CHAPTER VII

METHODOLOGY

Research Questions

1. Is an increased risk of drug resistance to anti-tuberculosis drugs associated with patient's compliance, concomitant illness(es), history of alcohol intake, previous contact with a known tuberculous patient, previous anti-tuberculosis drug intake and severity of pulmonary tuberculosis?

2. Are the risk factors for drug resistance the same in case of a single, 2-drug or multiple drug resistance?

Objectives

1. ULTIMATE OBJECTIVES:

    1.1. To devise a management scheme for high risk and low risk tuberculous patients.

    1.2. To reduce the morbidity and mortality rates of tuberculosis.

2. IMMEDIATE OBJECTIVES:

    2.1. To identify the risk factors in a tuberculous patient who developes resistance to anti-tuberculosis medicines.

2.2 To determine if the risk factors responsible for drug resistance are the same in a single drug, 2 drug or multiple drugs used in the treatment of pulmonary tuberculosis.

Hypotheses

1. Non-compliance with anti-tuberculosis drug is associated with drug resistance.
2. Concomitant illness(es) is(are) associated with drug resistance.
3. Alcohol intake is associated with drug resistance.
4. Previous exposure to a tuberculous patient is associated with drug resistance.
5. Previous anti-tuberculosis drug intake is associated with drug resistance.
6. Severity of pulmonary tuberculosis is associated with drug resistance.

Assumptions

1. Drug resistance-allowing growth at a prescribed concentration of such drug equal to or greater than a certain population of the control, usually 1%, rendering therapeutic success with it not likely to occur.

BACTEC method:

Resistance is defined based on growth index (GI) readings. The difference in GI values from the previous day is designated as △ GI.

△ GI (control) > △ GI (drug) = "susceptible"

△ GI (control) < △ GI (drug) = "resistant"

△ GI (control) = △ GI (drug) = "borderline"

The drugs and respective concentrations (ug/ml) used were as follows:

Isoniazid	0.2
Streptomycin	6.0
Rifampin	2.0
Ethambutol	7.5

2. Primary drug resistance occurs when tuberculous organisms isolated from a patient are resistant to antituberculosis drug or drugs that the patient has not yet used.

3. Secondary drug resistance means that the organisms isolated are resistant to anti-tuberculosis drugs which the patient has used previously. In the Philippines, this will be difficult to determine since anti-TB drug, specifically INH is incorporated in cough mixtures.

4. Compliance - adequate intake of drugs prescribed at a specific time or taking at least 80% of the total dose for the duration of therapy.

5. Risk factor - an attribute or agent that is suspected to be related to the occurrence of a particular disease.

Research Design

Although an experimental design or a randomized controlled trial is acknowledged to be the strongest study strategy for explaining a cause and effect relationship, this method for this research is definitely not possible for ethical reasons. Assigning patients randomly for exposure to factors under study certainly violates human rights and outrightly inhumane. For this reason, I believe that the end does not justify the means. A randomized controlled trial is in order when therapy and its effect or prophylaxis of a disease are the crux of the matter.

Initially, this research was conceived as a prospective cohort study which is the ideal design. However, given only 12 months to finish the research and at a limited resource, a case-control study is the next choice.

Justifications for Case-Control Design

Feasibility of carrying out the research in a limited time and budget is possible in case-control studies.

Another advantage of the design for this project is the fact that the investigator does not have to continue the use of an agent under investigation thereby subjecting the patients to potential dangers[30] as for example, the

biochemical effects of alcohol in the liver. The "cases" have already acquired drug resistance. Hence, the investigator merely gathers information on previous exposures.

Since the natural history of tuberculosis points to a long latency, a case- control design is more appropriate.

A lesser sample size is needed in a case-control study compared to that of a cohort design. Therefore, there is cost reduction in terms of costs for personnel and data processing.

In summary, the advantages of the design are numerous:

a.) Easier to conduct

b.) Less expensive

c.) Allows study of multiple potential causes of a disease with no risks to subjects included in the study

d.) Well suited to the study of tuberculosis and drug resistance

e.) Existing records of the patients can be used.

Research Methodology

The patients involved in the study were taken from the author's lists of the in and out patients of the Sto. Tomas University Hospital, Private and Clinical Divisions in Manila, Philippines.

A standardized, independent review of records and materials that formed the basis for the original diagnosis was done by an independent reviewer, a pulmonologist. The laboratory result was reviewed by another clinical pathologist. The chest x-rays were reviewed by a radiologist and a chest physician. Interobserver variations were computed using kappa error formula for the respective studies.

