METHODOLOGY

ETHICAL CONSIDERATIONS

The project proposal was presented to the members of Institutional Review Board (IRB) of JSS College of Pharmacy, Udhasamandalam and the approval for the conduct of the study was obtained. (JSSCP/DPP/IRB/009/2013-14 dated on 05/09/2013, Annexure 1) Followed by the approval from Government District Headquarters Hospital, Udhasamandalam was obtained (Annexure 2). Both the patient and healthy volunteers were recruited into the study after getting the written informed consent form from them (Annexure 3 and 4).

Study Period: 3 years

PROGRAM EVALUATION REVIEW TECHNIQUE CHART (PERT CHART)

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METHODOLOGY

1. A 5 year toxicoepidemiological surveillance of acute poisoning cases in Government District Head Quarters Hospital, Udhasamandalam

(I) Materials

- Patient data collection form (Annexure 5)
- Patient education leaflet on accidental poisoning in English (Annexure 6)
- Source of data: Patient medical records

(II) Method

i. Study design: Retrospective epidemiological surveillance

ii. Study site: Government District Headquarters Hospital, Udhasamandalam

iii. Study duration: 5 years (October 2008 to September 2013)

iv. Subjects: Poisoning patients

v. Sample size (n): 1860

vi. Study criteria:

Inclusion Criteria:

- All poisoning patients admitted in Government District Headquarters Hospital, Udhasamandalam from 2008 to 2013
Exclusion criteria:

- Food poisoning cases
- Snake bite cases

vii. Study procedure:

The demographic parameters of the poisoning patients such as age, gender, marital status, manner of poisoning, religion, time of poisoning and the causative agents for poisoning were gathered from the medical records in a structured data collection form.

viii. Statistical Analysis and interpretation of data:

- The association of descriptive measures on incidence of poisoning and fatality was analyzed by chi square test.
- The association of the confounding variables such as gender, age, manner of poisoning and poisoning agents on treatment cost was determined by chi-square test.
- The incidence rate, mortality rate and case fatality rate were calculated using following formula.
- Incidence rate = (Number of new cases of a disease occurring in the population during a specific period of time ÷ Number of persons who are at risk of developing the disease during that period of time) × 1000.
- Mortality Rate = (Total number of deaths from all causes in 1 year ÷ Number of persons in the population at mid-year) × 1000.
- Case fatality rate (%) = (Number of individuals dying during a specified period of time due to a specific condition ÷ Number of individuals affected with that condition) × 1000.

ix. Statistical Software: GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla, California, USA.

2. Survey of various agrochemicals available in Udhagamandalam region, preparation of booklets and updation of poison management protocol

(I) Materials:

- Data collection form (Annexure 7)

(II) Method:

i. Study design: Structured Interview

ii. Study duration: 6 months (November 2013 to April 2014)

iii. Study criteria:

Inclusion criteria:

- Selected agrochemical selling outlets in Udhagamandalam

Exclusion Criteria:

- The agrochemical vendors unwilling to provide information
iv. Study procedure:

Data collection: The agrochemical outlet centers in Udhagamandalam region were identified to collect the data. The purpose of the study was explained to the vendors and they were requested to give the required information to the researcher. The generic name, mode of purchase, price, chemical classification, physical description of the agrochemicals was collected by oral interview with them in a structured data collection form. Additional data were collected from the package inserts available with products.

Inference: The risk category, signs and symptoms, specific antidote, the treatment for each item were collected from primary, secondary and tertiary sources. An identification aid for the easy identification of agrochemicals was prepared and the poison management protocol was updated based on the collected data.

