*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 9, Issue, 11, pp.60511-60522, November, 2017*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## RESEARCH ARTICLE

# CORRELATION OF POPULATION PHARMACOKINETICS AND DYNAMICS OF COMMONLY USED AMINOGLYCOSIDE AMIKACIN WITH SNP'S (SINGLE NEUCLEOTIDE POLYMORPHISM)

***Dr. Maya Singha and Dr. Swanand S. Pathak**

Jawahrlal Nehru Medical College, DMIMS, Sawangi (Meghe) Wardha, Maharashtra, India

**ARTICLE INFO**

***Article History:***
Received 27th August, 2017
Received in revised form
19th September, 2017
Accepted 21st October, 2017
Published online 30th November, 2017

***Key words:***
Amikacin,
Population pharmacokinetics,
TDM (therapeutic drug monitoring),
SNP (single nucleotide polymorphism),
CYP3A4*22.

**ABSTRACT**

Amikacin is an Aminoglycoside group of antibiotic, it is a semi synthetic derivative prepared from Kanamycin A by acylation of the 1-amino group of the 2-deoxystreptamine moiety with 2-hydroxy-4-aminobutyric acid. Amikacin is an antibiotic used for a number of bacterial infections. Amikacin have the potential to produce reversible and irreversible vestibular, cochlear, and renal toxicity. Cytochrome P4503A4 (abbreviated CYP3A4), is an important enzyme in the body, mainly found in the liver and in the intestine. In this study, we have tried to evaluate if there is any relation between high serum Amikacin level and presence of CYP3A4*22 in an individual. Study population was patients admitted in Medicine and Paediatrics department receiving Amikacin as antimicrobial of all ages and both sexes. Each patient were observed for any kind of adverse drug reaction during the period of antimicrobial therapy with Amikacin. Therapeutic drug monitoring of Amikacin was done for the patients who had any complain during that period. In the last step, CYP3A4*22 status was estimated for those patients with ADRs to see whether there is any correlation of the SNP (CYP3A4*22) with high serum Amikacin level. Apparatus used were High Performance Liquid Chromatography and Real Time PCR. So from the observations, it can be concluded that high serum Amikacin level has a correlation with adverse reactions of Amikacin. But, as it is seen that CYP3A4*22 is present in both group of patients ie. High and normal serum Amikacin level. It can be concluded that CYP3A4*22 has no correlation with high serum level of Amikacin.



**Citation: Dr. Maya Singha and Dr. Swanand S. Pathak, 2017.** "Correlation of population pharmacokinetics and dynamics of commonly used *Aminoglycoside amikacin* with SNP'S (Single neucleotide polymorphism)", *International Journal of Current Research*, 9, (11), 60511-60522.

## INTRODUCTION

Amikacin is an Aminoglycoside group of antibiotic, it is a semi synthetic derivative prepared from Kanamycin A by acylation of the 1-amino group of the 2-deoxystreptamine moiety with 2-hydroxy-4-aminobutyric acid. Amikacin is an antibiotic used for a number of bacterial infections. This includes intra abdominal infections, meningitis, and urinary tract infections. It is also used for the treatment of multidrug-resistant tuberculosis. It is used either by injection into a vein or muscle. Amikacin have the potential to produce reversible and irreversible vestibular, cochlear, and renal toxicity. These side effects complicate the use of these compounds and make their proper administration difficult. (Bliziotis *et al*., 2005) Population pharmacokinetics in the drug development process to help identify differences in drug safety and efficacy among population subgroups. It summarizes scientific and regulatory issues that should be addressed using population pharmacokinetics. The guidance discusses when to perform a population pharmacokinetic study and/or analysis; how to design and execute a population pharmacokinetic guidance for industry focuses on population pharmacokinetics. Cytochrome P450 enzymes (CYPs) are implicated in many clinically relevant drug-drug interactions (DDI), metabolic reactions catalyzed by this enzyme family are the dominant route of elimination for the majority of drugs. Inhibition of the CYPs can lead to an unwanted elevation in the blood level of drugs administered concomitantly, which can result in life-threatening adverse drug reactions (Baciewicz *et al*., 2003; Appel, 1982). Induction of CYP expression can lead to inadequate drug efficacy (American Thoracic Society, 2005). For example, administration of rifampicin with cyclosporine results in excess metabolism of cyclosporine leading to allograft rejection in transplanted patients (Bailey *et al*., 1997; Banday *et al*., 2008). Cytochrome P4503A4 (abbreviated CYP3A4), is an important enzyme in the body, mainly found in the liver and in the intestine. It oxidizes small foreign organic molecules, such as toxins or drugs, so that they can be removed from the body. Many drugs are deactivated by CYP3A4, while there are some drugs which are *activated* by the enzyme. CYP3A4 is a member of the cytochrome P450 family of oxidizing enzymes. Several other enzymes of this family are also involved in drug metabolism, but CYP3A4 is the most

**Corresponding author:* **Dr. Maya Singha,**
Jawahrlal Nehru Medical College, DMIMS, Sawangi (Meghe) Wardha, Maharashtra, India.

common. Like all members of this family, it is a hemoprotein, i.e. a protein containing a heme group with an iron atom. In humans, the CYP3A4 protein is encoded by the *CYP3A4* gene. (American Thoracic Society, 2005) This gene is part of a cluster of cytochrome $P_{450}$ genes (Bailey *et al*., 1997). In this study, we have tried to evaluate if there is any relation between high serum Amikacin level and presence of CYP3A4*22 in an individual.

## MATERIALS AND METHODS

### Material

**Study design:** Cross sectional study

**Locus of the study:** The present study was conducted in the Department of Pharmacology, Jawaharlal Nehru Medical College and Acharya Vinobha Bhave Rural Hospital, Sawangi (Meghe), Wardha.

**Time frame:** 1st January 2016 to 31st December 2016

**Sample size:** 456

### Approval of institutional ethics commitee

The synopsis of the study protocol was submitted to the Institutional Ethics Committee of Datta Meghe Institute of Medical sciences, Wardha. The study was approved on 26.09.2015, vide ref. No. DMIMS(DU)/IEC/2015-16/1561.

### Presentation and interpretation of data

- The data was tabulated in the form of scientific tables and analyzed using Microsoft Excel for Windows
- Statistical analysis was done by using descriptive and inferential statistics using Chi square test. Software used in this analysis were SPSS17.0 and Graph pad prism 5.0 version and $p<0.05$ is considered as level of significance ($p<0.05$).
- The observations are presented in tables and figures at appropriate places.
- The results are expressed as frequency, means and percentages at the requisite places

**Study population:** Patients admitted in Medicine and Paediatrics department receiving Amikacin as antimicrobial of all ages and both sexes.

### Inclusion criteria for study patient

ALL age male and female patient admitted in Medicine and Paediatric department receiving treatment with Amikacin antibacterial AND willing to participate in the study

### Exclusion criteria for study patient

- Unconscious patient and patient in coma
- Any patient admitted in Medicine and Paediatric department not willing for to become part of study.

### Methods

After due written permission of Institutional ethics committee was obtained for the study on 26.09.2015, vide ref. No. DMIMS(DU)/IEC/2015-16/1561. A written informed consent was also obtained from all study participants/ patients/ legal gaurdians admitted in Medicine and Paediatrics dept. and was kept confidential. The study was proceeded forward only after written approval from the Institutional Ethical Committee. At the beginning, patients were divided according to department, then age wise distribution was done for both Medicine and Paediatrics patients. Sex distribution was done in case of both Medicine and Paediatric department Distribution of patients according to diagnosis category were done for which they were admitted in the hospital. Patients were then distributed according to total number of days stayed in the hospital. After which dose estimation and total number of doses administered were observed in each patients. Depending on that total cost of antibiotic therapy for each patient was calculated. Each patient were observed for any kind of adverse drug reaction during the period of antimicrobial therapy with Amikacin. Therapeutic drug monitoring of Amikacin was done for the patients who had any complain during that period. In the last step, CYP3A4*22 status was estimated for those patients with ADRs to see whether there is any correlation of the SNP (CYP3A4*22) with high serum Amikacin level. Outcome of each patient was monitored in context to discharge or death after completion of treatment.