The data were abstracted from patient's records by trained personnels who were not aware of the objectives of the study. Information on past exposures reported in a personal interview was personally done by the researcher. The variables included the following :

1. Demographic / personal Characteristics
    a. Age - stratified into seven groups
    b. Sex - male or female
    c. Smoking habits - number of cigarettes smoked per day ; duration of smoking
    d. Drinking habits - number of bottles or glasses of beer or alcohol taken per day and duration of drinking habits - drinker: if he has taken 100 gms. or more of alcohol for 5 years or more
    e. BCG status

2. Family history of PTB - relationship of the contact to the patient

3. History of relapse / retreatment

4. Compliance- adequate intake of drugs prescribed at a specific time or taking at least 80% of the total dose for the duration of therapy.

   - random pill count or random check on the color of the urine to monitor compliance.
   - request for a new prescription when the number of prescribed drugs are consumed. It is dichotomized into:

   a. Good - $\geq$ 80% of the total dose at the prescribed frequency and duration.

   b. Bad - drug intake is <80% of the required total dose for treatment

5. Kinds of anti-TB drugs taken - in patients who are aware of previous therapy; if they can not remember the name(s) of the drugs, he or she is asked to describe the color, size, shape of the pill; the number of times the tablet(s) are taken a day; the number of tablet(s) taken per day and how often it was taken; the price of the drug; time of treatment.

   - Old prescriptions of previous doctors are available at times.

6. Drugs taken / taking other than anti-TB drugs:
   - drug interaction is possible which may increase or decrease the bio-availability of the anti-tuberculosis drug.
7. Presence of Concomitant disease(s):
   - illness or illnesses other than tuberculosis of the lungs
   - for drug resistant patients, if and when there's a strong suspicion for HIV infection, HIV test is requested. This is one limitation because such a procedure is not performed on low risk groups.
   - history of intravenous drug use
   - history of contact with commercial sex workers, man or woman other than legal wife or husband; frequency
   - history of sexually transmitted disease(s)
8. Severity of pulmonary tuberculosis : stratified into:
   a. Minimal
   b. Moderately advanced
   c. Far-advanced

- radiologic classification defined previously is used since the chest x-ray is the tool used to indicate severity

- the chest films are reviewed by one radiologist and one pulmonologist who are both unaware of the objectives of the project. Inter-observer variation is measured using the formula for kappa error determination.

The information on record (Appendix A) was registered on a prepared data sheet (see Appendix B).

Inclusion Criteria

a. Patients diagnosed to have pulmonary tuberculosis by history and chest x-ray and/or (+) AFB smear and culture and sensitivity tests
b. Adult patients, 15-87 years old

Definition of Cases

Cases are patients diagnosed to have pulmonary tuberculosis who developed resistance to (any of the four) drugs (INH, RIH, SM, EM). They may either be primary or secondary drug resistant cases.

Since BACTEC method was used for culture and sensitivity testing, resistance is defined based on growth index (GI) readings. The difference in GI values from the previous day is designated as $\triangle$ GI. When the control vials reach a growth index of 30 or more, the results are interpreted as follows: if the $\triangle$ GI is less in the drug

vial than the control, the population is susceptible; if more, it is resistant. To summarize:

△GI (control) > △ GI (drug) = "susceptible"
△ GI (control) < △ GI (drug) = "resistant"
△ GI (control) = △ GI (drug) = "borderline"

Definition of Control

Control patients are patients who have pulmonary tuberculosis who did not develop resistance to any of the 4 drugs against tuberculosis (INH, RIF, SM, EM). They are patients who got well after completing the prescribed course of treatment.

