Methodology

III METHODOLOGY

The methodology adopted for the present study entitled **"Neural Tube Defects (NTD) and the Effect of Counseling and Folic Acid Supplementation"** consists of the following phases:

Phase 1 Assessment of the prevalence of NTDs in Erode town

Phase 2 Survey on the possible influence of maternal factors on NTDs and the problems faced by the pregnant mothers of NTD cases

Phase 3 Counseling women aspiring for pregnancy to prevent occurrence of NTDs and

Phase 4 Evaluation of the efficacy of periconceptional supplementation of folate alone as well as in combination with multi vitamin or iron in preventing the recurrence of NTDs

PHASE 1 ASSESSMENT OF THE PREVALENCE OF NTDs IN ERODE TOWN

A. Selection of Area and Hospitals

Erode town of Tamil Nadu state has been selected to study the prevalence of NTDs because information regarding the prevalence of NTDs in Erode town is not available and hence the investigator felt the need for this study. Moreover Erode town is the native of the investigator where she can carry out the study in an efficient manner. About 60 registered hospitals and one Government head quarters hospital in Erode town were included for the survey (Appendix I).

The details regarding the prevalence of NTDs was collected from the master birth files maintained by various hospitals in Erode town for a period of five years (2000 - 2004) since the hospitals maintain their records only for five years for administrative purposes. Secondary data refers to the data, which are not originally collected as primary data but rather obtained from published or unpublished sources (Gupta, 2004). The investigator used secondary data to assess the prevalence of NTDs in Erode town.

B. Collection of data

Necessary permission was obtained from higher authorities of the hospitals to collect data from the back volumes of hospital records. The available information collected from hospital records included age, literacy rate and weeks of gestation of mothers, parity, gender and birth weights of infants.

1) Age

Age of mothers have long been recognised as important determinants of infant morbidity and mortality (Institute of Medicine, 1990). Hence, details regarding the age of the mothers at the time of delivery were collected.

2) Literacy rate

Farley *et al* (2002) reported that low maternal education is an important predictor in having a child with NTDs. Hence, the literacy rate of the NTD affected subjects was included from the hospital records individually for each subject under prevalence survey.

3) Parity

Feldman *et al* (1982) reported significant association between the prevalence of NTDs and parity, gravidity and economic status. Hence the incidence of NTD was studied in relation to parity.

4) Weeks of gestation

Low folate status also increases the risk of preterm delivery, low birth weight and foetal growth retardation (Scholl and William, 2000). Hence weeks of gestation which was assessed by LMP of the mothers maintained in hospital records were included in the present study.

5) Birth weight

Birth weight of the newborns was collected since both neonatal and post neonatal mortality increase exponentially with decreasing birth weight and birth weight is taken as the single most important predictor of infant survival (Rabkins *et al* 1990).

In addition to the above, details regarding the period at which NTD was identified, type of complication or defect, type of delivery and registration of pregnancy were also collected.

C) Calculation and interpretation of rates and Confidence Intervals

The number of live births was obtained from the master birth files provided by various hospitals in Erode town and from the municipal birth register. The annual incidence rates (per 10,000 live births) for NTDs identified during 2000-2004 associated with foetal death were calculated based on the formula suggested by Fornoff *et al* (2004) as follows:

$$\frac{\text{Number of infants with selected congenital anomaly}}{\text{Number of live births}} \times 10{,}000$$

Occurrence of specific birth defects is assumed to be a rare event and hence exact confidence intervals were calculated for each rate (Armitage and Berry, 1987). Where there are a large number of cases of birth defects, the

confidence interval is narrow, indicating that the rate is stable. Where there are few birth defect cases, the confidence interval becomes very wide, indicating that the rate is not very stable and a small change in the number of infants born with the specific birth defect could result in a large change in the rate.

To compare two rates, it is important to look not just at their values, but also their confidence intervals. As a conservative approximation, if two confidence intervals overlap, then there is no evidence that the two rates are really different. If two confidence intervals do not overlap, then the rates are said to be statistically different. In this report, 95 per cent confidence intervals are used, where the confidence intervals do not overlap, the rates are statistically different at five per cent level ($p<0.05$).