### 1. Extraction of DNA

**Principle:** Extraction is based on spin column based nucleic acid purification method that will quickly purify nucleic acids.(1) The spin columns contain silica resin as the main component. It is called spin column because it uses the technique of augmenting the extraction by applying centrifugal force. Centrifugation method or spin column method includes four major steps; lysis, binding, washing and eluting.(2) The whole blood sample is first lysed by lysis buffer to release the DNA contents from membrane bound organelles. DNA binds to this silica resin membrane during centrifugation or under vacuum conditions. This binding is augmented in the presence of a chaotropic agent (usually guanidine hydrochloride) as it disrupts the hydrophobic effect of nucleic acids on water molecules and thus altering its tertiary structure at a particular pH. (3) It

### Materials required

a) Whole blood (200 $\mu$L)
b) DNA extraction kit
   - Specifications- spin column method, silica plate technology
   - Contents: Lysis buffer, Wash buffer, Elution buffer, spin column with silica
c) Microcentrifuge tubes (1.5 ml, 2 ml)
d) Proteinase K
e) Vortex
f) Micropipettes
g) Water bath
h) Absolute alcohol (100%)
i) Micro centrifuge
j) Collection tube
k) Distilled water

### Standard PCR procedure for CYP2C9*22

Polymerase chain reaction is the most widely used technique in molecular biology. It is a simple mean for producing relatively

large number of copies of DNA molecules from minute quantities of template DNA molecules from minute quantities of template DNA. PCR essentially comprises of preparation of master mix followed by thermo cycling. Which comprise a single "cycle" in the PCR amplification methodology. After each cycle the newly synthesized DNA strands can serve as templates for the subsequent cycle. After each cycle the newly synthesized DNA strands can serve as template for the subsequent cycle.

**Thermocycilng program for cyp2c9*22**

1. Initial denaturation – 94°C/5 min
2. Denaturation- 94°C/20 sec
   Annealing – 53°C/30 sec
   Extension – 72°C/5 min
   For 34 cycles
3. Final extension – 72° / 5min
4. Hold- 22°C/1min

**Requirements**

1. Equipments

- Master cycling gradient
- Micropipettes :0.1-2.5µl,2-20µl,10-100µl,100-1000µl
- Micro centrifuge
- Minicooler

2. Consumables

- PCR strips- 0.2ml
- Microtips (0.1-10µl,2-200µl,100-1000µl)
- Microcentrifuge tubes (0.5ml,2ml)

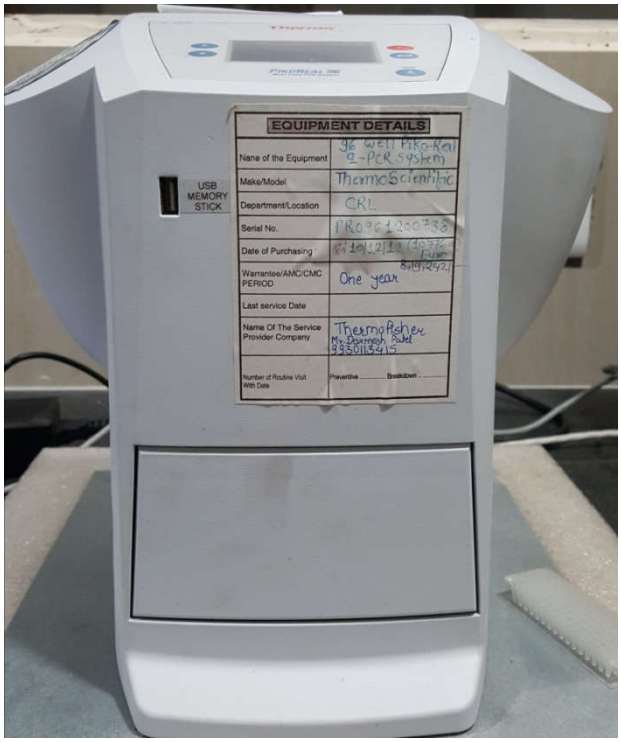
3. Reagents

1. Buffers (10× stock)
2. Primers
   - 2C9-F-5'TACAAATACAATGAAAATATCATG3'
   - 2C9-R-5'CTAACAACCAGACTCATAATG3'
3. dNTPs (stock solution-100Mm)
4. MgCl2(stock 1m)
5. Enzyme: TaqDNA polymerase(5U/µL)

PCR PRODUCT

Size: 690 base pairs

| | |
|---|---|
| TACAAATACAATGAAAATATCATGCTAAATCAGGCTTAG | FORWAR |
| CAAATGGACAAAATAGTAACTTCGTTTGCTGTTATCTCT | D PRIMER |
| GTCTACTTTCCTAGCTCTCAAGAGTCTATGGCCCTGTGT | (2C9-F) |
| TCACTCTGTATTTTGGCCTGAAACCCATAGTGGTGCTGC | |
| ATGGATATGAAGCAGTGAAGGAAGCCCTGATTGATCTTG | EXON 2 |
| GAGAGGAGTTTTCTGGAAGAGGCATTTTCCACTGGCTG | |
| AAAGAGCTAACAGAGGATTTGGTAGGTGTGCATGTGCCT | |
| GTTTCAGCATCTGTCTTGGGGATGGGGAGGATGGAAAAC | |
| AGAGACTTACAGAGCTCCTCGGGCCGAGCTTGGCCCATC | |
| CACATGGCTGCCCAAGTGTCAGTTCCTCTTTCTTGCCTG | |
| GGATCTCCCTCCTAGTTTCGTTTCTCTTCCTGTTAGGAA | |
| TTGTTTTCAGCAATGGAAAGAAATGGAAGGAGATCCGGC | EXON 3 |
| GTTTCTCCCTCATGACGCTGCGGAATTTTGGGATGGGGA | |
| AGAGGAGCATTGAGGAC GTGTTCAAGAGGAAGCCCG | SNP |
| CTGCCTTGTGGAGGAGTTGAGAAAAACCAAGGGTGGGTG | |
| ACCCTACTCCATATCACTGACCTTACTGGACTACTATCT | REVERSE |
| TCTCTACTGACATTCTTGGAAACATTTCAGGGGTGGCCA | PRIMER |
| TATCTTT | (2C9-R) |




**Real Time PCR**

**High performance liquid chromatography (HPLC ) is the technique that offers the highest resolution and best degree of specificity**

**Procedure :** Sample (Serum) 50µlit.

↓

To centrifuge tube

↓

Add50 ml of Trihydroxymethyl aminomethane

↓

Vertex for 15 sec

↓

200µl of acetonitrile & vertex for 15 sec

↓

Centrifuge at 2500 for 5 min

↓

Transfer 200µl to an ampule and add 20µl of FDNB

↓

close the ample for water bath at 80° C for 45 min

↓

Inject to the chromatograph 170 µl

**FDNB –** fluro 2,4 dinitrobenzene
**ACN –** acetonitrile
Apperatus –universal injector U6K, 2ml injection loop, 365nm filter, µ Bondapak C18 column
**Reagents:** water, HCL, FDNB, Trihydroxymethyl aminomethane, Gentamicin Sulfate, Human serum stored at 18® in 3days of collection.

**Apparatus used**

**High Performance Liquid Chromatography**

**Solutions**

All solutions and the acetonitrile were passed through a 0.2-µm filter before use.

Solution 1. Amikacin sulphate in water, containing the equivalent of 4mg of Amikacin per litre.
Solution 2. Tris 20g/l in water
Solution 3. FDNB 250 g/l in acetonitrile
Solution 4. FDNB 170g/l in acetonitrile

**Procedure**

**A.Derivatisation of aqueous Amikacin solutions**

Dispense into an ampule 50µl of solution 1, 50µl of solution 2, 200µl of acetonitrile and 20µl of solution 3. Close the ampoule and place in a water –bath at 80° for 45 min. Inject into the chromatograph.

**B. Derivatisation of serum samples**

Dispense 50µ of serum into a centrifuge tube add 50µl of solution 2 and vertex for 15 sec, then centrifuge at 2500g for 5min. Transfer 200µ of the supernatant into an ampoule, add 20 µl of solution 4. Close the ampoule and place in a water-bath at 80° for 45min. Inject 175 µl into the chromatograph.

## RESULTS

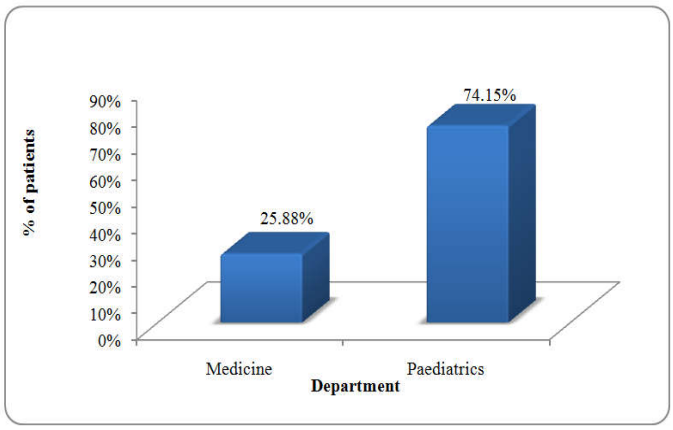
The present study was carried out in a tertiary teaching hospital in Central India, Vidharbha region of Maharashtra; AVBRH, Sawangi (Meghe), Wardha. 456 patients admitted in Medicine and Paediatrics Dept. were included in the study to find out the population pharmacokinetics (Therapeutic Drug Monitoring) of Amikacin and its relation with SNP (CYP3A4*22).

