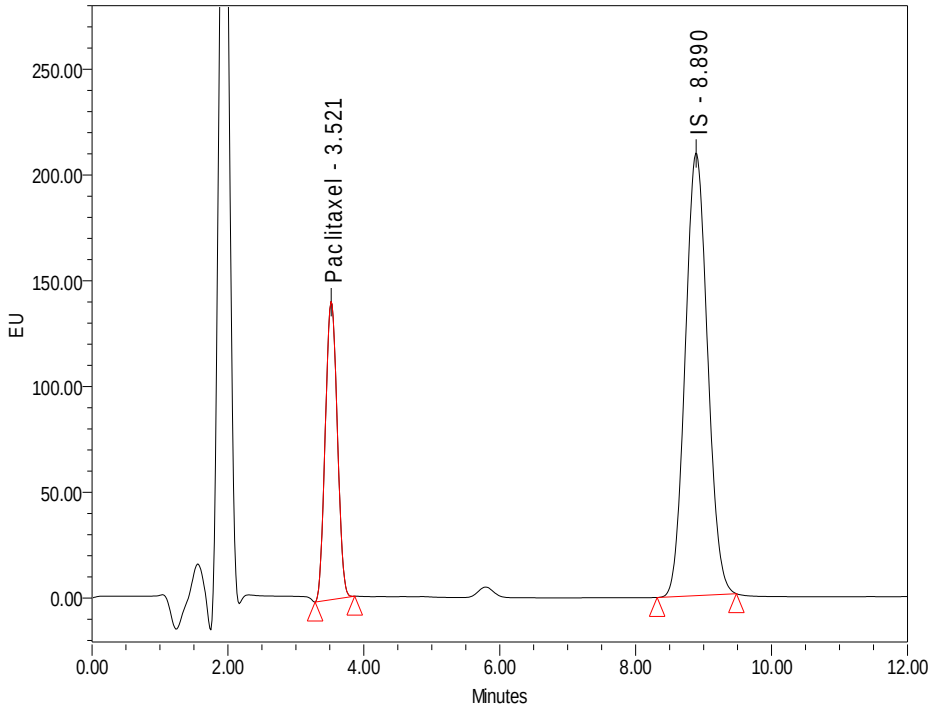


Figure. 6 Representative Chromatogram of Paclitaxel in patient plasma



5.4 Outcomes

Our primary outcome was to determine whether the plasma concentration of the drug has any influence on the liver and renal function test of the patients.

Adriamycin

The mean plasma concentration of Adriamycin was found to be 3460.50 ng/ml as determined by HPLC. (table 3) Plasma concentration of the drug did not have any influence on the liver function or renal function of the patient. However, we found a negative correlation between the plasma concentration of Adriamycin and the other parameters studied, though the p value was not significant. (table 4)

Table.3 Mean and standard deviation of various parameters - Adriamycin

Parameters	Mean	Standard deviation
Plasma concentration (ng/ml)	3460.0510	865.00122
Total bilirubin (mg/dl)	.4540	.23186
SGPT (U)	18.1100	5.28740
SGOT (U)	24.2200	9.51593
Urea (mg/dl)	21.0200	10.18559
Creatinine (mg/dl)	.6610	.26843

Table.4 Plasma concentration of Adriamycin vs Liver & Renal function tests

Parameters	Pearson correlation (r)	P value
Total bilirubin	-0.206	0.568
SGPT	-0.165	0.650
SGOT	-0.521	0.123
Urea	-0.449	0.193
Creatinine	-0.397	0.256

Cyclophosphamide

The mean plasma concentration of patients who received Cyclophosphamide was found to be 1592.44 ng/ml. (table 5) Similar to Adriamycin, Plasma concentration of Cyclophosphamide did not influence the liver function or renal functions of the patient. Though the p value was not significant, we found a weak positive correlation between plasma concentration and liver function tests (total bilirubin, SGPT and SGOT) and a negative correlation with renal function tests (urea, creatinine). (table 6)

Table.5 Mean and standard deviation of various parameters - Cyclophosphamide

Parameters	Mean	Standard deviation
Plasma concentration (ng/ml)	1592.4390	391.39907
Total bilirubin (mg/dl)	.5080	.22255
SGPT (U)	17.3000	6.63409
SGOT (U)	24.7000	10.96510
Urea (mg/dl)	21.6600	9.61714
Creatinine (mg/dl)	.7780	.34730

Table.6 Plasma concentration of Cyclophosphamide vs Liver & Renal function tests

Parameters	Pearson correlation (r)	P value
Total bilirubin	0.287	0.422
SGPT	0.319	0.369
SGOT	0.005	0.989
Urea	-0.264	0.462
Creatinine	-0.109	0.764

Paclitaxel

The mean plasma concentration of Paclitaxel in patients was found to be 3038.29 ng/ml. (table 7) None of the parameters studied seem to be influenced by the plasma concentration of the drug. Except for SGOT and SGPT which showed a weak positive correlation, the other parameters showed weak negative correlation though not statistically significant. (table 8)

Table.7 Mean and standard deviation of various parameters - Paclitaxel

Parameters	Mean	Standard deviation
Plasma concentration (ng/ml)	3038.2920	1872.70462
Total bilirubin (mg/dl)	.4740	.24874
SGPT (U)	26.3000	15.02627
SGOT (U)	28.2100	10.29222
Urea (mg/dl)	21.1000	7.78103
Creatinine (mg/dl)	.7280	.31460

Table.8 Plasma concentration of Paclitaxel vs Liver & Renal function tests

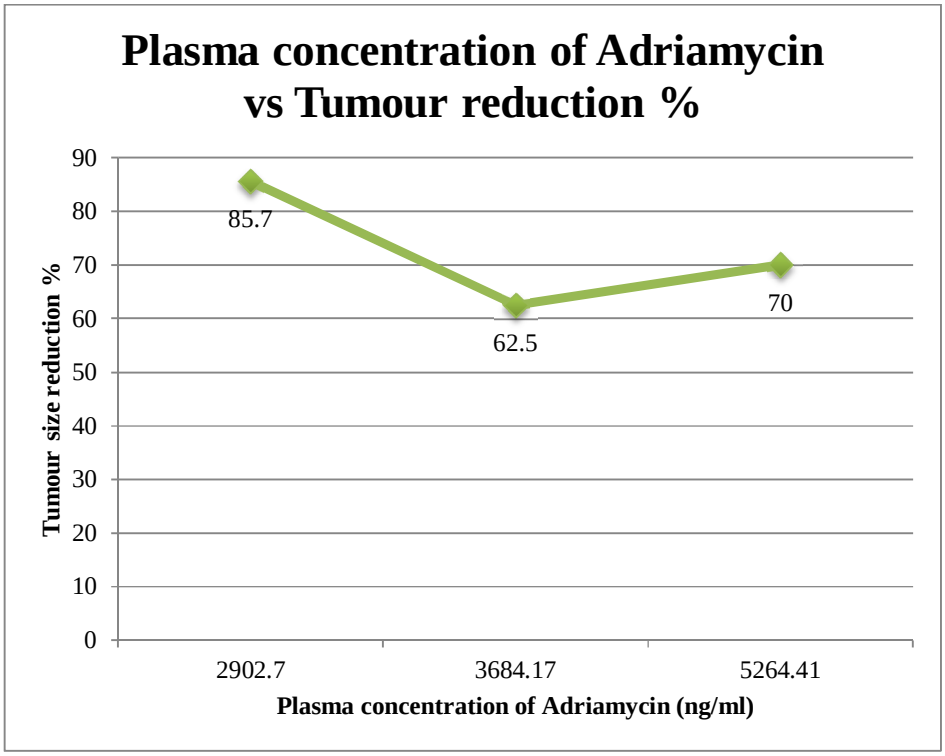
Parameters	Pearson correlation (r)	P value
Total bilirubin	-0.222	0.538
SGPT	0.236	0.512
SGOT	0.095	0.794
Urea	-0.035	0.924
Creatinine	-0.018	0.961

The other secondary outcomes include the influence of plasma concentration of the drugs on 1) clinical outcome as measured by reduction in tumour size in patients on neoadjuvant chemotherapy, 2) hormone receptors (ER, PR status) and 3) adverse drug reactions (ADRs).

Adriamycin

We had only three patients who were receiving Adriamycin as a part of neoadjuvant chemotherapy. All the 3 patients had reduction in the tumour size. Patient with a plasma concentration of 2902.7 ng/ml had a reduction in tumour size of 85.7% when compared to 70% reduction in a patient with a concentration of 5264.41ng/ml. (figure 7)

Figure.7 Plasma concentration of Adriamycin vs Tumour size reduction



We had 4 ER+ and 6 ER- patients receiving Adriamycin in our study. The mean plasma concentration in ER+ patients was 3180.76 ng/ml while that in ER- patients was 3646.2433 ng/ml (figure 8) which does not show any significant correlation.

There were 3 PR+ and 7 PR- patients in the Adriamycin group. The mean plasma concentration in PR+ patients was 2970.85 ng/ml while that in PR- patients was 3669.7086 ng/ml (figure 9) which does not show any statistically significant correlation thereby concluding that the ER/PR status does not have any influence on the plasma concentration of the drug in these patients.