PHASE 2 SURVEY ON THE POSSIBLE INFLUENCE OF MATERNAL FACTORS ON NTDs AND THE PROBLEMS FACED BY THE PREGNANT MOTHERS OF NTD CASES

A) Selection of area

This phase was also conducted in Erode town in the state of Tamil Nadu based on convenience sampling method. Convenience sampling is a method of selecting a fraction of the population neither by probability nor by judgement but by convenience (Gupta, 1995).

B) Selection of subjects

For this study, readily available 126 cases registered with NTDs in various hospitals during 2003 and 2004 were considered. The subjects were selected and information was obtained only from 116 based on the willingness and co-operation extended by the subjects with the help of physicians and with constant access with the hospitals during the study period by purposive sampling method.

C) Collection of data

According to Tripathi (1987) in the interview cum schedule method of data collection, the respondent is totally free to express the information needed in an unbiased manner. Cypel and Calkins (1990) have also stated that interview makes a process of inter stimulation and helps to secure data not obtainable by methods that do not involve any interpersonal relationship. Hence an interview schedule was designed by the investigator and pretested and used to collect relevant data from the selected samples(Appendix II).

Socio-economic, maternal factors and obstetric history of the expectant women

Details on the background information like age of the mother, age at marriage, type of family, family size, monthly family income and educational status were collected using an interview schedule. Details regarding consanguinity, parity, birth spacing , time of identification of complication, physiological and psychological problems faced, medical supplements taken, mode of delivery, weeks of gestation and cost of expenditure on previous pregnancy, survival of NTD affected child were also collected by enquiry from the selected subjects.

PHASE 3 COUNSELING WOMEN ASPIRING FOR PREGNANCY TO PREVENT THE OCCURRENCE OF NTDs

A) Selection of area and subjects

The study was conducted in Erode town since the investigator belonged to that area and is very familiar with the location of the hospitals. In addition, co-operation rendered by the subjects also formed the basis for the selection of the area. Fifty married women residents of Erode town in the age group of 20-30 years, aspiring to have the first child without an incidence of NTD

affected child and willing to co-operate were selected using convenience sampling for counseling. Gupta (1999) defined convenience sampling as the selection of population unit as per the convenience of the investigator.

B) Formulation of interview schedule

An interview schedule (Appendix III) was developed by the investigator to elicit information on general and socio economic status of the selected women.

C) Collection of socioeconomic data

The information regarding socio-economic data collected included details about present age, age at marriage, family type, total monthly income, educational status and type of activity using the developed schedule by interview method because this method makes possible a face to face interaction with respondents and there is greater chance for collecting reliable information (Saravanavel, 1998). The purpose of the study was explained to the subjects before collecting the needed information.

D) Assessment of nutritional status

1) Anthropometric measurements

Nutritional anthropometry deals with the measurement of the body at various ages and levels of nutritional status. It helps even in the assessment of sub-clinical stages of malnutrition. Piper *et al* (1996) reported that anthropometric measurements are the most widely used methods for groups to assess the nutritional status and are quick and easy to carry out.

a) Height

The height of an individual is influenced by genetic as well as environmental factors (Green and Hary, 1987). A non-flexible tape was fixed

to the wall. The subjects were made to stand on a flat floor adjacent to the wall with feet parallel and back of the head touching the wall. The head was held comfortably erect with arms hanging at the sides in a natural manner. A wooden scale was gently lowered crushing the hair and making contact with the top of the head and height was measured from the tape to 0.1 cm accuracy (Park, 2002) for all the subjects during the first visit of counseling in the premises of Aswin hospital, Erode.

b) Weight

The weight of a person is the most commonly used indicator of the body size which reflects adequate nutrition (Watson, 1994). The weight of the subjects was recorded with the help of a lever type of weighing balance to an accuracy of 0.1kg as recommended by Gordon *et al* (1988). Weight was taken with minimal clothing and without footwear. The weights of the subjects were recorded at Aswin hospital during the first visit and after three months of counseling.

The recorded heights and weights were used for calculating the Body Mass Index (BMI) using the formula

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height (m}^2\text{)}}$$

Subjects were categorized based on the BMI values as suggested by NNMB (2002).

2) Dietary pattern and habits

Diet surveys constitute an essential part of the complete study of nutritional status of individuals providing essential information on nutrient intake levels and food habits. Recall method has a major advantage of giving a

qualitative evaluation of diet in a short time and it is the most commonly used technique for dietary assessment (Mathan and Rees, 1994). Hence, a three day recall of food intake was done to find out the average of the daily intake by the selected sub sample (10 subjects) before counseling to assess the current food and nutrient intake pattern. The recall schedule also had questions related to food habits, frequency of consumption of folic acid rich foods with special reference to whole grain cereals, fruits, vegetables, green leafy vegetables and fleshy foods.