**1. Distribution of patients according to department**

Out of the 456 patients, 118 patients are from Medicine and rest 338 are from Paediatrics Dept. (Table No.1 and Figure No. 1)

**Table No.1**

| Department | No of patients | Percentage(%) |
|---|---|---|
| Medicine | 118 | 25.88 |
| Paediatrics | 338 | 74.15 |
| Total | 456(100%) | 100 |



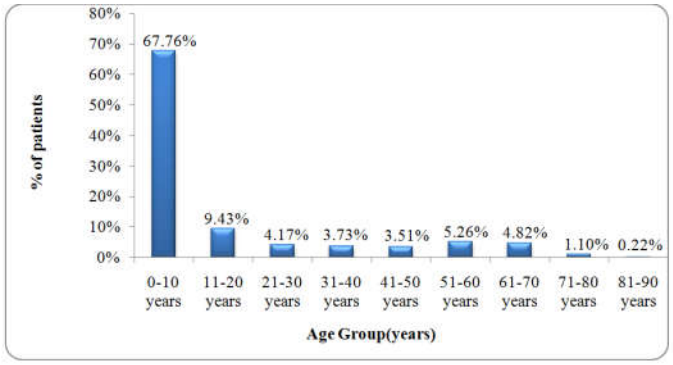

**Graph No.1**

**2. Age wise distribution of patients**

The 456 patients who were administered Amikacin divided nine age groups. Mean age of the patients was 32.84±22.94. Most the patients were of 0-10 years (67.76%), followed by 11-20 years (9.43%),51-60 years (5.26%) and least patients were of the age of 81-90 years (.22%).

**Table No 2**

| Age Group(yrs) | No of patients | Percentage(%) |
|---|---|---|
| 0-10 years | 309 | 67.76 |
| 11-20 years | 43 | 9.43 |
| 21-30 years | 19 | 4.17 |
| 31-40 years | 17 | 3.73 |
| 41-50 years | 16 | 3.51 |
| 51-60 years | 24 | 5.26 |
| 61-70 years | 22 | 4.82 |
| 71-80 years | 5 | 1.10 |
| 81-90 years | 1 | 0.22 |
| Total | 456 | 100 |
| Mean ± SD | 32.84±22.94(0-88 years) | |



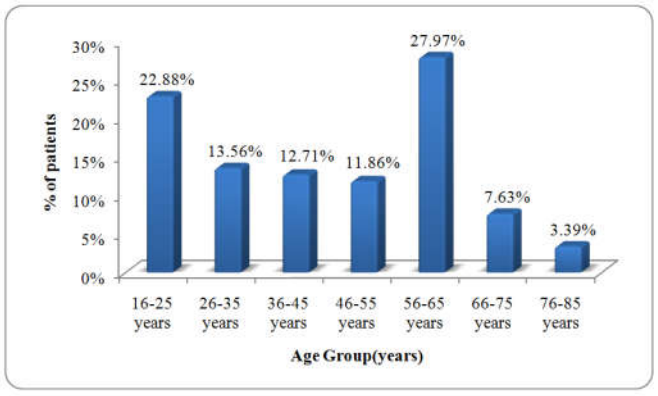

**Graph No. 2**

### 3. Age wise distribution of patients in Medicine department

Out of 118 patients receiving Amikacin treatment were divided into seven age groups. The mean age of the patient was 45.52±18.47. Maximum number of patients were of 56 to 65 years (27.97%), followed by 16-25years (22.88%) and least patients were of the age of 65 years and above (3.39%). (Table 3; Figure 3)

**Table no. 3**

| Age Group(yrs) | No of patients | Percentage(%) |
|---|---|---|
| 16-25 years | 27 | 22.88 |
| 26-35 years | 16 | 13.56 |
| 36-45 years | 15 | 12.71 |
| 46-55 years | 14 | 11.86 |
| 56-65 years | 33 | 27.97 |
| 66-75 years | 9 | 7.63 |
| 76-85 years | 4 | 3.39 |
| Total | 118 | 100 |
| Mean ± SD | 45.52±18.47(16-82 years) | |




**Graph No.3**

### 4. Age wise distribution of patients in Paediatrics department

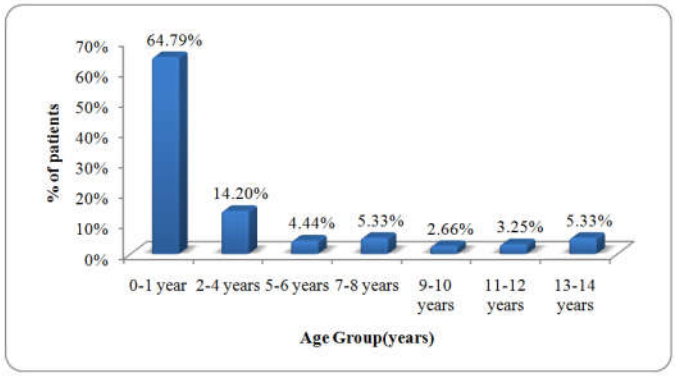
The 338 patients receiving Amikacin treatment were divided into seven age groups. The mean age of the patient was 2.45± 4.01. Most of the patients were of 0-1year (64.79%), followed by 2-4years (14.20%) and least patients were of the age of 9-10 years (2.66%). (Table no. 4; Figure no. 4)

### 5. Sex Distribution

Number of treated patients with Amikacin was predominantly seen more in males both in Medicine (66.95%) and Paediatric patients (59.76%)

**Table no 4**

| Age Group (yrs) | No of patients | Percentage(%) |
|---|---|---|
| 0-1 year | 219 | 64.79 |
| 2-4 years | 48 | 14.20 |
| 5-6 years | 15 | 4.44 |
| 7-8 years | 18 | 5.33 |
| 9-10 years | 9 | 2.66 |
| 11-12 years | 11 | 3.25 |
| 13-15 years | 18 | 5.33 |
| Total | 338 | 100 |
| Mean ± SD | 2.45±4.01(0-14 years) | |




**Graph No.4**

**Table No. 5**

| Department | Male | Female | Total |
|---|---|---|---|
| Medicine Department | 79(66.95%) | 39(33.05%) | 118(100%) |
| Paediatric Department | 202(59.76%) | 136(40.24%) | 338(100%) |

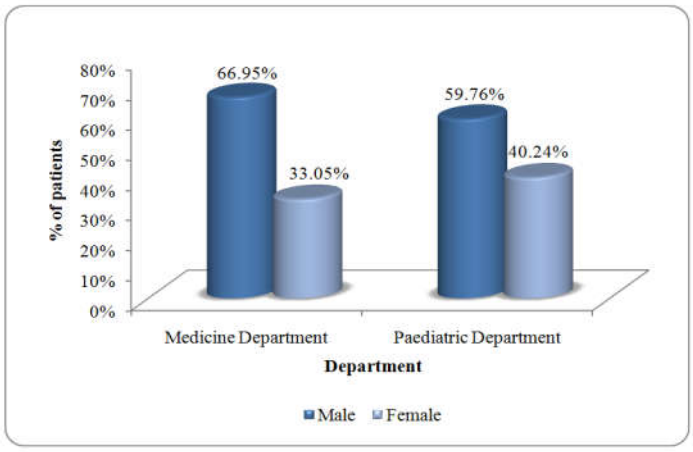



**Figure No. 5**

**Table No. 6**

| Type of discharge | Medicine(118) | Percentage |
|---|---|---|
| Respiratory Diseases | 44 | 37.29 |
| Gastro Intestinal diseases | 9 | 7.63 |
| Central Nervous System disorders | 18 | 15.25 |
| Haematological Disorder | 10 | 8.47 |
| Cardio Vascular diseases | 9 | 7.63 |
| Musculoskeletal Disorders | 6 | 5.08 |
| Diseases of Urinary Tract | 5 | 4.24 |
| Others | 17 | 14.41 |
| Total | 118 | 100.00 |

### 6. Distribution of patients according to diagnosis category in Medicine patients

All the patients treated with Amikacin were divided into eight categories system wise. Most of the patients were treated for

respiratory diseases (37.29%), followed by Central Nervous System disorders (15.25%) and least patients were with diseases of Urinary Tract (4.24%). (Table No. 6, Figure No.6)