Figure.8 Plasma concentration of Adriamycin vs Estrogen receptor (ER)

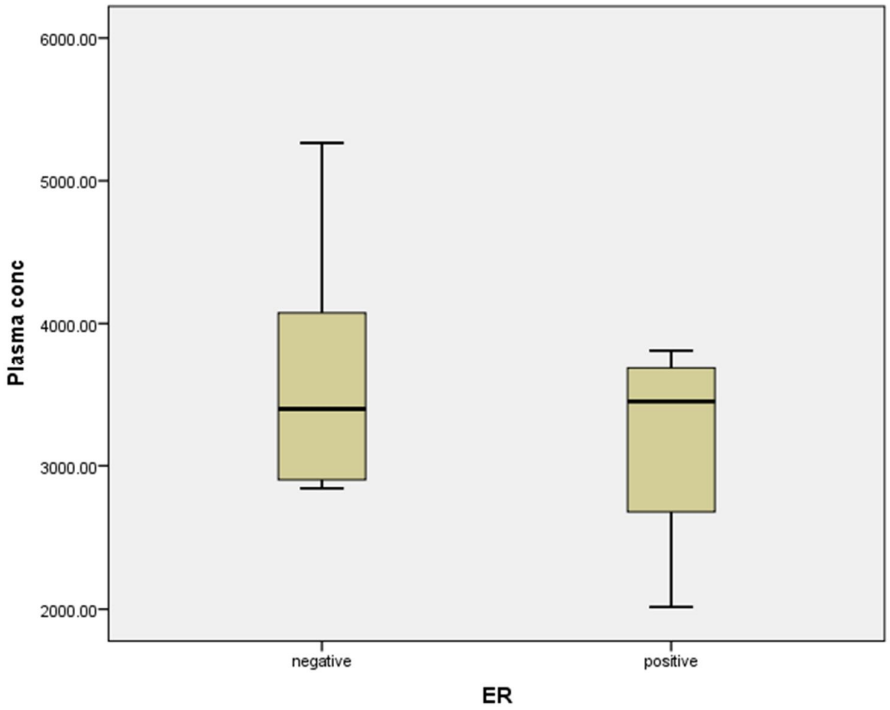
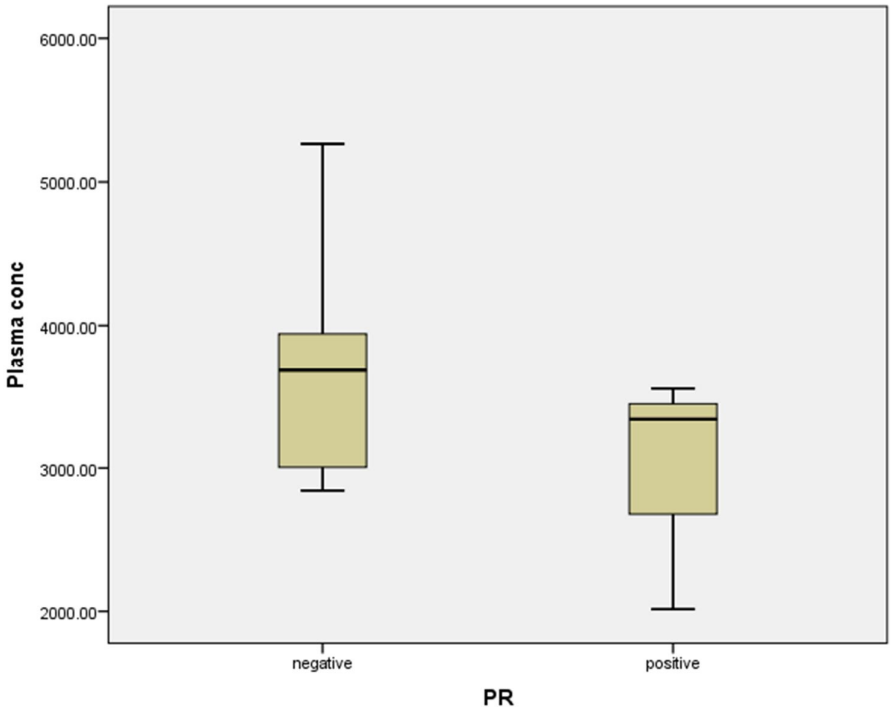
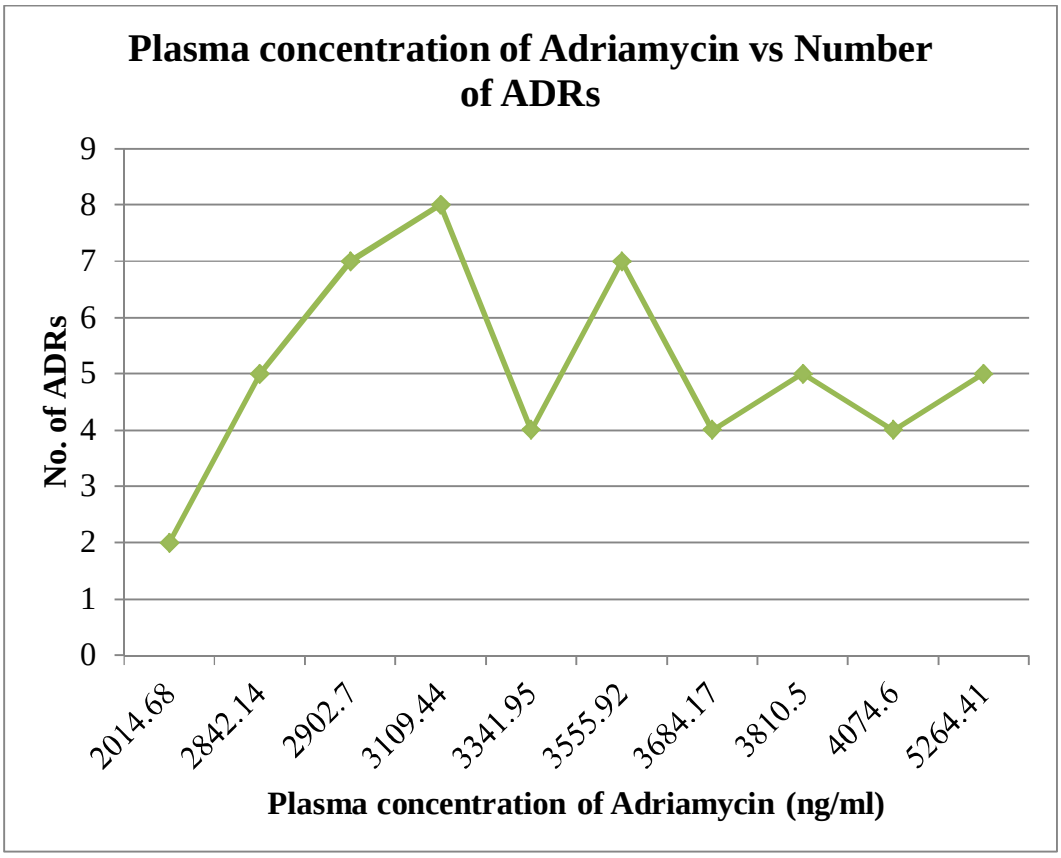


Figure.9 Plasma concentration of Adriamycin vs Progesterone receptor (PR)



It was also found that the plasma concentration of Adriamycin does not have any significant influence on the number of adverse drug reactions reported. (figure 10)

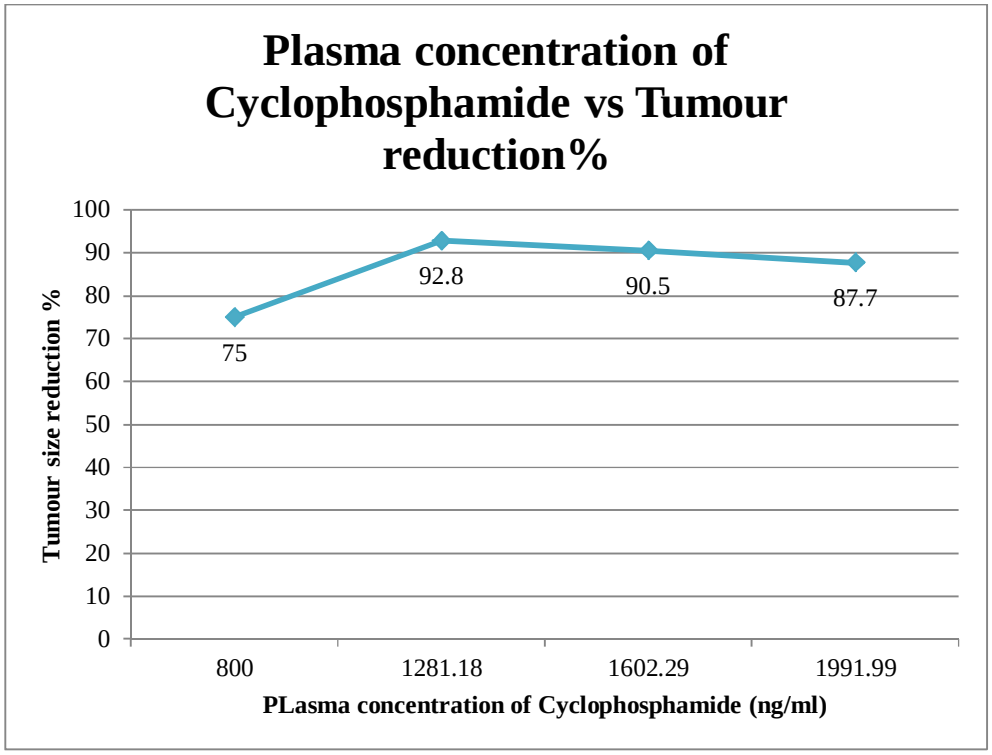
Figure.10 Plasma concentration of Adriamycin vs number of adverse drug reactions (ADRs)



Cyclophosphamide

We had only four patients who were receiving Cyclophosphamide as a part of neoadjuvant chemotherapy. All the 4 patients had reductions in their tumour size. The mean reduction in tumour size was 86.5%. Though there was a positive correlation between the plasma concentration and tumour size reduction, it was not statistically significant. (figure 11)

Figure.11 Plasma concentration of cyclophosphamide vs Tumour size reduction



We had 7 ER+ and 3 ER- patients receiving third cycle of Cyclophosphamide in our study. The mean plasma concentration in ER+ patients was 1688.9971 ng/ml while that in ER- patients was 1367.1367 ng/ml (figure 12) which does not show any significant correlation.

There were 5 PR+ and 5 PR- patients in the Cyclophosphamide group.The mean plasma concentration in PR+ patients was 1785.3660 ng/ml while that in PR- patients was 1399.5120 ng/ml, (figure 13) which does not show any significant correlation thereby concluding that the hormonal status does not have any influence on the plasma concentration of the cyclophosphamide in these patients.

