THERAPEUTIC DRUG MONITORING OF SELECTED DRUGS: AN APPROACH TO DRUG THERAPY OPTIMIZATION

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DOCTOR OF PHILOSOPHY

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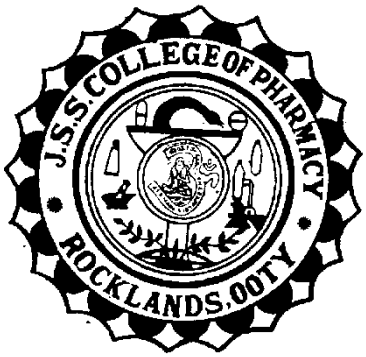

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

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"A desire to take medicine is the characteristic which distinguishes man from other animals." **WILLIAM OSLER**

1.1. BRIEF ON PHARMACIST ACTIVITIES[1]

The pharmacist's activities have moved from the traditional dispensing role to direct ambulatory patient care services since 1980s. Pursuing this patient care role in ambulatory care and primary care settings has increased job opportunities, positioned pharmacists in patient care areas, and changed the expectations and duties of pharmacists.

It is seen that among the pharmacists who work in ambulatory care positions 45% of the pharmacist's time is spent performing distributive functions, while 30% clinical and 21% of the pharmacist's time is spent performing administrative activities. Distributive functions of these pharmacists may be defined as filling and dispensing prescriptions, as well as preparing intravenous medications. The clinical portion of ambulatory care pharmacists includes a variety of activities, such as monitoring patient outcomes and compliance, conducting specialized clinics, providing *therapeutic drug monitoring* (TDM) services and, in some settings, practicing independently with prescriptive authority.

Clinical pharmacokinetics involves the application of pharmacokinetic principles to determine the optimal dosage regimens of specific drugs for specific patients to maximize pharmacotherapeutic effects and minimize toxic effects. Clinical pharmacokinetics emerged as a specialty in pharmacy practice in the late 1960s and early 1970s to provide clinical pharmacokinetic consultation or dosing service and has been growing in importance over the last 20 years[2]. The birth of clinical

pharmacokinetics as a discipline was spurred on by an increasing awareness of concentration- response relationships and knowledge of pharmacokinetic characteristics of various drugs, the advent of computerization, and advancements in analytical technology. Therapeutic drug monitoring is an important aspect of clinical pharmacokinetics that has helped many pharmacists to enter the clinical arena. Understanding the potential pharmacokinetic changes experienced by critically ill patients is essential for the optimal dosing and monitoring of drug therapy in the patient. Altered organ blood flow, dysfunction of drug-eliminating organs, and changes in fluid compartment volumes often dictate the need for individualized approaches for drug dosing. Pharmacists are ideally trained to provide comprehensive therapeutic drug monitoring and optimize expenditures for serum drug concentration. A health system can own and operate a centralized therapeutic drug monitoring service (TDMS) to focus on the application of clinical pharmacokinetics to the care of patients within the system. Its main objective is therapy optimization by achieving drug concentrations in the therapeutic range and thereby obtaining maximum efficacy with minimum adverse effect.

1.2. THERAPEUTIC DRUG MONITORING

1.2.1. Introduction

Therapeutic drug monitoring (TDM) is the process by which the treatment is optimized by ensuring that the plasma/blood drug concentrations lie within a therapeutic range, above which toxicity occurs and below which the drug is ineffective[3]. In other words it refers to the individualization of drug dosage, by maintaining plasma or blood drug concentrations within a targeted therapeutic range[2]. The notion of a therapeutic range is more a probabilistic concept then an absolute entity which represents a range of drug

concentrations within which the probability of a desired clinical response is relatively high and the probability of unacceptable toxicity is relatively low[4]. The concentrations above a previously determined target or therapeutic range are considered toxic or potentially toxic and levels below are subtherapeutic. The appropriate medical interpretation by TDM has a direct influence on drug prescribing procedures.

The close relationship between plasma levels of the drug and the clinical effects is the basis of the concept of TDM. The measurement of plasma level is justified only when the information provided is of potential therapeutic benefit. Therefore, in TDM the drug levels are an adjunct to the clinical picture and doses should be modified according to the individual's pharmacodynamic response (based on sound clinical judgement) using pharmacokinetic principles to aid titration of the dose to achieve the appropriate therapeutic end point or in other words optimal patient benefit. TDM demands knowledge of pharmacokinetics and the influencing factors and a knowledge of pharmacodynamic to assess the side effects and drug interactions which can result in apparent toxicity or lack of effect. In a nutshell the principle is that a stronger relationship exists between plasma concentration and effect (Ferguson principle) than between the dose and effect. TDM blends the knowledge of therapeutics, pharmacology, pharmacokinetics, laboratory technology, and clinical medicine and applies it to certain drugs that require determination of patient specific dosage regimens to maximize therapeutic effectiveness while minimizing toxicity.

1.2.2. Demand for Therapeutic Drug Monitoring[2,4,6]

TDM will be useful for drugs that satisfy the following criteria to a greater or lesser extent:

- The drug in question has a narrow therapeutic range; e.g. aminoglycosides, cyclosporine, carbamazepine, digoxin, lithium, phenytoin, phenobarbital, theophylline, etc.

- There is an unpredictable dose - response relationship; e.g. phenytoin, theophylline, amiodarone, warfarin, dabigatran, etc.

- The therapeutic effect cannot be readily assessed by the clinical observations - that is when there is no clear observable therapeutic or toxic endpoint; e.g. lithium.

- When toxicity or lack of effectiveness puts the patient at great risk; e.g. gentamicin.

- Dose adjustment is required in various disease states where individual variations in drug absorption, distribution, metabolism or elimination may be important; e.g. hepatic or renal failure.

- Large inter individual variability in steady state plasma concentration exist at any given dose; and

- Appropriate analytical techniques which should be accurate, precise, specific, inexpensive and readily available exist to determine the drug and metabolite levels.

1.2.3. Principles involved in Therapeutic Drug Monitoring[7]

The various disciplines followed for conducting a TDM process are as follows:

- Developing goals for patient therapy such as achievement of a target serum drug concentration (SDC).

- Obtain the blood specimens in which the concentration assessment has to be made after steady state concentrations have been achieved, usually after five half-lives for most of the drugs.

- Obtain trough blood concentrations and ensure that they are therapeutic for medication used to treat the medical conditions.

- Obtain peak and trough concentrations for selected agents for which the level have clinical implications relative to adverse effects and clinical effects.

- Obtain an accurate medical history of drug compliance, missed or delayed doses, and timing of administration of the last dose.

- Assess the patient for significant changes in physical function tests.

- Preventing or providing relief from acute exacerbations of a chronic disease.

- Recommending empiric loading and maintenance dosage regimens based on the pharmacokinetics and patients physiological factors such as body weight, body surface area, renal and hepatic function, and concurrent drug therapy.

- Ordering and interpreting SDCs and making recommendations or adjustments to the drug's dosage regimen to meet the target SDC.

- Observing the patient for signs and symptoms of toxicity, which might necessitate a recommendation to lower the dosage or provide less frequent dosing.

- Monitoring the patient's clinical progress by ensuring that the drug level is not subtherapeutic.

1.2.4. Clinical usefulness of Therapeutic Drug Monitoring[2,6]

TDM provides the clinician with greater insight into the factors determining the patients response to drug therapy, i.e., when a patient fails to respond to a usual therapeutic dose, measurement of the drug concentration in the plasma can help to identify whether the patient is a non – complier or is a true non – responder; it also provides information regarding individual variations as a consequence of altered physiological state or disease process. Scientists are also identifying, cataloging and studying small genetic variations among humans that will lead to more specialized and effective medical treatments. The newer challenge for scientists is to identify single nucleotide polymorphisms (SNPs) that correlate with a particular effect in patients. Reliable SNPs could serve as predictive markers that inform our decisions about numerous aspects of medical care, including specific diseases, effectiveness of various drugs and adverse reactions to specific drugs. This pharmacogenetic approach could save time, money, and discomfort for millions of patients through accurate diagnoses and matching patients with appropriate medicines.

1.2.5. Factors affecting therapeutic drug monitoring interpretation[8,9]

A number of factors may affect serum drug concentrations and need to be considered when interpreting TDM results. The following factors influence the serum drug concentrations:

- **Patient demographics:** The patient's age, sex, body weight and ethnicity should be considered when interpreting TDM results. Age, sex and lean body weight are particularly important for renally cleared drugs as knowledge of these allows calculation of creatinine clearance. Ethnicity may be an important consideration for TDM of some hepatically cleared drugs.

- **Dosage regimen and duration of therapy:** The duration of drug therapy, dosage, time since last dose, and dose-frequency must be known. For a drug which has recently been commenced, sufficient time should elapse to allow steady state to be achieved before TDM is performed. If a loading dose has not been given, this means at least 5 half- lives of the drug should elapse.
- **Active metabolites:** Some drugs form biologically active metabolites and the therapeutic effect of the prescribed drug may rely on the contribution from the metabolite. In such cases measurement of the metabolite instead of the parent compound is reasonable as the therapeutic effect correlates well with the metabolite concentration.
- **Sampling time:** The serum concentration of a drug depends on the time when blood drawn for a TDM assay was sampled in relation to the last dose. The time and date of blood sampling therefore need to be known. For drugs with a short half-life, samples should be drawn immediately before the next dose i.e. a trough level. For drugs with a long half-life, samples may be drawn at any time during the post distribution phase once steady – state has been achieved. As the time to reach peak concentrations shows great variability, peak levels are not performed routinely in clinical practice.
- **Patient compliance:** If the concentration of the drug is lower than expected, the possibility of non-compliance should be considered before a dose increase is recommended. The simplest way to check for non-compliance is to ask the patient in a non-judgmental way about their compliance. However in some situations, for example, a patient who is confused after a seizure, this may not be a reliable method.
- **Genetic factors:** Individual capacity to distribute/metabolize/excrete the drug may vary.

- **Altered protein binding:** Conditions such as malnutrition or nephropathy may reduce the concentration of plasma proteins. For drugs such as phenytoin which are strongly bound to plasma proteins, a reduced albumin level may result in higher concentration of unbound (free) drug. The measurement of both total drug concentration and free drug concentration can be useful in those situations.
- **Drug interactions/Combination therapy:** The concentration of one drug may be altered by the concurrent use of another. Considerations should be given to the possibility that the level may have been increased or decreased as a result of metabolic enzyme inhibition or induction, or that the concentration of the free (active) drug may have been increased by displacement from protein- binding sites. The effects may not be apparent for several weeks, but those relating to changes in protein binding usually occur in the first few days after a drug is added or withdrawn.
- **Pathological factors:** Conditions such as vomiting, diarrhea or inflammatory bowel disease can alter the absorption of drugs, which in turn can alter serum drug concentrations. Hepatic or renal problems can also show an elevated level of drug in serum.
- **Alcohol and tobacco use:** Chronic use of alcohol has been shown to cause non-specific hepatic microsomal enzyme induction, resulting in increased clearance and decreased serum concentrations of hepatically cleared drugs such as phenytoin; Cigarette smoking increases the hepatic clearance of theophylline and patients who have recently stopped smoking may have unexpectedly high theophylline concentrations.
- **Laboratory errors:** The reliability of the routine drug assay service cannot always be assumed. Inter-laboratory quality control schemes or repetition of the assay with a new sample should be encouraged.

1.2.6. Collection of biological samples for analysis[2,4]

The most readily accessible body fluids are blood, saliva and urine. While all these fluids are utilized for drug assays, plasma or serum measurements may yield a better correlation between drug concentration and effects. Whole blood analysis for drugs should not be encouraged, since the erythrocyte/plasma concentration ratio is dependent on a number of variables that may limit the interpretation of results.

In general blood samples should be taken, once steady state drug concentrations have been achieved, i.e. after at least five half-lives of the drug, unless a loading dose has been given. The blood specimens for drug monitoring can be taken at two different times: during the drugs' highest therapeutic concentration (peak level), or its lowest concentration (trough level). Trough levels - occasionally called as residual levels - show the threshold therapeutic levels, whereas peak level shows the toxicity.

1.2.7. Analytical techniques used for therapeutic drug monitoring[6,9]

The methodologies for determining drug concentration in the biological fluids have advanced dramatically. Methods now use micro samples and displays improved sensitivity, specificity and simplicity. The analytical methodology employed should ideally: a) distinguish between compounds of similar structure – unchanged drug and metabolites; b) detect small amounts of drug and metabolites; c) be simple enough to use as a routine assay; and d) be unaffected by other drugs administered simultaneously.

- **Spectrophotometry and Fluorimetry**: Prior to advent of gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC), drug samples were analyzed by spectrophotometric methods. Solvent extraction schemes

coupled with a spectrophotometric finish can still provide a much derived simplicity in assay procedure, when the level of sensitivity required is not too low. i.e. in the mcg/ml range. However the drawbacks are large volume of samples, complex extraction procedures and interference by other compounds.

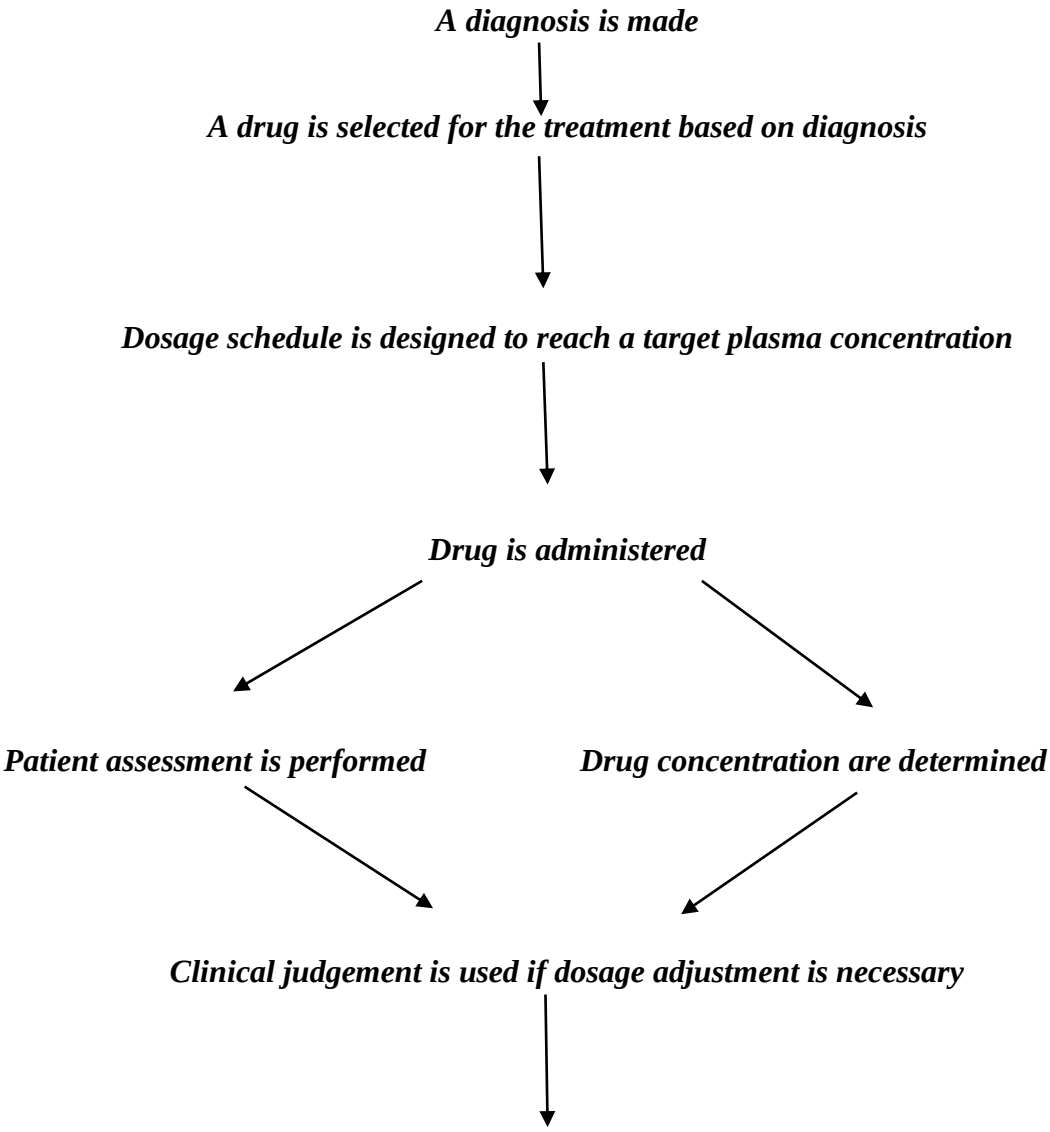
- **Thin layer chromatography (TLC):** TLC possesses adequate resolutions for identifying many drugs but it suffers from inability to quantify these drugs accurately and time consuming technique with inadequate sensitivity. However it is a useful technique in toxicology laboratory.
- **Gas liquid chromatography and High performance liquid chromatography:** These methods are highly specific, precise and sensitive and most frequently used. Besides multiple analyses can be done. The drawbacks are i) extraction steps required ii) slow, single serial analysis, iii) column degeneration with time and iv) complex analyses requiring considerable processing. Out of these two, HPLC technique is superior because thermolabile compounds can also be analyzed.
- **Radio immunoassay (RIA):** It is sensitive, reasonably precise but requires the use of radionucleides. Cross reactivity with other closely reacted drugs is a potential problem with this technique. Besides it is not possible to find out the optically active isomer. The hazards of using radioactive material are a considerable limitation of this method. RIA remains one of the most precise and sensitive methods for quantitation of digoxin in patients serum.
- **Enzyme multiplied immunoassay (EMIT)**: These techniques offer some advantages over RIA in that no radioactive tracer is required; also there is no need to separate the bound from the unbound fractions. However the potential for cross reactivity still exists. Interferences in EMIT assays are minimal and that Fluorescence

polarization immunoassay (FPIA) and HPLC determinations are to be cross checked for their agreements.

- **Fluorescence polarization immunoassay (FPIA)**: This assay procedure combines competitive protein binding with fluorescence polarization to give direct measurement without the need for a separation procedure. The advantages of this method are accuracy, precision and short turnaround time.

1.2.8. Process plan for optimizing drug dosage regimen using therapeutic drug monitoring[10]

There are two ways by which target levels can be utilized to optimize the individual drug dosage regimen. First, calculation of the dose based on predictive pharmacokinetic models for the individual patient; and second, inclusion of drug level monitoring to correct the predictive model for accountable intersubject variation. Predictive pharmacokinetic models are particularly useful for drugs that are mainly eliminated by renal excretion, since good correlations between clinical kidney function tests and drug clearance have been established. Thus, dosage adjustments, based on individual kidney function, are an integral part of modern drug therapy with drugs like digoxin and lithium. But the regulation of individual drug metabolizing capacity is too complex to allow the applications of predictive models. The second approach should be ideally based on a representative neuronal chart shown below which could be used for the process of reaching dosage decisions in therapeutic drug monitoring. The determination of drug levels allows the rapid and safe attainment of target plasma levels, and in conjunction with observations of the clinical drug effects, should provide the safest approach to drug therapy.



Representative neuronal chart for dosage decision in therapeutic drug monitoring

1.2.9. Pharmacoeconomic Impact of Therapeutic Drug Monitoring[2,11]

In recent years, TDM has developed a much more patient - oriented focus to include all the processes around drug therapy (patient response, adverse events, dosing information, blood sampling time, pharmacokinetic behavior, drug analysis, interpretation and dose adjustment). The pharmaceutical industries in general are not in

favour of TDM with reasons of increased therapeutic complexity and drug – associated costs thereby raising the barriers for the use of their drug. But studies have shown that a well conducted TDM is cost effective[6]; instead of just passive monitoring it has also earned the name of "proactive management". The cost factors in concern with the TDM of drugs includes the a) structure components such as laboratory facilities and personnel; b) process components such as sampling, interpretation and intervention based on results; and c) outcome components such as the clinical effects including recovery, duration of hospital stay, duration drug therapy, number of adverse events, morbidity and mortality, as well as cost savings of TDM.

Pressures continue within the healthcare system to provide services at the lowest possible cost. Thus, the role of many drug laboratories is to measure the concentration of therapeutic drug in blood sample and relate it to a therapeutic range published in the literature. As an intervention method TDM declares to improve patient responses to important life sustaining drugs and to decrease the adverse drug reactions. The studies show that the TDM has positive outcomes, including decreased hospitalizations and thus TDM is an appropriate candidate for an economic outcome evaluation.

1.3. DRUG PROFILE OF SELECTED DRUGS FOR THE STUDY

1.3.1. THEOPHYLLINE[12]

Chemistry: Theophylline is structurally classified as a xanthine derivative.

Therapeutic category: Bronchodilator

Uses: Theophylline is used as a bronchodilator in the symptomatic treatment of asthma and reversible bronchospasm that may occur in association with chronic bronchitis or emphysema.

Pharmacology: Theophylline competitively inhibits phosphodiesterase, the enzyme that degrades cyclic 3´,5´-adenosine monophosphate (cAMP). Increased concentrations of intracellular cAMP may mediate most of the pharmacologic effects of the drug. The actions of theophylline on the myocardium and on neuromuscular transmission may result from intracellular translocation of ionized calcium. The ubiquitous nature of calcium and cAMP accounts for the diversity of theophylline's pharmacologic actions.

• **Pulmonary Effects:** Theophylline directly relaxes smooth muscle of the respiratory tract, producing relief of bronchospasm and increasing flow rates and vital capacity. Theophylline also dilates pulmonary arterioles, reduces pulmonary hypertension and alveolar carbon dioxide tension, and increases pulmonary blood flow.

• **Nervous System Effects**: Stimulation of the vasomotor and vagal centers promotes vasoconstriction and bradycardia, respectively, but the overall effect of theophylline on heart rate and blood pressure depends on whether central nervous system (CNS) or peripheral effects predominate. In the medulla, theophylline also lowers the threshold of the respiratory center to carbon dioxide, but substantial increases in rate and depth of respiration occur only if respiration is depressed.

• **Cardiovascular Effects:** In doses larger than those required for bronchodilation, theophylline produces a positive inotropic effect on the myocardium and a positive chronotropic effect at the sino-atrial (SA) node. Although heart rate, force of contraction, cardiac output, and myocardial oxygen demand may be increased transiently, theophylline rarely alters heart rate to a substantial degree with usual doses.

• **Renal Effects:** Mild diuresis is produced by the combined effect of theophylline on renal hemodynamics and on tubular reabsorption. Increased cardiac output and dilation of efferent and afferent renal arterioles result in increased glomerular filtration rate

(GFR) and renal blood flow. In congestive heart failure, theophylline-induced changes in GFR are variable. Theophylline also inhibits sodium and chloride reabsorption at the proximal tubule. Potassium excretion is not markedly increased. Tolerance of a low magnitude may develop to the diuretic effect of theophylline.