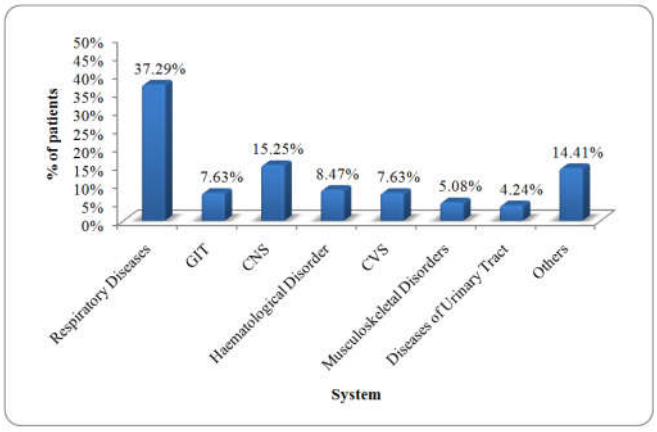



**Figure No. 6**

## 7. Distribution of patients according to diagnosis category in Paediatric patients

The patients receiving Amikacin in Paediatric department were divided into eight categories system wise. Out of 338 patients most of the patients were treated for Respiratory diseases (30.18%), followed by Gastro Intestinal diseases (8.28%), Cardio Vascular diseases (7.69%), CNS disorders (6.21%) and least patients were with Musculoskeletal Disorders (0.59%). (Table No. 7, Figure No. 7)

**Table No.7**

| Type of discharge | Paediatrics(338) | Percentage |
|---|---|---|
| Respiratory Diseases | 102 | 30.18 |
| Gastro Intestinal diseases | 28 | 8.28 |
| Central Nervous System Disorders | 21 | 6.21 |
| Haematological Disorders | 18 | 5.33 |
| Cardio Vascular disorders | 26 | 7.69 |
| Musculoskeletal Disorders | 2 | 0.59 |
| Diseases of Urinary Tract | 23 | 6.80 |
| Others | 118 | 34.91 |
| Total | 338 | 100.00 |

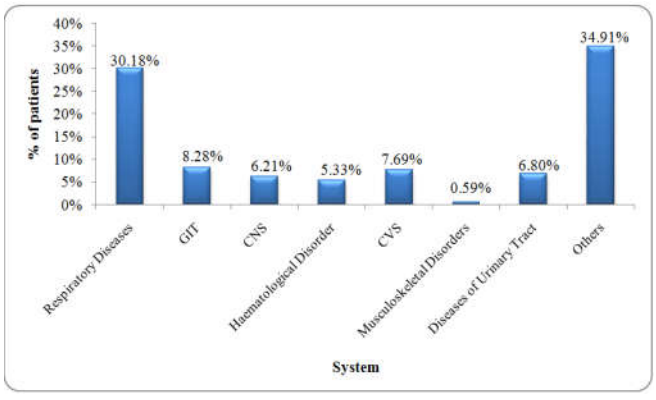



**Figure No. 7**

## 8. Days of stay in hospital

Distribution of patients according to number of days stayed in hospital were calculated. The mean stay in case of Medicine patients was 15.12±11.37. In case of Paediatric patients the mean stay was 10.72±8.39. (Table No. 8, Figure No.8)

**Table No. 8**

| Number of days | No of patients | Medicine | Paediatrics |
|---|---|---|---|
| 0-10 days | 265(58.11%) | 50(42.37%) | 215(63.61%) |
| 11-20 days | 116(25.44%) | 33(27.97%) | 83(24.56%) |
| 21-30 days | 57(16.86%) | 25(21.19%) | 32(9.47%) |
| 31-40 days | 9(2.66%) | 4(3.39%) | 5(1.48%) |
| 41-50 days | 6(1.78%) | 4(3.39%) | 2(0.59%) |
| >50 days | 3(0.89%) | 2(1.69%) | 1(0.30%) |
| Total | 456(100%) | 118(100%) | 338(100%) |
| Mean±SD | 11.86±9.40 | 15.12±11.37 | 10.72±8.39 |
| ×2-value | 26.22,P-value =0.0001,S | | |

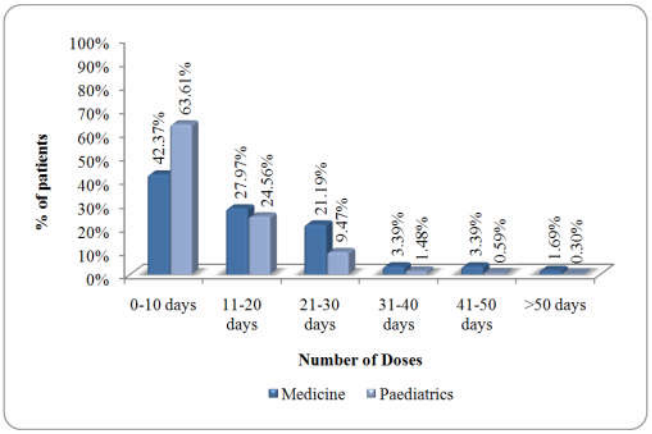



**Figure No. 8**

## 9. Dosing of Amikacin

Dosing of Amikacin was approximately 15mg/kg day twice daily for both Medicine & Paediatric patients. The mean number of given doses in case of Medicine was 15.25 with a standard deviation of 9.75. In case of Paediatrics the mean was 7.51 with a standard deviation 2.67.

**Table No. 9**

| No of doses | Medicine | Paediatrics | Total |
|---|---|---|---|
| 6 to 10 | 27(22.88%) | 93(27.51%) | 120(26.32%) |
| 11 to 15 | 72(61.02%) | 245(72.49%) | 286(69.52%) |
| 16 to 20 | 19(16.10%) | 0(0%) | 50(4.17%) |
| Total | 118(100%) | 338(100%) | 456(100%) |
| Mean | 15.25 | 7.51 | 12.63 |
| SD | 9.75 | 2.67 | 1.81 |

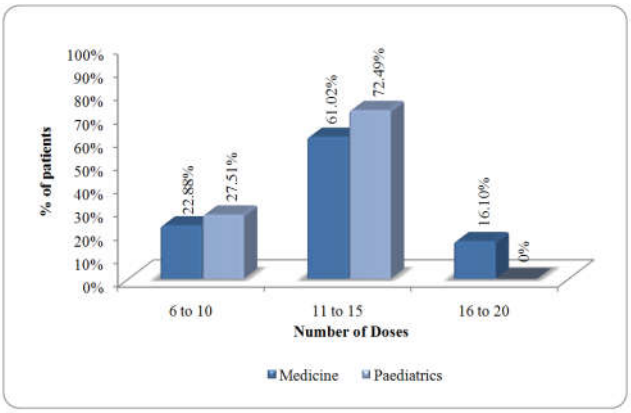



**Figure No. 9**

## 10. Comparison of cost of therapy:

Number of patients according to total cost of therapy were divided four categories viz. 101-200 `, 201-300 `, 301-400 `,

401-500`. Mean cost in case of Medicine patient was 476.35` with a standard deviation of 55.86`. In case of Paediatric patient the mean cost of therapy was 275.91` with a standard deviation of 45.29`. (Table No. 10, Figure No. 10)

**Table No. 10**

| Total Cost | Medicine | Paediatrics | Total |
|---|---|---|---|
| 101-200 ` | 1(0.85%) | 6(1.78%) | 7(1.54%) |
| 201-300 ` | 6(5.08%) | 326(96.45%) | 332(72.81%) |
| 301-400 ` | 70(59.32%) | 6(1.78%) | 76(16.67%) |
| 401-500 ` | 41(34.75%) | 0(0%) | 41(8.99%) |
| Total | 118(100%) | 338(100%) | 456(100%) |
| Mean | 476.35 | 275.91 | 276.03 |
| SD | 55.86 | 45.29 | 35.38 |

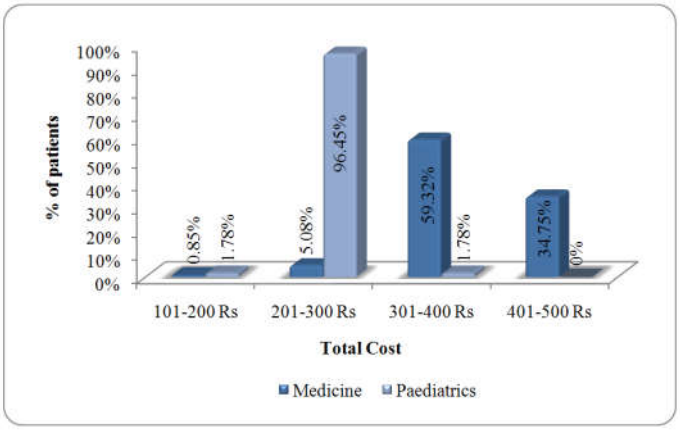



**Figure No. 10**

## 11. Comparison of outcome in Medicine department

Comparison of outcome in medicine patients were done, out 118 patients number of deaths were 13 (11.02%). (Table 11, Fig. 11)