3) Biochemical assessment

Biochemical tests form an integral part of modern medical diagnosis (Truswell, 1998). They are used to find out the levels of nutrients and other components in blood. They yield reliable data regarding the nutritional status of individuals with respect to the nutrients estimated. For a sub sample of ten subjects, blood haemoglobin by Cyanmethaemoglobin method (NIN, 2003) and serum folate by competitive immunoassay technique using IMMULITE Folic acid (PILKFO-4) kit were done respectively before counseling. For this purpose, 5ml of fasting blood was drawn from the selected subjects with the help of a laboratory technician and estimated by the procedure given in Appendix IV. More subjects could not be included for serum folate estimation because the method is very expensive.

4) Clinical Examination

Clinical examination is the process by which a health care provider investigates the body of a patient for signs of disease (Benson and Perry, 2004). Clinical examination was done for the fifty selected subjects with a help of a physician using a clinical examination schedule developed by the investigator (Appendix V).

E) DEVELOPMENT OF EDUCATIONAL MATERIALS

Audio visual aids have high potential for imparting and retention of knowledge (Joshi and Mehta, 1998). They help to improve concreteness, clarity and effectiveness of ideas and skills being transferred (Reddy, 1997).

A good poster arouses or urges people to immediate action and is highly suggestive (Reddy, 1997). A folder gives essential information relating to a particular topic and has long been in use for health communication. Leaflet is a single sheet of paper of information relating to a topic and can be shared by others in the family and community (Yadav, 1998).

Gopalan (1998) recommended a judicious use of available mass media and computers to popularize dietary guidelines. He emphasised that through computers these dietary guidelines should be internalized in hospitals.

Realising the significance of audio visual aids, the investigator developed educational materials like posters, folders, leaflets and CD scripts in both English and the local language, Tamil. The information for the development of the educational materials was obtained from libraries and health professionals at reputed hospitals. The health professionals were approached again to evaluate the content and quality of the educational materials developed (Plate 2). The doctors exhibited the visual aids in their hospital notice boards for the public to become aware about NTDs (Plate 3).

The educational materials developed contained details on concepts regarding the definition, classification, causes, consequences of NTDs, the role of folic acid in the prevention of NTDs, dietary folate, sources and recommended allowances for periconceptional consumption of folic acid. The developed posters, folders, leaflets and CD scripts are given in Plate 4 and Appendix VI.

DEVELOPMENT OF TEACHING AIDS WITH HEALTH PROFESSIONALS

PLATE - 2

FOLDERS EXHIBITED IN HOSPITAL NOTICE BOARD FOR THE PUBLIC

PLATE - 3

DEVELOPED TEACHING AIDS

PLATE - 4

F) COUNSELING USING EDUCATIONAL MATERIALS TO THE SELECTED SUBJECTS

The selected fifty women were requested to report at a common place of their convenience in order to assess the initial baseline knowledge about folic acid. For this purpose a questionnaire was developed and used and their knowledge was assessed for a maximum score of 20 (Appendix VII). The selected respondents were counseled regarding diagnosis, burden of the disease, risk of recurrence, nature of trial and efficacy of screening serum folate and ultra sonography for antenatal diagnosis of NTDs.

The subjects were counseled by using the developed posters, leaflets, folders and CD scripts which included simple tips regarding the basic aspects of folic acid, dietary guidelines and recommended allowances of folate so as to prevent the occurrence of NTDs (Plate 5). The selected fifty women were divided into five groups with ten members in each group at their convenience for counseling at a time in Aswin hospital premises, Erode for a period of one hour, followed by interactions with the subjects for half an hour. The investigator met each group three times during the course of counseling. The developed aids such as folder, leaflets on NTD understandable by even a lay person, prepared in the local language were given to the participants for effective counseling and follow-up.

G) IMPACT OF COUNSELING

In order to study the impact of counseling, the selected fifty subjects aspiring to have the first child were approached individually at their residences three months after counseling. After a brief discussion with them, post-counseling changes in their food choices, their post-counseling knowledge were assessed using the same questionnaire (Appendix VII), which was given earlier. The scores obtained for the pre and post counseling sessions were documented and analysed statistically.

