Chapter III

Materials and Methods

Materials

1. TDx® Gentamicin , Abbott Laboratories

1.1 Gentamicin Calibrators :

Six vials of accurately measured amounts of gentamicin in human serum at the following concentrations :

Vial	Gentamicin Concentration (mcg/ml)
A	0.0
B	0.5
C	1.5
D	3.0
E	6.0
F	10.0

Preservative : 0.1% Sodium azide

1.2 Gentamicin Controls :

Three vials of gentamicin in human serum should read within the following ranges:

Vial	Gentamicin Concentration (mcg/ml)
L	0.85 - 1.15
M	3.60 - 4.40
H	7.20 - 8.80

Preservative : 0.1% Sodium azide

1.3 Gentamicin Reagent Pack :

The reagent pack contains the following ready to use reagent :

Vial	Components
P	Pretreatment solution
S	< 1% Gentamicin Antiserum (Sheep) in buffer with protein stabilizer
T	< 0.01% Gentamicin Fluorescein Tracer in buffer containing surfactant and protein stabilizer

Preservative : 0.1% Sodium azide

1.4 Dilution Buffer

The dilution buffer contains 0.1M Phosphate buffer and 0.1% Sodium Azide as a preservative.

2. Apparatus

2.1 Automated Fluorescence Polarization Analyzer (Diagnostic Division ,Abbott Laboratories,Inc.,IL , USA)

2.2 Centrifuge

2.3 Freezer

Methods

1. Subjects

Patient Selection : The inclusion criteria were patients who were 18 years or older , were admitted to general wards of medical department or surgical department of Rajavithi Hospital, received at least three consecutive days of once-daily gentamicin dosage regimen for suspected or documented infection, had normal renal function or mild impaired renal function with serum creatinine level of less than 2 mg/dl, and had not received aminoglycosides within the 2 weeks prior to the study.

Sample size : At least 50 patients were treated with once-daily gentamicin either alone or in combination with other antimicrobials for a wide variety of indications.

All of available patients' data related to the study were recorded ; including age , gender , weight , height , medical history , diagnosis , drugs administered , dosage regimens, duration of gentamicin treatment, risk factors of nephrotoxicity , clinical responses to gentamicin treatment and other clinical and laboratory data.

2. Dosage Regimen and Administration

The loading dose was calculated based on the patient's body weight, the appropriate dose was calculated by applying the following nomogram (Gilbert,1995) and given by intravenous infusion for 30 minutes every 24 hours.

Estimated Creatinine Clearance (ml/min)	Dose (mg/kg/day)
100	5
90	5
80	5
70	4
60	4
50	3.5
40	2.5
30	2.5

Equation (Winter , 1994)

1. Ideal body weight (IBW)

for male = 50 + 2.3(height in inches - 60)

for female = 45 + 2.3(height in inches - 60)

2. Patient's dosing weight (DW)

If Total body weight (TBW) < IBW , DW = TBW

If TBW / IBW $\leq$ 1.3 , DW = IBW

If TBW / IBW > 1.3 , DW = IBW + 0.4(TBW - IBW)

3. Estimated Creatinine Clearance

for male $ClCr = \frac{(140-Age)DW}{72\ (SCr)}$

for female $ClCr = \frac{(140-Age)DW}{72\ (SCr)}$

3. Blood Sample Collection

After starting once-daily gentamicin treatment for 3 days (at 3rd dose), 3 ml. of blood samples for the determination of serum gentamicin concentration were drawn sequentially from patients at the following times :

Sample No.1 was obtained at 1 hr. after the beginning of gentamicin iv. infusion.

Sample No.2 was obtained at 2 hr. after the beginning of gentamicin iv. infusion.

Sample No.3 was obtained at 6 hr. after the beginning of gentamicin iv. infusion.

Sample No.4 was obtained at 8 hr. after the beginning of gentamicin iv. infusion.

Sample No.5 was obtained at 24 hr. after the beginning of gentamicin iv. infusion.