3. Assessment of healthcare expenditure burden on management of various poisoning cases to the secondary care hospital

(I) Materials
- Patient data collection form (Annexure 8)

(II) Method
i. Study design: A health economic study

ii. Study site: Government District Headquarters hospital, Udhagamandalam

iii. Study duration: Two years (April 2013 to March 2015)

iv. Subjects: All poisoning patients admitted in the hospital during the study period

v. Sample size (n): 994

vi. Study criteria:

Inclusion Criteria:
All poisoning patients admitted in the study site during the study period

Exclusion Criteria:

Pregnant poisoned patients

Poisoning patients with comorbidities like diabetes, hypertension, Myocardial infarction, peptic ulcer and renal failure

vii. Study procedure:

Healthcare expenditure includes direct medical cost, direct nonmedical costs and indirect costs. We considered expenses for drugs, hospital stay, healthcare provider remuneration and laboratory investigation expenses as direct medical cost. The direct nonmedical cost covers the cost of food, transportation and maintenance. Indirect cost includes the loss of wage of patient and patient caretaker. Treatment cost is calculated from direct medical and
direct nonmedical costs. Indirect costs were excluded from the treatment cost as these costs were not influencing the hospital healthcare budget.

The expenses for drugs were calculated based on price list of Tamil Nadu Medical Services Corporation (TNMSC), Govt. of Tamil Nadu. The healthcare provider’s remuneration, laboratory investigation expenses, hospital stay and food costs were calculated according to the norms provided by the hospital. The transportation cost included the expenses incurred for transferring patient from the study site to the higher center. Maintenance costs include expenses for the hospital cleaning, laundry and instrument maintenance.

A bed day is a day during which a person is confined to a bed and in which the patient stays overnight in a hospital. Day cases (patient admitted for a medical procedure/surgery in the morning and released before evening) were excluded\(^{(125)}\). The total number of bed days available in the hospital during the study duration and total number of bed days available in intensive care unit were obtained. Based on these data the total number of bed occupancy (in days) for poisoning cases during the study period was calculated.

vii. Statistical Analysis and interpretation of data:

- The association between the confounding variables such as gender, age, manner of poisoning and poisoning agents on treatment cost was found by chi-square test.
- The factors affecting length of stay in the hospital was determined by Logistic regression analysis.

viii. Statistical softwares Used:

- GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla, California, USA
- IBM SPSS Statistics for Windows, version 21.0, Armonk, NY:IBM Crp

4. Evidence (AchE level) based management of organophosphate poisoning and its economic impact on health care expenditure

(i) Materials

i. Tools used

- Patient data collection form (Annexure 9)
- Patient informed consent form (Annexure 3, 4)
- Translated version of informed consent form in Tamil (Annexure 10)
- Poisoning severity score (PSS) (Annexure 11)
- Peradeniya organophosphate scale (POP) (Annexure 12)

i. Chemicals and reagents

- Acetyl Thio choline iodide (Substrate): 0.156M, Molecular weight: 289.18g, 451 mg dissolved in aqueous buffer.
- 5,5'- dithio- bis – 2- nitro- benzoic acid (DTNB, Ellman’s/Colour reagent): 0.0002M, Molecular Weight: 396.36g, Dissolve 7.9272 mg DTNB dissolved in 100 ml of 0.05M Aqueous buffer.
Aqueous Phosphate Buffer (0.05M, pH7.4): Dissolve 1.369g Potassium di hydrogen phosphate (KH₂PO₄) dissolved in 200ml of distilled water. The pH was adjusted to 7.2 with required quantity of triethylamine.

iii. Instruments used
- Schimadzu UV Spectrophotometer (Model No: UV-1700)

(II) Method

i. Study design: Prospective Interventional study

ii. Study site:
- Government District Headquarters hospital, Udhagamandalam
- Department of Pharmacy Practice, JSS College of Pharmacy, Udhagamandalam

iii. Study duration: One year (April 2014 to March 2015)

iv. Subjects: Organophosphate poisoning patients

v. Sample size: 27

vi. Study criteria:

- **Inclusion Criteria:**
  - Organophosphate poisoning poisoned patients admitted during the study period

- **Exclusion criteria:**
  - Organophosphate poisoning patients with comorbidities such as diabetes, hypertension, myocardial infarction, peptic ulcer and so on
  - Psychological disorders
  - Who were lesser than 18 years old
  - Who got initial supportive care from any other Primary Healthcare Centre
  - Who were transferred to tertiary care hospital as per patient’s request

vii. Study procedure:

1. **Informed Consent Form (ICF)**
   
   The organophosphate poisoning patients admitted to the hospital or patients’ care takers were explained about the study and those who gave written informed consent to participate in the study were recruited into the study. The privacy and confidentiality norms in accordance with Good clinical practices (GCP) were followed.