Impact of counseling on food intake was also assessed by three day recall method of dietary assessment after three months of counseling for the same ten subjects. Plasma or serum folate is the most frequently used index of human folate status (Hine, 2003). Serum folate is considered as a sensitive indicator of the folate available to replicating cells with higher turn over rates (Bakker and Brandjes, 1997). Serum folate was also estimated for the same ten subjects by competitive immunoassay and haemoglobin by Cyanmethaemoglobin method and compared with initial values before counseling.

H) COUNSELING TO RECURRENT NTD SUBJECTS

Counseling was given individually in hospital premises to 30 women out of 116 who delivered babies with NTDs during the period of study were recruited for supplementation to prevent recurrence using the developed folders, leaflets and posters and with the guidance of the physician (Plate 6). Emphasis was given to clarify their doubts on diet and on the folate supplements advocated to improve their dietary folate intake.

COUNSELING SESSIONS USING POSTERS

PLATE - 5

COUNSELING MOTHERS WITH A PREVIOUS NTD AFFECTED PREGNANCY

PLATE - 6

PHASE 4 EVALUATION OF THE EFFICACY OF PERICONCEPTIONAL SUPPLEMENTATION OF FOLATE ALONE AS WELL AS IN COMBINATION WITH MULTIVITAMIN OR IRON IN PREVENTING THE RECURRENCE OF NTDs

A) Selection of subjects for supplementation

Among the 116 mothers who had one NTD affected child, 30 subjects in the age group of 20-30 years hoping to conceive again, were selected with the help of physician as a sub sample cautiously, to avoid wide variations in their haemoglobin levels and without any genetic defects in their previous family history. The selected sub samples were willing to cooperate and take nutritional supplements.

B) Collection of data

1) General and socio economic background

According to Whiteney and Rolfes (1993) socio economic factors profoundly affect the nutritional status. Purusothaman and Lakshmi (1985) have stated that there exists a positive correlation between income levels and diets chosen by the expectant women. With this background, the socio economic status was studied to collect details regarding age of the subject, educational and occupational status, family size, type and monthly family income using interview schedule (Appendix VIII).

2) Obstetric history of the previous pregnancy

Details regarding the consanguinity, age at first pregnancy, parity, number of live births, abortions, still births, birth spacing, type of NTD, period of survival of NTD child, physiological and psychological problems faced, time of identification of complication and termination of NTD child, details regarding the type, time and period of intake of medicinal supplements, outcome of pregnancy, sex of the NTD child and foetal biometrics of NTD child of previous pregnancy were collected using the interview schedule (Appendix VIII).

