CHAPTER 3
METHODOLOGY

3.1 STUDY DESIGN

A descriptive, clinical based study and cross-sectional design has been used to investigate the prevalence, common symptoms and risk factors of reproductive tract infections among women in reproductive ages suffering from rheumatoid arthritis who referred to Center for Rheumatic Disease (CRD) in Pune, India. The specific objectives of this study were:

- To determine prevalence of RTIs, among RA women in reproductive ages who referred to Center for Rheumatic Disease (CRD) in Pune, India.
- To identity common symptoms of RTIs among them.
- To determine the common cause of RTIs dependent on laboratory tests among sample population in this research. (focus of this study: Candida albicans, bacterial vaginosis, trichomoniasis and syphilis)
- To assess risk factors of RTIs, among selected population of this research.

This study has been conducted from June 2007 till May 2008. Quantitative data were collected with the use of questionnaire, clinical examination and laboratory tests to clinically and microbiologically identify RTIs / STIs. The sampling method used was systematic sampling method and the sampling frame for this study has been chosen depending on referred RA women to cover the sample size. The researcher prepared a list of married women referred to CRD who were 15-49 years of age and had rheumatoid arthritis disease for at least three months. Starting with the first patient randomly every 10th eligible women in the list was selected and this process was continued until the end of the list of patients. Women included in the study were given a multilingual flier outlining the study (Annexure No. I) and containing a series of questions to assess eligibility.
3.2 SAMPLE SIZE

Sample size was calculated based on the expected prevalence of RTIs as found in previous surveys. According to the survey of Indian government National Family Health Survey II, 1998-99 (NFHS- II) the prevalence of RTIs among Indian married women is 30%-40%. However there is no data or any study about RTI among RA women. For affirmation the researcher has estimated 50% proportion for calculating appropriate sample size. Because this study is a descriptive study the following formula has been used for calculation of sample size.

\[ N = \frac{p \cdot q \cdot z^2}{d^2} \]

i.e. if \( p = 0.50, q = 1-p, Z_{95\%} = 1.96, d = 0.05 \) error/distance value

\[ \therefore n = \frac{(0.50)^2 \times 1.96^2}{0.05^2} = \frac{0.9604}{.0025} = 384.16 \]

For reaffirmation 400 married women with RA in 15-49 years were selected randomly as sample size in this study.

3.3 STUDY SETTING

The study was carried out in the Center for Rheumatic Disease (CRD) in Pune, India. CRD is a research institute recognized by the Pune University and is a referral center for World Health Organization- International League of Associations for Rheumatology community oriented program for control of Rheumatic diseases (WHO - ILAR COPCORD), It is also the office for the Bone and Joint Decade - India: National Action Network (BJD-India: NAN) 2001-2010 (a program launched worldwide under support from WHO and UN, and endorsed by government of India). CRD is also the office for Mission Arthritis India (MAI), a patient support group launched in Pune.

3.4 STUDY PARAMETERS

RTI can be considered both as an independent and dependent variable. As an independent variable, RTI is recognized as the cause of adverse pregnancy outcomes such as abortion, premature labor, neonatal morbidity and mortality, ectopic pregnancy, and also infertility. As a dependent variable, RTI was seen as caused by
factors such as socio-demographic socio cultural, hygiene practices; history of disease and medicines. In this study, RTI was treated as a dependent variable.

3.5 PATIENT SELECTION
3.5.1 Inclusion criteria
The inclusion criteria of participants were:
1- Married women
2- Age: 15-49 years
3- Confirmed diagnosis of RA classified as per ACR 1987 criteria with minimum 3 months diseases duration.
4- Consenting to undergo a pelvic examination and further evaluation.
5- Consenting to give blood for laboratory testing

3.5.2 Exclusion criteria
The exclusion criteria of participants were:
1- Pregnant women
2- Reported missed periods
3- History of delivery in the previous 6 weeks
4- Women who had vaginal bleeding
5- Women treated with antibiotics or antifungal within one week
6- Women who had vaginal douché within 24 hours
7- Women who had intercourse within 24 hours.