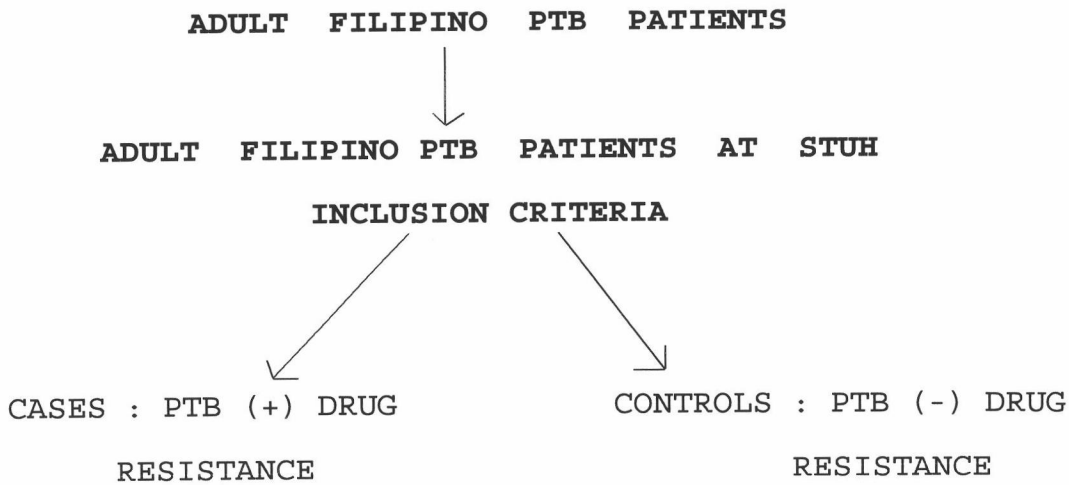
1. Population Sample

A. Target population: Adult Filipino pulmonary tuberculous patients
B. Sample population: Adult PTB patients seen at the Sto. Tomas University Hospital

1.1. Method of selection

Since eligible cases and controls were taken from my patients seen at the Sto. Tomas University Hospital from February, 1990 to May, 1992, hence, the groups in the study were selected from the target population and conclusions were drawn from the results of the study sample.

The patients were stratified according to the severity of pulmonary tuberculosis.



1.2. Possible Source of Bias

Selection bias is the limitation to this design. The problem lies in the selection of a control group.

An independent pulmonologist was employed to review the records and materials that formed the basis for the original diagnosis to minimize misclassification bias.

The persons who got the data from the records were not aware of the objectives of the study.

The data were taken from my own record of patients who consulted my clinic from February ,1990 to May, 1992. Hence, crucial data for the questions to be answered in my research were available on the existing records of our patients and therefore, missing data were avoided.

1.3. Sample Size

The number of subjects included in the investigation of disease-exposure relationship was a fundamental consideration in planning this research. The sample has to be large enough to avoid two sources of error:

(1) claiming that exposure is associated with the disease when in truth it is not (2) claiming that exposure is not associated with the disease when in fact it is.[31] When the frequency of exposure between cases and controls is compared by a statistical test, the probability of making the first error is called the level of significance, denoted by α ; the probability of making the second error is represented by β . The power of the study is 1-β . Assuming that the odds ratio in the target population differs from unity: Ho $\neq$ 1, the power (1-B) is the probability of finding that the sample estimate of relative risk (odds ratio) differs significantly from unity.[31]

Alpha is set at 0.05, two-tailed; beta is 0.20 with power of 80%. The relative frequency of exposure among controls in the target population is Po and a hypothesized relative risk associated with exposure of sufficient biologic or public health importance to warrant its detection is R.[31] Therefore, sample size was obtained by using the formula for case-control unmatched study:

$$n = \frac{2\,\bar{p}\bar{q}\,(Z + Z)^2}{(P_1 - Po)^2}$$ where : $P1 = PoR/[1 + Po\,(R-1)]$

$\bar{P} = {}^1/_2\,(P_1 + P_o)$

$\bar{q} = 1 - \bar{P}$ $q_1 = 1 - P_1$

$q_o = 1 - Po$

At a relative risk of 2 and P_o at 20% (obtained from literature)[26,20], n= 171 per group, using 1:1 case: control ratio. If we increase the number of controls per case, sample size of cases will decrease. Increasing or decreasing the odds ratio will also affect the sample size. These are seen on Fig 2.1 and Fig 2.2 below.

2. Observation and Measurement

Since the research attempts to identify risk factors for drug resistance, the investigator hopes to employ all the observational criteria for causation namely:

10.2.1 Temporal sequence

10.2.2 Consistency

10.2.3 Strength of association

10.2.4 Biological gradient (dose-response relationship)

10.2.5 Specificity of effect

10.2.6 Collateral evidence and biological plausibility

Measurement deals with measures of disease occurrence:

A. Relative risk: It represents how many times more (or less) likely disease occurs in the exposed group as

compared with the unexposed. For R > 1, the study factor is associated with the risk of disease. In a case - control design, this is not directly measured. Instead, odds ratio approximation is used.

B. Odds Ratio: This represents the ratio of the odds of disease in exposed individuals relative to the unexposed. This is particularly important for two reasons:

   a.) For rare diseases, it closely approximates the relative risk

   b.) It can be determined in a case - control study

C. Reliability and Validity

There is no way of assessing the reliability of the data since the information of interest is taken from the records, unless the patient is reinterviewed regarding vital information. Reliability is achieved by using independent observers and then compute for inter-observer variation using Kappa error formula. The validity of some data with hard outcome is better assured.