• **Endocrine and Metabolic Effects:** At therapeutic serum concentrations, theophylline may stimulate release of catecholamines from the adrenal medulla and increase the urinary excretion of epinephrine. Theophylline exhibits many of the beta adrenergic effects of epinephrine; their cardiac and hyperglycemic effects may be synergistic. Conversely, theophylline may potentiate corticotropin and catecholamine-induced insulin secretion. The net effect on blood glucose is variable. The lipolytic action of theophylline requires the presence of growth hormone or glucocorticoid to produce maximum increase in plasma free fatty acids. Theophylline may potentiate the calcemic response to parathyroid hormone and inhibit that of calcitonin. Theophylline may also increase basal metabolic rate.

• **Other Effects:** Theophylline relaxes smooth muscle of the biliary and gastrointestinal (GI) tract, and stimulates gastric secretion. Theophylline stimulates skeletal muscle in vitro, increasing the force of contraction and decreasing muscular fatigue; this action of theophylline may be mediated by acetylcholine.

Pharmacokinetics

• **Absorption:** Under the acidic conditions of the stomach, the theophylline salts and compounds release free theophylline. Although the rate of absorption is slower, extended-release preparations (capsules and tablets) of theophylline are generally absorbed to the same extent as uncoated tablets; however, the actual rate of absorption of extended-release preparations may differ. Serum theophylline concentrations of

about 10-20 mcg/ml are usually needed to produce optimum bronchodilator response. Some patients with mild pulmonary disease will experience relief of bronchospasm with serum theophylline concentrations of 5 mcg/ml. With serum concentrations ranging from 10-20 mcg/ml, a linear relationship exists between improvement in pulmonary function and the logarithm of serum theophylline concentration. In premature infants, serum theophylline concentrations of about 7-14 mcg/ml may be sufficient to reverse apnea. Adverse reactions to theophylline often occur when serum concentrations exceed 20 mcg/ml.

Intravenous (IV) theophylline produces the highest and most rapid serum theophylline concentration. Following a single IV dose of theophylline (as aminophylline) of about 5 mg/kg over 30 minutes to healthy adults, mean peak serum theophylline concentrations of about 10 mcg/ml are reached. Following oral administration of theophylline capsules or uncoated tablets, peak serum concentrations are usually reached in 1-2 hours. Peak serum theophylline concentrations are usually obtained after about 1 hour when theophylline oral solutions or microcrystalline tablets are administered. Enteric-coated theophylline tablets produce variable serum concentrations which usually peak at about 5 hours. Single doses of extended-release theophylline capsules or tablets usually produce peak serum concentrations after 4 hours, but commercial products vary in their rates and completeness of absorption. Extended-release theophylline preparations are generally associated with relatively small fluctuations in steady-state peak and trough serum concentration; however, clinically important steady-state peak-trough differences may occur in individuals who rapidly eliminate theophylline. Theophylline retention enemas usually produce peak serum concentrations in 1-2 hours.

• **Distribution:** Theophylline is rapidly distributed throughout extracellular fluids and body tissues with distribution equilibrium being reached 1 hour after an IV loading dose. The apparent volume of distribution of theophylline ranges from 0.3-0.7 l/kg and averages about 0.45 l/kg in children and adults. At serum concentrations of 17 mcg/ml, approximately 56% of theophylline in adults and children.

• **Metabolism:** Theophylline is metabolized by the liver to 1,3-dimethyluric acid, 1-methyluric acid, and 3-methylxanthine. Individuals metabolize theophylline at different rates; however, individual metabolism of the drug is generally reproducible.

• **Elimination:** In maintenance-dose theophylline schedules, serum concentrations among patients vary at least 6-fold and serum half-lives ($t_{1/2}$) exhibit wide interpatient variation because of differences in rate of metabolism. Serum $t_{1/2}$ ranges from about 3-12.8 (average 7-9) hours in otherwise healthy, nonsmoking asthmatic adults, from about 1.5-9.5 hours in children, and from about 15-58 hours in premature infants. Healthy, nonsmoking asthmatic adults, the serum $t_{1/2}$ of theophylline may be increased and total body clearance decreased in patients with congestive heart failure, chronic obstructive pulmonary disease, cor pulmonale, or liver disease, and in geriatric patients. In cigarette and/or marijuana smokers, theophylline serum $t_{1/2}$ averages 4-5 hours and total body clearance is increased compared with nonsmokers. Theophylline and its metabolites are excreted mainly by the kidneys. Renal clearance of the drug, however, contributes only 8-12% of the overall plasma clearance of theophylline. Small amounts of theophylline are excreted in faeces unchanged.

Dosage and Administration

• **General Administration:** Theophyllines (e.g., theophylline, aminophylline) are administered orally. For faster absorption, conventional oral theophylline dosage forms

(300 mg to 900 mg) may be taken with a full glass of water on an empty stomach 30-60 minutes before meals or 2 hours after meals; to minimize local gastrointestinal irritation, oral theophyllines may be taken with meals or immediately after meals, with a full glass of liquid, or with antacids.

• **Dosage:** Theophylline has a low therapeutic index; therefore, cautious dosage determination is essential. Because individuals metabolize theophylline at different rates, appropriate dosages must be determined for each patient by carefully monitoring patient response and tolerance, pulmonary function, and serum theophylline concentrations. Dosages required to achieve a therapeutic serum theophylline concentration vary fourfold among otherwise similar patients in the absence of factors known to alter theophylline clearance.

For maintenance therapy, serum theophylline concentrations should be obtained after a patient has received a given dosage for 3 days. Peak serum concentrations can be estimated by obtaining blood samples 30 minutes after administration of an IV loading dose, 1-2 hours after administration of an oral solution or uncoated tablet, or 3-12 (usually 3-8) hours (depending on the specific formulation) after administration of an extended-release preparation. Trough concentrations of theophylline can be determined by taking blood samples just before the next dose. When the recommended maximum dosage is exceeded, dosage adjustment should be based on measurement of peak serum theophylline concentrations. For dosage adjustments based on serum theophylline concentrations determined in such circumstances, it is important that dosage in the previous 48 hours be reasonably typical of the prescribed regimen and that the patient not have missed a dose nor taken an additional dose in this time period. Dosage adjustments based on serum theophylline concentration when these conditions have not

been fulfilled may result in dosages that present risk of toxicity to the patient. Therapeutic serum concentrations for bronchospastic disease generally range from 10-15 mcg/ml, although lower concentrations may provide beneficial effects in some patients with mild asthma and may be effective for neonatal apnea. When serum theophylline concentrations exceed 20 mcg/ml, toxicity often becomes apparent.

Adverse drug reactions: Uncommon at serum theophylline concentration $\leq$ 20 mcg/ml.

• **Gastrointestinal (GI) and Nervous System Effects**: The most common adverse GI effects (both locally and centrally mediated) include nausea, vomiting, epigastric pain, abdominal cramps, anorexia, and, rarely, diarrhea. Adverse central nervous system (CNS) effects, which are often more severe in children than in adults, include headache, irritability, restlessness, nervousness, insomnia, dizziness, reflex hyperexcitability, and seizures. Reduction of theophylline dosage usually reduces the incidence and severity of adverse gastric and CNS effects.

• **Cardiovascular Effects**: Adverse cardiovascular effects of theophyllines include palpitation, sinus tachycardia, extrasystoles, and increased pulse rate. These adverse cardiovascular effects are usually mild and transient. Flushing, hypotension, circulatory failure, and ventricular arrhythmias may also occur.

• **Other Adverse Effects:** Theophyllines may also produce transiently increased urinary frequency, dehydration, twitching of fingers and hands, tachypnea, and elevated serum aspartate transaminase concentrations.

In case of serum concentration above 20 mcg/ml the frequency of adverse reactions such as gastrointestinal upset, diarrhea, nausea, vomiting, anorexia, abdominal pain,

circulatory failure, nervousness headache, insomnia, tremor, agitation, dizziness, muscle cramp, tremor, tachycardia (>25 mcg/ml), seizures (>35 mcg/ml).

Acute Toxicity: Theophylline toxicity is most likely to occur when serum concentrations exceed 20 mcg/ml and becomes progressively more severe at higher serum concentrations. Tachycardia, in the absence of hypoxia, fever, or administration of sympathomimetic drugs, may be an indication of theophylline toxicity. Anorexia, nausea and occasional vomiting, diarrhea, insomnia, irritability, restlessness, and headache commonly occur. The distinguishing symptoms of toxicity may include agitated maniacal behavior, frequent vomiting, extreme thirst, slight fever, tinnitus, palpitation, and arrhythmias.

1.3.2. GENTAMICIN[12]

Chemistry: Gentamicin is an aminoglycoside antibiotic obtained from cultures of Micromonospora purpurea.

Therapeutic category: Aminoglycoside antibiotic.

Uses: Serious Bacterial Infections: Gentamicin is used for the treatment of serious bone and joint infections, respiratory tract infections, septicemia, skin and skin structure infections, and urinary tract infections caused by susceptible gram-negative bacteria, including Citrobacter, Enterobacter, Escherichia coli, Klebsiella, Proteus, Serratia, Pseudomonas aeruginosa or Staphylococcus aureus. The drug usually is used as an adjunct to an appropriate beta-lactam (e.g., ceftriaxone, cefotaxime, cefepime, piperacillin and tazobactam, ticarcillin and clavulanate) or carbapenem (e.g., imipenem, meropenem) for empiric treatment of these infections. Gentamicin is not usually indicated for initial treatment of uncomplicated infections (e.g., uncomplicated urinary

tract infections) unless the causative organism is susceptible and other less toxic anti-infectives cannot be used. Includes treatment of infections caused by susceptible staphylococci when other more appropriate anti-infectives are contraindicated (e.g., because of hypersensitivity) or would be ineffective because of resistance and for initial treatment of mixed infections when the causative organisms may be either gram-negative bacteria or staphylococci.

Mechanism of action: Interferes with bacterial protein synthesis by binding to 30s and 50s ribosomal subunits resulting in a defective bacterial cell membrane.

Pharmacokinetics

• **Absorption:** Gentamicin is poorly absorbed from the gastrointestinal (GI) tract and must be administered parenterally. Gentamicin is rapidly absorbed following intramuscular (IM) administration. Following IM administration of a single 1 mg/kg dose of gentamicin in adults with normal renal function, peak serum gentamicin concentrations of 4-7.6 mcg/ml are attained within 30-90 minutes. Serum concentrations attained following IV infusion over 20 minutes to 2 hours usually are similar to those attained when the same dose is given by IM injection. When gentamicin is administered by IV infusion over 2 hours, peak serum concentrations usually occur at 30-60 minutes and are measurable for 6-8 hours.

Accumulation of gentamicin does not appear to occur in patients with normal renal function receiving 1 mg/kg doses every 8 hours for 7-10 days. However, accumulation may occur with higher doses and/or when the drug is given for prolonged periods, especially in patients with renal impairment.

• **Distribution:** Following parenteral administration of usual dosages of gentamicin, the drug can be detected in lymph, subcutaneous tissue, lung, sputum, and bronchial, pleural, pericardial, synovial, ascitic, and peritoneal fluids. Only minimal concentrations of gentamicin are attained in ocular tissue following IM or IV administration. Gentamicin is distributed into Cerebrospinal fluid (CSF) in low concentrations following IM or IV administration. CSF concentrations of gentamicin following intrathecal administration depend on the dose administered, the site of injection, the volume in which the dose is diluted, and the presence or absence of obstruction to CSF flow. Gentamicin crosses the placenta. Gentamicin is distributed into milk following IM administration.

• **Elimination:** The plasma elimination half-life of gentamicin is usually 2-4 hours in adults with normal renal function and is reported to range from 24-60 hours in adults with severe renal impairment. The serum half-life of gentamicin averages 3-3.5 hours in infants 1 week to 6 months of age and 5.5 hours in full-term infants and large premature infants less than 1 week of age. In small premature infants, the plasma half-life is approximately 5 hours in those weighing over 2 kg, 8 hours in those weighing 1.5-2 kg, and 11.5 hours in those weighing less than 1.5 kg. Gentamicin clearance may be decreased in geriatric patients compared with other adults.

In adults with normal renal function, 50-93% of a single IM dose of gentamicin is excreted unchanged by glomerular filtration within 24 hours. Peak urine concentrations of gentamicin may range from 113-423 mcg/ml one hour after a single IM dose of 1 mg/kg in adults with normal renal function. Complete recovery of the dose in urine requires approximately 10-20 days in patients with normal renal function, and terminal

elimination half-lives of greater than 100 hours have been reported in adults with normal renal function following repeated IM or IV administration of the drug.

Dosage and Administration

Gentamicin sulfate is administered by intramuscular (IM) injection or intravenous (IV) infusion. IV administration may be preferred in patients with septicemia, shock, congestive heart failure, hematologic disorders, severe burns, or reduced muscle mass. Gentamicin sulfate has been administered intrathecally or intraventricularly as an adjunct to IM or IV administration of the drug for the treatment of meningitis and other CNS infections. Patients should be well hydrated prior to and during gentamicin therapy since dehydration increases the risk of toxicity.

Renal function should be assessed prior to and monitored during gentamicin therapy. Patients should be under close clinical observation because of the risk of ototoxicity and nephrotoxicity.

For IM injection, the appropriate dose of commercially available injection containing gentamicin in a concentration of 10 or 40 mg/mL should be withdrawn from the vial and given undiluted.

IV infusions for adults are prepared from commercially available injections containing gentamicin in a concentration of 10 or 40 mg/ml by diluting the appropriate dose of gentamicin with 50-200 ml of 0.9% sodium chloride or 5% dextrose injection. For pediatric patients, the volume of infusion fluid depends on the patient's needs, but should be sufficient to allow a gentamicin infusion period of 30 minutes to 2 hours. IV infusions of gentamicin should be given over 30 minutes to 2 hours.

General Adult Dosage. If IM or IV gentamicin is used for the treatment of serious infections caused by susceptible bacteria in adults with normal renal function, the usual adult dosage recommended by the manufacturers is 3 mg/kg daily given in 3 equally divided doses every 8 hours. For life-threatening infections in adults with normal renal function, IM or IV gentamicin dosage up to 5 mg/kg daily given in 3 or 4 equally divided doses may be used, but dosage should be reduced to 3 mg/kg daily as soon as clinically indicated.

If a once-daily gentamicin regimen is used in adults with normal renal function, some clinicians recommend a dosage of 4-5 mg/kg once daily. A once-daily regimen of 5-7 mg/kg once daily also has been recommended. It has been suggested that, if gentamicin is used alone for the treatment of serious infections (e.g., without concomitant use of a beta lactam), a dosage of 7 mg/kg once daily usually is required.

General Dosage for Neonates. When IM or IV gentamicin is used in premature or full-term neonates 1 week of age or younger, the manufacturers recommend 2.5 mg/kg every 12 hours. For neonates older than 1 week of age, the manufacturers recommend a dosage of 2.5 mg/kg every 8 hours. Neonates have received 4-5 mg/kg of gentamicin once daily by IV infusion over 30-60 minutes.

General Dosage for Infants and Children. The usual dosage of IM or IV gentamicin recommended by the manufacturers for older infants with normal renal function is 2.5 mg/kg every 8 hours. The manufacturers recommend that children receive gentamicin in a dosage of 2-2.5 mg/kg every 8 hours. Once-dailygentamicin regimens used in infants and children is 5-6 mg/kg once every 24 hours is investigational in children.

Warnings: Not intended for long term therapy due to toxic hazards associated with extended administration; pre-existing renal insufficiency, vestibular or cochlear

impairment, myasthenia gravis, hypokalemia, conditions which depress neuromuscular transmission. Parenteral aminoglycosides have been associated with significant nephrotoxicity or ototoxicity; the ototoxicity may be directly proportional to the amount of drug given and the duration of treatment.

In order to carry out the study of therapeutic drug monitoring in the study site, the drugs which can be used in the setup for the patients, which were prescribed predominantly in the study site and which satisfied the criteria for therapeutic drug monitoring were selected. The selected drugs for the study were – Theophylline and Gentamicin.

2.1. THEOPHYLLINE

Theophylline remains one of the most widely prescribed drugs for the treatment of airway diseases worldwide since it is inexpensive, although the development of newer bronchodilators has declined its use in industrialized countries. It's low cost and the easy administration (oral) still makes it a choice for the under developed and developing countries prescriptions for the poor. The choice of theophylline also counts due to its multiple mechanisms of actions and when breathing control have not been achieved by $beta_2$ agonist and corticosteroids. As the pharmacokinetic parameters for theophylline are completely characterized by large intra and inter individual variability and has a narrow therapeutic index of 10 - 20 mcg/ml in blood it necessitates for the therapeutic drug monitoring during theophylline therapy[4].

AIM: To study the pharmacokinetic and pharmacodynamic relationship of theophylline in asthmatic patients in the Government District Head Quarters Hospital, Ooty.

OBJECTIVES

- To estimate serum theophylline concentration in asthmatic patients prescribed with theophylline.

• To correlate the blood level concentration of theophylline with clinical progress of the patients using their pulmonary function and the quality of life.

• To assess the best possible therapeutic concentration for the patient receiving theophylline.

• To assess the adverse drug reactions with the use of theophylline.

• To study on the pharmacoeconomic outcomes of the therapeutic drug monitoring.

2.2. GENTAMICIN

Gentamicin has proven efficacy against may aerobic Gram – negative organisms and staphylococci. It is associated with low levels of resistance in common nosocomial pathogens and demonstrates rapid concentration dependent bactericidal activity and post antibiotic effect. The desirable peak serum concentrations of gentamicin are 4 – 12 mcg/ml and trough concentrations of the drug should not exceed 1 – 2 mcg/ml. Increased risk of toxicity is associated with prolonged peak serum gentamicin concentrations greater than 10 – 12 mcg/ml and/ or trough concentrations greater than 2 mcg/ml[33].

AIM: To compare the clinical efficacy and safety of different dosage regimens of gentamicin being used in Government District Head Quarters Hospital, Ooty.

OBJECTIVES

• To compare the present dosing strategy of gentamicin of once daily dosing and multiple dosing in Government District Head Quarters Hospital, Ooty.

- To study on the use of gentamicin in different clinical conditions.

- To identify the type of micro-organisms in the subjects where gentamicin is prescribed.

- To estimate gentamicin concentration in the blood and hence know about its safety and efficacy.

- To conduct adverse drug reaction monitoring of gentamicin.

The selection of drugs was based on facts that drugs should be satisfying the criteria for therapeutic drug monitoring and should be prescribed very commonly for the patients in the study site. Some of the studies which have been reviewed for the selected drugs study are given as follows:

3.1. THEOPHYLLINE

• Ken Ohta et al.[15] had conducted a prospective clinical study of theophylline safety in elderly patients with asthma or chronic obstructive pulmonary disease (COPD) who had been treated with sustained-release theophylline tablets for one to six months. The incidence of theophylline-related adverse events was higher in patients with hepatic disease and in patients with arrhythmia. Blood drug concentration measurements in seven hundred and thirty six patients indicated that the drug levels were $\leq$15 mg/ml in six hundred and forty one patients (87.1%), and no correlation was noted between dose and theophylline-related adverse events. These results suggest that sustained-release theophylline can be used safely in elderly patients with asthma or COPD.

• Rizzo A et al.[16] had conducted a study to determine the effect of body weight on the volume of distribution of theophylline. The study was conducted in forty acute asthmatic patients aged between 22 to 78 years weighing 45 – 176 kg. From the measurement of volume of distribution of theophylline it was found that it increases with the total body weight, and it cannot be accurately predicted from either total body weight or ideal body weight alone. The study helps to minimize the error in obtaining the target serum concentration of theophylline when giving a loading dose calculated form a predicted volume of distribution value.

• Cusack B et al.[17] had conducted study on single dose theophylline kinetics in groups of young and elderly smokers and non- smokers to assess the effect of age on

theophylline absorption and the effect of smoking on drug metabolizing enzyme activity in old age. The study found that the rate and absorption was not affected by age; distribution and elimination kinetics were similar in young and elderly non-smokers; in young subjects it was found that the elimination half life of theophylline was shorter and clearance was significantly greater in smokers than in non- smokers; and in the elderly the mean elimination half life was significantly shorter in smokers and their plasma clearance was 40 % higher than in non- smokers. The study concluded that the ageing does not affect the theophylline elimination and also that induction of theophylline metabolism due to smoking occurs in old age and smoking is a variable that should be taken account of when assessing drug metabolism in elderly patients.

- Wang et al.[18] had conducted a study on the comparison of inhaled corticosteroid combined with theophylline and double- dose inhaled corticosteroid in moderate to severe asthma. The study was done with 41 patients with asthma randomized into either beclomethasone dipropionate inhaler 500 mcg twice a day or a combination of beclomethasone dipropionate inhaler 250 mcg twice a day and sustained release tablet theophylline 200mg twice a day for 6 weeks. The results showed that the both treatment had the same effect on asthma control, improving symptoms and ameliorating lung function. Combining of sustained release theophylline may allow for the reduction in inhaled corticosteroid dose when treating asthma.

- Randolph WC et al.[19] had studied on the effect of age on theophylline clearance in normal subjects. Dose interval area under curve and clearance of theophylline at steady state were determined in healthy male subjects in each group of three age groups – 18 to 35, 36 to 54 and 55 to 70 years old. The mean area under curve in oldest group was significantly higher than in the youngest and clearance in both the middle and oldest groups was significantly lower than in the youngest. The study concluded that though

clearance was significantly correlated with age, age alone accounted for only 31 % of the variability in clearance.

- Wiggins J et al.[20] had conducted a study on the effect on calculated dose of knowledge of serum theophylline concentration on intravenous aminophylline in patients already taking oral theophylline. Fifty patients with worsening airflow obstruction, all of whom were taking oral theophylline and who had no contraindication to the use of parenteral aminophylline were randomly allocated into two groups before treatment was given. In one group the aminophylline dose was calculated without knowledge of serum theophylline concentration and other group was given calculated aminophylline dose with knowledge of serum theophylline concentration. The study concluded that although satisfactory use of parenteral aminophylline was achieved for most patients without knowledge of serum theophylline concentration at the time of admission to hospital, prompt measurement of serum theophylline concentration at the time of admission identified patients with either suboptimal, or potentially hazardous theophylline concentrations.

- Kupper TE et al.[21] had conducted a study on reduction of symptoms of acute mountain sickness (headache, nausea, and sleeplessness) at low dose theophylline. The study involved twenty healthy male volunteers who were randomized to receive either 300 mg of theophylline daily or placebo five days prior during ascent, and during a stay at a high altitude. The study concluded that low dose theophylline reduces symptoms of acute mountain sickness in association with alleviation of events of periodic breathing and oxygen desaturations.

- Otero MJ et al.[22] studied theophylline clearance values in adult patients using serum concentrations gathered from routine clinical care. Retrospective data from 204 asthmatic and COPD patients, with a total of 517-serum concentration were studied.

The authors concluded that the influence of the following factors on theophylline clearance was investigated- Body weight and age as continuous variables, gender, smoking habit and the presence of congestive heart failure as indicator variables. Thus measurement of serum theophylline concentration would only be required when other conditions known to alter theophylline metabolism exist, such as smoking or disease factors.