Every blood sample was allowed to clot and centrifuged immediately. The serum was separated and frozen until assayed. Usually, serum samples were assayed by fluorescence polarization immunoassay(TDx® Analyzer System) within 24 - 48 hours.

4. Pharmacokinetic analysis

The serum gentamicin concentrations were recorded . Calculated

pharmacokinetic parameters of volume of distribution and elimination hallf-life were calculated using a one-compartment intravenous infusion model(Sawchuk et al., 1977).

5. Evaluation of Efficacy

Patients who continued once-daily gentamicin for three days or more were included in the evaluation of clinical efficacy and bacteriologic efficacy.

The clinical efficacy was defined as favourable if there was clinical improvement with resolution of symptoms of infection , a normal body temparature (37.5°C) for at least 48 hours , and a normal leucocyte count or a 15% or more decreased in leucocyte count. All other outcomes were defined as unfavourable.

The bacteriologic efficacy was determined by follow up cultures after discontinuation of the antibiotic therapy. If the post-therapy culture was negative , or no specimens for follow-up culture were available (ie. no sputum in case of respiratory tract infection ,or no pus in patients with the skin or soft tissue infection) , or a new organism was cultured without clinical signs of infection , the response was defined as favourable. However, if the post-therapy culture was positive for the same organism , or a different organism was cultured with clinical signs of infection, or resistance to gentamicin developed, the response was defined as unfavorable. If no organism was cultured at the start of therapy , bacteriologic efficacy was considered indeterminate.

The efficacy was defined as favourable if there were both favourable clinical and bacteriologic efficacy.

6. Evaluation of Nephrotoxicity

Patients who continued once-daily gentamicin for three days or more were included in the evaluation of nephrotoxicity. The serum creatinine concentration was measured at the start of the therapy and was subsequently measured thrice

weekly (ie. day 0, 3 , 7 , 10 , 14,..) until 1 week after the discontinuation of gentamicin therapy.Nephrotoxicity was defined as a rise in the serum creatinine concentration of 0.5 mg/dl or more during this period.

Data on the following potential risk factors for nephrotoxicity were collected:

- Age
- Gender
- Baseline creatinine clearance
- Initial daily dose
- Duration of treatment
- Hypokalemia defined as the serum potassium level less than 3.5 mEq/L
- Hypomagnesemia defined as the serum magnesium level less than 1.7 mg/dl
- DM
- Liver dysfunction defined as noted by physician or the patient met any three of the following criteria for abnormal liver function
    a. albumin level less than 3 g/dl
    b. total bilirubin more than 2.5 mg/dl
    c. SGOT or AST more than two times the normal level
    d. a history of hepatic insufficiency
    e. the presence of ascites
- Shock defined as systolic blood pressure of less than 80 mmHg with a 24 hour urine output of less than 500ml. or a fall in systolic blood pressure of more than 50 mmHg .
- Volume depletion : fluid loss by history (ie. intake less than output , vomiting , diarrhea , overdiuresis) or the patient met any two of the following criteria for volume depletion.
    a. reduced skin turgor

b. postural hypotension (ie. BPdrop of at least 5-10 mmHg)

c. BUN : Scr ratio more than 20:1

- Concurrent pharmaceutical agents ie. amphotericin B , cisplatin , cyclosporin , furosemide (more than 160 mg/day) , radiocontrast , vancomycin.

7. Data Analysis

7.1 Characteristics data , clinical responses and serum gentamicin levels at different time points were displayed with descriptive statistic data.

7.2 Comparison of the associations between nephrotoxicity and serum gentamicin levels at different time points were performed by unpaired t-test. The relatively reference concentrations and nephrotoxicity were compared by Fisher's exact test. $P < 0.05$ was considered significant.

7.3 Comparison of the associations between clinical efficacy and serum gentamicin levels at different time points were performed by unpaired t-test. The relatively reference concentrations and efficacy were compared by Fisher's exact test. $P < 0.05$ was considered significant.