Figure.12 Plasma concentration of Cyclophosphamide vs Estrogen receptor (ER)

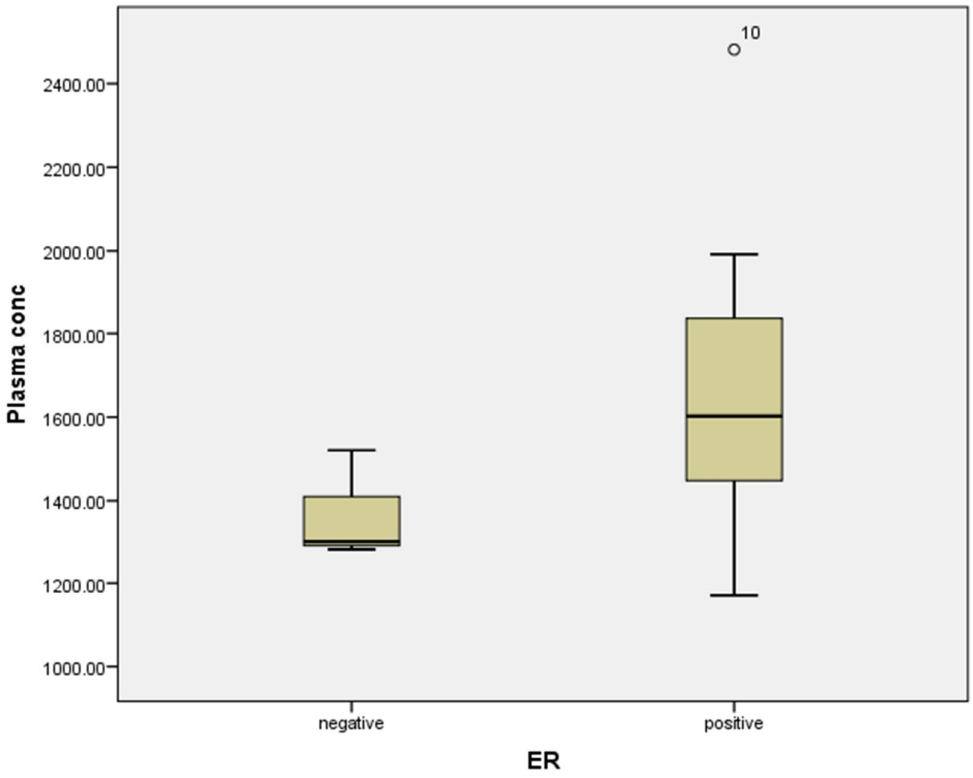
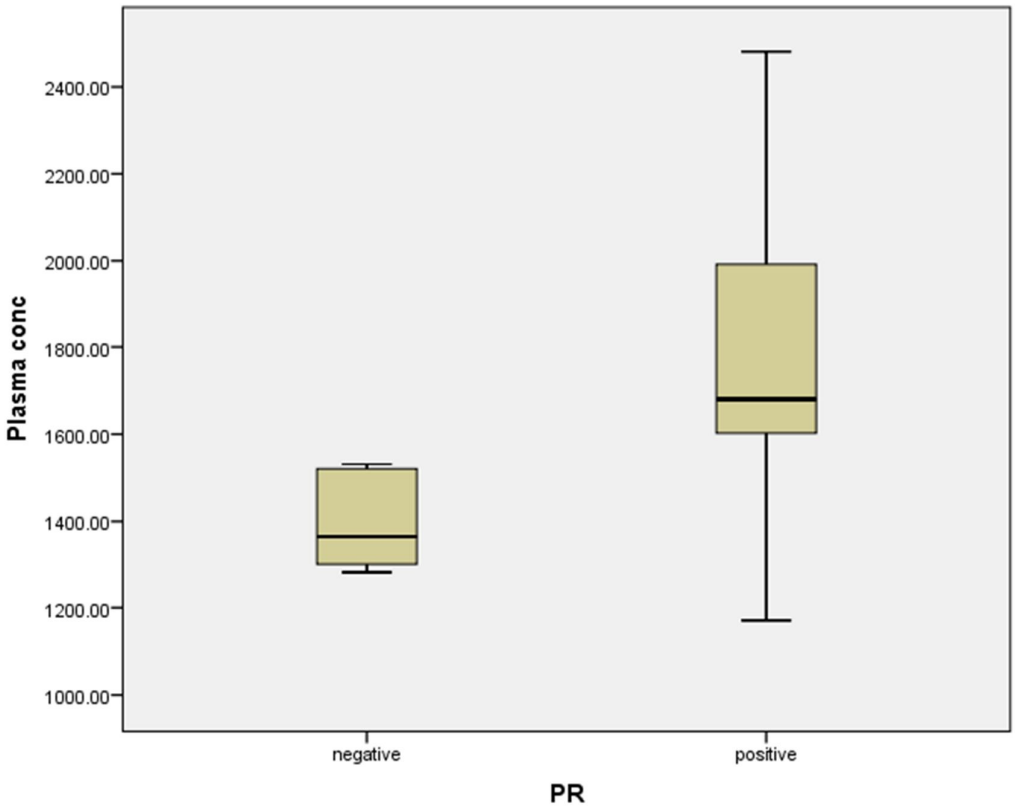
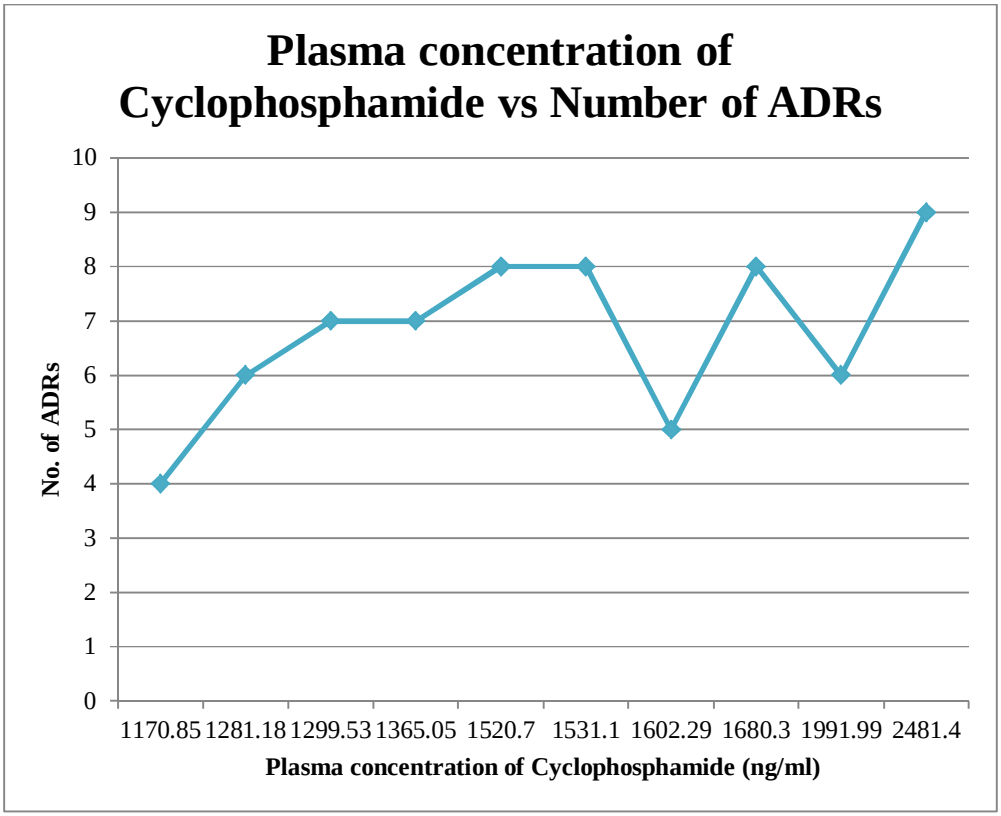


Figure.13 Plasma concentration of Cyclophosphamide vs Progesterone receptor (PR)



The plasma concentration of Cyclophosphamide was also found to have no influence on the number of adverse drug reactions reported in these patients. However, there was a positive correlation (r=0.529) with a p value of 0.115. (figure 14)

Figure.14 Plasma concentration of Cyclophosphamide vs number of adverse drug reactions (ADRS)



Paclitaxel

All the patients who were enrolled in Paclitaxel group were receiving only adjuvant chemotherapy. Hence tumour size reduction could not be assessed in these patients.

We had 7 ER+ and 3 ER- patients receiving third cycle of Paclitaxel in our study. The mean plasma concentration in ER+ patients was 3165.7657 ng/ml while that in ER- patients was 2740.8533 ng/ml (figure 15) which does not show any significant correlation.

There were 6 PR+ and 4 PR- patients in the Paclitaxel group.The mean plasma concentration in PR+ patients was 2754.3817 ng/ml while that in PR- patients was 3464.1575 ng/ml, (figure 16) which does not show any significant correlation thereby concluding that the hormonal status does not have any influence on the plasma concentration of the Paclitaxel as like the other two drugs in these patients.

Figure.15 Plasma concentration of Paclitaxel vs Estrogen receptor (ER)