- Micheal S et al.[23] conducted a longitudinal cohort study of patients with theophylline overdose. For a 125-month period, 356 patients were enrolled with a serum concentration of 30mcg/ml or more were followed up prospectively. The authors concluded that 162 patients had acute, 114 had chronic and 50 had acute – on - therapeutic poisoning. 74 patients developed cardiac arrhythmias and 29 patients developed seizures, 15 patients died, 11 of who had chronic over medication. Theophylline intoxication results in substantial morbidity and mortality, particularly in those with chronic over medication.

- Butts JD et al.[24] encountered two adult patients in whom nonlinear theophylline kinetics existed with the sub-therapeutic and therapeutic range of serum levels. In both cases were not immediately recognized by the clinician, resulted in prolonged use of sub therapeutic doses of theophylline, resulted in serious theophylline toxicity in one case. The authors concluded that to avoid such a potentially fatal complication supervised administration of oral theophylline, discontinuation of further empirical increased of the oral dose of theophylline and calculation of the appropriate maintenance dose of theophylline, for that individual patient essential.

- Makino S et al.[25] described a prospective survey on the safety of methyl xanthines administered to adult patients mainly with asthma. (The present review examines the efficacy and adverse effect of sustained- release theophylline and injectable methyl

xanthine in the treatment of chronic asthma.) In the prospective study, in the case of sustained - released theophylline, 3921 subjects reported by 66 medical centers were selected for analysis in the survey and in the case of intravenous methyl xanthine, 682 subjects reported by 55 medical centers were selected for analysis. The authors concluded that none of the subjects exhibited serious adverse drug reaction with sustained released theophylline or intravenous methyl xanthine. Methyl xanthine was effective for the treatment of asthma and was safe as long as the dose administered accords with the protocols recommended by asthma management guidelines

- Yamauchi K et al.[26] evaluated the efficacy and safety of intravenous administered theophylline (IAT) for the treatment of an acute exacerbation of bronchial asthma. The study subjects were 22 asthmatic patients with mild acute exacerbation of bronchial asthma. All patients had been taking oral, sustained - release theophylline and their serum concentrations of theophylline were measured. Pulmonary function and asthma severity before and after treatment were measured. The authors concluded that after intravenously administered theophylline (IAT) both peak expiratory flow and forced expiratory volume in one second (FEV_1) increased significantly. These results suggest that IAT is useful for patients with mild acute exacerbation of bronchial asthma and safe if serum theophylline concentrations are measured.
- Rivington RN et al.[27] studied a double blind, crossover comparison of morning versus evening dosing regimens with a new once – daily oral theophylline. The comparison was based upon steady-state theophylline pharmacokinetics, Spirometric measurements over 24 hours, the patient quantitative reporting of asthmatic symptoms and medication side effects. The authors concluded that evening dosing, but not morning dosing resulted in significant attenuation of the yearly morning dip in the pulmonary function.

• Mungall D et al.[28] studied two groups of patients in intensive care unit. In-group I consist of 19 male patients whose theophylline therapy was individualized by a clinical pharmacokinetics service and in-group II there were 34 male patients with empirically derived dosages. The authors concluded that patients in the pharmacokinetics group had fewer adverse reactions, shorter intensive care unit stay, shorter hospital stay and a shorter period of time to be placed on oral therapy then the group with empirically derived regimens. The pharmacokinetic method used to individualize theophylline therapy offered an accurate and efficient method of achieving therapeutic concentration. It reveals that the use of clinical pharmacokinetics to individualize theophylline therapy offers substantial benefits, over empirical assessments.

• Sin et al.[29] conducted a cost – effectiveness study using inhaled corticosteroids for different severities of chronic obstructive pulmonary disease. The cost-effectiveness of four treatment strategies involving inhaled corticosteroids were: a) no use regardless of chronic obstructive pulmonary disease severity; b) use in all disease stages; c) use in patients with stage 2 or 3 disease (forced expiratory volume in 1 second [FEV_1] <50% of predicted); and d) use in patients with stage 3 disease (FEV_1 <35% of predicted). Data from the literature were used to estimate mortality, exacerbation, and disease progression rates, as well as the costs associated with care and quality-adjusted life-years (QALYs), according to disease stage and use or nonuse of inhaled corticosteroids. A time horizon of 3 years was used. The study found that providing inhaled corticosteroids to all COPD patients was associated with a less favorable cost-effectiveness ratio. Use of inhaled corticosteroids in those with stage 2 or 3 disease for 3 years results in improved quality-adjusted life expectancy at a cost that is similar to that of other therapies commonly used in clinical practice.

- Ayres et al.[30] prospectively evaluated the cost effectiveness of fluticasone propionate (FP) treatment in patients with moderate to severe chronic obstructive pulmonary disease (COPD), who were symptomatic on regular bronchodilator therapy. The economic analysis was performed for a period of six months, randomized, double-blind clinical trial comparing FP 1000 mcg/day with placebo in 281 patients aged 45-79 years with symptomatic moderate to severe COPD. Data on clinical efficacy, health care resource use and productivity loss associated with the management of COPD prospectively collected. The main outcome measures were the incremental cost effectiveness of achieving a ≥10% improvement in FEV_1 and of remaining exacerbation-free throughout the study. Incremental cost-effectiveness analyses showed that the additional clinical benefits of FP relative to placebo, in terms of a ≥10% improvement in FEV_1 and an increased number of patients free of exacerbations, were achieved at minimal additional costs.

- Vatrella et al[31] reported the bronchodilating effects of a single dose of inhaled salmeterol (50μg) and oral slow-release theophylline (Theo-Dur, 600mg tablets), used either alone or in combination. Given in combination with salmeterol, theophylline elicited increase in airway calibre with respect to the bronchodilator action of the $beta_2$-agonist alone, with FEV_1 changes which resulted to be statistically significant at the fourth, sixth and eighth hour after administration ($p<0.05$, $p<0.03$ and $p<0.05$, respectively). At the fourth hour theophylline reached serum levels included within the therapeutic range, which were persistently maintained at least until the tenth hour. Their findings suggested that inhaled salmeterol and oral slow-release theophylline, the latter acting within the range of therapeutic plasma concentrations, exert additive bronchodilating effects in asthmatic patients with moderate to severe airflow limitation.

• Barnes PJ and Pauwels RA.[32] in their review stated that theophylline now considered to be a bronchodilator, has other anti-asthma activities, which may be more important. Theophylline, even at low plasma concentrations, inhibits the late asthmatic reaction following allergen challenge. These include the inhibition of cytokine synthesis and release, the inhibition of inflammatory cell activation and microvascular leakage, and the prevention of airway hyperresponsiveness induced by airway inflammation. Theophylline appears to have immunomodulatory effects, even at relatively low plasma concentrations. Based on these considerations, theophylline can be regarded as a useful alternative to other anti-inflammatory drugs for the chronic treatment of mild to moderate asthma. Theophylline should be used at lower doses to achieve plasma concentrations of 5-10 mcg/ml, which will avoid the risk of side-effects. They also recommended for further studies to evaluate the role of low-dose theophylline as an adjunct to low-dose inhaled steroids in the management of chronic asthma. It may now be appropriate to re-evaluate the role of theophylline in asthma management.

3.2. GENTAMICIN

• Buabang KO et al.[33] had conducted a study on the assessment of the efficacy, safety and quality of gentamicin being used in an infirmary. Fifty five patients who received gentamicin, once daily were studied. The protocol for administration and monitoring of gentamicin serum concentration was followed for twenty three patients. The study found that adherence to protocol improves the clinical efficacy of gentamicin and reduces the incidence of drug toxicity.

• James G Dahlgren et al.[34] had conducted a prospective study of gentamicin - dose blood - level relationships and the value of blood levels as a guide to prevent nephrotoxicity. The gentamicin blood concentrations were monitored in eighty six

patients. Twenty one patients had trough levels over 2 mcg/ml and 36% of these patients developed abnormal serum creatinine. The rise in the peak and trough levels during the therapy appeared to be dose related. The assessment of gentamicin in blood was useful in predicting accumulation of gentamicin which in turn may be correlated with early renal impairment, before potentially toxic serum levels of gentamicin develop.

- Stanford SJ et al.[35] had compared the aminoglycoside pharmacokinetics in Asian, Hispanic, and Caucasian patients by using population pharmacokinetic methods. The study did not find any statistical difference among the groups and so it was concluded that there was no difference in the aminoglycoside pharmacokinetics among the different patient groups.

- Rameis H et al.[36] had studied the relationship of endogenous creatinine clearance for the estimation of elimination half life of gentamicin. The study found that although there was good correlation between both parameters, satisfactory agreement between experimentally determined and calculated half life was found only in case of patients with normal or slightly reduced renal function. With decreasing creatinine clearance, the calculated value was constantly bigger than the measured value and the range of both values increased. From the study it can be concluded that the gentamicin treatment in patients with grossly decreased renal function should thus be performed under control of serum levels.

- Demczar DJ et al.[37] had compared the pharmacokinetics of two doses of gentamicin in healthy volunteers in crossover single dose study. The results show that the pharmacokinetics of gentamicin at a large dose differ significantly from those at the traditional dose. This information has direct implications for once daily aminoglycoside

literature when the C_{max} values reported are distributional and therefore show falsely high C_{max}/minimum inhibitory concentration ratio estimates.

- Thomson AH et al.[38] had conducted a study to evaluate the performance of dosage guidelines for starting gentamicin therapy in patients with suspected or proven Gram negative septicemia and the results were compared with a similar group of patients from whom the guidelines were not followed. The study found that the peak concentrations were significantly higher when the guidelines were followed but there was no difference in trough concentrations. Fifty eight percent of the patients had both peak and trough concentrations within target range when doses were followed empirically but this increased to ninety six percent when the guidelines were followed. The study concluded that the revised protocol with higher doses given less frequently and its performance indicated that satisfactory concentrations were obtained in ninety six percent of the patients.

- Monir Hossain M et al.[39] had conducted study on the simplified dosing of gentamicin for treatment of sepsis in Bangladeshi neonates. This prospective observational study was conducted among fifty nine neonates. Peak and trough concentrations of gentamicin and the presence of signs of nephrotoxicity and ototoxicity were determined. The study concluded that the favourable pharmacokinetic parameters found with the simplified dosing regimen to suggest that it is safe for the treatment of neonatal sepsis.

- Darwin E. Zaske et al.[40] had studied the pharmacokinetics and dosage requirement of gentamicin in one thousand six hundred and forty patients with Gram negative infections. A wide interpatient variation in the kinetic parameters of the drug occurred in all patients and in patients who had normal serum creatinine or normal creatinine clearance. Nearly 1% of the total patients had a significant change in baseline serum

creatinine occurring during or after treatment, which may have been gentamicin associated toxicity. Overt cochlear or vestibular toxicity did not occur in these patients. The study results suggest that the individualizing dosage regimens provided a clinically useful means of rapidly attaining therapeutic peak and trough serum concentrations.

- Ivan Matthews et al.[41] conducted a study on target concentration intervention – parameter variability and predictive performance using population pharmacokinetic models for aminoglycosides in 697 adult patients. The study concluded that using a fixed dose of aminoglycoside will achieve 35% of typical patients within 80–125% of a required dose. Covariate guided predictions can increase this up to 61% and target concentration intervention can potentially achieve safe and effective doses in 90% of patients.

- Crist KD et al.[42] evaluated the impact of a therapeutic drug-monitoring program on total aminoglycoside dose, cost of hospitalization, the duration of therapy, the number of serum concentrations determined, the length of hospital stay, and the potential cost reduction among 221 patients with proven or suspected gram-negative infections. Data's showed statistical significance between the aminoglycoside dose (study) and to the control on the basis of duration of therapy, length of hospital stay, and mean total dose. The type and site of infection, number of serum concentration determinations, and mortality were not statistically different for the groups. These data indicate that a therapeutic drug monitoring program can markedly reduce the total dose of aminoglycoside, which can potentially reduce tissue accumulation and toxicity.

- Nicolau DP et al.[43] the study focused on the use of once-daily aminoglycosides (ODA) in the medical literature and impact on therapeutic drug monitoring. In the first phase of implementation, therapeutic drug monitoring (TDM) was accomplished with the use of a random serum concentration and a nomogram that had been developed. In

the second phase, serum drug concentrations were eliminated on patients with normal renal function. The fully implemented program resulted in a 40% decrease in the request for gentamicin and tobramycin serum concentrations as compared with historic ordering patterns for conventional aminoglycoside dosing regimens. In addition, the incidence of nephrotoxicity was also reduced from 3 to 5% with conventional aminoglycoside dosing, to 1.2 and 1.3% for phases 1 and 2, respectively.

- Triggs E et al.[44] conducted a study on pharmacokinetics and therapeutic drug monitoring of gentamicin in the elderly with serious infection, particularly Gram-negative bacilli. The study conducted in particular; with the decline in renal function, after the aged of 65. Any differences in drug distribution with age are apparently not reflected in gentamicin disposition data, as patients of varying ages have similar volumes of distribution. The study concluded the data support the use of extended interval or once daily doses of gentamicin. It has been suggested that because of a lack of studies for this regimen in the elderly, specific recommendations cannot yet be made and procedures for the once daily administration of gentamicin include the use of the 'Hartford' nomogram and the targeted area under the concentration-time curve. The susceptibility of the elderly to aminoglycoside-related nephrotoxicity (and probably ototoxicity) may arise from a decline in renal function and an impaired capacity for cellular repair and regeneration.

- Soumya Tiwari et al.[45] conducted a study on comparisons of the clinical efficacy, pharmacokinetic profiles and safety of once-daily dosing (ODD) and multiple daily dosing (MDD) of gentamicin in hospitalized Indian children. 400 hundred childrens were studied prospectively. A higher number of patients in the ODD group showed favorable gentamicin peak concentrations as compared with the MDD group. The MDD group showed a higher number of trough concentrations in the undesirable range

as compared with the ODD group .The study supports extended-interval (single daily) dosing in hospitalized Indian children due to its efficacy and safety with the added advantage of needing fewer injections.

- Tobi Frymark et al.[46] Study evaluated the incidence and persistence of gentamicin-induced hearing loss and to determine the effects of dosage, route of administration, schedule of administration, and concomitant ototoxic drug use on the incidence of hearing loss. The intent is that audiologists will use information from this review to better understand the effects of gentamicin regimens on hearing and to advise physicians on the potential ototoxic. The study concluded meta-analyses examining the safety and efficacy of various aminoglycoside (including but not limited to gentamicin) dosing schedules. Similarly, dosage amount also did not appear to affect the likelihood of hearing loss. Based on the limited number of studies included in this review addressing the effects of route of administration, topical application of gentamicin may be associated with higher incidence of hearing loss than were other routes.
- Prins JM et al.[47] conducted a randomized trial in consecutive patients with serious infections for whom an aminoglycoside seemed warranted. For efficacy analysis only those patients were considered in whom treatment with the aminoglycoside was not stopped within 72 h toxicity was analyzed on patients receiving aminoglycosides for more than 48 h and not using other nephrotoxic medication. Gentamicin 4 mg/kg every day- once daily (OD) or gentamicin 1.33 mg/kg three times daily- multiple dose (MD) (with dose-reduction in case of renal dysfunction) were given intravenously. A good clinical response was observed in (91%) of the OD and in (78%) in the MD group. 2 patients in each group died with uncontrolled infection. Study concluded a once-daily dosing regimen of gentamicin is at least as effective as and is less nephrotoxic than more frequent dosing.

"Whether it is old or new the wise should not accept anything without investigation"

CHARAKA

4.1.1. THEOPHYLLINE

Although the development of newer bronchodilators has declined theophylline use in industrialized countries it remains one of the most widely prescribed drugs for the treatment of airway diseases worldwide in the primary and secondary care levels since it is inexpensive. The serum theophylline concentrations of about 10-20 mcg/ml is the target range needed to produce optimum bronchodilator response. The serum concentration levels on or above 20 mcg/ml have been associated with toxicity. Studies show that lower concentrations ranging from 5 – 15 mcg/ml are associated with anti-inflammatory, steroid sparing effects and also reduce the incidence of adverse effects[5,31,32]. There not many studies conducted in India to explore this concentration range effects in the asthmatic patients.

The asthmatic patients coming for consultation in the study site are financially poor patients. The patients are unable to afford for the first line choice on the basis of guideline for the treatment of asthma due to its high expense. The present study wanted to explore the alternative choice of treatment using theophylline which was affordable for the patient and was based on the Guidelines for Management of Bronchial Asthma in India at Primary and Secondary Levels of Health Care in India – a World Health Organization and Government of India collaborative program. Since the drug selected (theophylline) was a candidate of large inter-individual variability and narrow therapeutic index the therapeutic drug monitoring can help in deciding the safe and efficacious dose of theophylline for the patients.

PLAN OF WORK FOR THEOPHYLLINE TREATMENT

Literature Review

↓

Selection of the Study Topic

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Selection of drugs for the study

↓

Designing of the study protocol and documentation forms

↓

Approval from the Human Ethical Committee

↓

Enrolling patients based on the study criteria after their signed informed consent

↓

Estimation of study drug concentration in the target population and observation of the clinical response of the patients

↓

Changing the drug dose if necessary (if not in the therapeutic range)

↓

Observation of serum drug concentration and clinical response after the dose change

↓

Analysis of the observations made

↓

Conclusion and recommendation based on the analysis

4.1.2. GENTAMICIN

The dosing strategy for aminoglycosides has gone through significant changes in the last decade. The American Hospital Formulary System (AHFS) 2008[12]; the starting dose of gentamicin intravenously for patients with normal renal function are follows: Age 10-29 years: 6mg/kg/day; 30-60 years: 5mg/kg/day; above 60 years: 4 mg/kg/day. Our study site hospital prescribes gentamicin in doses 60 mg twice daily, 80 mg twice daily, 100 mg once daily, and 160 mg once daily for the adult population which is comparatively below to doses as compared to the American Hospital Formulary System guideline doses.

Once daily dosing based on target concentration strategy is the preferred method of aminoglycoside dosing in the developed world. However in our country, twice daily dosing has been the most commonly practiced dosing strategy. Also therapeutic drug monitoring of aminoglycosides is not commonly done in Indian hospitals. Another significant issue to consider is the dose used in our patients, which have been clearly lower than those used in western population and recommended in standard text books. Data are not available to know the serum drug concentrations reached with present dosing strategies of gentamicin in our patients. This study was designed to observe the safety and efficacy of the gentamicin dose prescribed for the patients in the study site.

PLAN OF WORK FOR GENTAMICIN TREATMENT

Literature Review

↓

Selection of the Study Topic

↓

Selection of drugs for the study

↓

Designing of the study protocol and documentation forms

↓

Approval from the Human Ethical Committee

↓

Enrolling patients based on the study criteria after their signed informed consent

↓

Observation of the gentamicin dose administered to the patients

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Estimation of study drug concentration in the target population and observation of the clinical response of the patients

↓

Other relevant tests, viz., renal function and audiogram

↓

Analysis of the observations made

↓

Conclusion and recommendation based on the analysis

5.1. THEOPHYLLINE

5.1.1. STUDY CENTER

The study was conducted in the Government District Head Quarters Hospital a secondary care hospital in the Nilgiris, Tamil Nadu, India after approval of the protocol by the Human Ethics Committee, JSS College of Pharmacy, Ooty.

5.1.2. STUDY DESIGN

The study was designed as a prospective open label intervention study. No blinding was adopted in the drug therapy and hence both patients as well as investigators involved knew the study medications.

5.1.3. STUDY DURATION

Each patient of the study was followed for a period of sixty days at regular predetermined intervals from the baseline.

5.1.4. STUDY CRITERIA

Inclusion criteria

- Mild, moderate and severe asthmatic patients who are experiencing both seasonal and perennial asthmatic disorders.
- Adult patients of 18 to 70 years of age.
- Patients who are able to undergo necessary tests to be conducted for the study and give their informed consent for the study.

Exclusion criteria

- Patients who are less than 80% compliant.
- Pediatric patients.
- Pregnant women and lactating mother.
- Patients with other respiratory problems such as chronic bronchitis and emphysema.

- Patients with clinically significant renal, respiratory (other than asthma) cardiac, gastrointestinal, hepatic, endocrine disorders, hematological disorders, acute infection, gram-negative sepsis, extensive burns, cancer or any other concurrent illness or patients who had undergone major surgery.
- Patients with respiratory infections.
- Patients taking drugs interacting with theophylline.

5.1.5. TREATMENT GUIDELINE FOLLOWED[48]

Guidelines for Management of Asthma at Primary and Secondary Levels of Health Care in India, World Health Organization - Government of India Collaborative Programme (2004 – 2005). Drug selected for the treatment and study according to the guideline for different severities was sustained release theophylline.

STAGING OF ASTHMA

	Mild	**Moderate**	**Severe**
Symptoms disturbing sleep	< Once per week	> Once per week	Daily
Daytime symptoms	< Daily	Daily	Daily
Limitation of accustomed activities	Nil	Some limitation	Severe limitation
Use of rescue medication (Inhaler Salbutamol)	< 1 dose per day	1-2 doses per day	>2 doses per day
Forced Expiratory Volume in one second (FEV_1) or Peak Expiratory Flow	Normal	60 – 80%	<60%

Mild asthma can be further divided into intermittent (symptoms for less than two days per week) and persistent (symptoms for more than two days per week) categories, and treatment given accordingly. Patients with intermittent or seasonal symptoms can be managed with only reliever medications (such as short acting $beta_2$ agonist) taken on an as needed basis.

MANAGEMENT OF ASTHMA

Stage	**Preferred choice**	**Alternative choice** **(Second line therapy)***
Mild	Low dose ICS ± LABA	Theophylline/Cromone
Moderate	Medium dose ICS + LABA and/ LTRA	Medium dose ICS + LTRA/Theophylline Or High Dose ICS
Severe	High dose ICS + LABA, LTRA, theophylline and/or oral steroids at the lowest dose	Oral steroids at the lowest dose to control symptoms (alternate day if possible) + therapy as above
In addition to daily controller therapy, reliever medications on as needed basis may be taken in all stages. ICS= Inhaled corticosteroids, LABA= Inhaled long acting $beta_2$ agonist, LTRA= Oral Leukotriene receptor antagonist *The treatment plan followed for the study.		

5.1.6. STUDY DRUG

- Commercially available brand of Theophylline Sustained Release Tablets

300 mg (Theobid), 400 mg and 600 mg (Theoday).

- **Additional Drugs as per guideline:**

Budesonide inhaler 200 mcg/puff (Pulmicort).

Prednisolone tablet 5 mg (Wysolone).

- **Reliever medication***:* Salbutamol inhaler 100 mcg/puff (Asthalin).

5.1.7. ESTIMATIONS DONE DURING THE STUDY

- Theophylline drug concentration in serum;
- Serum creatinine level;
- Body mass index;
- Pulmonary function;
- Quality of life;
- Adverse drug reaction of the drug selected for the study.