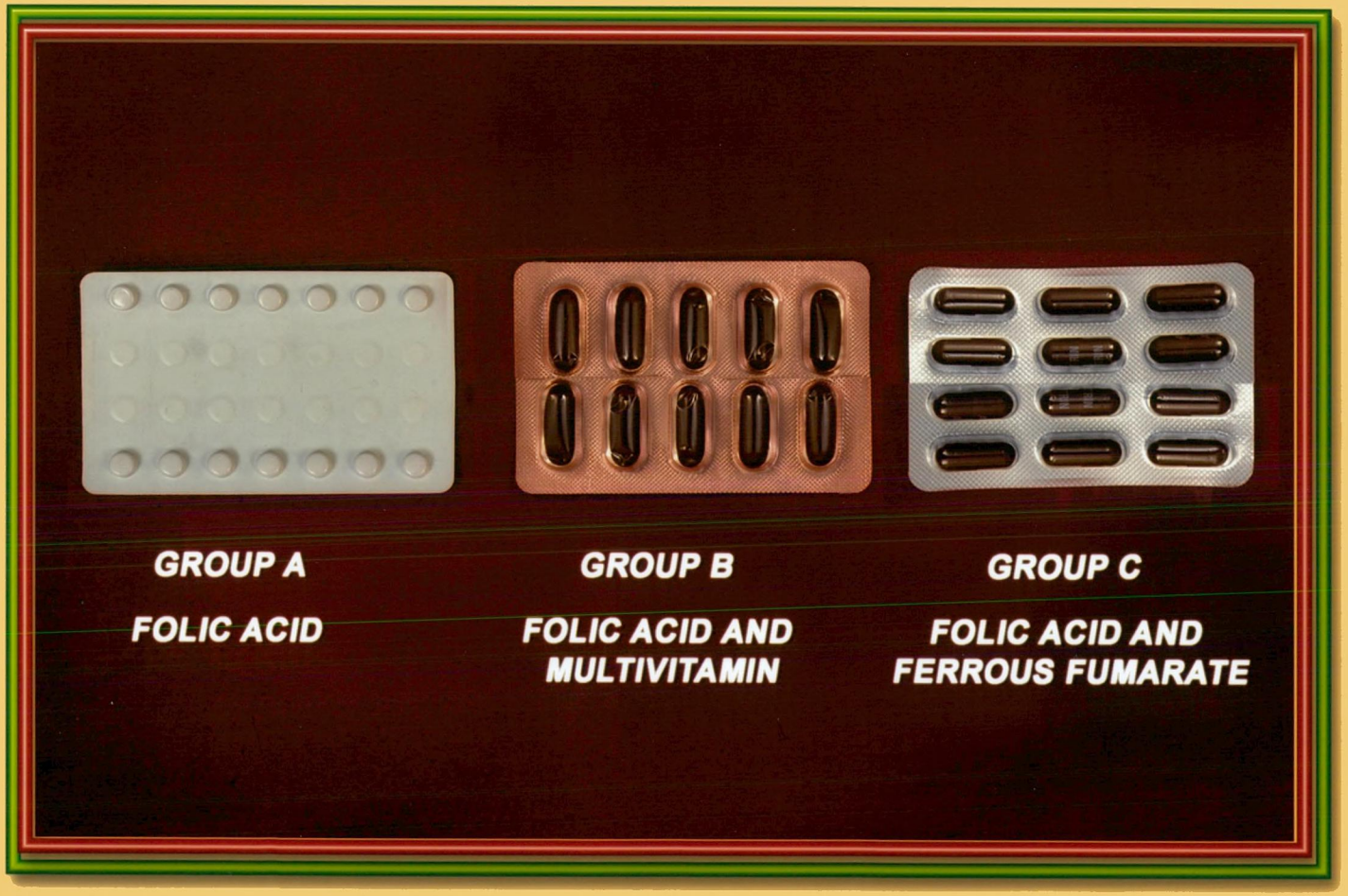
3) Dietary pattern and food and nutrient intake of the selected subjects

One of the best ways of finding out the nutritional status of the expectant women is to find out how much exactly of the various kinds of foods the mothers consume (Coutsaudis *et al* 1992). Diet surveys give information on nutrient intake levels, sources of nutrients, food habits and attitudes (Swaminathan, 1993). For this purpose, a three day recall method was carried out for the selected subjects initially before conception and in the sixth month of pregnancy. The raw equivalents of the foods were calculated and the nutrient intakes of the subjects were found out using the Food Composition Tables (Gopalan *et al* 2004). Information regarding consumption pattern of folic acid rich foods by the selected subjects was also collected using an interview schedule.

C) Supplementation of Folic Acid

Braekke and Staff (2003) recommended a daily supplementation of 400mcg of folic acid, a month before pregnancy and during the first two to three months of pregnancy in order to decrease the occurrence of NTDs. The Department of Health advises all the women considering pregnancy to take folate supplements for 12 weeks prior to conception and in the first trimester of pregnancy (Evans and Evans, 2002). Since most of the human pregnancies are neither preplanned nor such compliance on mass scale appears to be feasible, an attempt was made to supplement folic acid and its combinations after delivery of the NTD affected child.

The selected 30 subjects were divided into three groups each consisting of ten subjects and were supplemented daily with folic acid and combinations in the form of tablets (Plate 7), periconceptionally after the delivery of the NTD child and continued until three months after conception as follows:



FOLIC ACID SUPPLEMENTS

PLATE - 7

Group A - 5mg of folic acid

Group B - 5mg of folic acid +multivitamins

Group C - 5mg of folic acid + 60mg of ferrous fumarate.

It was considered unethical to form a control group since none of the mothers could be requested to volunteer to enter in to pregnancy without folic acid supplementation.