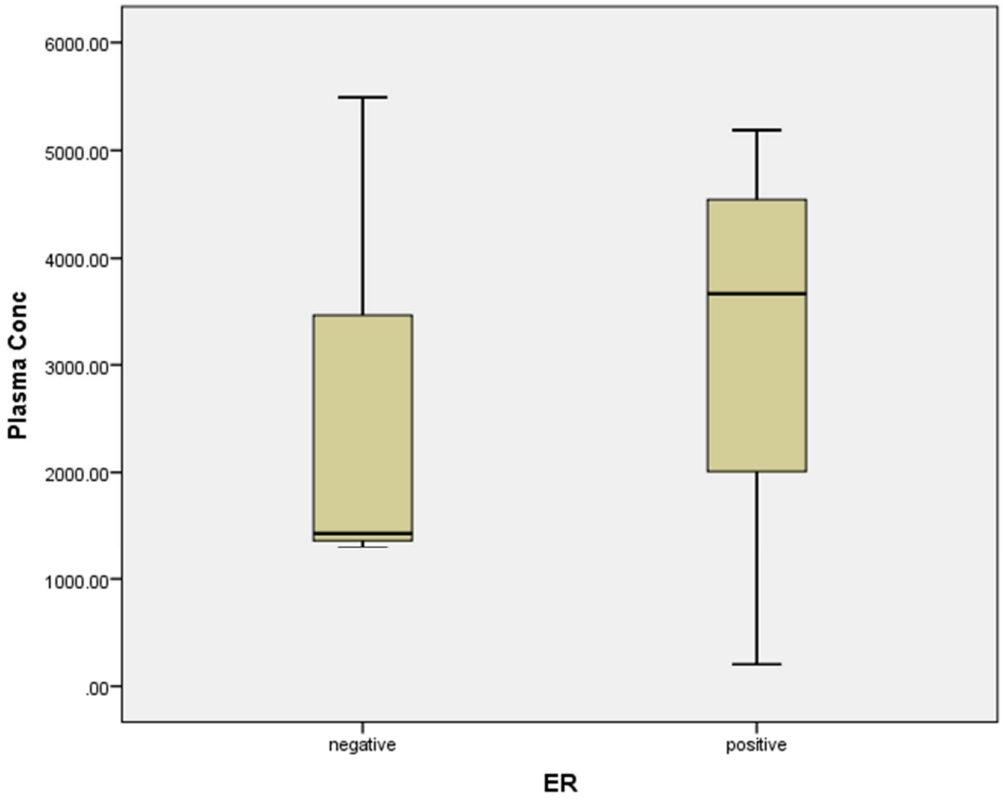
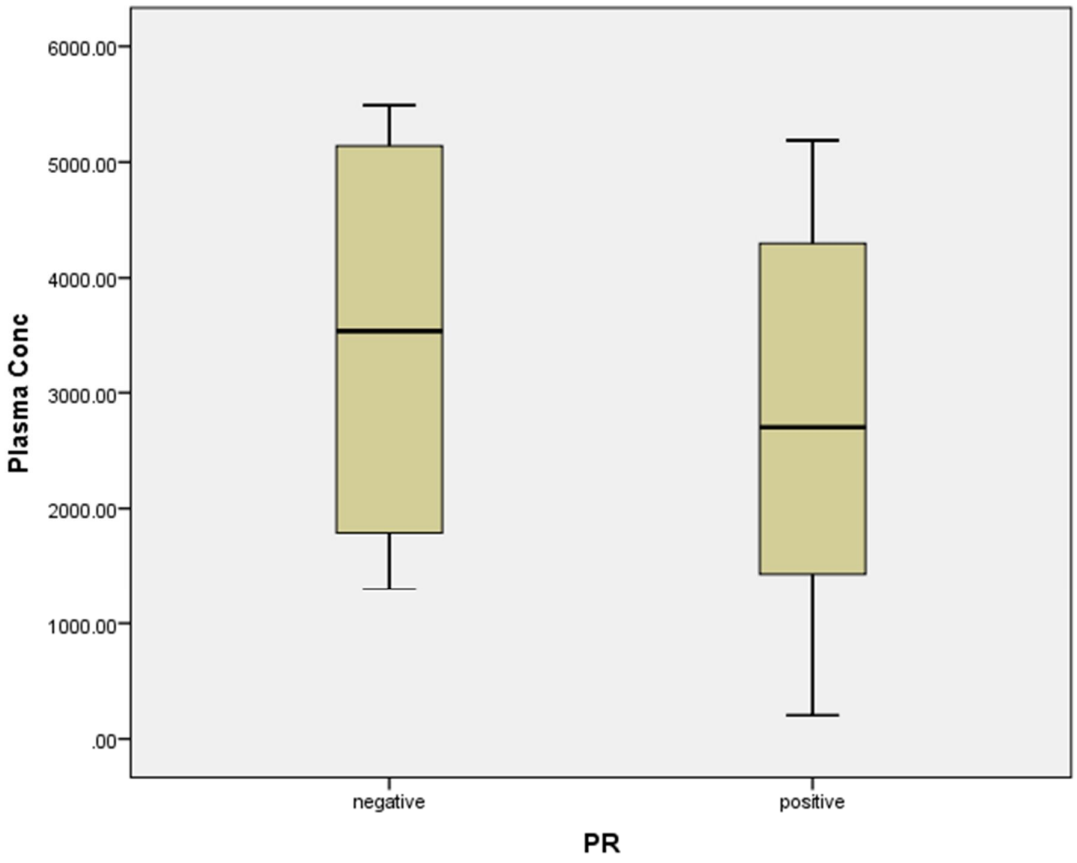
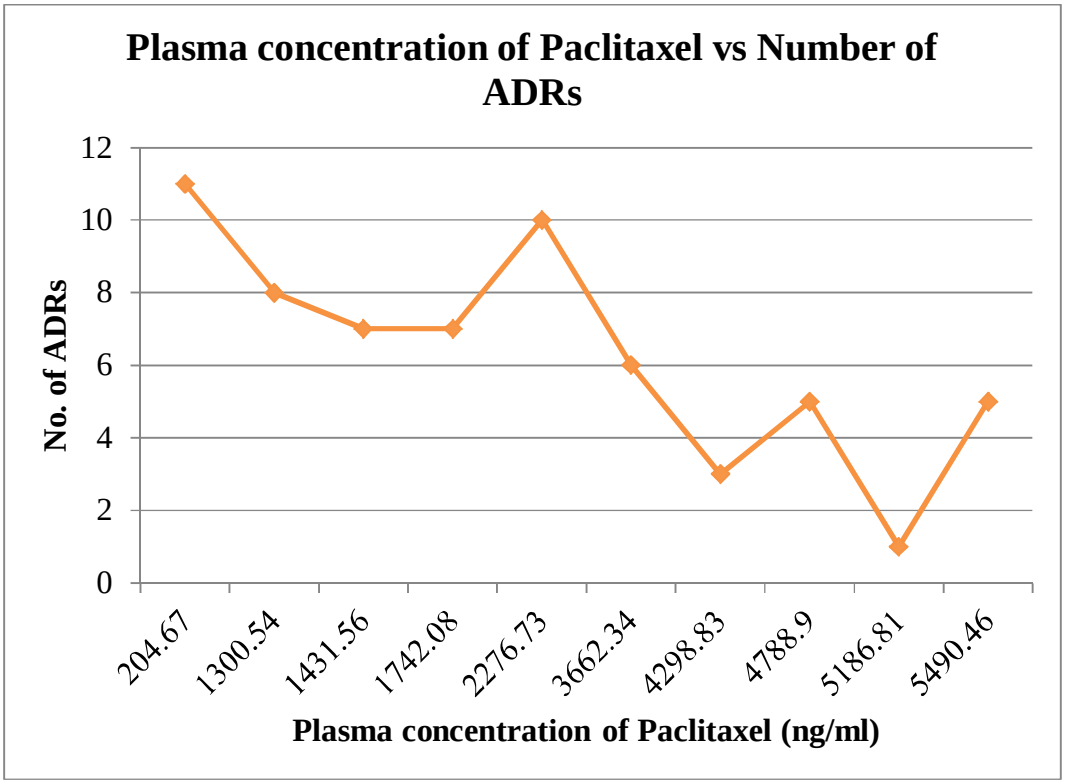


Figure.16 Plasma concentration of Paclitaxel vs Progesterone receptor (PR)



We found that there was significant negative correlation (r=-0.831, p= 0.003) between plasma concentration of Paclitaxel and the number of adverse drug reactions reported. Hence the plasma concentration of the drug has an influence on the number of adverse drug reactions in these patients. (figure 17)

Figure.17 Plasma concentration of Paclitaxel vs number of adverse drug reactions (ADRs)



We also studied the association of plasma concentration of these drugs with the dose administered and BSA which does not show any significant correlation expect for Adriamycin, which showed a better dose-concentration relationship (r= 0.693, p value = 0.026). and the common adverse drug reactions reported with each of the three drugs. (figure 18 - 26)