5.1.8. INSTRUMENTS USED

- Spirometer - Clemente Clarke VM_1 Mini Spirometer.
- Saint George's Respiratory Questionnaire (SGRQ) for Quality of Life (Annexure - IV).
- High performance liquid chromatography (HPLC) - Shimadzu LC –10 AT VP.
- Naranjo's causality assessment scale for adverse drug reaction (Annexure - V).

5.1.9. DATA COLLECTION

Data collection form was prepared (Annexure IIIa) which was used to record the vital information such as patient identity (I.D), age, gender, inpatient/outpatient (IP/OP) number, weight, height, past medical and medication history which included duration of disease and duration of drug use, co morbidities, allergies, over the counter medications used, pulmonary function, severity and quality of life. Social history like smoking habits, alcohol intake, educational, occupational and economic status were also assessed. The serum theophylline concentration levels assessed during the consequent visits were also recorded.

5.1.10. STUDY PROCEDURE

- The patients were selected on the basis of the study criteria.
- The patients were given information about the study
- After obtaining the signature of the patients in the consent form (Annexure - IIa) they were enrolled in the study.
- The pulmonary function of the patients was taken to observe the condition of the patient with the treatment followed till the day.
- The selected patients were given a run-in–period of seven days where they were advised not to take any medications for disease but were allowed to use salbutamol inhaler as a rescue medication during the crisis.
- The patients after their run-in–period was screened for the following: medical history, routine physical examination, laboratory and biochemical estimations, pulmonary function test using spirometry and reversibility test.
- The patients were diagnosed as asthmatic on the basis of pulmonary function test using spirometry by comparing the pre and post bronchodilation using salbutamol

inhaler 200 mcg – 400 mcg. If a 12% and/or 200 ml increase in the forced expiratory volume is observed between the pre and post bronchodilation the patient will be considered as eligible to be included for the study[49-51].

- Data relevant to the study were collected. Patients were educated regarding their disease and the use of spirometry and the best of the three pulmonary function test (PFT) values Forced expiratory volume in one second (FEV_1), Forced vital capacity (FVC), FEV1/FVC % and Peak expiratory flow (PEF) were documented.
- The quality of life using Saint George's Respiratory Questionnaire was also assessed on the first day.
- Further the study drug was prescribed for the patients based on the guideline followed for the severity assessed. The initial dose of theophylline sustained release was started with the dose of 300 mg per day as per the advice of the physician.
- The patients were educated about their severity and how to take their medications.
- The blood sampling was done on the third day (72 hours) after the administered study drug attained steady state level to assess the serum theophylline concentration. Both trough and peak level samples were collected for the serum theophylline concentration estimation. The pulmonary function assessment was also done on the third day.
- If the patients were not in the therapeutic range for the study drug the dose adjustment was done based on the patients severity. Further assessment was done on the seventh day for both pulmonary function and serum theophylline concentration level, and change in dose was done if there was an unacceptable serum theophylline concentration and unsatisfactory pulmonary function. The pulmonary function test, serum theophylline concentration, quality of life assessment, and adverse drug reaction monitoring was further done on the fifteenth, thirtieth, forty fifth, and sixtieth day.

- Telephonic interview was decided for the patients in case the patient misses a follow-up. Only assessments such as asthma control, adverse drug reactions, quality of life was possible with the telephonic interviews.
- The patients were also enquired on the episodes of poor asthma control to reflect the several dimensions of good asthma control, including physiology symptoms and healthcare use.

5.1.11. ASSESSMENTS DONE FOR THE STUDY

The assessments carried out during the study and the follow-up are given below

Assessments	**Baseline**	**3rd day**	**7th day***	**11th day***	**15th day**	**30th day**	**45th day**	**60th day**
Pulmonary function test	✓	✓	✓	✓	✓	✓	✓	✓
Blood sampling for Serum blood concentration assessment	-	✓	✓	✓	✓	✓	✓	✓
Quality of Life	✓	-	-	-	✓	✓	✓	✓
Adverse drug reaction assessment	-	✓	✓	✓	✓	✓	✓	✓
***In case dose change is given in the previous visit**								

5.1.12. SAMPLING PROCEDURE

Blood samples were collected after the steady state is reached at the trough and peak levels; Sterilized 5 ml tubes were used to collect the blood sample and were labeled with the patient I.P number/O.P number and sampling time; With the help of nurse 3 ml of blood sample was withdrawn from patient using disposable syringe and transferred in to the labeled blood collection tube (Ria vial); The blood sample was centrifuged at 4250 – 4300 revolutions per minute (rpm) for about 10-15 minutes. Upper layer (supernatant) serum was transferred from the centrifuged tube to the labeled serum collecting tube (Eppendorf Tube) without air bubbles using adjustable micropipette by placing the tip of the pipette under the surface of the serum[52]; and the serum - collected were analyzed by high performance liquid chromatography (HPLC) method.

5.1.13. SAINT GEORGE'S RESPIRATORY QUESTIONNAIRE FOR QUALITY OF LIFE[53]

The health related quality of life (QOL) has become an important outcome in respiratory patients as proved by the development of several respiratory diseases – specific questionnaires in the recent years. The Saint George's respiratory questionnaire (SGRQ) was designed to measure health impairment in patients with asthma and chronic obstructive pulmonary disease. It is also valid for use in bronchiectasis and has been used successfully in patients with kyphoscoliosis and sarcoidosis. It is not suitable for systic fibrosis.

The quality of life study for the patients was based on the three major domains – symptoms, activity and impact. These three domains are the most affected areas of the disease under study if appropriate treatment not followed. The "Symptoms" measures the distress caused by respiratory symptoms; "Activity," measures the effect of

disturbances to mobility and physical activity and "Impact," quantifies the psychosocial impact of the disease. A number of items in the symptoms component relate to the frequency of symptoms during the previous year, whereas the activity and impact components relate to the patient's current state. A "Total" score is also calculated from all component items, thus providing a global estimation of the patients respiratory health. The questionnaire is divided into two parts: Part 1 produced the *symptoms* score, Part 2 the *activity* and *impact* scores. For each subscale and for the overall questionnaire, scores range from zero (no impairment) for 100 (maximum impairment).

A difference of four units in the scores indicates a slight clinical effect, while a difference of eight or twelve units indicates moderate or very good clinical effects, respectively.

5.1.14. SPIROMETRY[51,53]

Pulmonary function tests (PFTs) are useful adjuncts in the diagnosis, evaluation, and monitoring of respiratory disease. PFTs can objectively quantify lung function and have been used as the standard evaluation of impairment of chronic lung disease. Spirometry is a procedure used to find the major pulmonary functions such as forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), percentage of FEV_1/FVC and peak expiratory flow (PEF) using an instrument called as spirometer.

Procedure: Mouthpiece and nose clips were secured in place; The patient was instructed to breath normally several times; The patient was instructed to inhale as fully as possible and exhale rapidly, forcefully and completely; The time for full exhalation takes about 5-6 seconds but may be prolonged by severe obstruction; The readings taken for at least three efforts should not vary by more than 5% or 0.1L.

The study used Clemente Clarke VM_1 Mini Spirometer (image given below).

5.1.15. ASSESSMENT OF BODY MASS INDEX

Body mass index (BMI) was calculated using BMI calculator involving the following formula:

$$\text{Body Mass Index} = \frac{\text{Weight of the patient}}{\text{Height}^2 \text{ of the patient}}$$

Weight in Kilogram, and Height in Metres.

Body Mass Index of 18.6 to 24.9 is considered as ideal, 25 to 29.9 is overweight with low health risk, 30 to 40 is considered as obese with moderate health risk and 40 and above is obese with high health risk[54].

5.1.16. ASSESSMENT OF SERUM CREATININE[55]

Method: Alkaline picrate method (Jaffe's method).

Principle: Creatinine present in serum reacts with picric acid in alkaline medium to produce reddish orange colour (Alkaline picrate complex). The rate of colour development is proportional to the creatinine concentration. The rate of reaction is measured photometrically at 510 nm (500-520 nm).

Reagents: Picric acid (Picrate), Sodium hydroxide and Creatinine Standard – 2 mg/dl

Procedure: A working solution was prepared by mixing equal volume of reagents 1 and 2, and set for 5 minutes. The resultant solution was stored in a dark glass bottle and was used on the same day. For the test, 200µl fresh, clear, unhaemolysed serum was mixed with 1000µl of standard reagent solution and the absorbance was measured after 20 seconds. Standard and sample reactions were proceeded at constant temperature and timing conditions. The test was performed using Merck test diagnostic kit (Merck, India) in semiautomatic auto analyzer model: Micro lab – 200, Merck. The Netherlands.

Creatinine was calculated using the following formula:

$$\text{Creatinine (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of standard (2 mg/dl)}$$

Normal Values:

Men : 0.7 to 1.1 mg/dl (0.62-0.97 mmol/l)

Women: 0.6 to 0.9 mg/dl (0.53-0.8 mmol/l)

5.1.17. ESTIMATION OF THEOPHYLLINE IN SERUM SAMPLES USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP - HPLC)

A reversed phase HPLC method was developed during the study. The materials and methods used for the estimation are given below.

Reagents and Chemicals: Acetonitrile of HPLC grade, ortho-phosphoric acid, Triethylamine of HPLC grade, Water of HPLC grade obtained from Milli-Q RO system. Reference Standards of Theophylline was obtained as gift sample. Caffeine (100 mcg/ml) was used as the internal standard.

Chromatographic Conditions:

A Shimadzu LC –10 AT VP HPLC system was used for the analysis.

Stationary Phase	Luna 5u C18 (2) 100A 250 X 4.60 mm
Mobile Phase	Triethylamine Buffer: Acetonitrile (pH 3.5)
Mobile phase ratio	85: 15% v/v
Flow rate	0.8 ml/min
Sample volume	50µl using Rheodyne injector (auto sampler)
Detection	275 nm using UV - Visible detector.
Data station	Class VP data station

The mobile phase was filtered through a 0.22µ membrane and degassed using ultrasonicator. The experiments were carried out at room temperature at about 20^0C.

Preparation of standard stock solutions: Standard solutions of 1mg/ml of theophylline were prepared using a mixture of triethylamine and acetonitrile (1: 1 v/v). From the standard solution, standard stock solutions were prepared to contain 50, 100, 200, 400 and 500 ng/ml of theophylline using mobile phase, respectively. Similarly, a standard solution of caffeine was also prepared.

Preparation of standard and sample solutions: ***Liquid-liquid extraction method:*** To 0.5 ml of standard stock solution (standard solution) or 0.5 ml of the serum sample (sample solution), 250 µl of internal standard caffeine and 5 ml of dichloromethane were added. The resulting solution was shaken for five minutes. Four milliliters of organic layer was separated and evaporated to dryness, to form residue and the residue dissolved in 250 µl of mobile phase.

Method: With the above chromatographic conditions, the standard solution and sample solution were injected and the chromatograms were recorded. The retention time of theophylline and caffeine were 6.7 minute and 9.8 minute respectively. The peak area of standard and the sample solution were calculated. The concentration of theophylline present in the serum sample solution was calculated. The response factors of the standard and sample solutions were calculated.

Recovery: The extraction efficiency was determined by comparing the peak heights of known amounts of theophylline (unextracted) in mobile phase directly injected to peak heights of samples containing the same amounts of theophylline in serum after extraction.

Calibration, accuracy and precision: Quantification was based on calibration curves constructed using peak height ratios of drug to internal standard versus nominal concentration. Intra-day reproducibility was tested by using five different concentrations (50, 100, 200, 400, 500 ng/ml).

The procedure was repeated on three separate days to allow determination of inter-day precision and accuracy. Intraday accuracy was estimated based on the mean percentage error, and the inter day accuracy was calculated as the mean of the intra–day accuracy determinations. The precision, expressed as a percentage, was evaluated by calculating the intra and inter-day relative standard deviation.

Linearity and range of the method: The standard drug solutions in varying concentrations ranging from 50 ng/ml to 500 ng/ml were examined by the assay procedure. The peak area was calculated. The calibration curve was plotted using peak area versus concentration of the standard solutions. The calibration curves show a linear response over the range of concentrations used in the assay procedure. The calibration curve passes through the origin, which justifies the use of single point calibration.

5.1.18. ADVERSE DRUG REACTION MONITORING[9,56]

The adverse drug reaction (ADR) monitoring of the study drug was done on the follow–up visits after baseline. Naranjo's algorithm scale was used for the causality assessment. Naranjo's algorithm consensual, content and concurrent validity considers several elements to assess the causality. Each question is weighed, with the total at the end of the question categorizing the adverse event as a definite (≥ 9), probable (5-8), possible (1-4) or doubtful (0) related to the suspected medication. The elements considered in this algorithm are as follows: previous conclusive report on this reaction,

time frame of the occurrence (after the administration of the suspected drug), improvement in patient after discontinuation of therapy, patient response with rechallenge, alternative causes for the reaction (other than the drug) for the reaction, recurrence with placebo, drug detected in the blood (or other fluids) in concentrations known to be toxic, relationship with severity and dose, occurrence of similar reaction to the same or similar drugs in any previous exposure and availability of objective evidence.

5.1.19. PHARMACOECONOMIC EVALUATION[9,57]

The pharmacoeconomic evaluation was done using cost effective analysis. Here the total cost related to the treatment and clinical response of the study subjects before getting enrolled into the study was compared with the total cost related to the treatment and clinical response of the study subjects at the end of the study. The total cost for the treatment was calculated with the direct medical cost, direct non-medical cost and indirect cost. The direct medical costs involved the medication cost, laboratory expenses and bed cost if any. The indirect medical costs involved the travel expenses and food expenses involved. The indirect medical costs involved the loss of wages of the patients and loss of wages for the patient's attenders if any. The clinical outcome assessed in the study was the percentage of predicted forced expiratory volume in one second for the treatment prior to the study and at the end of the study. The Average Cost Effectiveness Ratio (ACER) was calculated based on the following formula:

ACER = Healthcare Cost (in Rs.) ÷ Clinical Outcome (response to the treatment given)

As per the pharmacoeconomic principles, the least cost per outcome gained should be chosen as effective alternate.

5.1.20. STATISTICAL ANALYSIS

The data obtained through structured format were tabulated. The statistical analysis like, student t- test, Chi square test, and analysis of variance (ANOVA) were done using the statistical software Graph Pad Instat®. ANOVA followed by Tukey – Kramer multiple comparison post test was used in the analysis of the changes in variables from baseline to end of study in all the groups. Pair wise comparison between the groups was performed if the P value for the overall test was less than 0.05 at 95% confidence intervals of the means.

5.2. GENTAMICIN

5.2.1. STUDY CENTER

The study was conducted in the Government District Head Quarters Hospital a secondary care hospital in the Nilgiris, Tamil Nadu, India after approval of the protocol by the Human Ethical Committee, JSS College of Pharmacy, Ooty.

5.2.2. STUDY DESIGN

The study conducted was a prospective open label observational design conducted for a period of May 2010 to February 2011.

5.2.3. STUDY CRITERIA

Inclusion criteria

- Adult patients who were admitted to medical wards and who have not taken antibiotics prior to the admission. Clinical diagnosis was made based on the infections.
- Patient who are receiving parenteral injection gentamicin 40 mg/ml.

- Patient who are able to give informed consent for the study.

Exclusion criteria

- Patient with any major disorders of the hepatic, gastrointestinal or haemopoietic systems.
- The patient had a known history of allergy or hypersensitivity to gentamicin.
- Pregnant women.
- Patients with burns on > 20% of body surface.
- Patients on dialysis.
- The infection was severe enough to prevent patients from participating audiogram assessment.

5.2.4. ENROLMENT OF SUBJECTS

Patients satisfying the study criteria were enrolled after obtaining their signature in the consent form (Annexure IIb).

5.2.5. DRUGS AND DOSAGE REGIMEN UNDER THE STUDY

Study drug was Injection Gentamicin 40mg/ml.

Gentamicin Dose (Intravenous)	Frequency
60 mg	Twice daily
80 mg	Twice daily
100 mg	Once daily
160 mg	Once daily

5.2.6. DATA COLLECTION

Patient details were collected including, age, height, body weight, and base-line serum creatinine. Ideal body weight and body mass index were calculated. All the patient details were entered in a structured documentation form. The serum creatinine was also estimated using Jaffes method[55].

5.2.7. IDENTIFICATION OF MICROORGANISMS

When the patient was enrolled in the study, before treatment with antibiotic, sputum sample was collected using sterile screw cap vials. Procedure for specimen collection and identification of the pathogen is given in Appendix 3 of Indian Pharmacopoeia 1996[58].

Direct microscopic examination of the specimen was done as the first step in the laboratory diagnosis. Smear preparation was done by Dry Mount Technique. Gram staining technique was used on the smear in order to identify whether the specimen contains gram negative or gram positive organisms.

For the growth and isolation of the micro-organisms three media, namely, Blood Agar, Chocolate Agar and Mac-Conkey Agar (Hi-Media Laboratories Ltd., Mumbai) were used. The specimen was aseptically inoculated into the petri dish by discontinuous streaking method. Preliminary identification of the organisms was done macroscopically. Biochemical tests were carried out for identification of the microorganisms. Four different media were used for the purpose such as triple sugar-iron agar, peptone water, citrate utilization media and mannitol motility agar (Hi-Media Laboratories Ltd., Mumbai).

MICROBIOLOGICAL ASSAY

Microbiological assay for gentamicin was performed based on the procedure for Microbiological tests and assays described in Appendix 4 of Indian Pharmacopoeia 1996[58]. The method is as follows:

Principle: The inhibition of microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of antibiotics. The microbiological assay is based upon a comparison of the inhibition of growth of bacteria (zone of inhibition) by measured concentrations of the antibiotics to be examined with that produced by known concentrations of standard preparation of the antibiotics having a known activity.

Test organisms and inoculums: The test organism recommended for gentamicin was *Staphylococcus epidermidis* - 2493 was obtained from National center for industrial micro-organism (NCIM). A working standard of the antibiotic, *Staphylococcus epidermidis* was prepared.

Procedure: Two general methods are usually employed for microbial assay (cup-plate method and tube assay method). In the present study, the procedure was slightly modified for the microbial assay of gentamicin. Instead of cup plate method, disc diffusion method was followed as described by Heyward et al[59] and Rejean et al[60] the procedure is as follows:

A suspension of the test organism was prepared in the medium mentioned above with the inoculums composition of 0.03ml/100ml at 1:40 dilution to which 300mg/l of manganese sulphate was added. The suspension was kept for twenty four hours at 32^{o}C to 35^{o}C. After the suspension was prepared, it was added to each of several different

flasks containing 100 ml of the medium. For each petri dish, 16 ml of the inoculums having a microbial strength of 105 cfu/ml was used and was found to produce the optimum zones of inhibition for the median concentration of the antibiotic with respect to both clarity and diameter. Inter-day and intra-day variation were calculated by repeating the assay with standard solution on three different days and 3 different times of the day.

The standard concentrations used for the assay were 2, 4, 6, 8 and 10µg/ml. A stock solution of 1mg/ml of gentamicin was prepared in phosphate buffer solution. From the stock solution, sufficient solution was withdrawn and mixed with plain serum in 10 ml volumetric flask to make standard solutions. The standard thus prepared was stored at -20^{o}C and was used within a week. Peak serum samples collected from the patients were diluted with plain serum before the assay. To 0.5 ml of serum sample collected from the once daily dose and multiple dosing groups, 2 ml plain serum was added (Dilution factor : 5) and to 0.5 ml of serum sample collected from control group, 1ml of plain serum was added (Dilution factor : 3) before assay. Trough serum samples collected from all the study patients were used with-out any dilution.

Sixteen milliliters of the previously liquefied medium containing the suspension of micro-organism (prepared as mentioned above) at the temperature between 40^{o}C and 50^{o}C was immediately poured into petri-dishes to give a uniform layer of medium with a depth of 3 to 4 mm. After solidifying, discs containing one set of median standard solution and one set of one of the standard/test solution was placed. Plain, sterilized discs were impregnated with standard/test solution to allow saturation of the disc with the solution and were placed on the media. The plates were kept aside for one hour in the refrigerator at 4^{o}C and then incubated for twenty four hours at 35^{o}C to 37^{o}C. The

zone diameter was measured using zone reader and the results were calculated using one level factorial assay.

The average reading of solution S_3 in each set of plates and the average values of the all readings of solution S_3 was taken as the correction points for the curve. The average value obtained for each concentration (S_1, S_2, S_4, and S_5) was corrected accordingly. Thus in correcting the value obtained with any concentration, say S_1, if the average of all reading S_3 was for example 18.0 mm and the average of the S3 concentrations on one set of plates was 17.8 mm, the correction would be +0.2 mm. If the average reading of S_1 was 16.0mm, the corrected reading of S_1 would be 16.2 mm. These corrected values including the average of all the readings for solutions S_3 were plotted on cycle semi-log paper, using the concentration in units or μg per ml (as the ordinate logarithmic scale) and the diameter of the zones of inhibition as the abscissa. A straight response line was drawn by plotting the points for highest and lowest zone diameters obtained by means of the following equation.

$$L = \frac{3a + 2b + c - e}{5} \qquad H = \frac{3e + 2d + c - a}{5}$$

Where,

L = the calculated zone diameter for the lowest concentration of the standard curve response line.

H = the calculated zone diameter for the highest concentration of the standard curve response line.

C = Average zone diameter of 36 readings of the reference point solution.

a, b, d, e = Corrected average values for the other standard solutions. Lowest to highest concentration, respectively

The zone diameter for the sample solution and the solutions for S_3 on the plates used for the sample solution were averaged. If the sample gave a larger average zone size, than the average of the standard (solution S_3) the difference between them was added to the zone size of solution S_3 of the standard response line. If the sample gave a smaller average zone size than the average of the standard (solution S_3) the difference between them was subtracted from the zone size of solution S_3 of the standard response line. From the response line the concentration corresponding to these corrected values of zone sizes were read and from the dilution factor the concentrations of the samples were calculated.

5.2.8. BLOOD COLLECTION AND ASSAY

All blood samples were collected by following universal precautions for venipuncture (National Committee for Clinical Laboratory Standard 1990). After the third dose (steady state) two samples were collected from each of the patients. Peak concentration sample was collected thirty minutes to one hour after the intravenous administration of gentamicin and trough sample was collected before the next administration.

2 ml blood sample from each patient was collected in sterilized 5 ml tubes. The tubes were labeled with patient identification labels and were allowed to clot adequately before centrifugation to separate serum. All samples obtained from the different dosage group were immediately assayed or stored at -20^{o}C until use, in order to accumulate sufficient number of samples for assay. The serum gentamicin concentration was determined according to the microbiological assay described above. The sample plates denoting the method used is given in plates 1 to 3.