Women were excluded if they were pregnant, reported missed periods or had given birth in the previous 6 weeks, because of greater susceptibility to vaginal infection at these times. Those who had vaginal bleeding or were treated with antibiotics or antifungal within one week or women who had vaginal douché or intercourse within 24 hours was also excluded. Subjects who were not agreeable to undergo a pelvic examination were also excluded. For cultural and ethical reasons it was not felt appropriate to include unmarried women or girls in this study.
3.6 DATA COLLECTION

The prevalence of RTI can be assessed by self-reported symptoms and/or clinical examination and/or laboratory tests. Studies based on only self-reported symptoms did not have true prevalence estimation. Asymptomatic women were excluded and also culture of silence-especially among women in the developing countries shrouded RTI symptoms. Clinically diagnosed RTI prevalence differed depending on different diagnostic criteria and definitions employed by physicians in different settings. Laboratory assessments of RTI prevalence were conducted with different laboratory procedures and criteria for diagnosis. For a holistic approach to the problem under study three basic tools were used for the collection of data.

The methods for collecting data for this study were as follows:

1- Questionnaire (Annexure I) included socio economic characteristics, obstetric, gynecological and contraceptive history; hygiene practices, medical and surgical history, experiences regarding RTIs related to spouse extramarital relationships, health seeking behavior in case of experiencing RTI associated symptoms, and duration of RA, therapy taken complications, risk factors and Quality of life instruments such as CRD- Pune version of modified Stanford HAQ suitable and validated for Indian use (Annexure II) and Short form of health survey with 36 questions (SF – 36) (Annexure III).

2- Clinical examination was based on the Syndromic approach. Syndromic approach is based on identification of syndromes, which are a combination of symptoms, report by the client, and signs, observed during clinical diagnosis, following the algorithms by the WHO. Syndromic management refers to the approach of treating RTI symptoms and signs based on the organisms most commonly responsible for each syndrome. A more definite or etiological diagnosis may be possible in some settings with sophisticated laboratory facilities, but this is often problematic. Laboratory tests require resources, add to the cost of treatment, may require clients to make extra visits to the clinic and almost always result in delays in treatment. For these reasons, Syndromic management guidelines are widely used even in developed countries with advanced laboratory facilities. In Syndromic management the most up-to-date drugs are recommended and dosages
explained. Generally the treatment is provided during the patients' first visit. Thus; it was decided to use Syndromic management for decreasing chances of mistakes in diagnosing RTI cases and preventing of repetitive visit of RA women for treating their RTI especially for those women who were not referred for laboratory tests. This method is useful to avoid high costs of visiting and treating of participants even in developed countries with advanced laboratory facilities.

3- Laboratory diagnosis to ascertain the presence of *Candida albicans*, bacterial vaginosis, trichomoniasis and which are common causes of reproductive tract infections worldwide, have been selected in this study.

### 3.6.1 Pre testing

A very important part of the questionnaire construction process is pretesting. This involves testing research instrument in conditions as similar as possible to the research, but not in order to report results but rather to check for glitches in wording of questions, lack of clarity of instructions etc. - in fact, anything that could impede the instrument's ability to collect data in an economical and systematic fashion. Pretest were conducted systematically, with potential respondents and using the same method of administration.

A prior to the main study pilot study was conducted to obtain approximate prevalence of RTI examine the feasibility and accuracy of methodology and validate the questionnaire. In this pilot study 150 married healthy women (age ranging from 15-49 years) who did not suffer from any chronic diseases were included. The questionnaire in local language Marathi and in English was used to collect information about the participants. On pelvic examination a vaginal examination was done and a wet mount was prepared. The prevalence of RTI among this cohort of 150 non-RA women was 20%. Prevalence of candidiasis was highest (11.3%) among all infections diagnosed. It was followed by bacterial vaginosis (8.7%); None of the subjects were positive for trichomoniasis and syphilis.
3.6.2 Interview

Eligible women who expressed interest in participation were explained the study in greater detail, answered questions and obtained informed consent (Annexure IV). Questionnaire in Marathi and English, the languages widely spoken locally, were used to collect information about the participants. Consenting women were interviewed in a private room- both auditory and visual privacy was ensured—to obtain information. The interviews with Marathi speaking women were conducted with the help of two trained female interpreters who worked for CRD, Pune. Women who knew English language was interviewed by researcher herself.