Figure.18 Dose vs Plasma concentration of Adriamycin

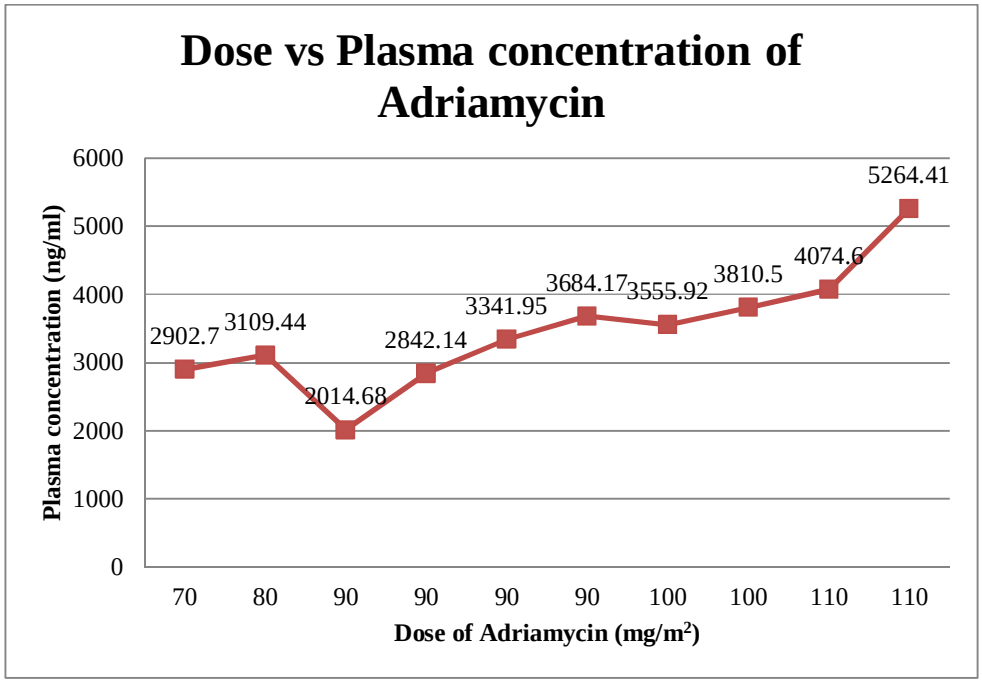


Figure.19 BSA vs Plasma concentration of Adriamycin

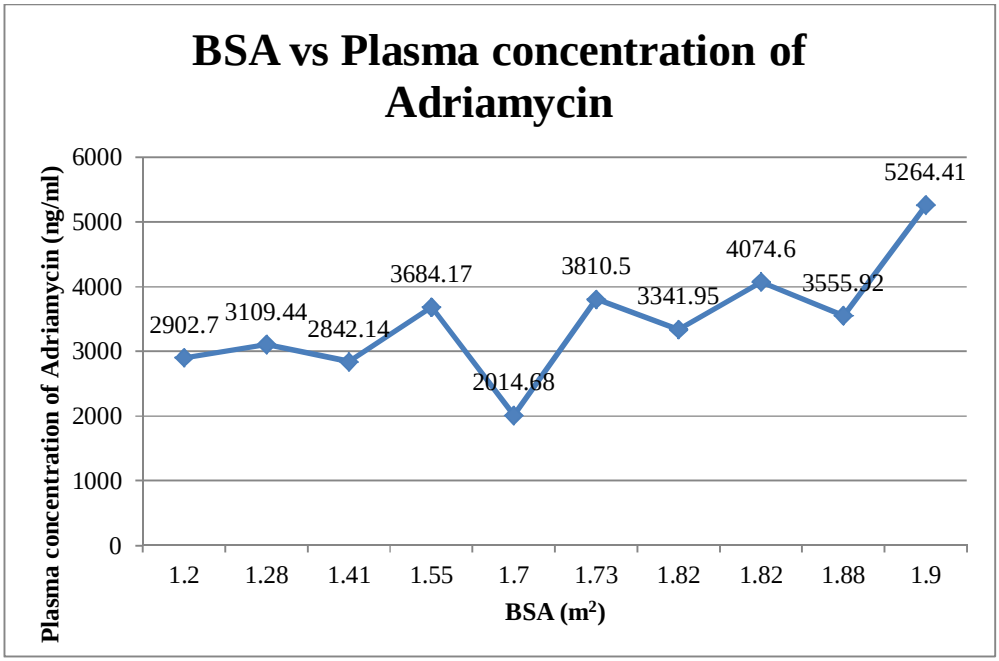


Figure.20 Common adverse drug reactions (ADRs) observed with Adriamycin

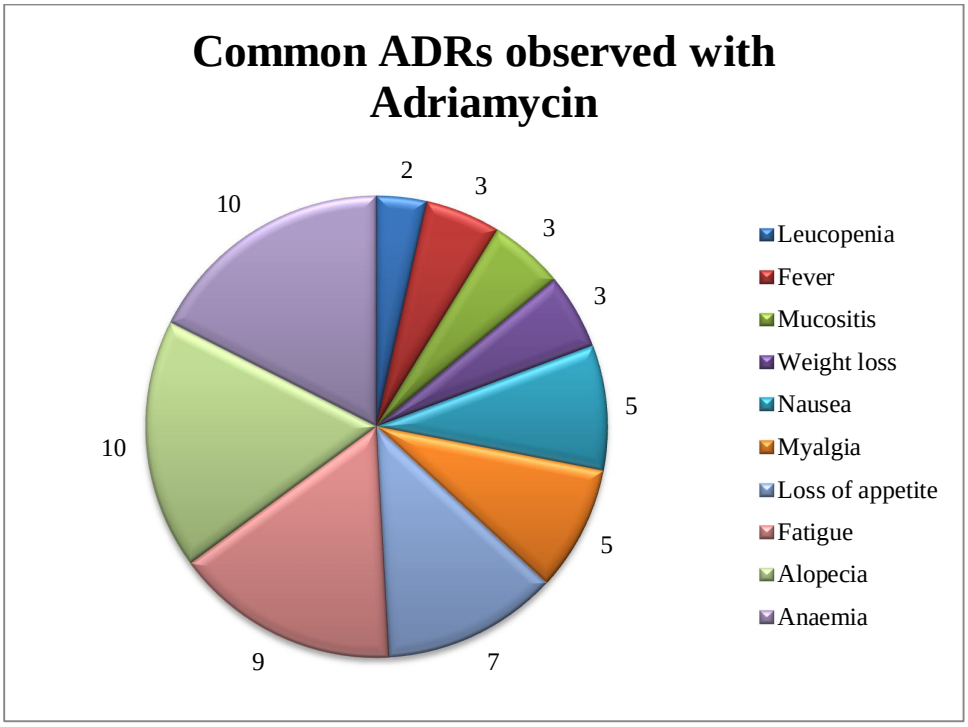


Figure.21 Dose vs Plasma concentration of Cyclophosphamide

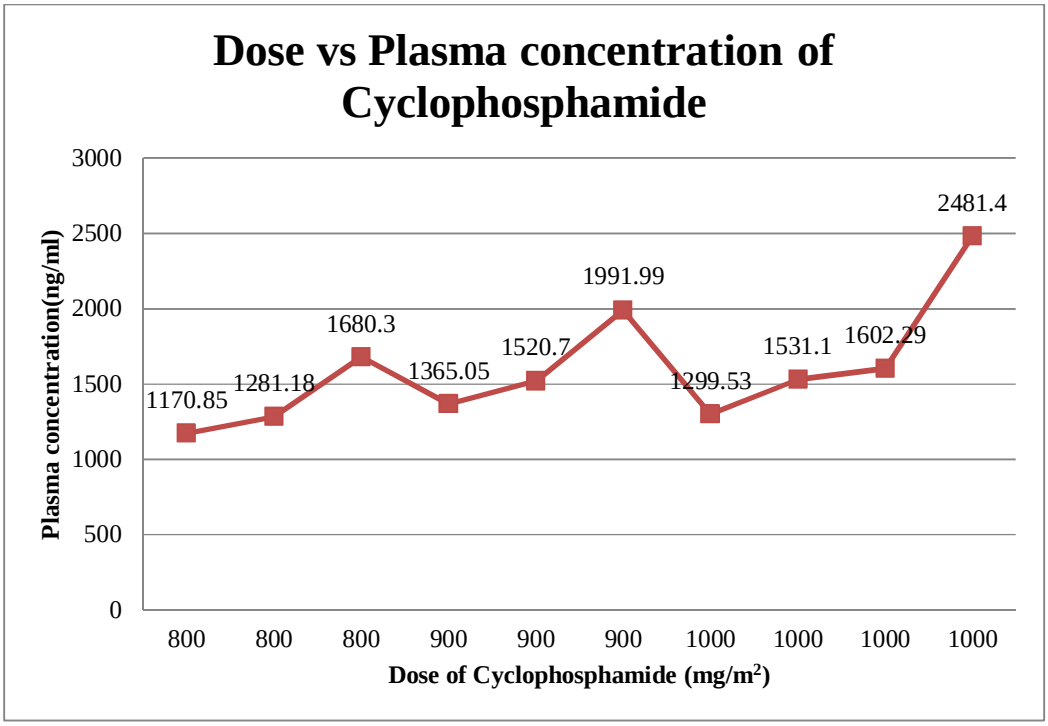


Figure.22 BSA vs Plasma concentration of Cyclophosphamide

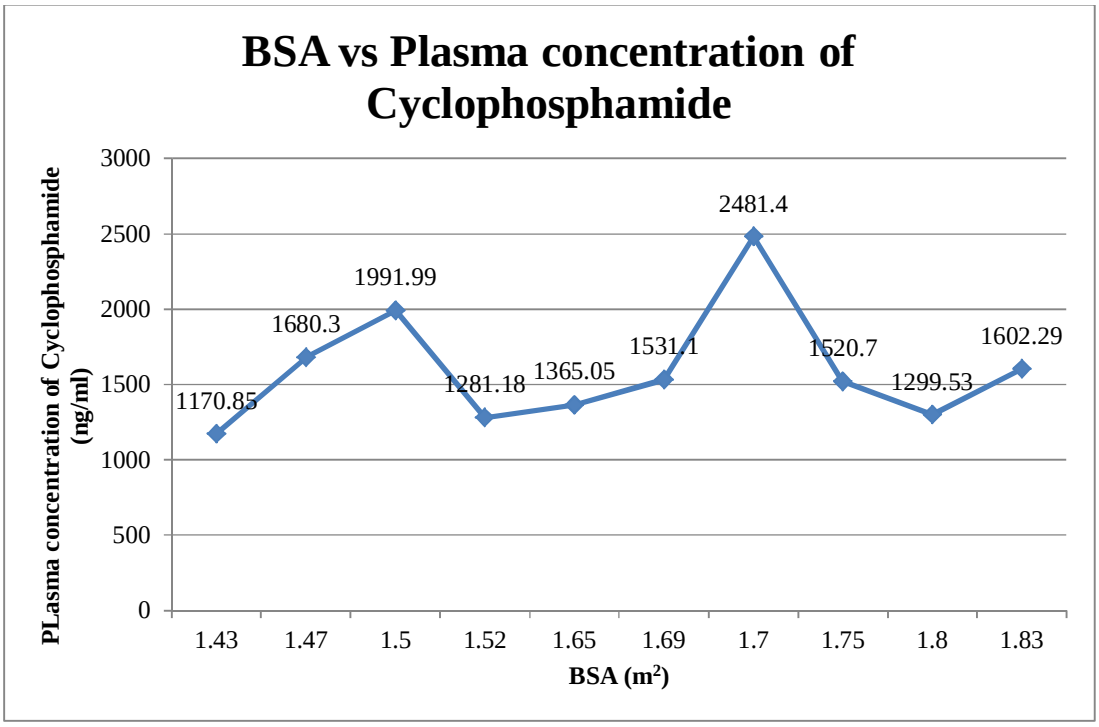


Figure.23 Common adverse drug reactions (ADRs) observed with Cyclophosphamide

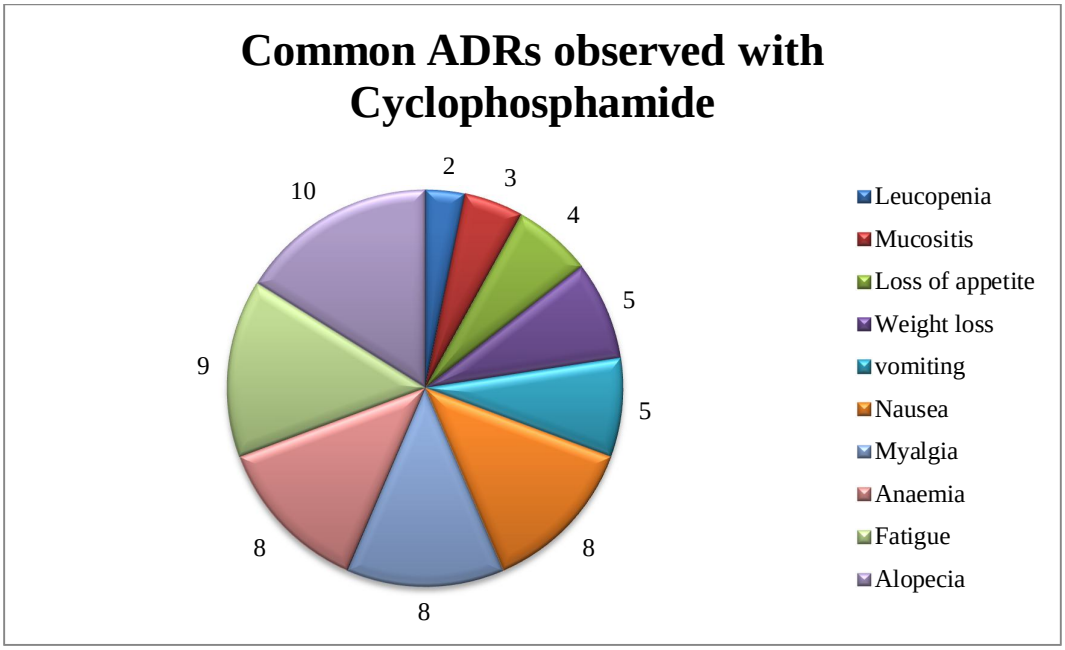


Figure.24 Dose vs Plasma concentration of Paclitaxel

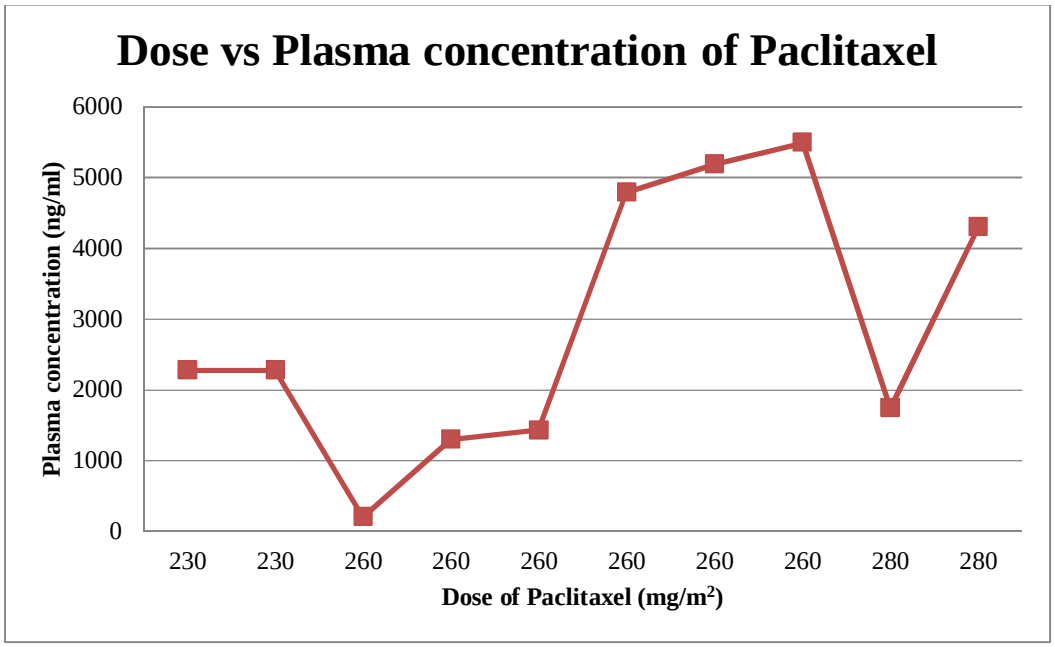


Figure.25 BSA vs Plasma concentration of Paclitaxel

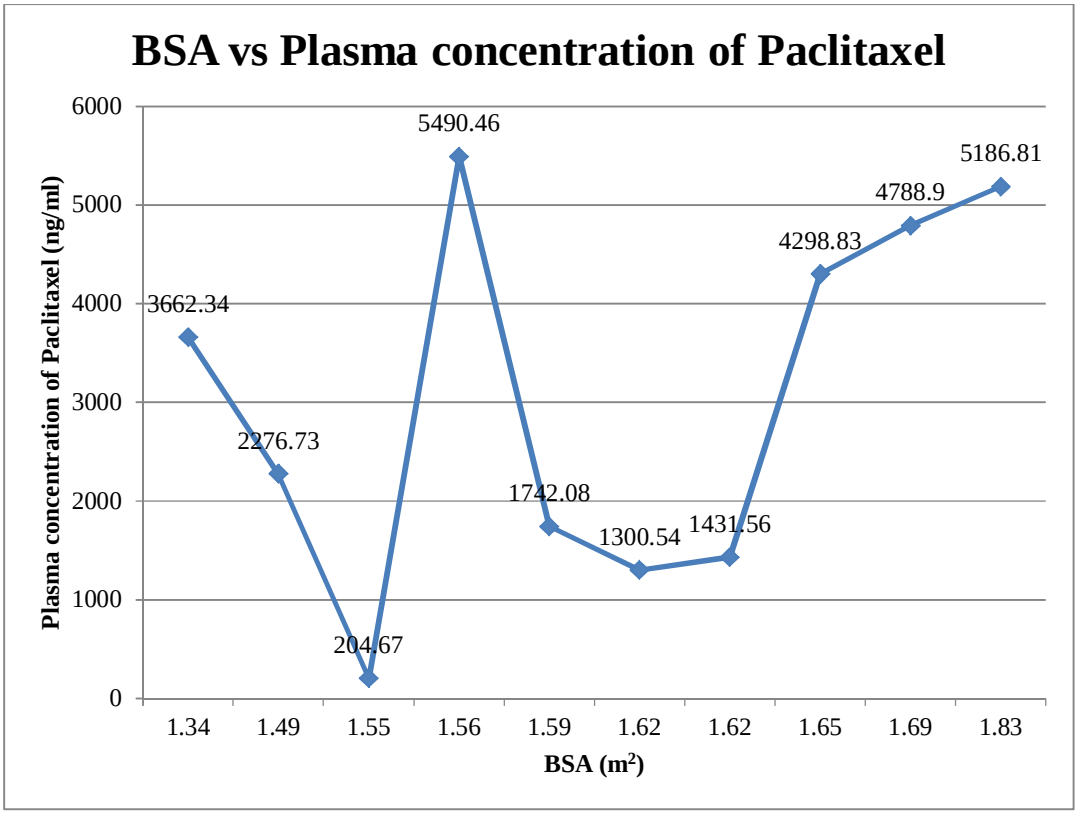
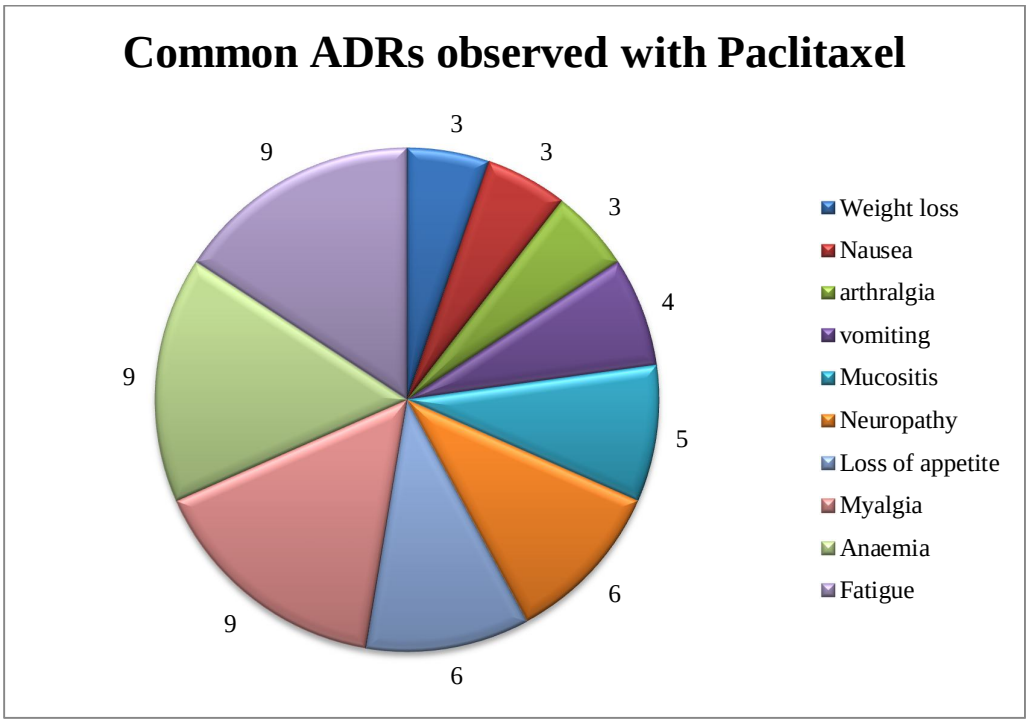


Figure.26 Common adverse drug reactions (ADRs) observed with Paclitaxel



Hence in this study we found that the plasma concentration of the drugs Adriamycin, Cyclophosphamide and Paclitaxel in breast cancer patients showed inter-individual variations which were consistent with the previous studies. No significant influence of plasma concentration of these chemotherapeutic drugs was found with the other clinical, biochemical and hormonal parameters.

6. DISCUSSION

In our study all the patients (n=30) were given the respective chemotherapeutic drugs based on the body surface area (BSA). The mean BSA was almost similar for the 3 different groups (1.60, 1.63 & 1.59 m^2 for Adriamycin, Cyclophosphamide and Paclitaxel respectively). (table 2) And all the venous blood samples were collected at the same point of time i.e at the end of infusion of each drug which was 15 minutes for Adriamycin, 60 minutes for Cyclophosphamide and 3 hours for Paclitaxel. (table 1)

Adriamycin

The plasma concentration of patients on Adriamycin ranged from 2014.68 to 5264.41 ng/ml and the mean plasma concentration was 3460.05 ng/ml. (table 3) Since the patients were dosed based on BSA, the patients were administered five different doses of drug ranging from 70 – 110 mg/m^2. Hence relating the dose and BSA to the plasma concentration, we found that there was a statistically significant correlation between dose and plasma concentration (r= 0.693, p value = 0.026). (figure 18) Though there was a correlation between BSA and plasma concentration, it was not statistically significant. (r =0.548, p value = 0.101). (figure 19)

The statistical analysis of plasma concentration with LFT & RFT parameters was done by using Pearson correlation which does not show any significant correlation between these parameters (p value >0.05) (table 4) thus rejecting

the null hypothesis, indicating that there is no influence of plasma concentration of Adriamycin on the liver and renal functions of these patients. In our study, all the 10 patients in the Adriamycin group had normal liver function tests, except for one patient, who had a marginal rise in SGOT (39 U) and had a plasma concentration of 2842.14 ng/ml.

A study by Christopher J et al., has shown that patients with isolated increase in SGOT levels had reduced Adriamycin clearance and increased exposure, which though not significant is consistent with our results. Several other studies have reported no correlation of liver dysfunction on Adriamycin pharmacokinetics.[115]