5.2.9. AUDIOMETRY ASSESSMENT

Audiogram was obtained using arphi clinical diagnostic audiometer model 2001 digital. The hearing loss attenuator (intensity dial) was calibrated for hearing loss of 10 dB to 100 db in 5 db steps. The audiogram assessment was done as follows:

The earphones were plugged in to the jack socket on the side panel. The audiometer was switched by operating the switch to "ON". The instrument was set by starting the test with 1000 Hz and then to each higher frequency and upto 8000 Hz. The procedure was repeated to find out the reliability and validity of the patient's response. After this, the test was done at frequencies lower than 1000 Hz. The intensity of the tone was controlled by rotating the hearing loss attenuator. While testing each frequency the intensity of the tone was presented at a higher level to make the patient aware of the tone and then gradually brought down to the threshold of the patient, which was 50% response.

5.2.9. OUTCOME ASSESSMENT

Independent assessment of the patients by a clinician who did not receive information regarding the study arm of the patients was made to avoid bias in the assessment. Clinical efficacy was defined as cure, improvement or failure. Nephrotoxicity was defined as an increase in serum Creatinine concentration by at least 25% during the study period. The patients were assessed to have clinical auditory toxicity based on the report on tinnitus, reduced hearing or deafness.

5.2.10. ADVERSE DRUG REACTION MONITORING

The adverse drug reaction (ADR) monitoring of gentamicin was done for the patients during the study duration. Naranjo's algorithm scale was used for the causality assessment. Naranjo's algorithm consensual, content and concurrent validity considers

several elements to assess the causality. Each question is weighed, with the total at the end of the question categorizing the adverse event as a definite (≥ 9), probable (5-8), possible (1-4) or doubtful (0) related to the suspected medication. The elements considered in this algorithm are as follows: previous conclusive report on this reaction, time frame of the occurrence (after the administration of the suspected drug), improvement in patient after discontinuation of therapy, patient response with rechallenge, alternative causes for the reaction (other than the drug) for the reaction, recurrence with placebo, drug detected in the blood (or other fluids) in concentrations known to be toxic, relationship with severity and dose, occurrence of similar reaction to the same or similar drugs in any previous exposure and availability of objective evidence.

6.1. THEOPHYLLINE

The study was conducted from January 2009 to June 2011 in the Government District Headquarters Hospital, Ooty, Tamil Nadu which is a 420 bedded secondary care hospital. A total of 146 asthmatic patients were screened for the study. Among them, 125 patients were eligible to be included in the study based on the study criteria. Out of 125 eligible patients, 15 denied to participate in the study. Pulmonary function test was done for rest of the 110 patients after getting their informed consent to participate in the study. This was to assess their response to the antiasthmatic drugs they were treated with till then. Further the patients were subjected to a run-in-period of seven days in which the antiasthmatic medications they were taking were stopped and the use of salbutamol inhaler as a rescue medication was allowed in case of crisis. After the run-in-period, the pulmonary function test was done with pre and post bronchodilation using salbutamol inhaler (200 - 400 mcg) to observe the reversibility. Accordingly, 8 patients did not show any reversibility after post bronchodilator use, while rest of the 102 patients showed a reversibility of 12% increase in forced expiratory volume in one second (FEV_1) after bronchodilation and were enrolled for the follow up phases.

All the 102 patients completed the study period of sixty days. The patients were categorized into mild, moderate and severe category by comparing their predicted pulmonary function value with their observed pulmonary function on the baseline day. The observations and analysis of the 102 patients who were enrolled and completed the study are discussed here.

6.1.1. DEMOGRAPHIC DATA

The demographic data of the patients in the study are given in table 7.1.1 (page no. 84). Among the 102 patients enrolled in the study the majority of the patients were male gender (n= 80, 78.43%).

The age group of 41 to 60 years comprised about 60.78% (n=62) of the patients followed by 32.35% (n=33) patients in the 21 to 40 years of age group and only 6.86% (n=7) patients were in the age group above 60 years. The mean age of the patients enrolled in the study was 45.17 ± 9.04 years.

Among the 102 patients, 9.8% (n=10) were illiterates and 90.2% (n=92) were literates. 85 among the literate patients had attained school level education and rest of the 7 patients had attained graduate level education. 21.57% (n=22) patients were unemployed; 58.82% (n=60) patients were daily wagers; and 19.61% (n=20) patients were office workers. 38.23% (n=39) patients had an income of less than Rs.1000 per month, 25.49% (n=26) patients had monthly income between Rs.1000 to 3000 and 14.71% (n=15) patients had monthly income between Rs.3000 to 6000.

72.55% (n=74) of the patients were non- smokers; 16.67% (n=17) were past smokers and 10.78% (n=11) were smokers. The mean body mass index of the enrolled patient was 22.15 ± 1.93 kg/m^2. 6.86% (n=7) patients had the problem of asthma for less than 5 years; 27.45% (n=28) patients had the asthma for past 5 to 10 years, 34.31% (n=35) patients had asthma for past 11 to 15 years and 31.37% (n=32) patients had asthma problem for more than 15 years. The mean creatinine level of the patients was found to be 0.74 ± 0.11 mg/dl.

6.1.2. SEVERITY AND PULMONARY FUNCTION OF THE PATIENTS AT BASELINE

Among the study patients, 20 of them (19.61%) were mild asthmatic patients, 50 of them (49.02%) were moderate asthmatic patients and 32 of them (31.37%) were severe asthmatic patients. The mean forced expiratory volume in one second (FEV_1) observed among the mild, moderate and severe patients at the baseline were 1.97 ± 0.38, 1.93 ± 0.27 and 1.25 ± 0.27 litres respectively. The overall average FEV_1 value was 1.73 ± 0.43 litres. The mean forced vital capacity (FVC) of mild, moderate and severe patients were observed to be 2.45 ± 0.26, 2.31 ± 0.39 and 1.79 ± 0.37 litres respectively. The overall mean FVC was 2.17 ± 0.45 litres. The mean peak expiratory flow (PEF) of mild, moderate and severe patients were observed to be 244.45 ± 63.54, 234.14 ± 50.20 and 159.25 ± 49.70 litres/minute respectively. The overall mean PEF was found to be 212.67 ± 63.82 litres/minute. Table 7.1.2 (Page no. 86) represents the baseline data of the mean pulmonary function.

6.1.3. QUALITY OF LIFE OF THE STUDY PATIENTS AT BASELINE

The average symptom score for the mild, moderate and severe patients at baseline were observed to be 39.76, 59.98 and 88.74 units respectively. The overall symptom score for the 102 patients enrolled was 65.04 units. The average activity score of mild, moderate and severe patients were observed to be 55.03, 49.87 and 84.02 units respectively. The overall activity score of the total patients enrolled was 61.60 units. The average impact score of mild, moderate and severe patients were observed to be 65.41, 62.23 and 77.58 units respectively. The overall impact score of the total patients was found to be 67.67 units. The average total score of mild, moderate and severe patients were observed to be 58.00, 55.59 and 82.85 units respectively. The overall total

score of the total patients was found to be 64.62. Table 7.1.3 (Page no. 87) represents the mean quality of life of the patients enrolled at baseline.

6.1.4. SERUM THEOPHYLLINE CONCENTRATION DURING THE STUDY

The patients in the study started treatment with theophylline sustained release tablet of 300 mg once per day as per the physician's advice. The additional drugs were given as per the severity guideline. The trough and peak serum concentration was assessed on the third day after the steady state was reached. Among the 102 patients, only 5 patients were observed to be in the therapeutic range (5.19 ± 0.09 and 6.94 ± 0.09 mcg/ml of trough and peak level respectively) with 300 mg sustained release theophylline tablet. Among the 5 patients who were in the therapeutic range, 4 were moderate asthmatic patients and 1 was severe asthmatic patient.

Out of the 97 patients who were not in the therapeutic range, 20 patients were mild patients (3.70 ± 1.08 and 5.30 ± 1.12 mcg/ml of trough and peak level respectively), 46 patients were moderate (3.40 ± 0.94 and 5.10 ± 0.91 mcg/ml of trough and peak level respectively), and 31 were severe patients (4.02 ± 0.78 and 5.65 ± 0.85 mcg/ml of trough and peak level respectively). The mild patients' dose was increased to 400 mg of sustained release theophylline tablet per day and the moderate to severe patients the dose was increased to 600 mg of sustained release theophylline tablet per day as per the physician's advice.

Table 7.1.8a (Page No. 91) represents the mean serum theophylline peak concentration of the patients received different doses of theophylline during the study. The mean steady state peak concentration of theophylline 300 mg ranged between 6.94 to 7.16 mcg/ml over the study duration. The percentage coefficient of variation ranged between 1.20 to 4.52. The patients who were on 400 mg of theophylline sustained release tablet

have shown the mean steady state peak concentration in the range of 9.65 to 10.14 mcg/ml with the percentage coefficient variation between 20.04 to 20.44. 600 mg of the theophylline attained the mean steady state peak concentration in the range of 11.78 to 12.20 mcg/ml with the percentage coefficient variation between 19.95 to 20.49. The mean theophylline peak concentration of these three doses showed good linearity (correlation coefficient, r^2= 0.94).

The mean steady state trough concentration of theophylline 300 mg ranged between 5.19 to 5.28 mcg/ml over the study duration. The percentage coefficient of variation ranged between 0.89 to 2.68. The patients who were on 400 mg of theophylline sustained release tablet have shown the mean steady state trough concentration in the range of 7.13 to 7.94 mcg/ml with the coefficient variation between 21.58 to 23.10%. 600 mg of the theophylline attained the mean steady state trough concentration in the range of 8.20 to 8.52 mcg/ml with coefficient variation between 27.38 to 27.94%. The mean theophylline trough concentration of these three doses showed linearity (correlation coefficient, r^2= 0.80). Table 7.1.8b (Page No. 92) represents the mean serum theophylline trough concentration of the patients receiving different doses of theophylline during the study.

The trough concentration ranges of mild, moderate and severe category of patients were found to be 5.64 to 9.96, 5.65 to 10.88 and 6.08 to 11.18 mcg/ml respectively. Similarly, the peak concentration ranges of mild, moderate and severe category of patients were found to be 7.07 to 12.23, 9.19 to 15.06 and 8.63 to 15.17 mcg/ml respectively. From these it may be considered that the minimum effective concentration of theophylline in all the three severities should be set as 6 mcg/ml whereas the peak

concentration for mild patients should not exceed 13 mcg/ml and for moderate and severe patients it should not exceed 16 mcg/ml.

6.1.5. QUALITY OF LIFE OF THE STUDY PATIENTS DURING THE STUDY

6.1.5a. Symptom Score

Table 7.1.9a (Page no. 93) represents the mean symptom domain scores of the patients during the study. The symptom scores observed has shown very good clinical effect throughout the study for the patients receiving the different dose of theophylline when the follow up score was compared to the baseline score. Figure 3 (Page No. 98) represents the clinical improvement observed in the symptom. Statistical analysis showed that symptom scores of the patients receiving different doses of theophylline during the study were considered extremely significant among the follow ups (P value < 0.0001).

6.1.5b. Activity Score

Table 7.1.9b (Page no. 94) represents the mean activity domain scores of the patients during the study. The activity scores showed that the patients receiving the 300 mg of theophylline had moderate clinical improvement at the 30^{th} day and very good clinical improvement on the 45^{th} and 60^{th} day when compared to the baseline. The activity score of patients receiving 400 mg of theophylline showed very good clinical improvement throughout the follow up when compared to the baseline. The activity score of the patients receiving 600 mg of theophylline showed slight clinical improvement on the 15^{th} day, moderate clinical significant improvement on the 30^{th} day and very good clinical significant improvement on the 45^{th} and 60^{th} day when

compared to baseline. Figure 4 (Page No. 99) represents the clinical improvement observed in activity.

Statistical analysis showed that activity score of the patients receiving 300 mg theophylline treatment did not show any quite significant difference (P value = 0.508) during the study and the activity score of the patients receiving 400 mg and 600 mg of theophylline during the study were considered extremely significant (P value < 0.0001).

6.1.5c. Impact Score

Table 7.1.9c (Page no. 95) represents the mean impact domain scores of the patients during the study. The impact scores observed has shown very good clinical effect throughout the study for the patients receiving the different dose of theophylline when the follow up score was compared to the baseline score. Figure 5 (Page No. 100) represents the clinical improvement observed for impact.

Statistical analysis showed that impact score of the patients receiving 300 mg theophylline treatment was considered extremely significant (P value < 0.0002) and the patients receiving 400 mg and 600 mg of theophylline were also considered extremely significant (P value < 0.0001).

6.1.5d. Total Score

Table 7.1.9d (Page no. 97) represents the mean total quality of life scores of the patients during the study. The total scores observed have shown very good clinical improvement throughout the study for the patients receiving the different dose of theophylline when the follow up score was compared to the baseline score. Figure 6 (Page No. 101) represents the clinical improvement observed in the total score.

Statistical analysis showed that total scores of the patients receiving different doses of theophylline during the study were considered extremely significant (P value < 0.0001).

6.1.6. PULMONARY FUNCTION OF THE STUDY PATIENTS DURING THE STUDY

6.1.6a. Forced Expiratory Volume in One Second (FEV_1) in Litre

Table 7.1.11a (Page no.102) represents the mean FEV_1 of the patients receiving different dose of theophylline during the study duration. The patients receiving 300 mg of theophylline showed a clinically significant improvement in the FEV_1 (5% increase) from the 15^{th} day when compared to the baseline. Patients receiving 400 mg of theophylline showed a clinically significant improvement from the 45^{th} day and patients receiving 600 mg showed a clinically significant improvement from the 15^{th} day when compared to the baseline.

Statistical analysis showed that FEV_1 of the patients receiving 300 and 600 mg of theophylline treatment was considered extremely significant (P value < 0.0001) and FEV_1 of the patients receiving 400 mg theophylline treatment was considered not quite significant (P value 0.3902).

6.1.6b. Forced Vital Capacity (FVC) in Litre

Table 7.1.11b (Page no.105) represents the FVC of the patients at different doses of theophylline. In case of forced vital capacity the statistically significant difference was only seen with the 300 mg theophylline treated patients on the baseline to 15^{th} day (P value <0.05). Other doses of theophylline statistical significant difference were not observed between the baseline and follow – up days.

6.1.6c. Peak Expiratory Flow (PEF) in Litre/Minute

Table 7.1.11c (Page no. 105) represents the PEF of the patients at different doses of theophylline. The statistical analysis of the PEF has indicated extreme significance (P value < 0.0001) in all doses of theophylline used in the study.

6.1.7. COST EFFECTIVE ANALYSIS

The various types of costs like direct medical cost, direct non medical cost and indirect cost for each patients of this study was calculated for one month based on their prescription. As the study was carried out in government hospital, the treatment costs were calculated according to the cost of the effective proprietary products available in the market. Table 7.1.12 (Page no. 109) represents the total cost of the study for patients related to treatment prior to the study and at the end of the study. The drugs which were prescribed empirically to the study patients before the study included Tab. Deriphylline 100 mg, Tab. Salbutamol 4 mg and Chlorpheniramine maleate cough syrup (50 ml).

The average cost effectiveness ratio (ACER) was analyzed based the costs related to the treatment given the patients and the response observed in the study patients at the last follow - up. The therapeutic response which was taken into account was the average percentage of predicted forced expiratory volume in one second. The ACER for the treatment of the study subjects who were receiving prior to the study and at the end of the study were compared to assess the cost effective treatment.

- ACER of the treatment prior to the study enrolling =

  Cost per day per patient ÷ Percentage of FEV_1 predicted value = 27.10/64.79 = **0.42**

- ACER of the treatment at the end of the study =

Cost per day per patient ÷ Percentage of FEV_1 predicted value = 17.83/73.77 = **0.24**

As per the pharmacoeconomic principles, the least cost per outcome gained should be chosen as effective alternative. The study results show that the guideline treatment used in our study had the least cost per outcome and is the effective alternative when compared to the empirical treatment for the patients involved in the study.

6.1.8. ADVERSE DRUG REACTION MONITORING

Safety and tolerability of study medications were assessed by physical examination including oropharyngeal inspection, heart rate and blood pressure measurements. There were no significant changes in such assessments recorded in all the clinical visits compared to baseline values. Since the study patients were not given with high dose of theophylline the peak and trough level therapeutic concentrations of the patients did not reach the higher ranges. Therefore, there were very less adverse effects of the drug observed in this study.

Among the 77 patients receiving theophylline dose of 600 mg per day 4 reported adverse drug reactions (ADRs). Table 7.1.13 (Page No. 110) represents the ADR observed with theophylline. Headache, insomnia and palpitation were the most reported ADRs among the patients. According to Naranjo's Scale used for the causality assessment, it was confirmed that most of the ADRs were probable to oral theophylline tablet treatment since the total score ranged from 5 to 8. The drugs were continued till the end of the study since it was observed as non–serious in nature by the physician.

6.2. GENTAMICIN

During the study period, a total of 60 patients who satisfied the study criteria were enrolled in the study from the Government District Headquarters Hospital, Ooty. Gentamicin is the aminoglycoside which are prescribed more often by the physicians due to their effectiveness in treating Gram negative infection and their low cost. It is also used in combination with other broad spectrum antibiotics in case of empirical treatment for mixed infection. The patients were categorized based on the different dosage regimen of intravenous gentamicin as follows:

1. Multiple Daily Dose (MDD) – a) 60 mg twice daily; b) 80 mg twice daily, and

2. Once Daily Dose (ODD) - a) 100 mg once daily; b) 160 mg once daily.

Clinical data collected for the patients included the complete blood profile, especially the erythrocyte sedimentation rate, white blood cell count, serum creatinine level and audiogram report. Microbiological assay method was followed for the estimation of gentamicin concentration in the blood.

6.2.1. DEMOGRAPHIC DATA

Table 7.2.1 (Page no. 111) represents the demographic details of the patients enrolled. Among the 60 patients enrolled in the study 33 were male and 27 were female patients. 46 patients received multiple dose of gentamicin per day (twice daily) and 14 patients received the once daily dose of gentamicin. The patients were treated with gentamicin for a minimum period of 4 days. The patient mean age group treated was between 30 to 46 years. The physician did not continue the prescribing of gentamicin beyond this period to avoid toxic effects related to the drug.

6.2.2. DIAGNOSIS OF THE PATIENTS TREATED WITH GENTAMICIN DURING THE STUDY

Table 7.2.2 (Page no. 112) represents the diagnosis of the patients enrolled. The data observed show that gentamicin was mostly prescribed in case of respiratory problems such as chronic obstructive pulmonary disease and respiratory tract infection.

6.2.3. ADDITIONAL ANTIBIOTICS PRESCRIBED WITH GENTAMICIN

Table 7.2.3 (Page no. 113) represents the additional antibiotics which were prescribed with gentamicin injection. It was observed that injection cefotaxime (beta-lactam antibiotic) was prescribed frequently in adjunct with gentamicin for the empirical treatment of infections.

6.2.4. PATHOGENS ISOLATED FROM THE SPUTUM SAMPLES OF THE PATIENTS

Sputum samples from 20 patients were collected during the study. The samples were collected before the initiation of the gentamicin therapy. The pathogens identified in the sputum samples among the 20 patients were *Klebsiella pneumonia* (70%), *Pseudomonas aureginosa* (10%), *Escherichia coli* (15%) and *Haemophilus influenza* (5%). Table 7.2.4 (Page no. 114) represents the pathogen isolated from the sputum samples of the 20 patients.

The outcome assessments found in the study subjects were based on the cure with the therapy. The study has found cure to have been attained with the dosage regimen which was concluded with the observation of the micro-organism presence in the biological sample (sputum) before and absence of pathogens after the treatment. The micro–organisms were not observed in the sputum samples collected after the treatment with gentamicin in the patients.

6.2.5. SERUM GENTAMICIN CONCENTRATION

The serum gentamicin concentrations were estimated at the peak and trough level, i.e., after reaching the steady state (after the third dose), the concentration half an hour prior to the dose (trough) and one hour after the dose (peak). Table 7.2.5 (Page no.115) represents the peak and trough concentrations of the different doses. The peak and trough gentamicin concentration observed in the patients with different dosage regimen of gentamicin did not show any significant difference. The average peak concentration of the patients treated with 60 mg twice daily, 80 mg twice daily, 100 mg once daily and 160 mg once daily were 4.45, 4.67, 4.63 and 4.83 mcg/ml respectively. The average trough concentration of the patients treated with 60 mg twice daily, 80 mg twice daily, 100 mg once daily and 160 mg once daily were 0.58, 0.60, 0.64 and 0.78 mcg/ml respectively.

6.2.6. ADVERSE DRUG REACTION MONITORING

The adverse drug reaction (ADR) monitoring of the gentamicin was done throughout the study duration of the patient while he or she was receiving gentamicin. The patients were checked for the renal function and audiogram after the treatment with gentamicin. The creatinine level was in normal for all the patients after the gentamicin treatment. It was found that 2 (3.3%) patients among the 60 patients enrolled showed a suspected adverse drug reaction of ototoxicity. The causality assessment using Naranjo's algorithm scale showed a score of 6, indicates that it comes under probable category. Table 7.2.7 (Page no. 116) represents the ADR observed due to gentamicin in the study patient.