3.6.3 Clinical examination

After history taking, a speculum and vaginal examination was done by researcher under supervision of a gynaecologist. During every speculum examinations, specimens were taken from the posterior vaginal fornix and cervical canal and wet mount were prepared by mixing 1 drop of vaginal discharge with 1 drop of normal saline for diagnosing motile trichomonas and bacterial vaginosis and for *Candida albicans* 1 drop of vaginal discharge mixed with 1 drop of 10% KOH. Also the presence of vaginal discharge and the appearance of the cervix were noted. After removing of the speculum bimanual examination was carried out to determine the dimensions of the internal genital organs and the presence of adnexal tenderness. A venous blood sample was collected from all study participants for syphilis serology. All tests in this study were free of charge. All speculum and vaginal examinations were done by researcher under supervision of a gynaecologist.

3.6.4 Laboratory methods

The specimens were immediately transported to the laboratory of CRD for etiological diagnosis. *Candidia* was diagnosed by the visualization of budding yeasts of pseudohyphae on microscopy of vaginal wet mount, *Trichomonas* was determined by visualization of motile *Trichomonas* and bacterial vaginosis was determined by presence of clue cells - epithelial cells coated with bacteria - in microscopic wet mount. Syphilis was screened by rapid plasma regain (RPR) test in the blood draw from 400 RA women. Rapid plasma reagin is a screening test for syphilis to detect
antibodies against *Treponema pallidum*. The presence of flocculation indicates a reactive (positive) test result for syphilis. If the screening test was reactive, then a Treponemal test different from that used in screening should be performed as confirmation. The Fluorescent Treponema Antibody (FTA-ABS) test is currently the standard confirmatory test. So the next step after a reactive test would be to confirm the diagnosis with a more specific test for syphilis, such as FTA-ABS\textsuperscript{110}. But a Treponemal test was not required in this research because none of the subjects tested reactive in the RPR test. To avoid testing bias, a qualified microbiologist and pathologist performed the laboratory tests in CRD: Research and Diagnostic Laboratory. Training on the laboratory testing was also given to the researcher.

**3.6.5 Management of RTIs / STIs among target population**

Treatment for RTIs/ STIs were prescribed based on the initial treatment on guidelines on Syndromic RTI/ STI case management free of charge.- under supervision of gynecologist - A follow up appointment was recommended to the patients for any further treatment of RTIs/ STIs subsequently detected by laboratory tests. All treatment was given together with the 4 Cs: Counseling / education, Correct condom use, Contact treatment and Compliance with treatment.

**3.7 DATA ANALYSIS**

Data were analyzed using Statistical Program for the Social Science (SPSS 13.0) for windows. Data were coded and entered into personal computer for analysis. Descriptive statistics were used for data checking and correction. Frequency distribution and measures of central tendency and variances were assessed for the relevant continuous variables. Demographic variables were examined for relationship with between independent variable (age group) and dependent variable (RTI). Pearson’s chi-square, parametric and non-parametric analysis were used in this study. Alpha was set at p ≤ 0.05 for all data analyses. The odds ratio (OR) and logistic regression analysis was performed to obtain the independent determinates of RTI.
3.8 ETHICAL CONSIDERATIONS

The research project was approved by the ethical committee of CRD, Pune. Before starting the interview, each participant woman was given a fair idea of the study and informed consent was obtained from all subjects (400 RA women) emphasizing the fact that they could refuse participation or leave the study at any, and thus would not adversely affect treatment or care provided by CRD. Participation was voluntary. At the time of interview and clinical examination, privacy was maintained and collected data were kept confidential for ethical issues.