As with many anti-cancer drugs, plasma pharmacokinetics of Adriamycin also exhibits inter-individual variability which is also evident in our study. The pharmacokinetic variability of drugs may be clarified based on elucidation of metabolic pathways.[116] About 3 to 10-fold variations in systemic exposure have been reported, even in patients with normal liver and renal functions.[117]

A similar multicentre study by Gilles F et al., on population pharmacokinetics of Adriamycin, Etoposide and Ifosfamide in patients with small cell lung cancer showed no significant impact of covariates like age, BSA, creatinine, SGOT, SGPT, total bilirubin on individual pharmacokinetics of drugs and all patients had normal parameters.[117]

The number of patients who received neoadjuvant chemotherapy was very small. Only 3 out of 10 patients were analysed for reduction in tumour size, which does not show any significant correlation with plasma concentration, probably because of the small effect size. However, all 3 patients showed reduction in tumour size. Patient with a plasma concentration of 2902.7 ng/ml had a reduction in tumour size of 85.7% when compared to 70% reduction in a patient with a high concentration of 5264.41ng/ml. (figure 7)

There is a paucity of data on the association of plasma concentration with the status of hormone receptors (ER/PR) of the patient. We had 4 ER+ and 6 ER- patients receiving Adriamycin and mean plasma concentration in ER+ patients was 3180.76 ng/ml while that in ER- patients was 3646.2433 ng/ml (figure 8). There were 3 PR+ and 7 PR- patients on Adriamycin and the mean plasma concentration in PR+ patients was 2970.85 ng/ml while that in PR- patients was 3669.7086 ng/ml. (figure 9) It was found that the ER/PR status does not have any significant influence on the plasma concentration of the drug in these patients ($r = 0.278$ & p value = 0.437 for ER and $r = 0.390$ & p value = 0.265 for PR).

The average number of adverse drug reactions reported in patients receiving Adriamycin was 6. The most common adverse effects reported were alopecia, fatigue, anaemia and loss of appetite. (figure 20) No case of cardiotoxicity was reported. Further, there was no significant effect of the plasma concentration of Adriamycin on the number of adverse drug reactions reported. (figure 10)

Cyclophosphamide

The plasma concentration of patients on Cyclophosphamide ranged from 1170.85 to 2481.40 ng/ml and the mean plasma concentration was 1592.44 ng/ml.(table 5) Since the patients were dosed based on BSA, the patients were administered three different doses of drug ranging from 800 - 1000 mg/m^2.(table 5) Hence relating the dose and BSA to the plasma concentration, we found that there was only a weak correlation between these parameters which was was not significant. (figure 21,22)

The statistical analysis of plasma concentration of Cyclophosphamide with liver and renal function parameters was done using Pearson correlation which does not show any significant correlation between these parameters (p value >0.05) (table 6) thus rejecting the null hypothesis stating that there is no influence of plasma concentration of Adriamycin on the liver and renal functions of these patients.

