# CHAPTER III

## METHODOLOGY AND PROCEDURE

**Research design :**

This is a descriptive, cross-sectional study. The data collection and measurement were carried out on a single visit of each patient.

**Target population and Study population :** Sexually active childbearing age females(*) in PH community.
(*) The sexually active childbearing age females in this study were the women, aged 15-49 years, have ever had sexual partner(s), (Howard W.Jones III, 1988 ).

**Population to be sampled:** Sexually active childbearing age females in PH community fulfilled for the eligible criteria, under the systematic sampling selection during the study period, from the 21st to 25th of July, 1992.

**Eligibility Criteria:**

1. Inclusion Criteria:

In order to be included, each woman must agree to have:

a). Systematic review.

b). Standard interview on medical background about past illness, current symptoms, and information collected in the study.

c). Pelvic examination.

d). Three endo-cervical swab specimens taken for study.

e). Three vaginal swab specimens taken (from the posterior fornix).

2. Exclusion Criteria:

Women who had taken antibiotics or douched within 48 hours prior to examination.

**Sample size calculation:**

The prevalence of these three common vaginal infections (i.e., bacterial vaginosis, candidiasis, and trichomoniasis) is estimated about 32% (Sobel J.D., 1990). With the alpha-error and clinical acceptable-error of 0.05 and 0.06, respectively, the smallest sample size can be expressed by the following formula:

$$n = (Z\alpha)^2 . P . Q \; / \; (\delta)^2 .$$

Where $Z\alpha = 1.96$ (for 95% confidence level)

$P = 0.32$ (prevalence estimated of vaginal infections)

$Q = 1 - P = 0.68$

$\delta = 0.06$ (clinical acceptable-error)

Therefore, the calculated sample size was 232 subjects.

It was estimated about 70-75% of the community inhabitants were willing to participate the study, so the number of people were needed to be approached were about 310-330 subjects.

**Sampling procedure:**

By using the census profile, the total women qualified for the eligible criteria were stratified into three age groups: under 20 years old, from 20 to 40 years old, and over 40 years old. The sampling procedure was carried out as follow:

| Age groups | Population | Sample |
|---|---|---|
| (1) Under 20 years old | N1 = 24 | n1 = 8 |
| (2) From 20-40 years old | N2 = 785 | n2 = 255 |
| (3) Over 40 years old | N3 = 207 | n3 = 67 |
| Total | N = N1 + N2 + N3 = 1016 | n = n1 + n2 + n3 = 330 |

Thus, in this study, the subjects were selected systematically in each age group at every 3 people.

**Observation and Outcome measures:**

All women in the study were underwent pelvic examination by using bimanual palpation and internally examined by speculum.

The questionnaires were constructed to collect demographic variables, socioeconomic and sexual history, contraception and details of menstrual cycle, lifestyle, health status data, and all hygienics materials that have come in contact with the perineum of women. The questionnaires also covered attributes, attitudes toward vaginitis prevention, daily behaviors in their work as well as hygienics behaviors, and beliefs in preventing vaginitis. The questionnaire administration was organized by following the standardized procedures (Ian McDowell, Claire Newell, 1987). Enquiries were made concerning symptoms and signs of genital infection including discharge (information will be recorded on the amount of discharge if any, its smell, colour and consistency), vaginal irritation, soreness or smell, the presence or absence of vaginal inflammation and the presence of any cervical abnormalities, pelvic, abdominal or back pain and dyspareunia. In addition to those mentioned data, the questionnaires were also recorded physical findings of vaginitis, and collected the laboratory data if any, detected microorganisms.

Because of the reasons mentioned previously in the

chapter II, the laboratory methods being used in this study were wet smear and Gram's stain.

Three slides were prepared for microscopic examination from the swabs, if the secretions were scant. In case of secretions being moderate or profuse, a drop of secretions was collected from the examination glove. The first slide was examined with normal saline, the second slide with 10% potassium hydroxide, the third slide was reserved for Gram's stain examination.

The two direct wet smears were examined by using the saline microscopic examination with a x20 objective for pus cells, clue cells, Trichomonas vaginalis and Candida albicans. Pus cells were recorded as present if there were > 5/field. Given the low sensitivity of the saline examination in detecting Candida species, a 10% KOH microscopic examination were always performed, even if the saline wet mount identified other causes of vaginitis because mixed infections are common.

After microscopic examination, a gram's stain of secretions was prepared for permanent record, with the standardized Gram's stain procedure.

The diagnosis of bacterial vaginosis was based on the predominant presence of bacteria (cocci or bacilli or a mixed bacterial population) and the absence of Lactobacilli (Doderlein bacilli) in the background of Gram-stained vaginal

smears (Blackwell A, Barlow D, 1984).

The diagnosis of candidiasis and trichomoniasis were based on the presence of Candida albicans, such as the demonstration of filaments (pseudo-hyphae) and spores , and the presence of Trichomonas vaginalis on microscopic examination with or without the signs of abnormal discharge.

The diagnosis of the vaginal infection caused by the three common infective agents was based on the appearance of either bacterial vaginosis, or candidiasis, or trichomoniasis.

**Validity and Reliability:**

Validity and reliability of measurement are of interest and importance in clinical and epidemiological studies. However, there is sometimes still confusion about these terms.

Validity, accuracy, conformity or lack of bias is a constant property of measurement. It can be determined only by comparison with " truth " or an accepted standard known as the " gold standard " , in this case it is called criterion validity. In many circumstances such a gold standard does not exit or may be too expensive, risky or impossible to use. Thus, validity may be difficult to assess, one has to use the other measures for assessing the measurement validity, such as one of the following types: face validity , content validity, consensual validity, predictive validity, construct validity.