2. **Blood sample collection**
   
   Immediately after the admission of patients and before initiation of treatment, blood samples were collected in a blood collection tube.

3. **Analytical procedure for estimation of serum Acetyl cholinesterase (AchE)**
   
   The separated serum was used to estimate the AchE level by Boehringer method using UV Spectrophotometer. The unit of enzyme activity was expressed as milli units/ml serum.

Boehringer method

**Principle:** The Boehringer Method developed by Ellman et al was used for the determination of serum AChE activity. This is a colorimetric method utilizing acetyl thio...
choline iodide as substrate. The thiocholine released by the enzyme coupled with 5,5'-dithio- bis – 2- nitro- benzoic acid (DTNB) to form a yellow product with an absorbance maximum at 405 nm.

Procedure: Individual assays were performed at 25º C by pipetting 3ml of 0.0002M DTNB in 0.05M aqueous phosphate buffer (pH 7.1 to 7.8) and 20µl serum sample into the Ria vial. The substrate 0.1ml of 0.056M of acetyl thio choline iodide was added and rapidly mixed in a vortex mixer for 3 minutes. The resultant solution was taken in cuvette and the absorbance was measures at 405nm. The increase in absorbance at 405nm was recorded at every 30 seconds for 3 minutes. If change in absorbance exceeds 0.2 in 30 seconds, the sample was diluted to 1 in 10 with normal saline and the measurements were repeated (The reading were multiplied with dilution factor 10)

Calculation: Enzyme concentration was measured using the following equation.

The concentration of the Acetyl cholinesterase is calculated as Cholinesterase = The mean value of changes in absorbance in 30 seconds every 3 minutes× 23400]

4. Severity grading

Based on the AchE level from the blood sample collected at the time of admission, patients were categorized into any of the 4 groups viz. latent, mild, moderate and severe based on grading methods developed by Namba et al as follows\(^{(39)}\).

Latent:  <10  % inhibition from the normal value
Mild:  ≥10<20 % inhibition from the normal value
Moderate:  ≥20<50 % inhibition from the normal value
Severe:  ≥50<90 % inhibition from the normal value

In order to monitor the prognosis of the treatment, 24h, 48h and 72h samples were also collected.

5. Assessment of normal value of AchE in Udhagamandalam town:

i. Background:

The extent of inhibition can be determined by comparing the AchE level of an organophosphate patient with the normal value of AchE. The AchE levels are highly influenced by race, religion and extraneous health problems. Therefore, it is not possible to compare the acetyl cholinesterase values with normal acetyl cholinesterase values set in another region/population. So, in order to get a mean normal range of acetyl cholinesterase in this particular region, a preliminary study was done.

ii. Sample Size: 12

iii. Study criteria:

Inclusion criteria:

- Healthy individual of either gender
- The subjects not undergone any kind of moderate or severe exercise at least for 24 hours prior to the study. [Because, vigorous muscular exercise even of short duration (4-
5 mts) can cause temporary rise in cholinesterase, which can become normal after rest. This may be because of the shift of cholinesterase from erythrocytes to plasma.

**Exclusion Criteria:**
- Those who had known exposure to organophosphorous compounds
- Presence of signs and symptoms of the OP poisoning
- Pregnancy: During pregnancy enzyme status may vary (decreased level of AchE)
- Alcohol: Serum acetyl cholinesterase activity may vary (increased) by chronic ethanol consumption and increased activity was observed even after 24h of ethanol withdrawal. Cholinesterase activity returned to control levels at 72th h of ethanol withdrawal.
- Malnutrition: Generally decreased serum level activity was observed in malnutrition; which may be due to extreme protein depletion and impairment of hepatocellular function.
- All types of anemia.