Out of 10 patients, 8 patients had normal liver and renal function tests. One patient had SGOT of 49 U, with a low plasma concentration of 1281.18 ng/ml on a dose of 800 mg. The same patient had developed moderate anaemia with the haemoglobin level of 9.4 g/dl which could be explained on the basis of the increased rate of conversion of the drug into its active metabolites, causing hepatotoxicity and hemotological toxicity.[118]

We also had one patient with marginally raised serum creatinine with a plasma concentration of 1531.10 ng/ml. However, all other biochemical parameters were within normal limits.

It has been shown that large inter-patient variation in clinical effect exist with cyclophosphamide therapy and has been proposed that these variations in both efficacy and toxicity may reflect inter-patient differences in metabolism and distribution of the drug which is incompletely understood because of its complexity.[118] Randomised studies on cyclophosphamide-based standard chemotherapeutic regimens have demonstrated conflicting results while correlating dose and efficacy.[119]

We had only four patients who were receiving Cyclophosphamide as a part of neoadjuvant chemotherapy. All the 4 patients had reduction in the tumour size. The mean reduction in tumour size was 86.5%. Though there was a positive correlation between the plasma concentration and tumour size reduction, it was not statistically significant. (figure 11)

As mentioned earlier, the establishment of dose–response relationship is not only hampered by inter-individual variability in pharmacokinetics & pharmacodynamics, but also by the complex metabolism of Cyclophosphamide. Further, there are only a few data relating the plasma concentration of drug to efficacy and toxicity.[120]

We had 7 ER+ and 3 ER- patients receiving third cycle of Cyclophosphamide in our study and the mean plasma concentration in ER+ patients was 1688.9971 ng/ml while that in ER- patients was 1367.1367 ng/ml. (figure 15) There were 5 PR+ and 5 PR- patients and the mean plasma concentration in PR+ patients was 1785.3660 ng/ml while that in PR- patients was 1399.5120 ng/ml. (figure 16) We did not find any significant correlation between the two parameters, thereby concluding that the hormonal status does not have any influence on the plasma concentration of the cyclophosphamide in these patients. As with Adriamycin, we could not find any literature evidence to support our results.

The mean number of adverse drug reactions reported in patients receiving Cyclophosphamide was 7. The most common adverse effects reported were alopecia, fatigue, anaemia and myalgia. (figure 23) Further, there was no significant effect of the plasma concentration of Adriamycin on the number of adverse drug reactions reported (r= 0.529 & p value = 0.115).(figure 14) We had one patient reported with hyperpigmentation of dermal creases.

Paclitaxel

The plasma concentration of patients on Paclitaxel ranged from 204.67 to 5490.46 ng/ml and the mean plasma concentration was 3038.29 ng/ml. (table 7) Since the patients were dosed based on BSA, the patients were administered four different doses of drug ranging from 230 - 280 mg/m^2. Hence relating the dose and BSA to the plasma concentration, we did not find any statistically significant correlation between these parameters. (figure 24,25)

There was a large inter-patient variability in plasma concentration of Paclitaxel which was consistent with few other studies.[121] We had one patient with a unexpected low plasma concentration of 204.67 ng/ml, inspite of the repeated and verified assays. she did not have any co-morbidities nor had any drug-drug interactions lowering the plasma concentration of the drug. The sampling schedule was also respected. This could be explained by rapid distribution of the drug into body fluids and tissues due to its large volume of distribution[122] or by genetic polymorphisms in CYP enzymes involved in the metabolism of the drug.[123]

The correlation between plasma concentration of Paclitaxel and liver and renal function of patients was done using Pearson correlation which failed to show any significance. Hence the plasma concentration of Paclitaxel does not show any influence on these two parameters. (Table 8)

All the patients had normal liver and renal function tests except one patient who had raised serum transaminases (SGPT – 61U & SGOT 54U) but with normal bilirubin levels and her plasma concentration was 4298.83 ng/ml. Data on influence of liver dysfunction and pharmacokinetics is limited. However, few studies have shown that patients with hepatic failure have increased incidence of haematological and non-haematological toxicities since the major route of elimination of Paclitaxel is through hepato-biliary pathway.[124]

All the patients who were in Paclitaxel group were receiving only adjuvant chemotherapy. Hence tumour size reduction could not be assessed in these patients.

We had 7 ER+ and 3 ER- patients receiving third cycle of Paclitaxel in our study. The mean plasma concentration in ER+ patients was 3165.7657 ng/ml while that in ER- patients was 2740.8533 ng/ml (figure 15). There were 6 PR+ and 4 PR- patients in the Paclitaxel group.The mean plasma concentration in PR+ patients was 2754.3817 ng/ml while that in PR- patients was 3464.1575 ng/ml, (figure 16). Both ER and PR status does not show any significant correlation thereby concluding that the hormonal status does not have any influence on the plasma concentration of the Paclitaxel as like the other two drugs in these patients.

The mean number of adverse drug reactions reported in patients receiving Paclitaxel was 7. The most common adverse effects reported fatigue, anaemia, myalgia and neuropathy. (figure 26) We found a strong negative correlation (r = -0.831) between the plasma concentration of the drug and number of adverse drug reactions reported which was statistically significant (p value = 0.003) (figure 17) which could be explained by rapid distribution of the drug into body fluids and tissues due to large volume of distribution as mentioned earlier.[122]

We acknowledge that the major limitation of our study was a small sample size which failed to explain the influence of plasma concentration of the drugs on the biochemical, immunological and toxicity profiles of these patients.

Furthermore, in order to get a clearer picture about the relevance of pharmacokinetic parameters to other clinical covariates, we need 1) sampling at various time intervals, 2) development of assay techniques to simultaneously measure the metabolite concentrations, which are known to influence both the therapeutic response and toxicity and 3) simultaneous pharmacogenetic studies to determine the influence of genetic polymorphisms on these PK-PD parameters and other clinical covariates. When studying a polychemotherapy regimen, several PK parameters must be considered with greater number of patients to reach an acceptable level of significance.

However, as mentioned earlier there are only a limited number of studies addressing the pharmacokinetics of chemotherapeutic drugs and their relationship with other clinical and biochemical parameters especially in south Indian population. Though there are few studies on pharmacokinetics of individual drugs, there is a paucity of studies on pharmacokinetics of AC/T regimen as a whole, linking the plasma concentration of all three drugs with the other parameters. Further larger studies are needed with more pharmacokinetic and pharmacodynamic parameters to optimise and individualize chemotherapeutic drug dosages in these patients.

6. CONCLUSION

Our study shows that the plasma concentration of the chemotherapeutic drugs Adriamycin, Cyclophosphamide and Paclitaxel shows inter-individual variations in breast cancer patients, which was consistent with the previous studies. The results indicate that plasma concentration alone is not a determinant of clinical outcome or toxicities in these patients. Dose optimisation has to be validated prospectively in a large group of patients with more pharmacokinetic-pharmacodynamic parameters.

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ABBREVIATIONS

AC/T	-	Adriamycin, Cyclophosphamide, Paclitaxel
ADR	-	Adverse drug reaction
BSA	-	Body surface area
CBC	-	Complete blood count
EDTA	-	Ethylene diamine tetra acetic acid
ER	-	Estrogen receptor
GBD	-	Global burden of disease
HER 2	-	Human epidermal growth factor receptor 2
HPLC	-	High performance liquid chromatography
HTCA	-	Human tumor clonogenic assay
NK	-	Natural killer cells
PR	-	Progesterone receptor
QC	-	Quality control
SDI	-	Succinic dehydrogenase inhibition test
SGPT	-	Serum glutamic pyruvic transaminase
SGPT	-	Serum oxaloacetic transaminase
SIOG	-	Society of geriatric oncology
TIA	-	Thymidine incorporation assay

A STUDY ON THE INFLUENCE OF PLASMA CONCENTRATIONS OF ADRIAMYCIN, CYCLOPHOSPHAMIDE AND PACLITAXEL ON CLINICAL OUTCOME OF PATIENTS WITH BREAST CANCER IN A TERTIARY CARE HOSPITAL

Dr. M. S. Yamuna devi., Dr. K. Bhuvaneswari.

CASE REPORT FORM

Patient name: IP/OP no:

Age/Gender: Address:

Contact number:

Height: Weight: BMI:

Occupation (Current &Past):

Ethnicity (Religion/caste)

Past History:

H/o Hypertension, Diabetes mellitus, Cardiovascular, Liver and Renal disorders, other cancers.

H/o radiation to chest

Personal History:

Smoking/Alcohol -

Other Substance abuse-

Family History:

H/O breast cancer in relatives (especially in first degree relatives – mother, sister, daughter)

Menstrual History:

LMP:

(Including age at menarche, menopause attained/not)

Marital History:

Married since _____ years

Consanguinous marriage –

Obstetric History:

Obstetric code:

First child birth at:

Breastfeeding H/o:

Use of OCPs:

Disease related details:

Ca breast:

Complaints of:

Duration of disease:

Size of the tumour at the start of the regimen (by clinical examination):

Size of the tumour at present:

% reduction in tumour size:

Surgery done:

No. of cycles of chemotherapy completed (with the drug):

Adverse drug reactions to previous cycles:

- Nausea
- Vomiting
- Fatigue

- Myalgia
- Arthralgia
- Fever
- Mouth sores
- Urticaria
- Bronchospasm
- Alopecia
- Neuropathy
- Loss of appetite
- Others (specify)

Laboratory investigations:

LFT:

RFT:

Hormone receptors:

Others:

PSG INSTITUTE OF MEDICAL SCIENCE AND RESEARCH, COIMBATORE

Institutional Human Ethics Committee

INFORMED CONSENT FOR RESEARCH PROJECTS

I **Dr.M.S.Yamuna devi** am carrying out a study on the topic**: A study on the influence of plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel on clinical outcome of patients with breast cancer in a tertiary care hospital** as part of my research project being carried out under the aegis of the Department of Pharmacology.

My research guide is**: Prof. Dr. K. Bhuvaneswari**

The justification for this study is: Determining the plasma concentration of the drugs (Adriamycin, Cyclophosphamide and Paclitaxel) and correlating it with the clinical outcome and adverse drug reactions allows individualisation of drug dosage thereby reducing its adverse effects.

The objectives of this study are:

Primary Objective:

To study the influence of liver and renal functions of patients with Ca breast on the plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel.