7.1. THEOPHYLLINE

7.1.1. DEMOGRAPHIC DATA OF THE STUDY SUBJECTS

Sl. No.	Characteristics			N=102 (%)	Mean ± SD
1.	Gender	Male		80 (78.43%)	-
		Female		22 (21.57%)	-
2.	Age (in years)	21 – 40		33 (32.35%)	34.45 ± 3.76
		41 – 60		62 (60.78%)	49.38 ± 4.82
		> 60		7 (6.86%)	62 ± 1.00
3.	Educational status	Illiterate		10 (9.8%)	-
		Literate	School level (85)	92 (90.2%)	-
			Graduate (7)		-
4.	Occupational status	Unemployed		22 (21.57%)	-
		Daily wagers		60 (58.82%)	-
		Office workers		20 (19.61 %)	-
5.	Income status (in rupees)	No income		22 (21.57%)	-
		< 1000		39 (38.23%)	-
		1000 - 3000		26 (25.49%)	-
		3000 - 6000		15 (14.71%)	-

6.	**Smoking status**	Non– smokers	74 (72.55%)	-
		Past Smokers	17 (16.67%)	-
		Smokers	11 (10.78%)	-
7.	**Weight**	Male	80 (78.43%)	61.43 ± 5.90
	(in kg)	Female	22 (21.57%)	54.36 ± 6.43
8.	**Body Mass Index**	Male	80 (78.43%)	21.87 ± 1.91
	(in kg/m^2)	Female	22 (21.57%)	23.15 ± 1.70
9.	**Duration of disease condition**	< 5 years	7 (6.86%)	-
		5 – 10 years	28 (27.45%)	-
	(in years)	10 – 15 years	35 (34.31%)	-
		> 15 years	32 (31.37%)	-
10.	**Creatinine level**	Male	80 (78.43%)	0.76 ± 0.11
	(in mg/dl)	Female	22 (21.57%)	0.68 ± 0.05

7.1.2. BASELINE PULMONARY FUNCTION AND SEVERITY OF THE STUDY SUBJECTS

Sl. No	Severity	Number of patients (%)	Pulmonary function (Mean ± SD)			
			FEV_1 (in litres)	**Percentage Predicted FEV_1 (%)**	**FVC (in litres)**	**PEF (in litres/minute)**
1.	**Mild**	20 (19.61 %)	1.97 ± 0.38	87.26 ± 4.97	2.45 ± 0.26	244.45 ± 63.54
2.	**Moderate**	50 (49.02 %)	1.93 ± 0.27	64.38 ± 4.37	2.31 ± 0.39	234.14 ± 50.20
3.	**Severe**	32 (31.37 %)	1.25 ± 0.27	42.01 ± 7.34	1.79 ± 0.37	159.25 ± 49.70
4.	**Total Patients**	102	1.73 ± 0.43	61.85 ± 16.92	2.17 ± 0.45	212.67 ± 63.82

FEV_1 – Forced expiratory volume in one second

FVC – Forced vital capacity

PEF – Peak expiratory flow

7.1.3. Baseline Quality of Life of study subjects

Sl. No	Severity	Number of patients (%)	Quality of life domain scores (Mean ± S.D)			
			Symptom	**Activity**	**Impact**	**Total**
1.	**Mild**	20 (19.61 %)	39.76 ± 5.02	55.03 ± 5.01	65.41 ± 19.70	58.00 ± 12.32
2.	**Moderate**	50 (49.02 %)	59.98 ± 9.87	49.87 ± 27.32	62.23 ± 19.11	55.59 ± 22.10
3.	**Severe**	32 (31.37 %)	88.74 ± 5.59	84.02 ± 7.59	77.58 ± 10.79	82.85 ± 6.01
4.	**Total Patients**	102	65.04 ± 19.46	61.60 ± 24.91	67.67 ± 18.24	64.62 ± 20.76

7.1.4. LINEARITY OF THEOPHYLLINE IN SERUM BY HPLC METHOD

System suitability studies

Parameters	Theophyline
Linearity range	50-500 (ng/ml)
Regression equation Y = mx + c	0.007x -0.0015
Correlation coefficient	0.9976
Theoretical plate/meter	25132
Resolution factor	2.71
Asymmetric factor	0.85
Limit of detection (ng/ml)	5.0
Limit of quantification (ng/ml)	10.0

7.1.5. INTRADAY AND INTERDAY ACCURACY AND PRECISION OF THEOPHYLLINE IN HUMAN SERUM

Concentration added (ng/ml)	Intraday			Interday		
	Measured concentration (Mean ± S.D)	CV (%)	Accuracy	Measured concentration (Mean ± S.D)	CV (%)	Accuracy
50	48.56 ± 0.54	1.11	97.23	48.80 ± 0.58	1.19	97.61
200	198.32 ± 0.87	0.87	99.15	199.09 ± 0.64	0.32	99.54
500	498.86 ± 0.43	0.08	99.77	499.14 ± 0.53	0.10	99.82

CV = Percentage of coefficient of variation

7.1.6. CALIBRATION CURVE FOR THEOPHYLLINE USING HIGH PERFORMACE LIQUID CHROMATOGRAPHY

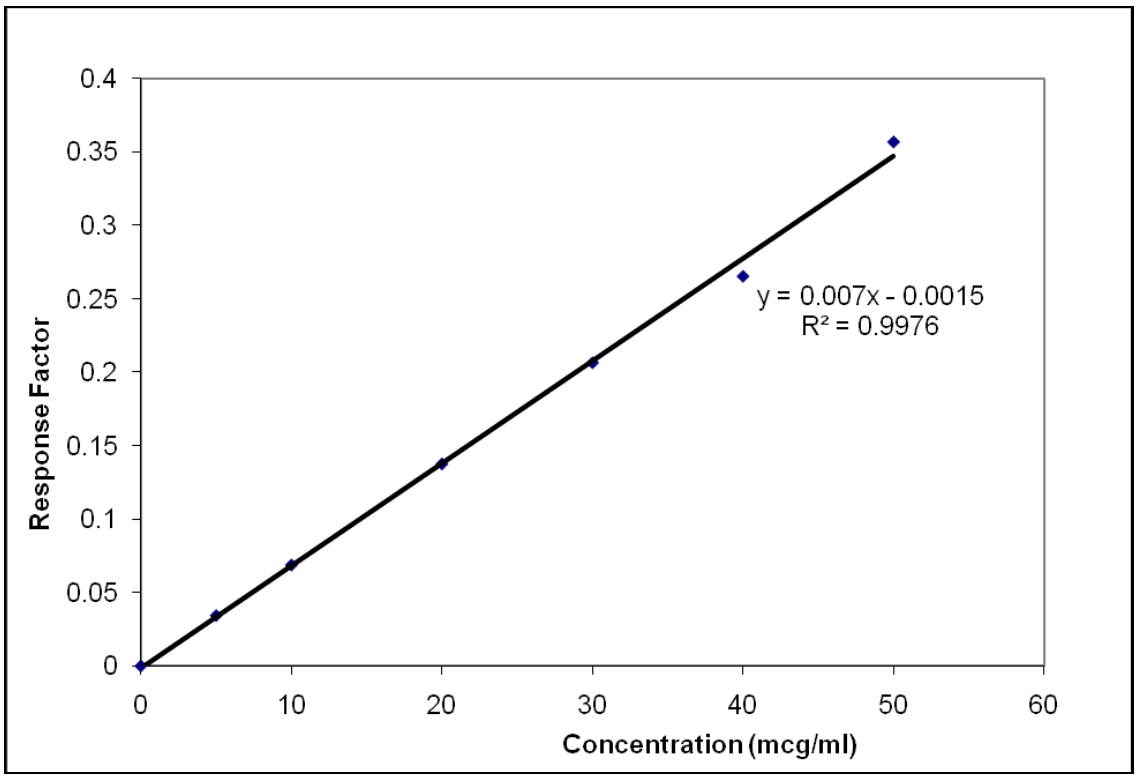


Figure 1: CALIBRATION CURVE FOR THEOPHYLLINE

7.1.7. THEOPHYLLINE CHROMATOGRAM

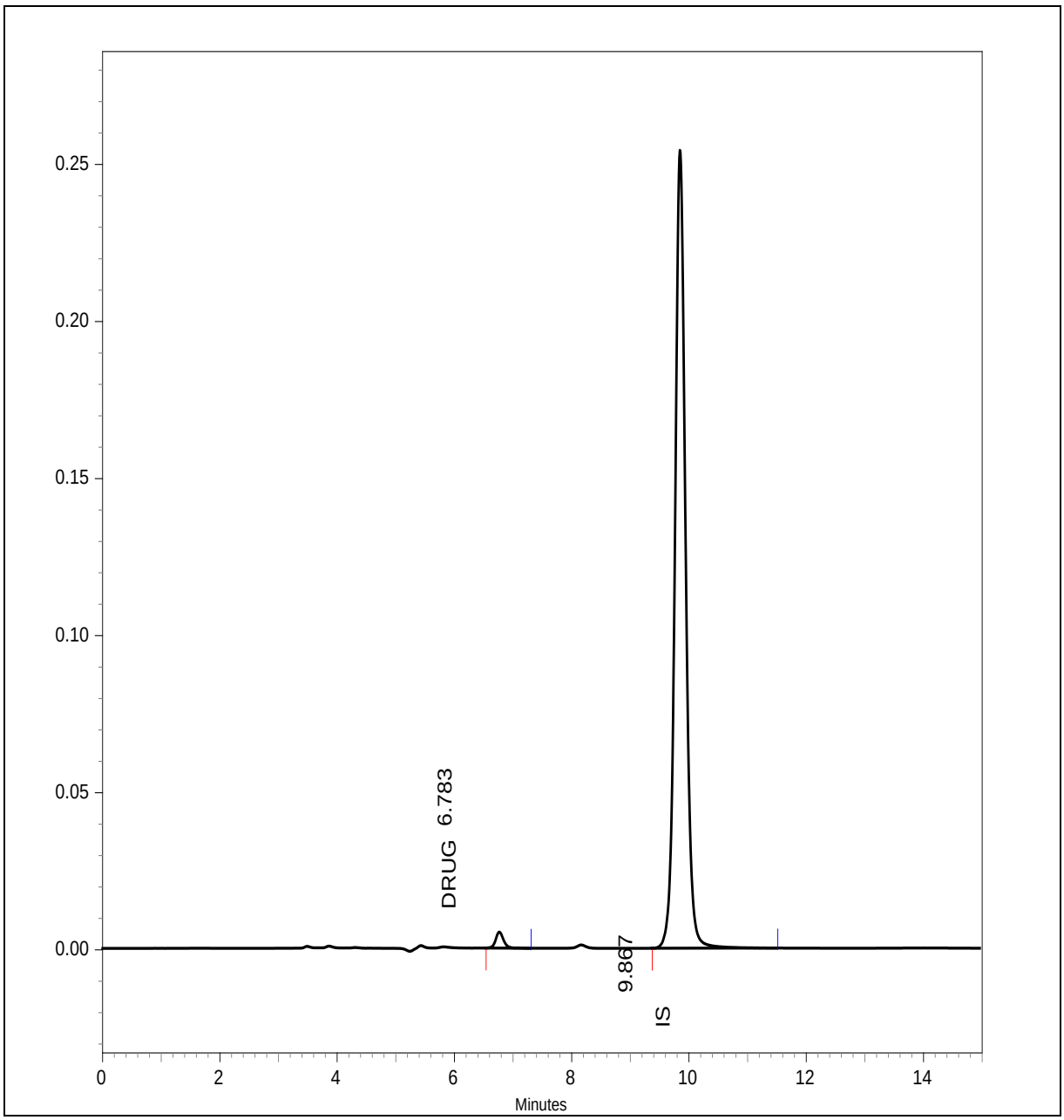


Figure 2: A typical chromatogram of theophylline (drug) given to the patient and internal standard (IS) caffeine

7.1.8. SERUM THEOPHYLLINE CONCENTRATION DURING FOLLOW UP

7.1.8a. Serum Theophylline Peak Concentration

Theophylline dose	**Serum Theophylline Concentration (in mcg/ml) Mean ± SD (Percentage coefficient of variation)**					
	3rd day (300 mg)	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	6.94 ± 0.09 (1.29)	7.00 ± 0.14 (2.00)	7.16 ± 0.09 (1.20)	7.06 ± 0.06 (1.33)	7.16 ± 0.32 (4.52)	7.15 ± 0.16 (2.25)
400 mg (n= 20)	5.30 ± 1.12 (21.12)	9.65 ± 1.97 (20.44)	9.75 ± 1.95 (20.06)	10.14 ± 2.04 (20.12)	9.91 ± 1.99 (20.04)	9.76 ± 1.97 (20.17)
600 mg (n= 77)	5.21 ± 0.88 (16.83)	11.78 ± 2.41 (20.49)	11.92 ± 2.40 (20.16)	12.17 ± 2.45 (20.15)	12.20 ± 2.43 (19.95)	11.90 ± 2.44 (20.46)

7.1.8b. Serum Theophylline Trough Concentration

Theophylline dose	**Serum Theophylline Concentration (in mcg/ml)** **Mean ± SD (Percentage coefficient of variation)**					
	3rd day (300 mg)	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
300 mg **(n= 5)**	5.19 ± 0.09 (1.73)	5.28 ± 0.11 (2.04)	5.26 ± 0.14 (2.68)	5.21 ± 0.05 (0.89)	5.20 ± 0.06 (1.13)	5.22 ± 0.06 (1.22)
400 mg **(n= 20)**	3.70 ± 1.08 (29.13)	7.13 ± 1.54 (21.64)	7.63 ± 1.70 (22.28)	7.94 ± 1.83 (23.10)	7.86 ± 1.77 (22.57)	7.59 ± 1.64 (21.58)
600 mg **(n= 77)**	3.53 ± 0.90 (25.33)	8.20 ± 2.29 (27.94)	8.47 ± 2.35 (27.71)	8.52 ± 2.33 (27.38)	8.48 ± 2.34 (27.56)	8.37 ± 2.33 (27.82)

7.1.9. QUALITY OF LIFE SCORES OF THE PATIENTS

7.1.9a. Symptom domain score

Dose	Symptom score (Mean ± SD)				
	Baseline	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	63.81 ± 13.93	48.05 ± 14.72	19.78 ± 27.87	15.95 ± 25.53	11.93 ± 17.56
400 mg (n= 20)	39.76 ± 5.02	1.74 ± 1.03	0.58 ± 1.03	0.58 ± 1.03	0.58 ± 1.03
600 mg (n= 77)	71.69 ± 16.55	51.80 ± 15.21	22.20 ± 29.13	19.59 ± 26.01	18.23 ± 24.28

Statistical analysis of symptom score

	300 mg	**400 mg**	**600 mg**
Baseline vs 15th day	*P<0.05	***P<0.001	*** P<0.001
Baseline vs 30th day	***P<0.001	*** P<0.001	*** P<0.001
Baseline vs 45th day	*** P<0.001	*** P<0.001	*** P<0.001
Baseline vs 60th day	*** P<0.001	*** P<0.001	*** P<0.001
15th day vs 30th day	*** P<0.001	ns P>0.05	*** P<0.001
15th day vs 45th day	*** P<0.001	ns P>0.05	*** P<0.001
15th day vs 60th day	*** P<0.001	ns P>0.05	*** P<0.001

30th day vs 45th day	ns P>0.05	ns P>0.05	ns P>0.05
30th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
45th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
* Significant *** Extremely Significant ns – Not Significant			

7.1.9b. Activity domain score

Dose	**Activity score (Mean ± SD)**				
	Baseline	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	50.93 ± 26.88	49.71 ± 26.91	40.80 ± 16.19	35.93 ± 13.26	34.74 ± 13.49
400 mg (n= 20)	55.03 ± 5.01	33.91 ± 2.62	32.35 ± 5.37	32.35 ± 5.37	32.35 ± 5.37
600 mg (n= 77)	63.99 ± 27.49	58.84 ± 25.23	48.94 ± 19.19	45.10 ± 16.84	42.07 ± 16.24

Statistical analysis of activity score

	300 mg	**400 mg**	**600 mg**
Baseline vs 15th day	ns P>0.05	*** P<0.001	ns P>0.05
Baseline vs 30th day	ns P>0.05	*** P<0.001	*** P<0.001
Baseline vs 45th day	ns P>0.05	*** P<0.001	*** P<0.001

Baseline vs 60th day	ns P>0.05	*** P<0.001	*** P<0.001
15th day vs 30th day	ns P>0.05	* P<0.05	*** P<0.001
15th day vs 45th day	ns P>0.05	* P<0.05	*** P<0.001
15th day vs 60th day	ns P>0.05	* P<0.05	*** P<0.001
30th day vs 45th day	ns P>0.05	ns P>0.05	ns P>0.05
30th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
45th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
*Significant *** Extremely Significant ns – Not Significant			

7.1.9c. Impact domain score

Dose	**Impact score (Mean ± SD)**				
	Baseline	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	54.07 ± 16.28	33.14 ± 17.98	19.85 ± 7.05	17.45 ± 4.21	16.75 ± 3.00
400 mg (n= 20)	65.41 ± 19.70	29.11 ± 14.08	21.09 ± 7.77	21.09 ± 7.77	21.09 ± 7.77
600 mg (n= 77)	69.14 ± 17.76	46.27 ± 22.37	33.04 ± 21.46	29.46 ± 19.93	27.19 ± 18.35

Statistical analysis of impact score

	300 mg	**400 mg**	**600 mg**
Baseline vs 15th day	* P<0.05	*** P<0.001	*** P<0.001
Baseline vs 30th day	*** P<0.001	*** P<0.001	*** P<0.001
Baseline vs 45th day	*** P<0.001	*** P<0.001	*** P<0.001
Baseline vs 60th day	*** P<0.001	*** P<0.001	*** P<0.001
15th day vs 30th day	ns P>0.05	*** P<0.001	*** P<0.001
15th day vs 45th day	ns P>0.05	*** P<0.001	*** P<0.001
15th day vs 60th day	ns P>0.05	*** P<0.001	*** P<0.001
30th day vs 45th day	ns P>0.05	ns P>0.05	ns P>0.05
30th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
45th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05

*Significant

*** Extremely Significant

ns – Not Significant

7.1.9d. Total score

Dose	**Total score (Mean ± SD)**				
	Baseline	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	55.92 ± 18.31	40.64 ± 19.35	26.19 ± 11.36	22.80 ± 6.96	21.40 ± 4.58
400 mg (n= 20)	58.00 ± 12.32	26.02 ± 8.29	21.28 ± 5.35	21.10 ± 5.65	21.10 ± 5.65
600 mg (n= 77)	66.90 ± 22.26	51.02 ± 21.05	36.46 ± 20.31	32.77 ± 16.97	30.43 ± 15.67

Statistical analysis of total score

	300 mg	**400 mg**	**600 mg**
Baseline vs 15th day	ns P>0.05	*** P<0.001	*** P<0.001
Baseline vs 30th day	*** P<0.001	*** P<0.001	*** P<0.001
Baseline vs 45th day	*** P<0.001	*** P<0.001	*** P<0.001
Baseline vs 60th day	*** P<0.001	*** P<0.001	*** P<0.001
15th day vs 30th day	ns P>0.05	*** P<0.001	*** P<0.001
15th day vs 45th day	* P<0.05	*** P<0.001	*** P<0.001
15th day vs 60th day	* P<0.05	*** P<0.001	*** P<0.001
30th day vs 45th day	ns P>0.05	ns P>0.05	ns P>0.05
30th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
45th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05

*Significant

*** Extremely Significant

ns – Not Significant

7.1.10. CLINICAL SIGNIFICANCE OBSERVED IN THE QUALITY OF LIFE

A difference of four units in the scores indicates a slight clinical effect, while a difference of eight or twelve units indicates moderate or very good clinical effects, respectively.