**Table No. 11**

| | No of patients | Percentage(%) |
|---|---|---|
| Discharge | 105 | 88.98 |
| Death | 13 | 11.02 |
| Total | 118 | 100 |

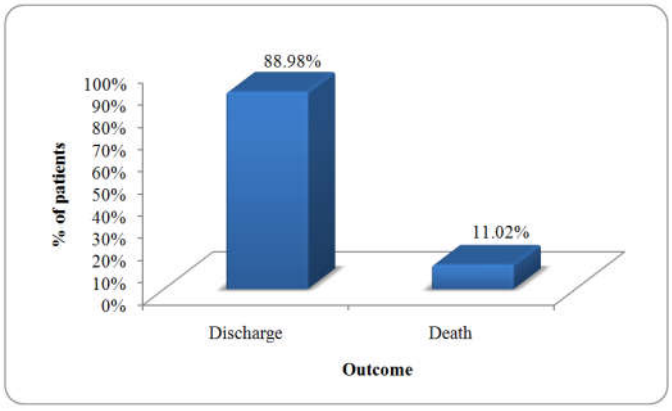



**Figure No. 11**

## 12. Comparison of outcome in Paediatrics department

Comparison of outcome in paediatric patients were done, out of 338 patients number of deaths were 22 (6.51%).

**Table No. 12**

| | No of patients | Percentage (%) |
|---|---|---|
| Discharge | 316 | 93.49 |
| Death | 22 | 6.51 |
| Total | 338 | 100 |

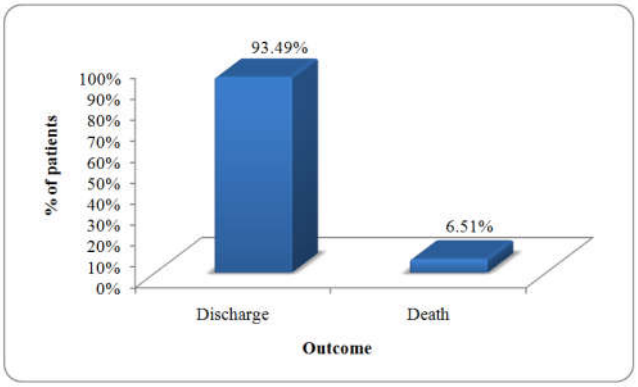



**Figure No. 12**

**Table No. 13**

| | Total Patients | No of patients having adverse drug reaction | Percentage (%) |
|---|---|---|---|
| Medicine | 118 | 6 | 5.08% |
| Paediatrics | 338 | 32 | 9.46% |

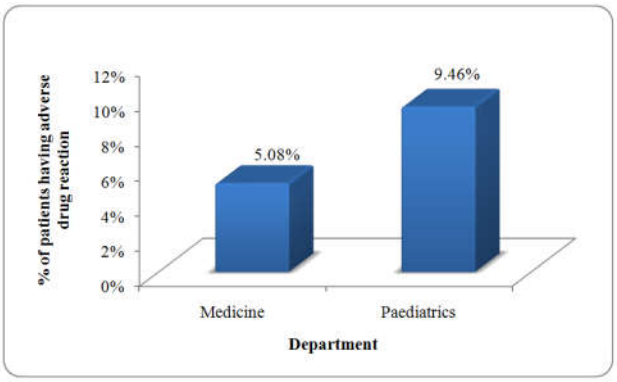



**Figure No. 13**

**Table No. 14**

**Z-test for difference between two means**

| | N | Mean serum Amikacin | Std. Deviation | Std. Error Mean | z-value |
|---|---|---|---|---|---|
| Medicine | 6 | 12.05 | 3.91 | 1.59 | 1.27 |
| Paediatrics | 33 | 10.05 | 3.44 | 0.59 | P=0.20,NS |

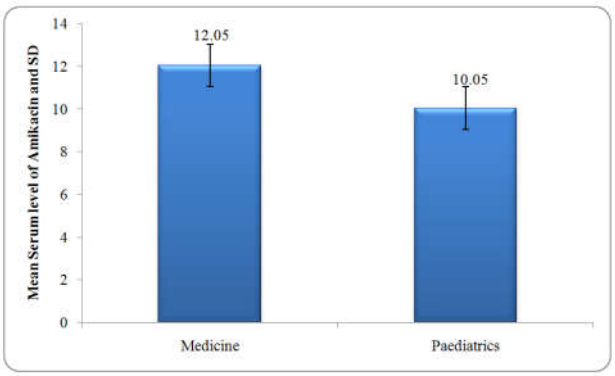



**Figure No. 14**

**Table No. 15**

| | Total Patients | No of patients having high serum level of Amikacin | No of patients having normal Serum level of Amikacin |
|---|---|---|---|
| Medicine | 6 | 4(66.67%) | 2(33.33%) |
| Paediatrics | 33 | 24(72.73%) | 9(27.27%) |

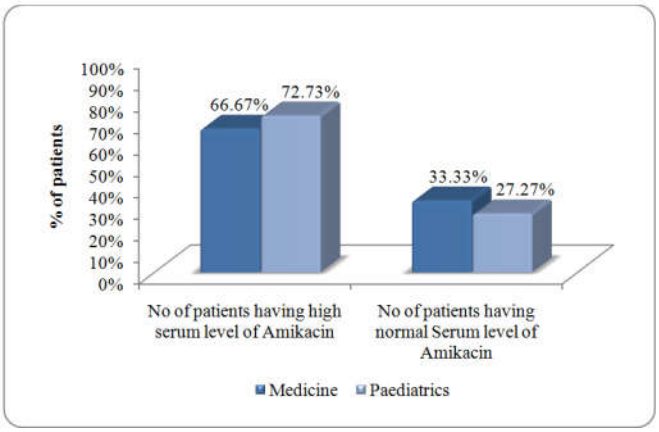



**Figure No. 15**

**Table No. 16**

| | Total Patients | No of patients having CYP 3A4*22 status | Percentage (%) |
|---|---|---|---|
| Patients with ADR and high level of Amikacin | 4 | 0 | 0% |
| Patients with ADR with normal level of Amikacin | 2 | 0 | 0% |

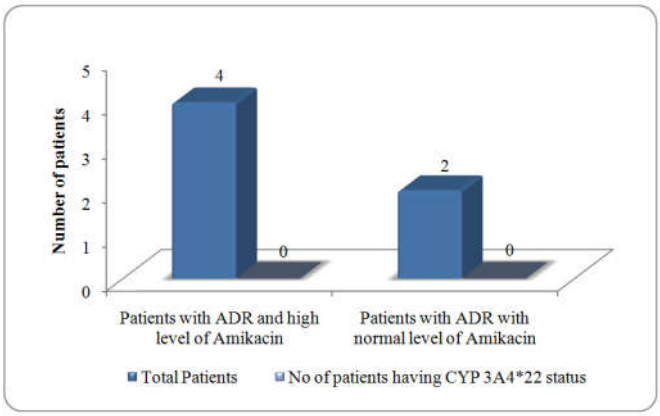



**Figure No. 16**

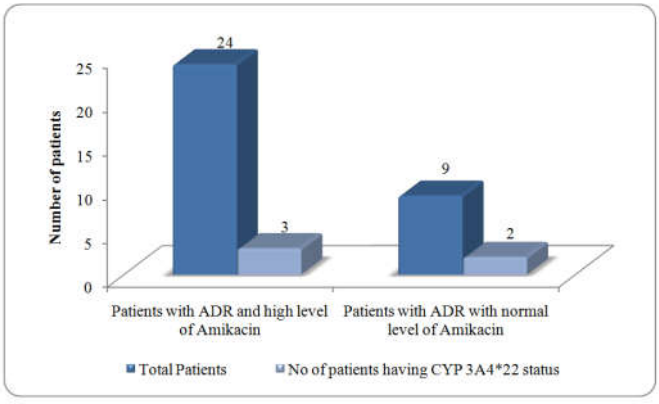



**Figure No. 17**

**Table No.17**

| | Total Patients | No of patients having CYP 3A4*22 status | Percentage (%) |
|---|---|---|---|
| Patients with ADR and high level of Amikacin | 24 | 3 | 12.5% |
| Patients with ADR with normal level of Amikacin | 9 | 2 | 22.22% |

### 13. Comparison of number of patients with adverse drug reaction

Out of 118 patients of Medicine, six patient had some kind of adverse drug reaction, (5.08%) whereas out of 338 patients of Paediatrics 32 patient had shown adverse drug reaction (9.46%).