Secondary Objective:

To study the relationship between the plasma concentrations of Adriamycin, Cyclophospamide and Paclitaxel and the clinical reduction in tumour size of patients with Ca breast.

Tertiary Objective:

To study the role of estrogen and progesterone receptors on plasma concentrations of Adriamycin, Cyclophosphamide and Paclitaxel and related ADRs in patients with Ca breast.

Sample size: 30 patients

Study participants: Ca breast patients on AC/T regimen attending Oncology OP and IP in PSG Hospital

Location: PSGIMSR, Coimbatore

We request you to kindly cooperate with us in this study. We propose to collect background information and other relevant details related to this study. We will be carrying out:

Initial interview: 10 to 15 minutes.

Data collected will be stored for a period of fifteen years. We will / will not use the data as part of another study.

Blood sample collection: 2 ml directly from patient or from left over samples in Pathology and Biochemistry labs, these collected samples will not be used for any other purposes

No. of times it will be collected: **Once**

Whether blood sample collection is part of routine procedure or for research (study) purpose: **Research purpose**

Specify **purpose**, discomfort likely to be felt and side effects, if any: **To do Liver and Renal function tests and to determine plasma concentration of drugs (Adriamycin, Cyclophospamide and Paclitaxel). No discomfort or side effects.**

Whether blood sample collected will be stored after study period**: No**

Case details and data will be stored for **5 yrs**

Whether blood sample collected will be sold: **No**

Whether blood sample collected will be shared with persons from another institution: **No**

Medication given, if any, duration, side effects, purpose, benefits**: No medications**

Benefits from this study: Determining the plasma concentration of the drugs allows individualisation of drug dosage thereby reducing its adverse effects

Risks involved by participating in this study: **No risks**

How the **results** will be used: the results will be used for **further researches and publications**

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at any time.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study,

developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer /

Legal Representative:

Signature of the Interviewer with date:

Witness:

பூ. சா. கோ மருத்துவக் கல்லூரி மற்றும் ஆராய்ச்சி நிறுவனம், கோவை

மனித நெறிமுறைக் குழு

ஒப்புதல் படிவம்

தேதி:

மரு. எம், எஸ். யமுனாதேவி, ஆகிய நான் PSG மருத்துவக் கல்லூரியின் / மருத்துவமனையின் மருந்தியல் துறையின் கீழ், "ஒரு மூன்றாம் நிலை மருத்துவ கல்லூரி மருத்துவமனையில், மார்பக புற்றுநோயால் பாதிக்கப்பட்ட நோயளிகளின் அட்ரியாமைசின், சைக்லோபாஸ்பமைட் மற்றும் பாக்லிடாக்சல் ஆகிய மருந்துகளின் ப்ளாஸ்மா அளவு மற்றும் அதனால் நோயின் தன்மையில் ஏற்படும் மாற்றம்" என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி: மரு. கே. புவனேஸ்வரி, மரு. விக்னேஷ் கந்தகுமார்

ஆய்வு மேற்கொள்வதற்கான அடிப்படை:

அட்ரியாமைசின், சைக்லோபாஸ்பமைட் மற்றும் பாக்லிடாக்சல் மருந்துகளின் ப்ளாஸ்மா அளவை கண்டறிந்து, அதனால் நோயின் தன்மையில் ஏற்படும் மாற்றத்தை அறிதல்.

ஆய்வின் நோக்கம்:

அட்ரியாமைசின், சைக்லோபாஸ்பமைட் மற்றும் பாக்லிடாக்சல் மருந்துகளின் ப்ளாஸ்மா அளவை பொருத்து,

1. கல்லீரல் மற்றும் சிறுநீரக செயல்பாடுகளில் ஏற்படும் மாற்றம்.
2. புற்றுநோய் கட்டியின் அளவில் ஏற்படும் மாற்றம்.
3. ஈஸ்ட்ரோஜன் மற்றும் ப்ராஜஸ்ட்ரோன் ரிசப்டார்கள் இம்மருந்துகளின் ப்ளாஸ்மா அளவில் ஏற்படுத்தும் மாற்றங்கள், மற்றும் இம்மருந்துகளின் பக்க விளைவுகளைப் பற்றிய ஆய்வு.

ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை: 30

ஆய்வில் பங்கு பெறுவோர்மற்றும் வயது: மார்பக புற்றுநோயால் பாதிக்கப்பட்ட நோயாளிகள் (30 வயதிலிருந்து 65 வயது வரை)

ஆய்வு மேற்கொள்ளும் இடம்: பூ. சா. கோ மருத்துவ கல்லூரி மருத்துவமனை, கோயமுத்தூர்

இந்த ஆய்வில் எங்களுடன் ஒத்துழைக்குமாறு கேட்டுக்கொள்கிறோம். நாங்கள் சில தகவல்களை இந்த ஆய்விற்காக சேகரிக்க உள்ளோம்.

ஆய்வு செய்யப்படும் முறை:

முதன்மை நேர்காணல்: **10-15 நிமிடங்கள்**

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் **5 வருடங்கள்** பாதுகாக்கப்படும். இந்த தகவல்கள் வேறு ஆய்விற்குப் பயன்படுத்தப் படும்/பயன்படுத்தப் பட மாட்டாது.

மருத்துவ பரிசோதனைகள்:

இரத்த மாதிரி சேகரிப்பு: 2 மிலி, ஒருமுறை

இரத்த மாதிரி எடுப்பது வழக்கமான சிகிச்சைக்காகவோ அல்லது இந்த ஆய்விற்காகவோ: குறிப்பிட்ட ஆய்விற்காக

இதனால் ஏற்படக் கூடிய அசௌகரியங்கள் / பக்க விளைவுகள்: இதனால் எந்த அசௌகரியமோ, பக்க விளைவுகளோ ஏற்படாது.

இரத்த மாதிரிகள் ஆய்விற்குப் பின் பாதுகாத்து வைக்கப்படுமா? ஆம் / இல்லை, அழிக்கப்படும்: **இல்லை**

சேகரிக்கப்பட்ட இரத்தம் விற்கப்படுமா? ஆம் / இல்லை **இல்லை**

சேகரிக்கப்பட்ட இரத்தம் வேறு நிறுவனத்துடன் பகிர்ந்து கொள்ளப்படுமா? ஆம் / இல்லை: **இல்லை**

மருந்துகள் ஏதேனும் கொடுக்கப் பட்டிருந்தால் அவை பற்றிய விவரம் (கொடுக்கப்பட்ட காரணம், காலம், பக்க விளைவுகள், பயன்கள்) **இல்லை**

மருந்துகள் கொடுக்கப்படுவது வழக்கமான சிகிச்சை முறையா? ஆம் / இல்லை (ஆம் என்றால் இந்த குறிப்பிட்ட மருந்து கொடுக்கப்பட்டதன் கரணம்)

ஆய்வில் பங்குபெறுவதால் ஏற்படும் பலன்கள்:
இம்மருந்துகளின் ப்ளாஸ்மா அளவுகளை கண்டறிவதன் மூலம் நோயின் தன்மையில் ஏற்படும் மாற்றத்தை அறிய்லாம். மேலும் பக்க விளைவுகள் வராதவாறு சரியான முறையில் மருந்துகளை செலுத்தலாம்.

ஆய்வின் முடிவுகள் எந்த முறையில் பயன்படுத்தப்படும்?
ஆய்வின் முடிவுகள், அடுத்தகட்ட ஆராய்ச்சிகளுக்கும், மருத்துவ ஆய்வு பத்திரிக்கைகளில் வெளியிடுவதற்கும் பயன்படுத்துப்படும்.

இந்த ஆய்வின் கேள்விகளுக்கு பதிலளிப்பதோ, இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுப்பதிலோ உங்களுக்கு ஏதேனும் அசௌகரியங்கள் இருந்தால், எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக்கொள்ளும் உரிமை உங்களுக்கு உண்டு. எப்பொழுது வேண்டுமானாலும் ஆய்விலிருந்து விலகும் உரிமை உங்களுக்கு உள்ளது. ஆய்விலிருந்து விலகிக்கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சை முறையில் எந்த வித பாதிப்பும் இருக்காது என்று உங்களுக்கு உறுதியளிக்கிறோம். மருத்துவ மனையில் நோயாளிகளுக்கு அளிக்கப்படும் சேவைகளை நீங்கள் தொடர்ந்து பெறலாம். இந்த ஆய்வில் பங்கேற்க ஒப்புக்கொள்ளுவதால் வேறு எந்த விதமான கூடுதலான பலனும் உங்களுக்குக் கிடைக்காது. நீங்கள் அளிக்கும் தகவல்கள் இரகசியமாக வைக்கப்படும். ஆய்வில் பங்கேற்பவர்கள் பற்றியோ அவர்கள் குடும்பத்தைப் பற்றியோ எந்தத் தகவலும் எக்காரணம் கொண்டும் வெளியிடப்படாது என்று உறுதியளிக்கிறோம். நீங்கள் அளிக்கும் தகவல்கள் / இரத்த மாதிரிகள் / திசு மாதிரிகள் அங்கீகரிக்கப்பட்ட ஆய்விற்கு மட்டுமே பயன்படுத்தப்படும். இந்த ஆய்வு நடைபெறும் காலத்தில் குறிப்பிடத்தகுந்த புதிய கண்டுபிடிப்புகள் அல்லது பக்க விளைவுகள் ஏதும் ஏற்பட்டால் உங்களுக்குத் தெரிவிக்கப்படும். இதனால் ஆய்வில் தொடர்ந்து பங்கு பெறுவது பற்றிய உங்கள் நிலைப்பாட்டை நீங்கள் தெரிவிக்க ஏதுவாகும்.

ஆய்வுக்குட்படுபவரின் ஒப்புதல்: இந்த ஆய்வைப் பற்றிய மேற்கூறிய தகவல்களை நான் படித்து அறிந்து கொண்டேன் / ஆய்வாளர் படிக்கக் கேட்டுத் தெரிந்து கொண்டேன். ஆய்வினைப் பற்றி நன்றாகப் புரிந்து கொண்டு இந்த ஆய்வில் பங்கு பெற ஒப்புக்கொள்கிறேன். இந்த ஆய்வில் பங்கேற்பதற்கான எனது ஒப்புதலை கீழே கையொப்பமிட்டு . கை ரேகை பதித்து நான் தெரிவித்துக் கொள்கிறேன்.

பங்கேற்பாளரின் பெயர், முகவரி:

பங்கேற்பாளரின் கையொப்பம் / கை ரேகை / சட்டப்பூர்வ பிரதிநிதியின் கையொப்பம்:

தேதி :

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வாளரின் தொலைபேசி எண்:

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண்:

அலுவலக நேரத்தில்0422 2570170 Extn.: 5808

அலுவலக் நேரத்திற்குப்பின்: 9865943043