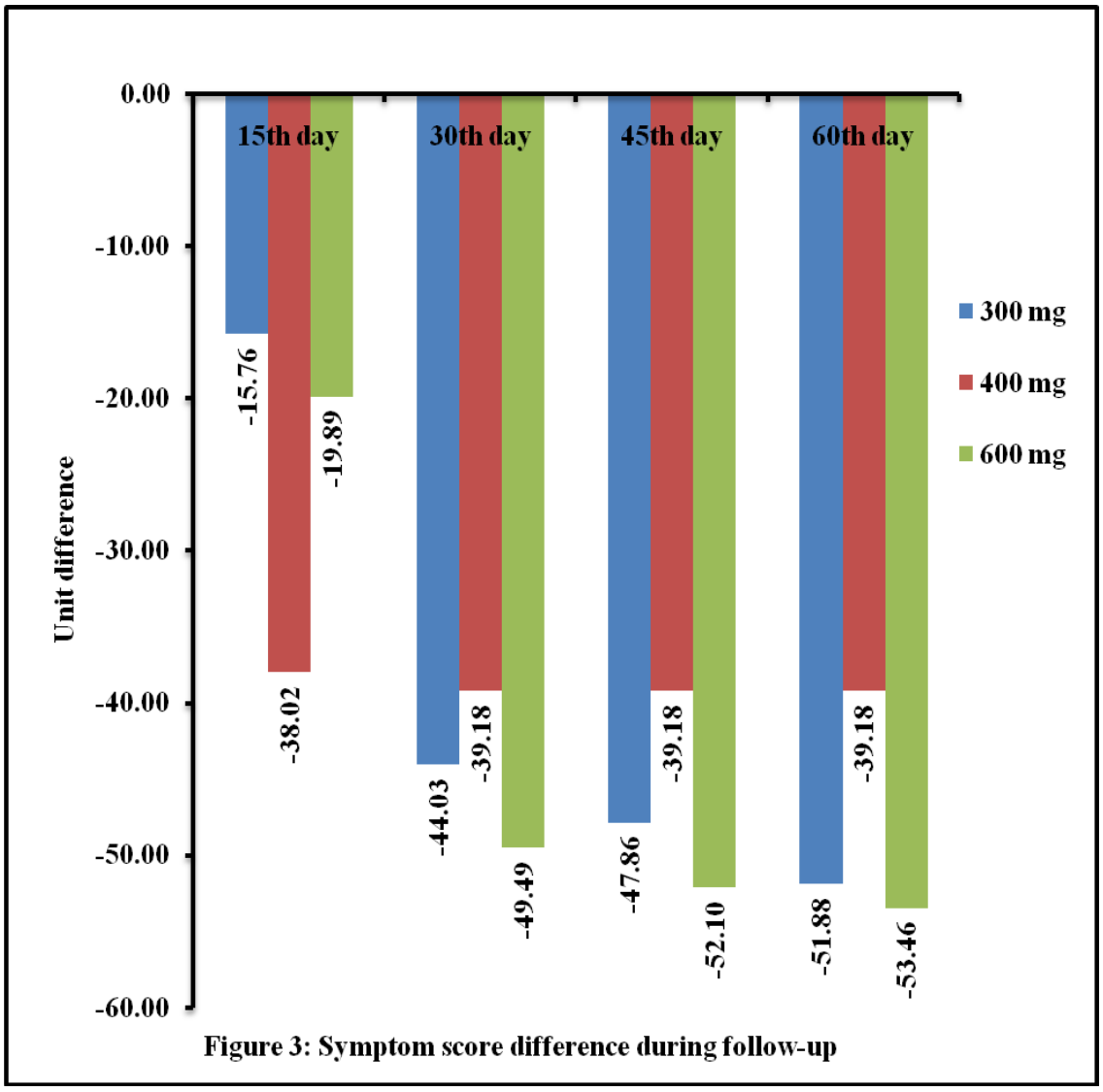


Figure 3: Symptom score difference during follow-up

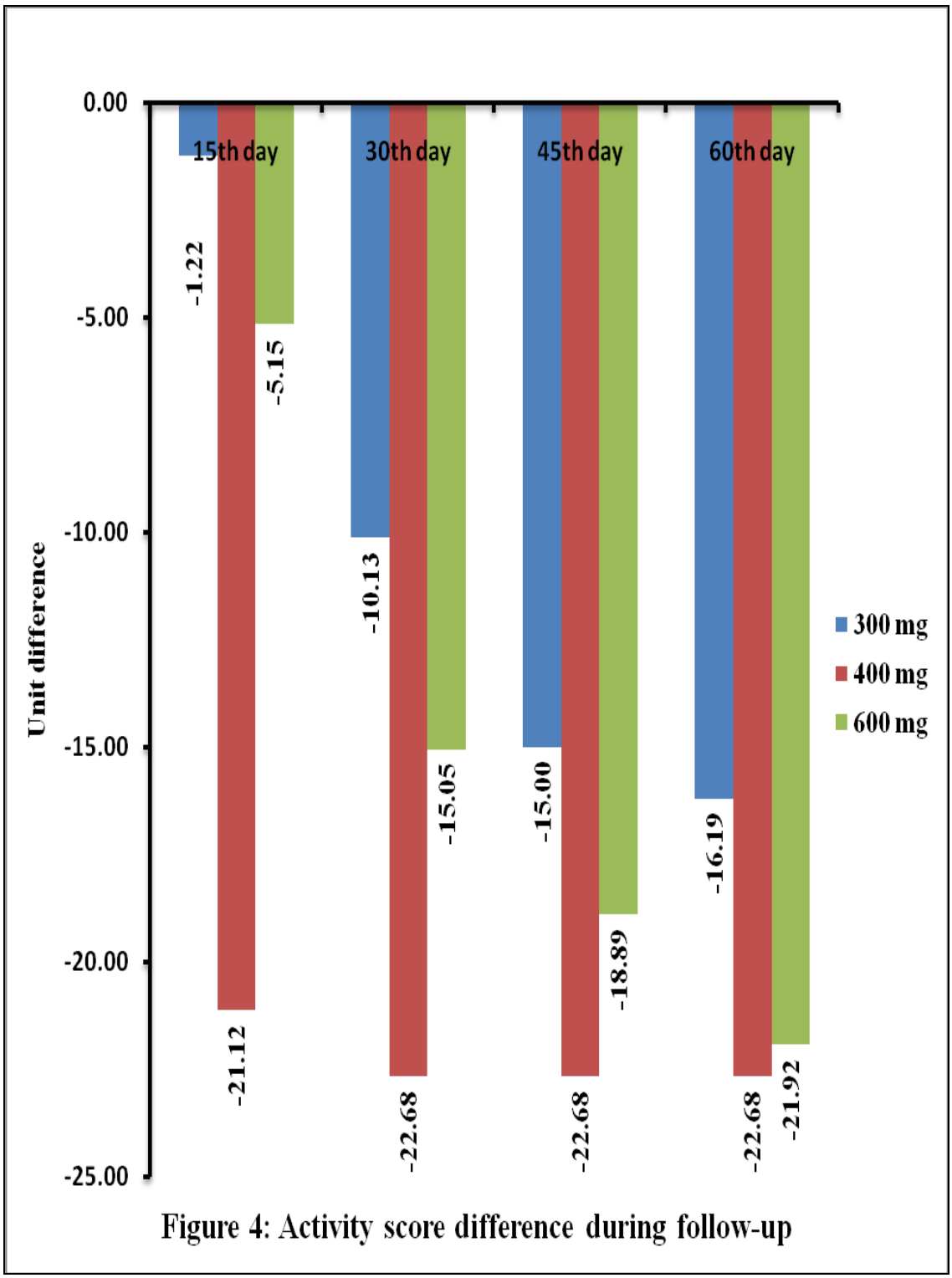


Figure 4: Activity score difference during follow-up

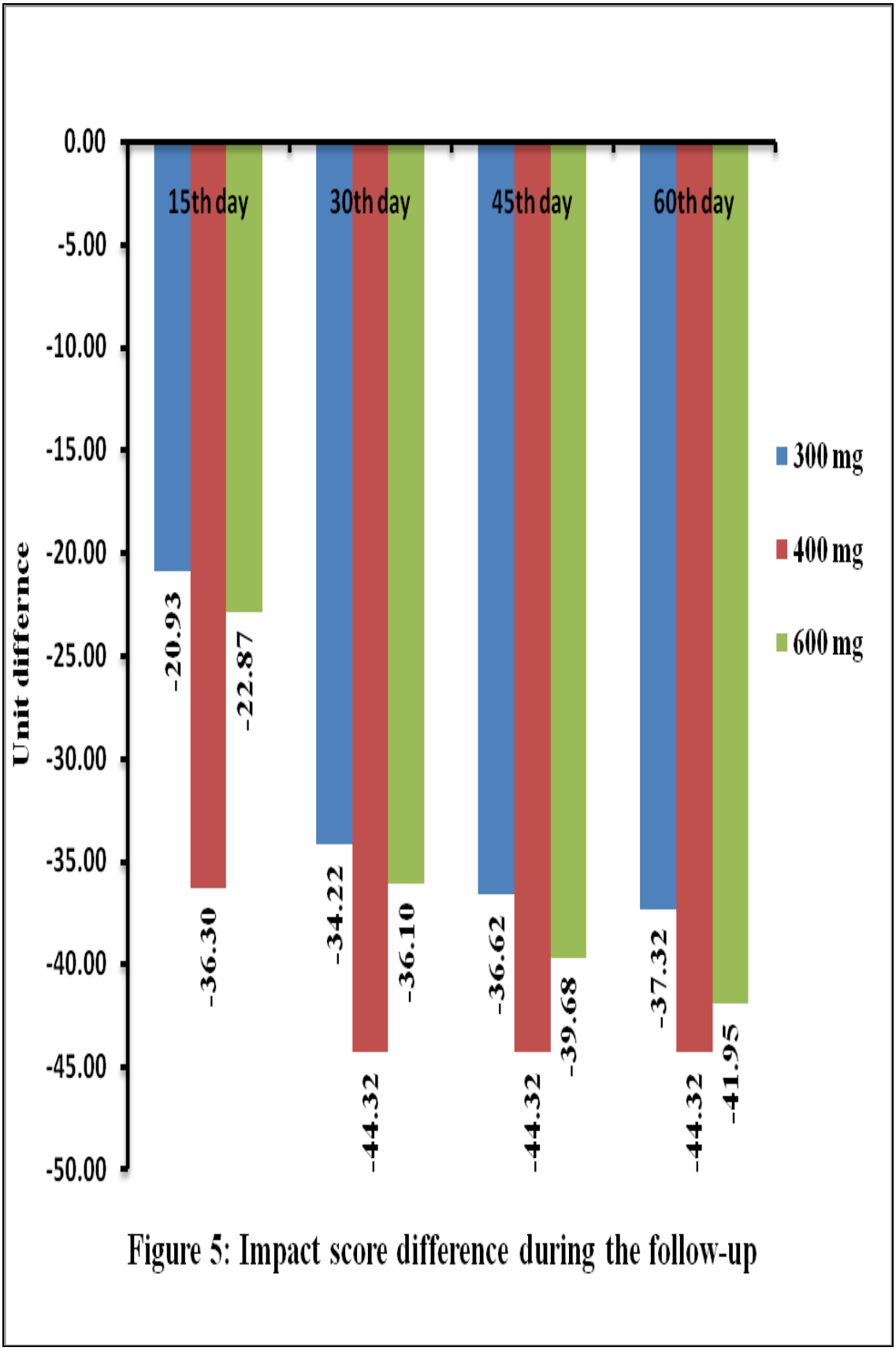


Figure 5: Impact score difference during the follow-up

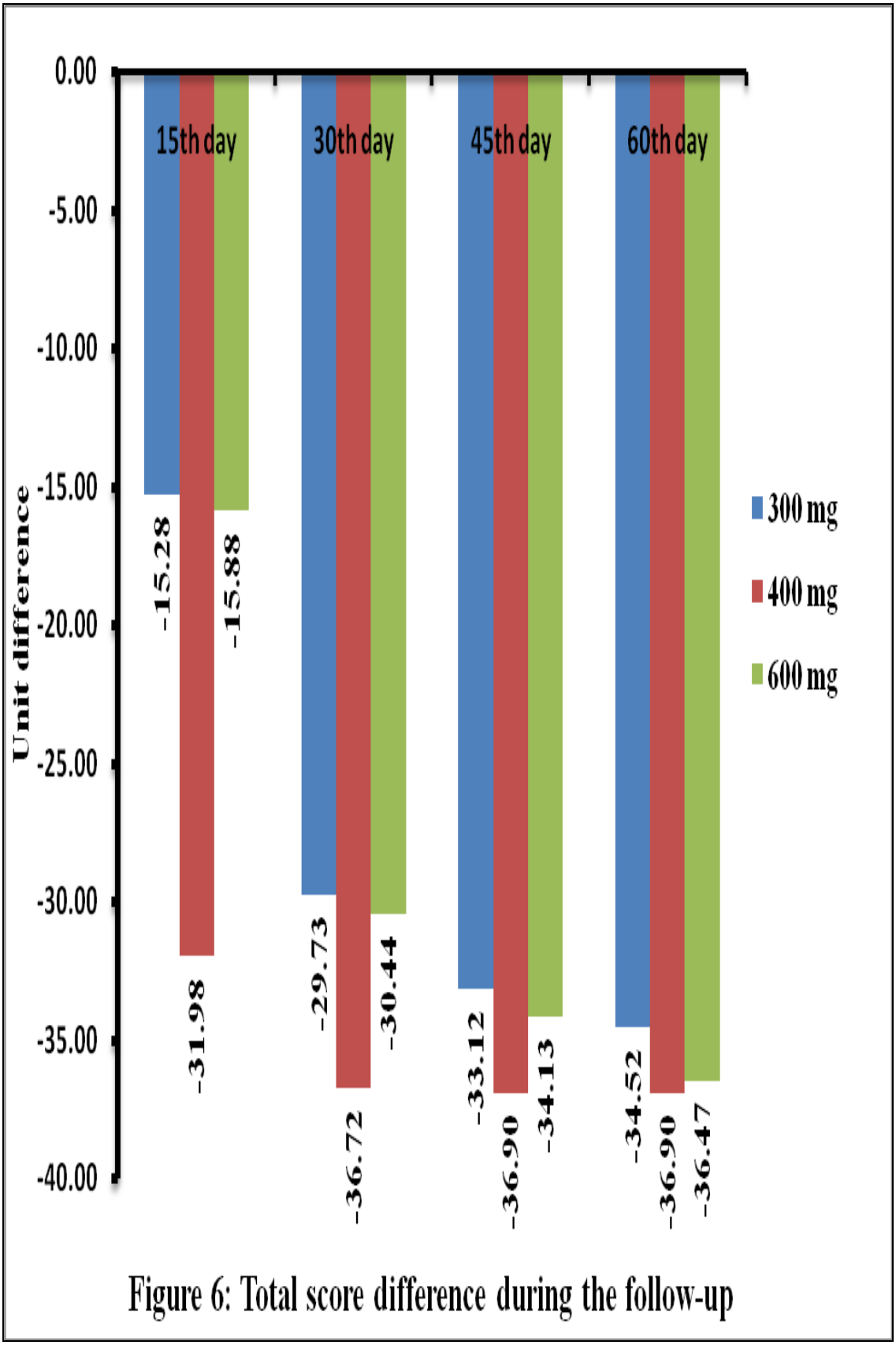


Figure 6: Total score difference during the follow-up

7.1.11. PULMONARY FUNCTION OF THE STUDY PATIENTS DURING STUDY

7.1.11a. Forced expiratory volume in one second (FEV_1) in Litres

Dose	FEV_1 (Mean ± S.D)						
	Baseline (300 mg)	3rd day	7th day	15th day	30th day	45th day	60th day
300 mg (n= 5)	1.97 ± 0.22	1.99 ± 0.22	2.02 ± 0.21	2.08 ± 0.21	2.21 ± 0.25	2.32 ± 0.29	2.38 ± 0.30
400 mg (n= 20)	1.97 ± 0.38	1.99 ± 0.38	2.01 ± 0.38	2.06 ± 0.37	2.06 ± 0.37	2.18 ± 0.36	2.19 ± 0.36
600 mg (n= 77)	1.65 ± 0.43	1.67 ± 0.43	1.68 ± 0.43	1.74 ± 0.45	1.85 ± 0.45	1.97 ± 0.48	2.01 ± 0.50

Statistical analysis of FEV_1

	300 mg	400 mg	600 mg
Baseline vs 3rd day	ns P>0.05	ns P>0.05	ns P>0.05
Baseline vs 7th day	ns P>0.05		ns P>0.05
Baseline vs 15th day	* P<0.05		ns P>0.05
Baseline vs 30th day	*** P<0.001		ns P>0.05
Baseline vs 45th day	*** P<0.001		*** P<0.001
Baseline vs 60th day	*** P<0.001		*** P<0.001
3rd day vs 7th day	ns P>0.05		ns P>0.05

3rd day vs 15th day	ns P>0.05		ns P>0.05
3rd day vs 30th day	*** P<0.001		ns P>0.05
3rd day vs 45th day	*** P<0.001		** P<0.01
3rd day vs 60th day	*** P<0.001		*** P<0.001
7th day vs 15th day	ns P>0.05		ns P>0.05
7th day vs 30th day	*** P<0.001		ns P>0.05
7th day vs 45th day	*** P<0.001		** P<0.01
7th day vs 60th day	*** P<0.001		*** P<0.001
15th day vs 30th day	** P<0.01		ns P>0.05
15th day vs 45th day	*** P<0.001		* P<0.05
15th day vs 60th day	*** P<0.001		** P<0.01
30th day vs 45th day	* P<0.05		ns P>0.05
30th day vs 60th day	*** P<0.001		ns P>0.05
45th day vs 60th day	ns P>0.05		ns P>0.05

*Significant

** Very Significant

*** Extremely Significant

ns – Not Significant

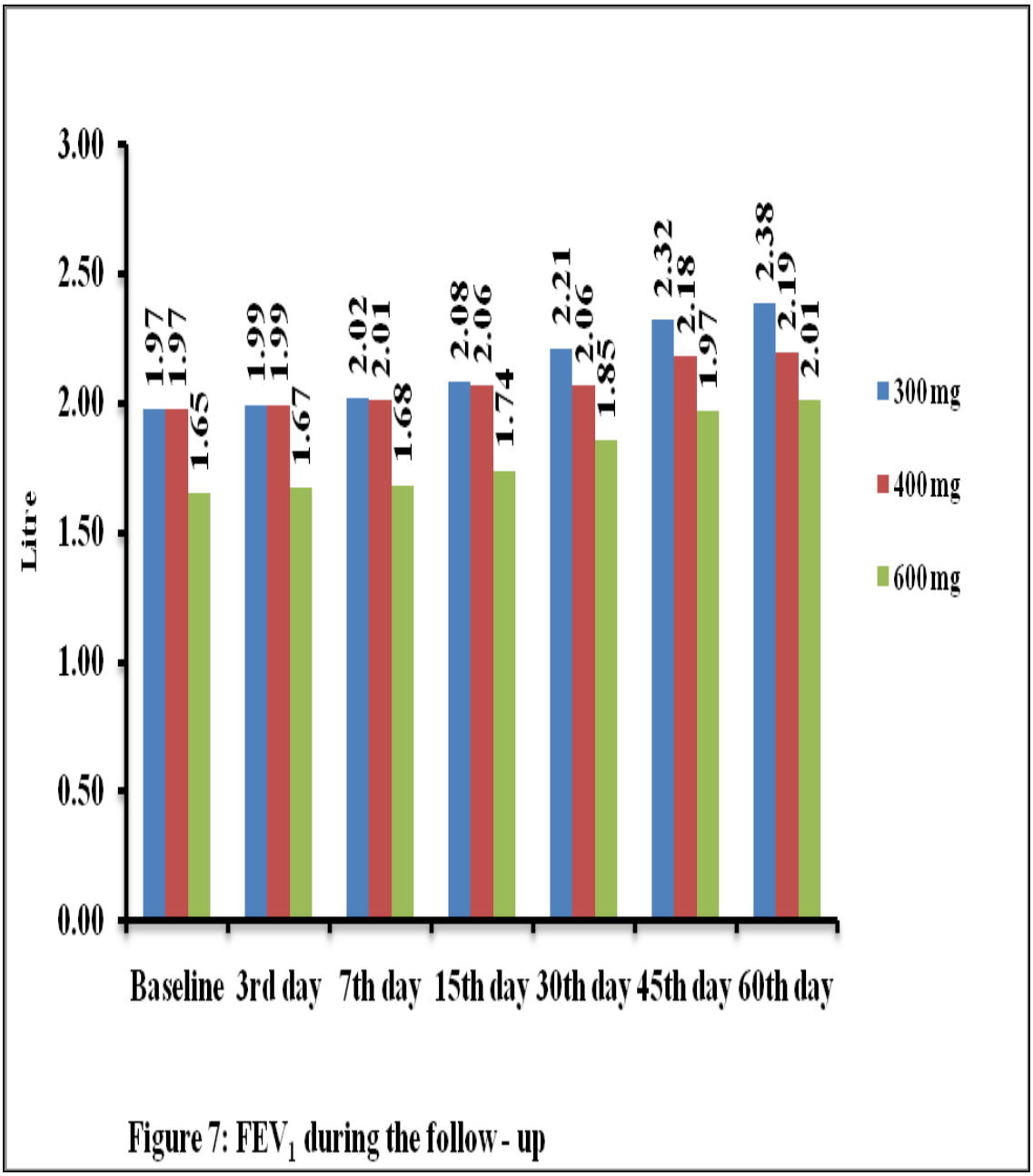


Figure 7: FEV_1 during the follow - up

7.1.11b. Forced vital capacity (FVC) in litres

Dose	FVC (Mean ± S.D)						
	Baseline	**3rd day**	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	2.59 ± 0.55	2.18 ± 0.21	2.41 ± 0.22	2.20 ± 0.27	2.43 ± 0.36	2.51 ± 0.29	2.57 ± 0.35
400 mg (n= 20)	2.45 ± 0.26	2.23 ± 0.29	2.46 ± 0.32	2.39 ± 0.26	2.50 ± 0.26	2.56 ± 0.19	2.64 ± 0.18
600 mg (n= 77)	2.08 ± 0.44	1.84 ± 0.45	2.09 ± 0.43	1.97 ± 0.44	2.16 ± 0.44	2.14 ± 0.50	2.27 ± 0.46

7.1.11c. Peak expiratory flow (PEF) in litres per minute

Dose	PEF (Mean ± S.D)						
	Baseline	**3rd day**	**7th day**	**15th day**	**30th day**	**45th day**	**60th day**
300 mg (n= 5)	246.20 ± 54.00	247.80 ± 53.42	250.00 ± 54.41	259.20 ± 51.48	269.60 ± 53.99	288.60 ± 51.52	289.00 ± 54.00
400 mg (n= 20)	244.45 ± 63.54	250.70 ± 62.08	254.35 ± 60.62	276.85 ± 56.32	344.56 ± 43.82	360.68 ± 34.93	359.90 ± 36.79
600 mg (n= 77)	202.23 ± 61.64	205.36 ± 62.17	205.16 ± 66.25	217.60 ± 61.02	228.94 ± 55.72	245.71 ± 57.95	246.25 ± 60.87

Statistical analysis of PEF

	300 mg	**400 mg**	**600 mg**
Baseline vs 3rd day	ns P>0.05	ns P>0.05	ns P>0.05
Baseline vs 7th day	ns P>0.05	ns P>0.05	ns P>0.05
Baseline vs 15th day	*** P<0.001	ns P>0.05	ns P>0.05
Baseline vs 30th day	*** P<0.001	*** P<0.001	ns P>0.05
Baseline vs 45th day	*** P<0.001	*** P<0.001	ns P>0.05
Baseline vs 60th day	*** P<0.001	*** P<0.001	*** P<0.001
3rd day vs 7th day	ns P>0.05	ns P>0.05	ns P>0.05
3rd day vs 15th day	*** P<0.001	ns P>0.05	ns P>0.05
3rd day vs 30th day	*** P<0.001	*** P<0.001	ns P>0.05
3rd day vs 45th day	*** P<0.001	*** P<0.001	ns P>0.05
3rd day vs 60th day	*** P<0.001	*** P<0.001	*** P<0.001
7th day vs 15th day	** P<0.01	ns P>0.05	ns P>0.05
7th day vs 30th day	*** P<0.001	*** P<0.001	ns P>0.05
7th day vs 45th day	*** P<0.001	*** P<0.001	ns P>0.05

7th day vs 60th day	*** P<0.001	*** P<0.001	** P<0.01
15th day vs 30th day	*** P<0.001	** P<0.01	ns P>0.05
15th day vs 45th day	*** P<0.001	*** P<0.001	ns P>0.05
15th day vs 60th day	*** P<0.001	*** P<0.001	** P<0.01
30th day vs 45th day	*** P<0.001	ns P>0.05	ns P>0.05
30th day vs 60th day	*** P<0.001	ns P>0.05	ns P>0.05
45th day vs 60th day	ns P>0.05	ns P>0.05	ns P>0.05
*Significant ** Very Significant *** Extremely Significant ns – Not Significant			

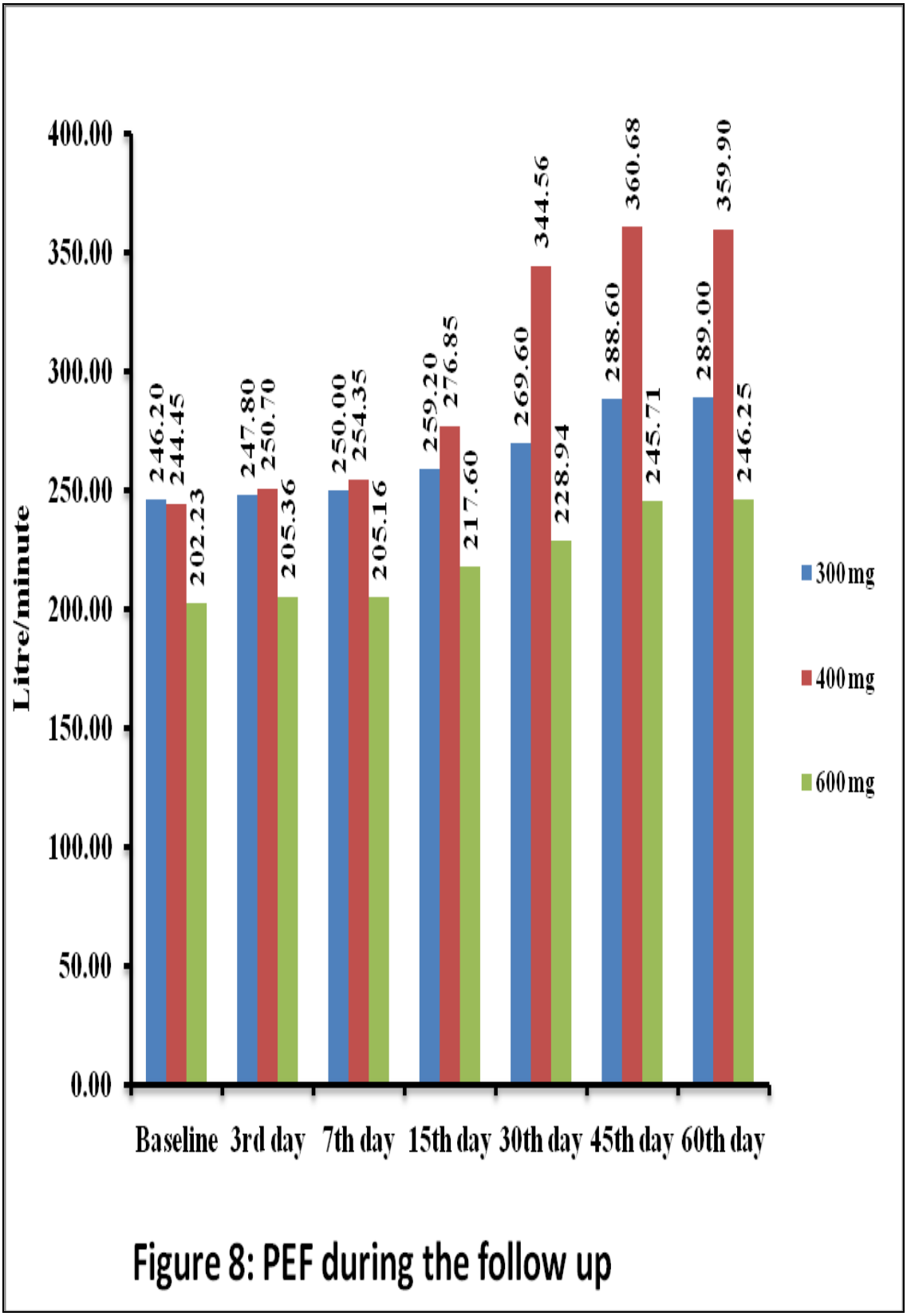


Figure 8: PEF during the follow up

7.1.12. PHARMACOECONOMIC EVALUATION

Category		Treatment prior to the study (in Rs)	Study treatment (in Rs)
Direct medical cost	**Medication cost**	10519.44	29914.47
	Laboratory charges	17920	20400
	Hospital charges (Bed cost)	7910	-
Direct non medical costs	**Travel expenses**	7040	2434
	Food expenses	9630	-
Indirect non-medical costs	**Loss of wages of patients**	26400	1600
	Loss of wages for the patients attenders	3400	200
Total cost		82819.44	54548.47
Total cost/day		2760.65	1818.28
Total cost/day/patient		27.10	17.83
Percentage predicted value of forced expiratory volume in one second (Mean ± SD)		64.79 ± 17.65	73.77 ± 18.14