### 14. Comparison of Serum level of Amikacin

Patients who had any adverse reaction for those patients Serum Amikacin level was estimated. The mean Serum Amikacin level was 12.05 with a standard deviation 3.91 for Medicine patients and in case of Paediatrics the mean was 10.05 with standard deviation 3.44.

### 15. Comparison of High Serum level of Amikacin (>10)

Out of 6 patients, 4 had high serum level of Amikacin (66.67%) in Medicine patients. Out of 33 patients, 24 had high Serum level of Amikacin (72.73%) in Paediatric patients. Rest of the patients had normal Serum Amikacin level.

### 16. Comparison of CYP 3A4*22 status in Medicine patients with adverse drug reaction

Out of six patients who had adverse drug reaction, no one had CYP3A4*22 present in blood.

### 17. Comparison of CYP3A4*22 status in Paediatric patients with adverse drug reaction

Patients having high serum level of Amikacin ie. 24, were tested for presence of CYP3A4*22 and 3 patients had positive results (12.5%). Whereas, patients with normal serum Amikacin level ie. 9, out of which 2 patient had positive results (22.22%).

## DISCUSSION

The present study was an observational study and attempted to evaluate the relation between presence of SNP (CYP3A4*22) and adverse drug reaction with Amikacin (Aminoglyciside). Our study strength was 456 patients, out of which 118 patients were from Medicine department and 338 from Paediatric department. The discussion of findings are as follows. The age group of maximum number of patients in our study was among (0- 10yr). The most likely reason for this could be, children are more prone to various infections in our country. There were variations in the mean age of Amikacin treated patients in various studies. In a study in Maxico by *ANA* MARIA CONTREAS *et al*, the mean age of 50.2 + 18.5yrs as the study was mainly focused on nephrotoxicity by Amikacin. In that age group there were underlying Diabetes Mellitus patients, which was the inclusion criteria for the study. The parameters from our study, revealed number of male patient was higher in case of both Medicine (66.95%) and Paediatrics (59.76%). The

most likely for this reason could be, in rural India the male population has more access to health services as compared to the female population. But it is seen that in case of Paediatric age group the difference is very less. It was also observed that the patients who received Inj. Amikacin as treatment were mostly admitted for diseases of Respiratory system. It may be because the population is more prone to respiratory infection than any other infection. There were variations in the type of infections treated with Amikacin in various studies, as it is commonly in use for variety of bacterial infections of lungs, skin, abdomen and blood caused by E. coli, Streptococci, Enterococci.

**The mean length of stay in hospital, in case of patients**

In Medicine department it was 15.12±11.37 and in Paediatrics it was 10.72±8.39. It can be explained as most of the patient's condition was stable after 7-10 days of treatment. The dosing of Inj. Amikacin included in the study was approximately 15mg/kg per day in 2-3 divided doses daily for both Medicine & Paediatric patients. The mean number of given doses in case of Medicine was 15.25 with a standard deviation of 9.75. In case of Paediatrics the mean was 7.51 with a standard deviation 2.67. In a similar study, by Hideo Kato *et al* used the conventional dosing of (15-20mg/kg) for evaluation of pharmacokinetics. However, there is discrepancy regarding Amikacin initial dosage, with some reports recently recommending >25mg/kg. (Hideo Kato *et al*.) In our study, it was observed that the number of deaths in case of Medicine was 13(6.51%) and in Paediatrics it was 22(11.02%). It can be explained as many of the patients were admitted with severe infections along with other life threatening diseases, cardiac complications, etc. Serum level of Amikacin was estimated for the study for those patients who had any kind of adverse reactions during the period of treatment. It included adverse reactions like skin rashes, nausea vomiting, eosinophilia, vertigo, tingling of skin, temporary hearing loss and few cases with high serum creatinine level. Like in a study, by Hideo Kato *et al* (6$^{th}$ January 2017) it was mentioned that his work which was a single-center retrospective study, included all patients admitted to Aichi Medical University Hospital, between September 2009 and December 2014 who were treated with Amikacin for at least 3 days because of several types of infections. The blood concentrations of Amikacin from patients were obtained as routine practice as therapeutic drug monitoring (TDM). Exclusion criteria were burns, pregnancy, or the use of continuous renal replacement therapy at the onset of Amikacin therapy. After estimation of serum Amikacin level for the patients with adverse drug reactions, it was observed that in most of the cases, serum Amikacin level was high (>10 µg/ml). After which we have looked for presence of CYP3A4*22 in both groups, the one with high Serum Amikacin level and other which had normal Serum Amikacin level. As the CYP3A4 enzyme contributes to the disposition of more than 60 therapeutically important drugs and displays marked person to person variability of catalytic function.

In a study by Shufeng Zhou *et al*, it was mentioned that mechanism-based inhibition of cytochrome P450 (CYP) 3A4 is characterized by NADPH-, time-, and concentration-dependent enzyme inactivation, occurring when some drugs are converted by CYPs to reactive metabolites. Such inhibition of CYP3A4 can be due to the chemical modification of the heme, the protein, or both as a result of covalent binding of modified heme to the protein. The inactivation of CYP3A4 by drugs has important clinical significance as it metabolizes approximately 60% of therapeutic drugs, and its inhibition frequently causes unfavorable drug–drug interactions and toxicity. The clinical outcomes due to CYP3A4 inactivation depend on many factors associated with the enzyme, drugs, and patients. Clinical professionals should adopt proper approaches when using drugs that are mechanism-based CYP3A4 inhibitors. These include early identification of drugs behaving as CYP3A4 inactivators, rational use of such drugs (eg, safe drug combination regimen, dose adjustment, or discontinuation of therapy when toxic drug interactions occur), therapeutic drug monitoring, and predicting the risks for potential drug–drug interactions. A good understanding of CYP3A4 inactivation and proper clinical management are needed by clinical professionals when these drugs are used. The human cytochrome P450 (CYP) 3A subfamily, includes CYP3A4, 3A5, 3A7 (Nelson *et al* 1996), and 3A43 (Domanski *et al* 2001). CYP3A4 is most abundant in the human liver (~ 40%) and metabolizes more than 50% of clinically used drugs. After completion of the study, we have observed that the SNP, CYP3A4*22 absent in the 6 patients who had adverse drug reaction. Out of 24 patients in Paediatric department who had high serum Amikacin level 3 had CYP3A4*22 present in their blood. Whereas out of the 9 patients who had normal serum Amikacin level 2 had shown presence of CYP3A4*22 as well.

**Summary**

This study was designed, executed and concluded to analyze the correlation of Amikacin induced adverse drug effects with SNP, particularly CYP3A4*22. The study was cross sectional study carried out in the Medicine and Paediatric ward of AVBRH, Sawangi (M), Wardha; a tertiary care teaching hospital in Central India. The study took into account 456 data of patients who were administered Inj. Amikacin as antibacterial therapy and who fulfilled inclusion/exclusion criteria. Patient demographics and treatment details were collected. The collected data was evaluated and analyzed to reveal various demographic and pharmacotherapy related results. All the 456 patients, 118 from Medicine and 338 from Paediatrics were screened for adverse effects. Out of 118 patients of Medicine, 6 patients had some kind of adverse drug reaction, (5.08%) whereas out of 338 patients of Paediatrics 32 patient had shown adverse drug reaction (9.46%). Out of 6 patients, 4 had high serum level of Amikacin (66.67%) in Medicine patients and out of 33 patients , 24 had high Serum level of Amikacin (72.73%) in Paediatric patients where CYP3A4*22 estimation was done for the same number of patients .Out of 6 patients who had adverse drug reaction, no one had CYP3A4*22. Patients having high serum level of Amikacin ie. 24 in Paediatrics, were tested for presence of CYP3A4*22 and 3 patients had positive results (12.5%). Whereas, patients with normal serum Amikacin level ie. 9, out of which 2 patient had positive results (22.22%). So from the observations, it can be concluded that high serum Amikacin level has a correlation with adverse reactions of Amikacin. But, as it is seen that CYP3A4*22 is present in both group of patients ie. high and normal serum Amikacin level. It can be concluded that CYP3A4*22 has no correlation with high serum level of Amikacin.

**Conclusion**

The study illustrates the relationship of CYP3A4*22 and serum level of Amikacin and adverse drug reactions as well in

a tertiary care teaching hospital. The study was executed and data collected was thoroughly analyzed under the guidance of our objectives to reveal various observations.