3. Intervention

Since the protocol aimed to detect risk factors, intervention measures were employed after the factors were identified.

Data Collection

1. Pilot testing

A small scale feasibility study was carried out prior to the actual operation. This allowed for any possible last-minute alteration or revision in the protocol. The problem that came out during the pilot test was lack of control subjects. Some changes were made in the coding sample. Since there were only few samples taken, an actual logistic regression analysis was not done. Pilot-testing was done to rescue an otherwise problematic research effort and in the long run was cost-efficient.[31] Descriptive analysis and a simple 2 x 2 table was instead done.

2. Preparing for Data gathering

The personnel hired to abstract the data was trained in the criteria used for the diagnosis of the disease in question and in the pertinent factors that was taken from the records. They were fully supervised and regularly monitored by the principal investigator.

Computer programming for data editing and statistical analysis had to be prepared early, before the receipt of the data. (See Appendix B.)

Approval from the Medical Director was obtained after the research protocol passed the approval of the Pharmacy, Therapeutics and Research Committee of the Sto. Tomas University Hospital. A pre-formed application for

approval was furnished by the secretary of the committee for the applicant.

3. Preparing for Data Analysis

Weekly, all the data entered in the computer were rechecked for any possible error in transcription.

Dummy tables and skeletons of factors and drug resistance were drawn. These helped the author to specify the important comparisons and categories the analysis required.

Data Analysis

The statistical test used was multiple logistic regression. This was the appropriate test since the research involved exploration of the tuberculous patient and the joint effects of a number of variables.

However, before plunging into the more sophisticated test of logistic regression, simple steps were applied first. A simple descriptive statistical analysis was in order. Then, relationships between variables are explored by means of simple tabulation and measures of association like the crude and adjusted estimates of odds ratio. Gaps, patterns and inconsistencies were checked; when found, they led to additional tabulations.[14] After full exploration using the simpler statistical techniques, multiple logistic regression was now utilized.

These steps were done to clarify the research design, prevent failure to collect crucial information and promote rigorous thinking about the relationships between variables.[14]

The result of logistic regression is on page 68.

Ethical Consideration

The identity of the patients which was kept in confidence and permission to gather data was obtained from the hospital Medical Director. There were no other ethical problems encountered in the conduct of the research.

Limitation and Obstacle

As previously stated, the outstanding limitation to the study was patient selection. This involved the selection of a control group. Attempts to minimize bias were discussed earlier in the topic of "Research Methodology".

Confounders may mask an underlying true association. A confounder satisfies these conditions:

1) it is a risk factor for a study disease
2) it is associated with the study exposure but is not a consequence of exposure.

The problem of confounding variables could be dealt with by stratification technique or adjustment procedures or

multivariate analysis provided one had data on the confounder in question. Hence, in this study design, the author regarded any known risk factor for drug resistance as a potential confounder. Multiple logistic regression assessed or eliminated the effects of such variables.

Since this is a case-control study, the reliability and validity of some information were dependent on patient's ability to remember history on past exposures. The rates of disease in exposed and unexposed individuals could not be determined. A detailed study of mechanism is rarely possible.

Expected Benefit and Application

Merits from identifying the risk factors that predict drug resistance will certainly be numerous. The risk factors serve as guide to the clinician as he or she designs his or her therapeutic scheme when confronted by a tuberculous patient. If resistance has to be overcome in a treatment program that does not call for pre-treatment tests, a treatment standard known to consistently achieve success in its presence should be used.[9]

High risk patients will necessitate successive culture and sensitivity analysis, before and during treatment. Proper drug combination and length of treatment are chosen based on the individual's category as a high-risk or a low-risk case of drug resistant tuberculosis. Compliance monitoring is especially implemented among patients who are at high risk of harboring drug resistant tubercle bacilli.

Hopefully, the problem of treatment failure will be minimized and prevention of spreading TB in the community will be achieved.

Thus, we look forward to seeing a healthier, more efficient, more productive population in the days to come.

Administration and Time Schedule

Training of personnel was done in the first month of the study. Pilot testing and planning which included approval from the hospital committee in charge of research took two months since the committee met only once a month.

Data collection began on the third month and ended on the sixth month, roughly 6 months or 168 working days . The number of days was computed according to the present energy crisis in the Philippines where we have electric power interruption for 2-6 hours a day and the records section of the hospital is closed on Sundays, public and university holidays.