7.1.13. ADVERSE DRUG REACTION (ADR) MONITORING OF THEOPHYLLINE

Patient Characteristics		**Trough serum concentration (in mcg/ml**	**Peak serum concentration (in mcg/ml)**	**ADR Observed**		
Age (in years)	**Sex**			**30th day**	**45th day**	**60th day**
40	**Female**	11.50	15.21	Insomnia		-
57	**Male**	11.83	15.22	-	Headache	-
37	**Male**	11.82	15.06	-	-	Headache
62	**Male**	11.06	15.01	-	-	Palpitation

7.2. GENTAMICIN

7.2.1. DEMOGRAPHIC DATA OF STUDY SUBJECTS

Patient characteristics		**Multiple daily dose (Twice daily) (n=46)**		**Once daily dose (n=14)**	
		60 mg (n= 7)	**80 mg (n= 39)**	**100 mg (n= 8)**	**160 mg (n= 6)**
Gender	**Male (n= 33)**	4	21	5	3
	Female (n= 27)	3	18	3	3
Age (in years) (Mean ± SD)		45.40 ± 15.65	34.10 ± 15.14	30.60 ± 4.67	31.50 ± 9.50
Body Mass Index (in kg/m^2) (Mean ± SD)		46.94 ± 9.46	49.31 ± 9.40	43.62 ± 8.46	54.90 ± 4.70

7.2.2. DIAGNOSIS OF THE PATIENTS TREATED WITH GENTAMICIN DURING THE STUDY

Diagnosis	**Multiple daily dose (Twice daily)**		**Once daily dose**		**Total (n=60)**
	60 mg (n= 7)	**80 mg (n= 39)**	**100 mg (n= 8)**	**160 mg (n= 6)**	
Lower respiratory infection	2	5	1	-	**8**
Assault	-	3	-	-	**3**
Dysentery	2	-	-	-	**2**
Pyrexia of unknown origin	-	7	-	-	**7**
Chronic obstructive pulmonary disease	3	11	2	3	**19**
Urinary tract infection	-	5	-	-	**5**
Appendectomy	-	2	-	-	**2**

Corpulmonale	-	1	-	-	**1**
Bronchial Asthma	-	1	1	-	**2**
Acute pharyngitis	-	2	2	1	**5**
Upper respiratory infection	-	2	2	2	**6**

7.2.3. ADDITIONAL ANTIBIOTICS GIVEN WITH GENTAMICIN

Antibiotic	**Dose and frequency of administration**	**Multiple daily dose**		**Once daily dose**		**Total**
		60 mg	**80 mg**	**100 mg**	**160 mg**	
Injection Cefotaxime	**250 mg twice daily**	1	1	-	4	6
	500 mg twice daily	-	-	2	-	2
	1g twice daily	4	23	3	-	30
Injection Ampicillin	**250 gm twice daily**	1	6	-	-	7
Injection Metronidazole	**250 mg once daily**	-	-	1	-	1

	500 mg twice daily	1	5	-	-	6
Injection Ciprofloxacin	**200 mg twice daily**	-	5	-	-	5

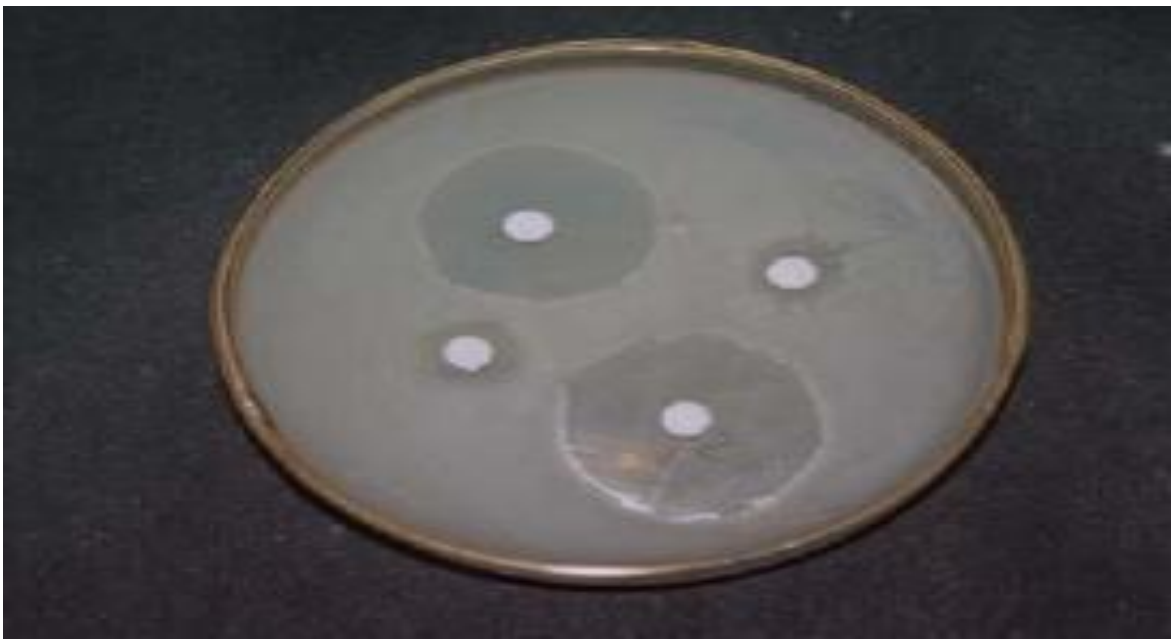
7.2.4. PATHOGENS ISOLATED FROM THE SPUTUM SAMPLES OF STUDY SUBJECTS

Micro – organism isolated	**Multiple daily dose (Twice daily)**		**Once daily dose**		**Total**
	60 mg	**80 mg**	**100 mg**	**160 mg**	
Klebsiella pneumonia	1	11	1	1	**14**
Pseudomonas aureginosa	-	2	-	-	**2**
Escherichia coli	-	3	-	-	**3**
Haemophilus influenzae	-	1	-	-	**1**

7.2.5. SERUM GENTAMICIN CONCENTRATION

Dosage regimen	**Dose (mg)**	**Dose (mg/kg) Mean ± SD**	**Serum Gentamicin Concentration (in mcg/ml) Mean ± SD**	
			Peak level concentration	**Trough level concentration**
Multiple daily dose (Twice daily)	**60 mg**	1.19 ± 0.21	4.45 ± 0.37	0.58 ± 0.08
	80 mg	1.64 ± 0.30	4.67 ± 0.44	0.60 ± 0.16
Once daily dose	**100 mg**	2.00 ± 0.50	4.63 ± 0.31	0.64 ± 0.55
	160 mg	2.81 ± 0.21	4.83 ± 0.38	0.78 ± 0.05

7.2.6. Figure 9: ZONE OF INHIBITION BY GENTAMICIN



7.2.7. ADVERSE DRUG REACTION MONITORING

Dosage regimen of gentamicin		**Adverse drug reaction observed**
Multiple daily dose (Twice daily) **(n=46)**	**60 mg** **(n= 7)**	-
	80 mg **(n= 39)**	Ototoxicity (n=1)
Once daily dose (n=14)	**100 mg** **(n= 8)**	Ototoxicity (n=1)
	160 mg **(n= 6)**	-

8.1. THEOPHYLLINE

As per the guideline for management of asthma at primary and secondary level of health care in India, theophylline has been recommended as the alternative choice of treatment for asthmatic patients in all the three severity categories such as mild, moderate and severe patients. Theophylline is a narrow therapeutic index drug and has large inter- individual variability, therefore requires therapeutic drug monitoring. For these reasons theophylline is not prescribed widely in spite of the recommendations in the guideline. The present study was carried out to study the pharmacokinetic and pharmacodynamic relationship of theophylline used as the alternative choice of treatment in asthmatic patients. Therapeutic drug monitoring of theophylline was done to quantify trough and peak level concentrations and the treatment outcome was assessed by pulmonary function test and health related quality of life. In addition to that the pharmacoeconomic analysis was also done to observe the advantage of theophylline treatment in the study set-up.

8.1.1. INTERPRETATION OF THE PATIENT CHARACTERISTICS

Demographic data showed that in this study the number of male patients was more when compared to the female patients. The majority of the patients were in the age group of 41 – 60 years. The majority of the study subjects, irrespective of the severity conditions and the treatments given, had education up to the school/college level and the number of illiterates been comparatively less. As this study population had more number of educated people, it resulted in better patient cooperation. Smokers were comparatively very low in number when compared to the non- smokers in the study population. Smokers present a challenge in the control of asthma as they need specific

care in their disease management in terms of not only pharmacotherapy, but also a motivation for cessation of smoking.

Another major observation made was on the employment and economic status of the patients, where it was seen that the patients were mainly daily wagers and mostly the patient's income status was not supporting the choice of first line therapy for asthma. The study showed that the majority of the patients had asthma for 10 to 15 years followed by more than 15 years and further 5 to 10 years. The patients who had disease condition for less than 5 years were comparatively very less.

Severity and Pulmonary function: The study found that among the patients enrolled, the majority of the patients were in the moderate condition, followed by severe and mild respectively. The severity assessment was done based on the percentage predicted forced expiratory volume in one second (FEV_1).

8.1.2. THERAPEUTIC DRUG MONITORING

As per the advice of the physician the patients were initially given with low dose of sustained release tablet of theophylline (300 mg per day) to avoid toxicity and to determine whether the lowest dose available could reach the desired therapeutic range. Only 5 patients among the 102 patients were in the target therapeutic range of 5 – 15 mcg/ml at steady state for 300 mg/day dose. For the 97 patients who were not in the therapeutic range the dose of theophylline was increased in accordance with the severity viz., for the mild patients the dose was increased with 400 mg of sustained release theophylline whereas in the moderate to severe patients the dose was increased to 600 mg of sustained release theophylline tablet. The additional drug/s for the patients along with theophylline were also given based on the guidelines followed.

The therapeutic range was attained in all the 97 patients after the change in dose of theophylline. It was observed that there was no statistically significant difference (P value > 0.05) between the follow-up values of serum theophylline peak concentrations as well as trough concentrations, which showed that the steady state concentration was achieved.

More than 20% of coefficient of variation was observed for the peak and trough theophylline concentrations among the patients treated with 400 mg and 600 mg of theophylline sustained release tablet. This shows that large inter individual variation exist in the study population. This observation was comparable with the study reported by Williams et al[61]. This supports the need of conducting the therapeutic drug monitoring for theophylline.

The study observed that the patients receiving the different doses of theophylline such as 300 mg, 400 mg, and 600 mg showed linearity in the peak and trough serum concentrations i.e., the serum theophylline concentration increased with the increase in dose. This suggests that the phenomenon of non-linear pharmacokinetics is of relatively small importance when dealing with serum concentrations in the lower and middle therapeutic range of theophylline observed in the study patients. This finding was very much correlating with the study reported by Koeter et al[62].

The mean difference between the peak and trough concentration of theophylline doses given in the study at the final visit was found to be about 40%. The study by Williams et al[61] reported this value as about 76%, where higher doses from 600 mg to 1200 mg per day was used. The increase in the mean percentage difference may be observed in higher doses.

Since this was the first study conducted based on Indian guidelines, the therapeutic range of theophylline for the different severity conditions was not available in the literature for comparison. Therefore, the trough and peak concentration of different doses of this study were compared with the other population data. The study patients were maintained at doses to achieve concentration at lower or middle range of therapeutic window to avoid the therapeutic failure and adverse effect of the drug.

8.1.3. Pulmonary function

The forced expiratory volume in one second (FEV_1) is generally considered as efficacy parameters in asthma clinical trials. This study has shown that there was a clinically significant improvement in FEV_1 (increase by 5%) with the treatment given in the patients[53]. The mild patients who received theophylline 400 mg showed improvement from the forty fifth day whereas the moderate and severe patients treated with 300 mg and 600 mg of theophylline and additional drugs according to the guideline showed the improvement from the fifteenth day. The results were comparable with the study reported by American lung association asthma clinical research centers[63] and Ukena et al[64].

8.1.4. Quality of life

The quality of life assessment using the Saint Georges' Respiratory Questionnaire in this study has shown very good clinical significance improvement in the total score throughout the follow-ups when compared to the baseline. Previous studies related to the quality of life based on the guideline treatment with theophylline were not available for the comparison with this study results for assessing the quality of life.

8.1.5. Pharmacoeconomic evaluation

The present study uses cost effective analysis by comparing the guideline treatment used in this study versus the empirical treatment of the patients which were being followed prior to the study. The result showed that the guideline treatment used had the least cost per outcome and is the effective alternative. The medical cost and laboratory charges are more in case of the study treatment due to the costly inhaled corticosteroids and reliever medication used as per the guideline for the moderate and severe patients and due to the laboratory charges for the conduct of therapeutic drug monitoring. But the overall cost was comparatively more for the patients when they were following the empirical treatment for the asthma prior to the study. The reason observed is that the patients, while in the empirical therapy had more number of indirect medical and non-medical costs due to the more number of hospital visits, hospital admissions, loss of working days, food expenses, etc. In case of the guideline treatment using theophylline and additional drugs which was in the alternative choice and of lesser cost, it was found that the indirect medical and non-medical costs were very less. The efficacy parameter measured using the FEV_1 showed a better response by the guideline treatment with theophylline, when compared to the empirical treatment before the study.

8.1.6. Adverse drug reaction monitoring

Our study showed that the doses of theophylline used for the treatment in the patients were relatively well tolerated. There was no serious adverse events reported and the number of adverse reactions reported was comparatively low since the serum concentration was maintained at the lower and moderate range of therapeutic window. Headache, insomnia and palpitation were the adverse drug reactions (ADRs) reported by the patients. As per Naranjo's scale the total score range for these ADRs ranged

from 5 to 8, which fell in the probable category. These findings of ADRs were similar to the study reported by Tyagi et al[56].

8.2. GENTAMICIN

The present study observed that the prescribing of gentamicin was on a prophylactic basis and was empirical. Cefotaxime was the antibiotic which was widely prescribed for respiratory tract infections in the hospital. The hospital did not follow any framed antibiotic policy for the prescribing practice of antibiotic, or, in the alternative, for gentamicin it was not followed through any biological therapeutic failure.

8.2.1. Antibiotics prescribed in adjunct to gentamicin

This study has found the use of injection cefotaxime as an adjunct to the study drug gentamicin followed by injection ampicillin when compared with a previous study by Sowmya Tiwari et al[45] which showed ampicillin was the drug prescribed mostly followed by the cefotaxime. The reason might be that the latter was done on the pediatrics population whereas our study was conducted in adult population.

8.2.2. Pathogen identified

In the present study isolation of Gram negative micro organisms such as *Klebsiella pneumonia, Pseudomonas aureginosa, Escherichia coli and Haemophilus influenza* from the sputum samples of the patient who were provisionally diagnosed to have respiratory infection. But no Gram positive micro organisms were identified in patients enrolled. The results were comparable with study conducted previously by Siber et al[65] where similar micro organisms were identified.

8.2.3. Therapeutic drug monitoring

The peak and trough serum gentamicin concentrations were estimated after reaching the steady state level using the microbiological assay method. The study gave a positive result, where the peak concentration was found to be complying with the peak concentration therapeutic range of 4 – 12 mcg/ml. The trough concentration in the study was found to be below 2 mcg/ml. The peak and trough concentrations observed in the study were comparable with the values reported by Meunier et al[66] but the trough levels were measured 8 hours post dose in their study whereas the same was measured at 12 hours post dose. The trough concentrations achieved with thrice daily dosage regimen in the western population were achieved with twice daily regimen given in the study patients. This may be due to difference in the gentamicin disposition in the study population.

This study showed positive outcome at the lower range (4.45 – 4.83 mcg/ml) of peak concentrations. This finding was different from the result finding of the study conducted by Moore et al[67] where the study finding was that 7 mcg/ml or more are likely to have positive outcome in gram negative patients. The findings of the present study supports for the dose which is prescribed by the physician in the study site.

8.2.4. Adverse drug reaction monitoring

A high rate of ototoxicity has been reported by Anaizi et al[68] with aminoglycosides administration. Significant nephrotoxicity was reported to a range of 3.8 to 21 percent with the use of gentamicin in various study conducted by Smith et al[69], Hottendorf et al[70] and Kirkpatrick et al[71]. In this study, no patient showed nephrotoxicity, one of the prime adverse drug reactions of gentamicin and the other adverse drug reaction of the drug, ototoxicity was comparatively less during the treatment course with gentamicin.

This may be due to the fact that gentamicin was administered in lesser dose with longer interval than recommended in the study patients.

This study did not find any significant differences in the patient outcomes in the different dose administered using gentamicin. This study finding was contradictory to the work reported by Rajendran et al[72]. There was no increased risk involved with once daily dosing of gentamicin over multiple dosing. The findings in the present study were similar to the study reported by Nordstrom et al[73].

Elisa et al[74] study findings indicated that bolus intravenous dosing with gentamicin could maximize bactericidal activity when delivered in long interval of 12 to 24 hours between doses. The present study complies with this finding as the dosage regimens used were either twice daily or once daily.

9.1. THEOPHYLLINE

The study conducted here aimed in the therapeutic drug monitoring of theophylline which is a drug candidate having large interindividual variability and having narrow therapeutic index. Since theophylline was the affordable alternative choice for treatment as per guidelines in the study setup, it was chosen for the study. The study assessed the efficacy and safety by estimating the steady state serum theophylline concentrations at the trough and peak levels and the pharmacodynamic response of the study patients to the treatment.

The patients were given with the dose of 300 mg sustained release tablet of theophylline per day initially. The dose of theophylline was increased according to the severity of the patients, if the concentration observed was not in the therapeutic range. The dose adjustment was in accordance to the severity of the patients and resulted in the theophylline concentration within the therapeutic range.

The patients did not show any significant difference in the serum peak concentration as well as trough concentration after the dosage adjustments made. The pulmonary function assessment and quality of life were the outcome measurements done for the study patients. In case of the forced expiratory volume in one second, the mild patients who were receiving 400 mg per day showed clinically significant improvement (5% increase) from the forty fifth day from the baseline, whereas the moderate and severe patients receiving 600 mg per day showed the clinically significant improvement on the fifteenth day onwards. The overall total score of quality of life has showed very good improvement from the fifteenth day when compared to the baseline.

The pharmacoeconomic evaluation found that the medical cost and the laboratory expenses were comparatively more when the study patients were taking the guideline treatment using theophylline. But the overall expenses was more for the empirical treatment, followed by the patients before the study, since there was more number of hospital admissions, consultations, travel expenses, food expenses and more of loss of wages for the patients. The clinical response of the study patients while taking the guideline treatment using theophylline was more effective when compared to the empirical treatment followed prior to the study. Since the drug was in the lower and moderate concentration level of the therapeutic window it was well tolerated and very few patients showed an adverse drug reaction which were within the probable score of causality assessment. The adverse drug reactions reported were headache, insomnia and palpitation.

9.2 GENTAMICIN

The study conducted here aimed in the therapeutic drug monitoring of gentamicin. Since gentamicin is effective against Gram negative bacterial infections and is an affordable treatment choice, it is widely prescribed in the study setup. But gentamicin is a drug candidate of narrow therapeutic index, in which the higher concentrations can lead to toxicity. The study assessed the safety and efficacy, by estimating the serum gentamicin concentrations at the trough and peak levels after it had reached the steady state and the pharmacodynamic response of the study patients to the treatment.

The present study assessed the serum drug concentrations reached in the patient's population, with the different gentamicin dosing and observed the clinical efficacy and safety.

The study observed that gentamicin was prescribed mostly in respiratory infections. The present study observed prescribing of an additional antibiotic (either one broad spectrum antibiotic or

antibiotic acting against the Gram positive micro-organism) along with gentamicin as prophylaxis and was empirical. Cefotaxime was the antibiotic widely prescribed with gentamicin in the hospital. The hospital did not follow any antibiotic policy for the prescription of antibiotics in addition to gentamicin.

The present study showed isolation of Gram negative micro organisms such as *Klebsiella pneumonia, Pseudomonas* aureginosa, *Escherichia coli and Haemophilus influenza*. But no Gram positive micro organisms were identified in patients where the sputum samples were analyzed. Complete cure was observed in the study, which shows that the antibiotic therapy given was successful. The study results were found to be satisfactory, since the clinical efficacy and safety levels achieved in the patients supports the use of gentamicin in the dose as prescribed by the physicians in the study site. Gentamicin showed a low incidence of adverse drug reaction in the study patients. Ototoxicity was the adverse drug reaction reported to occur due to the drug use.

10.1. THEOPHYLLINE

The study results found with the use of theophylline suggests that the dose at 400 mg to 600 mg per day showed the concentration at the lower and moderate level of therapeutic range. The theophylline dose showed a good clinical response and a better cost effectiveness. The study here recommends for the guideline treatment using sustained release theophylline as a more economic alternative acceptable for the asthmatic patients in the study set-up and can be more widely applied.

10.2. GENTAMICIN

The study results were found to be satisfactory since the clinical efficacy and safety achieved in the patients supports the use of gentamicin in the dose as prescribed by the physicians in the study site. Hence the same dosing strategy for gentamicin can be followed, subjecting the patients for ototoxicity and nephrotoxicity tests after four days of gentamicin treatment which is recommended therapeutic regimen.

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