**The study concludes with the following observations and inferences:**

- Out of the 456 patients, 118 patients are from Medicine and rest 338 are from Paediatrics Dept (74.15%).
- Mean age of the patients receiving Amikacin was 32.84±22.94. Most the patients were of 0-10 years (67.76%).
- Number of treated patients with Amikacin was predominantly seen more in males both in Medicine (66.95%) and Paediatric patients (59.76%)
- Most of the patients were treated for respiratory diseases. In Medicine it was (37.29%) and (30.18%) in Paediatrics.
- The mean stay in case of Medicine patients was 15.12±11.37 & 10.72±8.39 in Paediatrics.
- Dosing of Amikacin was approximately 15mg/kg day twice daily for both Medicine & Paediatric patients.
- Mean cost in case of Medicine patient was 476.35. In case of Paediatric patient the mean cost of therapy was 275.91.
- Out of 118 patients of Medicine, six patients had some kind of adverse drug reaction, (5.08%) whereas out of 338 patients of Paediatrics 32 patient had shown adverse drug reaction (9.46%).
- Out of 6 patients, 4 had high serum level of Amikacin (66.67%) in Medicine patients.
- Out of 33 patients, 24 had high Serum level of Amikacin (72.73%) in Paediatric patients.
- Out of six patients who had adverse drug reaction, no one had CYP3A4*22 present in blood.
- Patients having high serum level of Amikacin ie. 24 in Paediatrics, were tested for presence of CYP3A4*22 and 3 patients had positive results (12.5%). Whereas, patients with normal serum Amikacin level ie. 9, out of which 2 patient had positive results (22.22%).

So from the observations, it can be concluded that high serum Amikacin level has a correlation with adverse reactions of Amikacin. But, as it is seen that CYP3A4*22 is present in both group of patients ie. high and normal serum Amikacin level. It can be concluded that CYP3A4*22 has no correlation with high serum level of Amikacin.

**Recommendation**

It is recommended that therapeutic drug monitoring of Amikacin should be practiced to reduce the chances of adverse drug reactions.

## REFERENCES


American Thoracic Society. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Resp Crit Care Med*, 2005, *171:*388–41

Appel GB. 1982. Aminoglycoside nephrotoxicity: Physiologic studies of the sites of nephron damage. In: *The Aminoglycosides: Microbiology, Clinical Use, and Toxicity* (Whelton A, Neu HC, eds.), Marcel Dekker, New York, pp. 269–282.

Baciewicz AM, Sokos DR, Cowan RI. 2003. Aminoglycoside-associated nephrotoxicity in the elderly. *Ann Pharmacother*, *37:*182–186.(PMID: 12549943) (Full Text)

Bailey TC, Little JR, Littenberg B, *et al*. 1997. A meta-analysis of extended-interval dosing versus multiple daily dosing of aminoglycosides. *Clin Infect Dis*, *24:*786–795.(PMID: 9142771) (Full Text)

Banday AA, Farooq N, Priyamvada S, *et al*. 2008. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. *Life Sciences*, *82:*450–459.(PMID: 18201728) (Full Text)

Barclay ML, Kirkpatrick CM, Begg EJ. 1999. Once-daily aminoglycoside therapy: Is it less toxic than multiple daily doses and how should it be monitored? *Clin Pharmacokinet*, *36:*89–98.(PMID: 10092956) (Full Text)

Barnes BJ, Wiederhold NP, Micek ST, *et al*. 2003. *Enterobacter cloacae* ventriculitis successfully treated with cefepime and gentamicin: Case report and review of the literature. *Pharmacotherapy*, *23:*537–542. (PMID: 12680484) (Full Text)

Bartal C, Danon A, Schlaeffer F, *et al*. 2003. Pharmacokinetic dosing of aminoglycosides: A controlled trial. *Am J Med*, *114:* 194–198.(PMID: 12637133) (Full Text)

Bergeron MG, Bastille A, Lessard C, Gagnon PM. 1982. Significance of intrarenal concentrations of gentamicin for the outcome of experimental pyelonephritis in rats. *J Infect Dis*, *146:* 91–96.(PMID: 7045256) (Full Text)

Blair DC, Duggan DO, Schroeder ET. 1982. Inactivation of amikacin and gentamicin by carbenicillin in patients with end-stage renal failure. *Antimicrob Agents Chemother*, *22:*376–379.(PMID: 7137981) (Full Text)

Bliziotis IA, Samonis, G, Vardakas KZ, *et al*. 2005. Effect of aminoglycoside and beta-lactam combination therapy versus beta-lactam monotherapy on the emergence of antimicrobial resistance: a meta-analysis of randomized, controlled trials. *Clin Infect Dis*, *41:*149–158.(PMID: 15983909) (Full Text)

Boulanger LL, Ettestad P, Fogarty JD, *et al*. 2004. Gentamicin and tetracyclines for the treatment of human plague: Review of 75 cases in New Mexico, 1985–1999. *Clin Infect Dis*, *38:* 663–669.(PMID: 14986250) (Full Text)

Brummett RE, Morrison RB. 1990. The incidence of aminoglycoside antibiotic-induced hearing loss. *Arch Otolaryngol Head Neck Surg*, *116:*406–410.(PMID: 2317321) (Full Text)

Bryan LE. 1989. Cytoplasmic membrane transport and antimicrobial resistance. In: *Microbial Resistance to Drugs: Handbook of Experimental Pharmacology*, Vol. 91 (Bryan LE, ed.), Springer-Verlag, Berlin, pp. 35–57.

Buijk SE, Mouton JW, Gyssens IC, *et al*. 2002. Experience with a once-daily dosing program of aminoglycosides in critically ill patients. *Intensive Care Med*, *28:*936–942.(PMID: 12122533) (Full Text)

Busse HJ, Wöstmann C, Bakker EP. 1992. The bactericidal action of streptomycin: Membrane permeabilization caused by the insertion of mistranslated proteins into the cytoplasmic membrane of *Escherichia coli* and subsequent caging of the antibiotic inside the cells due to degradation of these proteins. *J Gen Microbiol*, *138:*551–561.(PMID: 1375623) (Full Text)

Chambers HF, Hadley WK, Jawetz E. 1998. Aminoglycosides and spectinomycin. In: *Basic and Clinical Pharmacology*, 7th ed. (Katzung BG, ed.), Appleton & Lange, Stamford, CT, pp. 752–760.

Chambers HF, Miller RT, Newman MD. 1988. Right-sided *Staphylo coccus aureus* endocarditis in intravenous drug abusers: Two-week combination study. *Ann Intern Med*, *109:*619–624.(PMID: 3421575) (Full Text)

Contopoulos-Ioannidis DG, Giotis ND, Baliatsa DV, *et al*. 2004. Extended-interval aminoglycoside administration for children: A meta-analysis. *Pediatrics*, *114:*e111–118.

Cosgrove SE, Vigliani GA, Fowler VG Jr, *et al*. 2009. Initial low-dose gentamicin for *Staphylococcus aureus* bacteremia and endocarditis is nephrotoxic. *Clin Infect Dis*, *48:*713–721.(PMID: 19207079) (Full Text)

Davies J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science*, *264:*375–382. (PMID: 8153624) (Full Text) de Jager P, van Altena R. Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. *Int J Tuberc Lung Dis*, 2002, *6:*622–627.

Dupont H, Menlec H, Sollet JP, Bleichner G. 2001. Impact of appropriateness of initial antibiotic therapy on the outcome of ventilator-associated pneumonia. *Intensive Care Med*, *27:* 355–362.(PMID: 11396279) (Full Text)

Eliopoulos GM, Farber BF, Murray BE, *et al*. 1984. Ribosomal resistance of clinical enterococcal to streptomycin isolates. *Antimicrob Agents Chemother*, *25:* 398–399.(PMID: 6326668) (Full Text)

Fischel-Ghodsian N. 2005. Genetic factors in aminoglycoside toxicity. *Pharmacogenomics*, *6:*27–36.(PMID: 15723603) (Full Text)

Geller DE, Pitlick WH, Nardella PA, *et al*. 2002. Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest*, *122:*219–226.(PMID: 12114362) (Full Text)

Gosden PE, Bedford KA, Dixon JJ, *et al*. 2001. Pharmacokinetics of once-a-day netilmicin (4.5 mg/kg) in neonates. *J Chemother, 13:*270–276.(PMID: 11450885) (Full Text)

Guthrie OW. 2008. Aminoglycoside-induced ototoxicity. *Toxicology, 249:*91–96.(PMID: 18514377) (Full Text)

Hansen A, Forbes P, Arnold A, O'Rourke E. 2003. Once-daily gentamicin dosing for the preterm and term newborn: Proposal for a simple regimen that achieves target levels. *J Perinatol*, *23:*635–639.(PMID: 14647159) (Full Text)

Humes HD, Sastrasinh M, Weinberg JM. 1984. Calcium is a competitive inhibitor of gentamicin-renal membrane binding interactions, and dietary calcium supplementation protects against gentamicin nephrotoxicity. *J Clin Invest*, *73:* 134–147.(PMID: 6690474) (Full Text)

Hummel H, Böck A. 1989. Ribosomal changes resulting in antimicrobial resistance. In: *Microbial Resistance to Drugs. Handbook of Experimental Pharmacology*. Vol. 91. (Bryan LE, ed.), Springer-Verlag, Berlin, pp. 193–226.