**iv. Method:**

The acetyl cholinesterase estimation tests were performed for two times not less than 3 days and not greater than 14 days apart. Averages of the two closest values were considered as the normal value. For those who had more than 15% variation in the above 2 tests, a third test was also be performed within 3 to 14 days.

**6. Empirical treatment being given in GHQH for organophosphate patients:**

The empirical treatment includes atropine and pralidoxime apart from the general supportive measures. Atropine was administered as intravenous infusion or bolus and dose tapering was done based on pupil size and pulse rate. The atropine administered to each patient was in the range of 50 to 100mg. Similarly, pralidoxime was administered as loading dose of 1g to 2g IV bolus followed by 1g to 2g up to 3 days.

**7. Proposed treatment guidelines for OP poisoning:**

Namba et al developed standard treatment guidelines for the treatment of organophosphate poisoning patients and that was based on extent of inhibition of acetyl cholinesterase. The treatment pattern required in each severity category was given as follows.

**Latent poisoning:**

**Cholinesterase level:** In this category nearly 50-90% of the cholinesterase levels were maintained even after the exposure to organophosphate.

**Clinical manifestations:** The usual signs and symptoms of OP poisoning cannot be seen in this grade. The cholinesterase level to this range is the indication to the physicians that the patients can be discharged immediately.

**Treatment:** The patients should be observed for few hours without any treatment.

**Mild poisoning:**

**Cholinesterase level:** In this category nearly 30-50% of the cholinesterase levels were maintained after the exposure to organophosphate.
**Clinical manifestations:** The mid symptoms such as head ache, dizziness, fatigue, and diarrhea can be seen with these patients.

**Treatment:** The treatment includes 1 g of pralidoxime and 1 mg of atropine sulphate.

**Moderate poisoning:**

**Cholinesterase level:** In this category nearly 10-20% of the cholinesterase levels were maintained after the exposure to organophosphate.

**Clinical manifestations:** The muscular fasciculation, miosis, generalized weakness are usually present in this patients.

**Treatment:** The treatment includes 1-2 mg of atropine sulphate (every 20-30 minutes) until the dilation of pupil or decrease in the secretions and 1 g of pralidoxime.

**Severe poisoning:**

**Cholinesterase level:** In this category less than 10% of the cholinesterase levels were only maintained after the exposure to organophosphate.

**Clinical manifestations:** The breathing difficulty, cyanosis, unconsciousness, miosis are usually present in this patients. The absence of treatment in this category surely leads to the death of patient.

**Treatment:** The treatment should start with 2g pralidoxime. An intravenous administration of pralidoxime with the rate of 0.5g/hour can be suggested if no improvement is seen. Similarly, 5mg of atropine sulphate every 20-30 minutes can be given. Both these drugs should continue until the decrease in the symptoms.

8. Pharmacoeconomic Analysis

Alteration in the treatment plan was recommended based on the above guidelines which is based on AchE levels of the patients. The outcomes of empirical treatment and the hypothetical interventional treatment were compared by performing pharmacoeconomic analysis (cost effective analysis).

(1) Cost – Effective Analysis:

The results of CEA were expressed as a ratio, either as an average cost-effectiveness ratio (ACER) or as an incremental cost-effectiveness ratio (ICER). An ACER represents the total cost of a program or treatment alternative divided by its clinical outcome to yield a ratio representing the dollar cost per specific clinical outcome gained independent of comparators.

\[
ACER = \frac{\text{health care costs (})}{\text{clinical outcome (not in $)).}}
\]

Note: This allows the costs and outcomes to be reduced to a single value to allow for comparison. Using this ratio, the clinician would choose the alternative with the least cost per outcome gained. The most cost-effective alternative is not always the least costly alternative for obtaining a specific therapeutic objective. Instead of comparing the ACERs of each treatment alternative, the additional cost that a treatment alternative imposes over another treatment is compared with the additional effect, benefit, or outcome it provides.
(2) Cost-minimization Analysis:

The cost-minimization analysis was done by calculating and comparing the total cost of treatment for both empirical and proposed treatment pattern.

Note: Cost minimization is the most accurate method when comparing cost between two therapeutically equivalent medicines. This is the most used cost evaluation method.