The content validity (by reviewing literature), consensual validity (by consulting four senior gynaecologists), and face validity (by examining and interviewing directly subjects), were used in the study.

Reliability, precision, is the capacity of a measuring procedure or observer to produce closely similar results in a series of observations conducted under identical conditions. Kappa is a statistical index for concordance with ordinal data. In a 2x2 table for Kappa statistic, the formula of Kappa is as follows:

| | | Observer 1 + | Observer 1 - | Total |
|---|---|---|---|---|
| Observer 2 | + | a | b | R1 |
| | - | c | d | R2 |
| Total: | | C1 | C2 | N |

Kappa = (Po - Pc) / (1 - Pc)

Where: Po = Proportion of observed agreement

= (a + d) / N

Pc = Proportion of chance-expected agreement

= (E(a) + E(d)) / N = (R1.C1/N + R2.C2/N) / N

Po - Pc= Observed agreement beyond chance

1 - Pc = Maximal possible agreement beyond chance

The interpretation of the values of kappa is somewhat arbitrary. Landis and Koch 1977 interpreted the Kappa level as follows:

| Kappa level | Interpretation |
|---|---|
| Less than 0 | Poor |
| 0.00 to 0.20 | Slight |
| 0.21 to 0.40 | Fair |
| 0.41 to 0.60 | Moderate |
| 0.61 to 0.80 | Substantial |
| 0.81 to 1.00 | Almost perfect to perfect |

Interpretation of Kappa level. Source: Landis R.J., Koch G.G. The measurement of observer agreement for categorical data. Biometrics 1977; 33:159-174.

The inter-rater agreement (inter-observer agreement) was assessed in the study, by using the Kappa level, with the interpretation of Landis R.J. and Koch G.G., 1977. Two observers (two laboratory technicians) were blinded, and they applied the same measurements, i.e., wet smear and Gram's stain, to the same subjects. The calculated Kappa level were 0.84, 0.76, 0.62, and 0.55 for diagnosis Trichomonas vaginalis, Candida albican, Cocci, and Bacilli infection, respectively. The data for calculating Kappa level is shown

in the following table:

| | | Observer 1 + | Observer 1 - | |
|---|---|---|---|---|
| Observer 2 | + | 3 | 1 | Kappa= 0.84, for diagnosis Trichomonas vaginalis infection |
| | - | 0 | 31 | |
| Observer 2 | + | 7 | 2 | Kappa= 0.76, for diagnosis Candida albican infection |
| | - | 1 | 24 | |
| Observer 2 | + | 3 | 1 | Kappa= 0.62, for diagnosis Cocci infection |
| | - | 2 | 29 | |
| Observer 2 | + | 6 | 3 | Kappa= 0.55, for diagnosis Bacilli infection |
| | - | 3 | 22 | |

**Data analysis and Statistical methods :**

The collected data was summarized according to the proportion of these three common types of vaginitis. This sample proportion ($\hat{p}$) was used as the point estimator of the population proportion p (the prevalence of these three common vaginitis), then a confidence interval was obtained by the general formula:

estimator ± (reliability coefficient) x (standard error)

In this study, both np and n (1 - p) were greater than 5, we considered the sampling distribution of $\hat{p}$ to be close to the normal distribution. Therefore, our reliability coefficient was some value of Z from the standard normal distribution, the standard error was estimated by $\sqrt{[\ \hat{p}\ (1 - \hat{p})/n\ ]}$ and the 100 $(1 - \alpha)$ percent confidence interval for p was given by

$$\hat{p} \pm Z_{(1-\alpha/2)} \sqrt{[\ \hat{p}\ (1 - \hat{p})/n\ ]}$$

Hence, the 95% confidence interval for p was

$$\hat{p} \pm 1.96 \sqrt{[\ \hat{p}\ (1 - \hat{p})/n\ ]}$$

By using the formula mentioned above we obtained the 95% confidence interval for the prevalence of bacterial vaginosis, candidiasis, and trichomoniasis.

The participants were stratified into age groups to control confounders.

The examination of associated factors with the three common infective agents was analyzed statistically using a 2x2 contingency table analysis with calculation of the Chi-square statistic, using Yate's correction.

Apart from the stratification to control for confounding effects and Chi-square tests for analyzing vaginitis and its associations, stepwise multiple logistic regression was used, this method can simultaneously control for the potential confounding effects, to determine the

relative contributions of variables associated with infection.

In all statistical analyses, differences were considered significant when p-value < 0.05.

The Statistical Package for the Social Sciences version 4.0 (SPSS version 4.0; McGraw-Hill Book Co., Chicago, Illinois) was used to carry out those statistical tests mentioned above.

## ETHICAL CONSIDERATIONS :

Before subjects were recruited, the aims of the study were explained and verbal consent was obtained.

Pap's smears were done for all the participants, focusing on those who are 35 years old or over, because it is the high risk group for cervical cancer (K.R.Peel, 1986).

ADMINISTRATION AND TIME SCHEDULE:

May, 1992: Looking for Collaboration.
Recruiting research associates.
Community leaders contacted.
Production of Posters, Pamphlets, and contruction of Questionnaires.

June, 1992: Trainning research associates.
Pretesting of questionnaires.
Organizing the health examination team.
Preparing chemical substances for laboratory methods.
Completing operations manual.

July, 1992: Carrying out sampling procedure.
Doctors meetings, health examination team meetings before the study.
Informating community of the programme.
Data collection: Recruiting, examining, and interviewing subjects.
Doctors meetings, health examination team meetings during the study.

August, 1992: Data entry .

September, October, 1992: Analyzing data.

November, December, 1992: Preparing pertinent in thesis.

January, February, March, 1993: Writing thesis.