Kearney BP, Aweeka FT. 1999. The penetration of anti-infectives into the central nervous system. *Neurol Clin*, *17:* 883–900.(PMID: 10517933) (Full Text) Le T, Bayer AS. Combination antibiotic therapy for infective endocarditis. *Clin Infect Dis*, 2003, *36:*615–621.(PMID: 12594643) (Full Text)

Knoderer CA, Everett JA, Buss WF. 2003. Clinical issues surrounding once-daily aminoglycoside dosing in children. *Pharmacotherapy*, *23:*44–56.(PMID: 12523459) (Full Text)

Lerner AM, Reyes MP, Cone LA, *et al*. 1983. Randomised, controlled trial of the comparative efficacy, auditory toxicity, and nephrotoxicity of tobramycin and netilmicin. *Lancet*, *1:* 1123–1126.(PMID: 6133153) (Full Text)

Lietman PS, Smith CR. 1983. Aminoglycoside nephrotoxicity in humans. *J Infect Dis*, *5*(suppl. 2):S284–S292.

LoBue PA. 2005. Inhaled tobramycin. *Chest, 127:*1098–1101.(PMID: 15821180) (Full Text)

Luzzatto L, Apirion D, Schlessinger D. 1969. Polyribosome depletion and blockage of the ribosome cycle by streptomycin in *Escherichia coli*. *J Mol Biol*, *42:*315–335.(PMID: 4896026) (Full Text)

Mann HJ, Canafax DM, Cipolle RJ, et al. 1985. Increased dosage requirements of tobramycin and gentamicin for treating *Pseudomonas* pneumonia in patients with cystic fibrosis. *Pediatr Pulmonol*., *1:*238–243.(PMID: 4069813) (Full Text)

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. 1999. Aminogly cosides: Activity and resistance. *Antimicrob Agents Chemother*., *43:*727–737.(PMID: 10103173) (Full Text)

Moore RD, Smith CR, Lietman PS. 1984. Risk factors for the development of auditory toxicity in patients receiving aminoglycosides. *J Infect Dis*, *149:*23–30.(PMID: 6693788) (Full Text)

Nestaas E, Bangstad HJ, Sandvik L, Wathne KO. 2005. Aminoglycoside extended interval dosing in neonates is safe and effective: A meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. *90:*F294–300.

Neu HC, Bendush CL. 1976. Ototoxicity of tobramycin: A clinical overview. *J Infect Dis*., *134:*S206–S218.

Olaison L, Schadewitz K. 2002. Swedish Society of Infectious Diseases Quality Assurance Study Group for Endocarditis. Enterococcal endocarditis in Sweden, 1995–1999: Can shorter therapy with aminoglycosides be used? *Clin Infect Dis*, *34:*159–166.(PMID: 11740702) (Full Text)

Panidis D, Markantonis SL, Boutzouka E, *et al*. 2005. Penetration of gentamicin into the alveolar lining fluid of critically ill patients with ventilator-associated pneumonia. *Chest*, *128:* 545–552.(PMID: 16100136) (Full Text)

Panwalker AP, Malow JB, Zimelis VM, Jackson GG. 1978. Netilmicin: Clinical efficacy, tolerance, and toxicity. *Antimicrob Agents Chemother*, *13:*170–176.(PMID: 348092) (Full Text)

Paul M, Benuri-Silbiger I, Soards-Weiser K, Leibovici L. 2004. Beta lactam monotherapy versus beta lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: Systematic review and meta-analysis of randomised trials. *BMJ*, *328:*668.(PMID: 14996699) (Full Text)

Paul M, Soares-Weiser K, Leibovici L. 2003. Beta lactam monotherapy versus beta lactam-aminoglycoside combination therapy for fever with neutropenia: Systematic review and meta-analysis. *BMJ*, *326:*1111.(PMID: 12763980) (Full Text)

Peloquin CA, Berning SE, Nitta AT, *et al*. 2004. Aminoglycoside toxicity: Daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clin Infect Dis,* 38:1538–1544.(PMID: 15156439) (Full Text)

Philips JB III, Satterwhite C, Dworsky ME, Cassady G. 1982. Recommended amikacin doses in newborns often produce excessive serum levels. *Pediatr Pharmacol (New York)*, *2:*121–125.(PMID: 12760404) (Full Text)

Powell SH, Thompson WL, Luthe MA, *et al*. 1983. Once-daily vs. continuous aminoglycoside dosing: Efficacy and toxicity in animal and clinical studies of gentamicin, netilmicin, and tobramycin. *J Infect Dis*, *147:*918–932.(PMID: 6860416) (Full Text)

Queener SF, Luft FC, Hamel FG. 1983. Effect of gentamicin treatment on adenylate cyclase and $Na^+$, $K^+$-ATPase activities in renal tissues of rats. *Antimicrob Agents Chemother*, *24:*815–818.(PMID: 6318658) (Full Text)

Rastogi A, Agarwal G, Pyati S, Pildes RS. 2002. Comparison of two gentamicin dosing schedules in very low birth weight infants. *Pediatr Infect Dis J*, *21:*234–240.(PMID: 12005088) (Full Text)

Safdar N, Handelsman J, Maki DG. 2004. Does combination antimicrobial therapy reduce mortality in gram-negative bacteraemia? A meta-analysis. *Lancet Infect Dis*, *4:*519–527.(PMID: 15288826) (Full Text)

Sarkar A, Koley BN, Koley J, Sarkar R. 1992. Calcium as a counteractive agent to streptomycin induced respiratory depression: an in vivo electrophysiological observation. *Acta Physiol Hungar*, *79:*305–321.(PMID: 1340087) (Full Text)

Schentag JJ, Gengo FM, Plaut ME, *et al*. 1979. Urinary casts as an indicator of renal tubular damage in patients receiving aminoglycosides. *Antimicrob Agents Chemother*, *16:*468–474.(PMID: 518076) (Full Text)

Smith CR, Lietman PS. 1983. Effect of furosemide on aminoglycoside-induced nephrotoxicity and auditory toxicity in humans. *Antimicrob Agents Chemother*, *23:*133–137.(PMID: 6830203) (Full Text)

Smithivas T, Hyams PJ, Matalon R, *et al*. 1971. The use of gentamicin in peritoneal dialysis: I. Pharmacologic results. *J Infect Dis*, *124*(suppl):77–83.

Spera RV Jr, Farber BF. 1992. Multiply-resistant *Enterococcus faecium:* The nosocomial pathogen of the 1990s. *JAMA*, *268:*2563–2564.(PMID: 1308665) (Full Text)

Tange RA, Dreschler WA, Prins JM, *et al*. 1995. Ototoxicity and nephrotoxicity of gentamicin vs. netilmicin in patients with serious infections: A randomized clinical trial. *Clin Otolaryngol*., *20:*1118–1123.

Toschlog EA, Blount KP, Rotondo MF, *et al*. 2003. Clinical predictors of subtherapeutic aminoglycoside levels in trauma patients undergoing once-daily dosing. *J Trauma*, *55:*255–260; discussion 260–262.(PMID: 12913634) (Full Text)

Vandebona H, Mitchell P, Manwaring N, *et al*. 2009. Prevalence of mitochondrial 1555A->G mutation in adults of European descent. *N Engl J Med*, *360:*642–644.(PMID: 19196685) (Full Text)

Vemuri RK, Zervos MJ. 1993. Enterococcal infections: The increasing threat of nosocomial spread and drug resistance. *Postgrad Med J*, *93:*121–124, 127–128.

Ward K, Theiler RN. 2008. Once-daily dosing of gentamicin in obstetrics and gynecology. *Clin Obstet Gynecol*, *51:*498–506.(PMID: 18677142) (Full Text)

Wilson WR, Wilkowske CJ, Wright AJ, *et al*. 1984.Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. *Ann Intern Med*, *100:*816–823.(PMID: 6426359) (Full Text)

Wood CA, Kohlhepp SJ, Kohnen PW, *et al*. 1986.Vancomycin enhancement of experimental tobramycin nephrotoxicity. *Antimicrob Agents Chemother*, *30:*20–24.(PMID: 3752981) (Full Text)

Yow MD. 1977. An overview of pediatric experience with amikacin. *Am J Med*., *62:*954–958.(PMID: 868913) (Full Text)

*******