D) Evaluation of the impact of supplementation

The folic acid supplements and their combinations were issued to the selected subjects during the first week of each month under the guidance of the physician and in the presence of the investigator. Each respondent was asked to keep the record of supplements on a diary card. Further the intake of folic acid supplements was ensured through frequent telephone contacts with the selected subjects. The women were also advised not to take any vitamin preparation during the supplementation period other than the capsules provided to her. They were informed about the safety and risks of the trial. At each visit, compliance of the drug intake was checked with the help of the diary card maintained by the women and also assessed from the blister packs.

E) Assessment of the nutritional status of mothers

The heights of the subjects were recorded during their first visit to the physician after conception to the nearest 0.1cm accuracy using a stadiometer at the Aswin hospital premises. The weights of the subjects were recorded during their first visit to the doctor and thereafter in the final trimester as per the guidelines of Jelliffe (1996).

F) Assessment of nutritional knowledge

A proforma was prepared and administered to assess the initial knowledge of the selected subjects. Counseling was done using the developed materials and the impact of counseling was studied through the scores obtained and analysed statistically.

G) Serum folate levels

Serum folate levels of the selected sub samples (30 subjects) were analysed by collecting 5ml of fasting blood sample soon after the delivery of the NTD child as and when intimated by the hospital authorities using competitive immunoassay technique using IMMULITE folic acid (PILKO-4) kit and was taken as the initial serum folate levels of the experimental group. The impact of supplementation of folic acid and its combinations was studied after three months of conception by the three groups as and when the period was completed and estimating the final levels of folate at the end of the three months.

H) Haemoglobin

Surveys carried out in various parts of India have indicated that 10 to 40 per cent of the Indian pregnant women have haemoglobin levels less than 10g/dl. Such mothers are more prone to have a high incidence of complications during pregnancy (Rangnekar and Darbari, 1993). In order to study the prevalence of iron deficiency anemia among the selected women, the haemoglobin levels were estimated using 0.02 ml of blood drawn from the samples after delivery of a NTD affected child and after three months of conception to see the impact of supplementation of folic acid and its combinations on the selected sub sample and analysed by Cyanmethaemoglobin method (NIN, 2003).

I) Identification of factors associated with serum folate level by Factor Analysis

Factor Analysis was carried out based on the method developed by Thurstone (1959). A total of six socio economic and maternal factors were identified which would help to elucidate association with serum folate levels (in the three experimental groups).

J) Foetal Biometrics

The details regarding foetal biometrics such as bioparietal diameter, head circumference, abdominal circumference and femur length of foetus were recorded through sonogram reports during the 22^{nd} week of gestation to study the impact of the supplementation (Plate 8).

K) Pregnancy outcome

Details regarding weeks of gestation, nature of delivery, sex of new born were collected and recorded.

1) Anthropometric assessment of the new born

a) Crown heel length

The crown heel length of the infant was measured using an infantometer. The infant was made to lie down on the infantometer. The head was positioned firmly against the fixed head piece, with eyes looking vertically. The knees were extended by firm pressure and the feet were flexed at right angles, to the lower legs. The upright sliding foot-piece was moved to obtain firm contact with the heels and length was read to the nearest 0.1cm (Jelliffe , 1996).

b) Weight

The weight of the infant was measured soon after delivery using a beam balance which is less likely to be inaccurate (Jelliffe, 1996). The weight of the infant was recorded to an accuracy of 0.1kg (Plate 8). The balance was checked for accuracy using standard weights before taking the weight of the infants.

c) Head circumference

The measurement of head circumference is a standard procedure in pediatrics, usually to detect pathological conditions accompanied by a large or one of increasing size as in hydrocephaly or too small a skull as with microcephaly (Jelliffe, 1996) (Plate 8).

Head circumference was taken using a narrow (less than 1 cm wide), flexible, non stretchable, PVC coated fibre glass tape. The child's head was made steady and the greatest circumference was measured by placing the tape firmly round the frontal bones just superior to the supra orbital ridges, passing it round the head at the same level on each side and laying it over the maximum occipital prominence at the back. Head circumference of infants soon after delivery was taken to the nearest 0.1 cm.

d) Chest circumference

Chest circumference was recorded by fibre glass tape passing over both the nipples in mammary gland posteriorly. The chest measurements of infants were taken to the nearest 0.1cm (Jelliffe, 1996) after delivery (Plate 8).

ULTRA SCANNING

CHEST CIRCUMFERENCE

BIRTH WEIGHT

HEAD CIRCUMFERENCE

MID ARM CIRCUMFERENCE

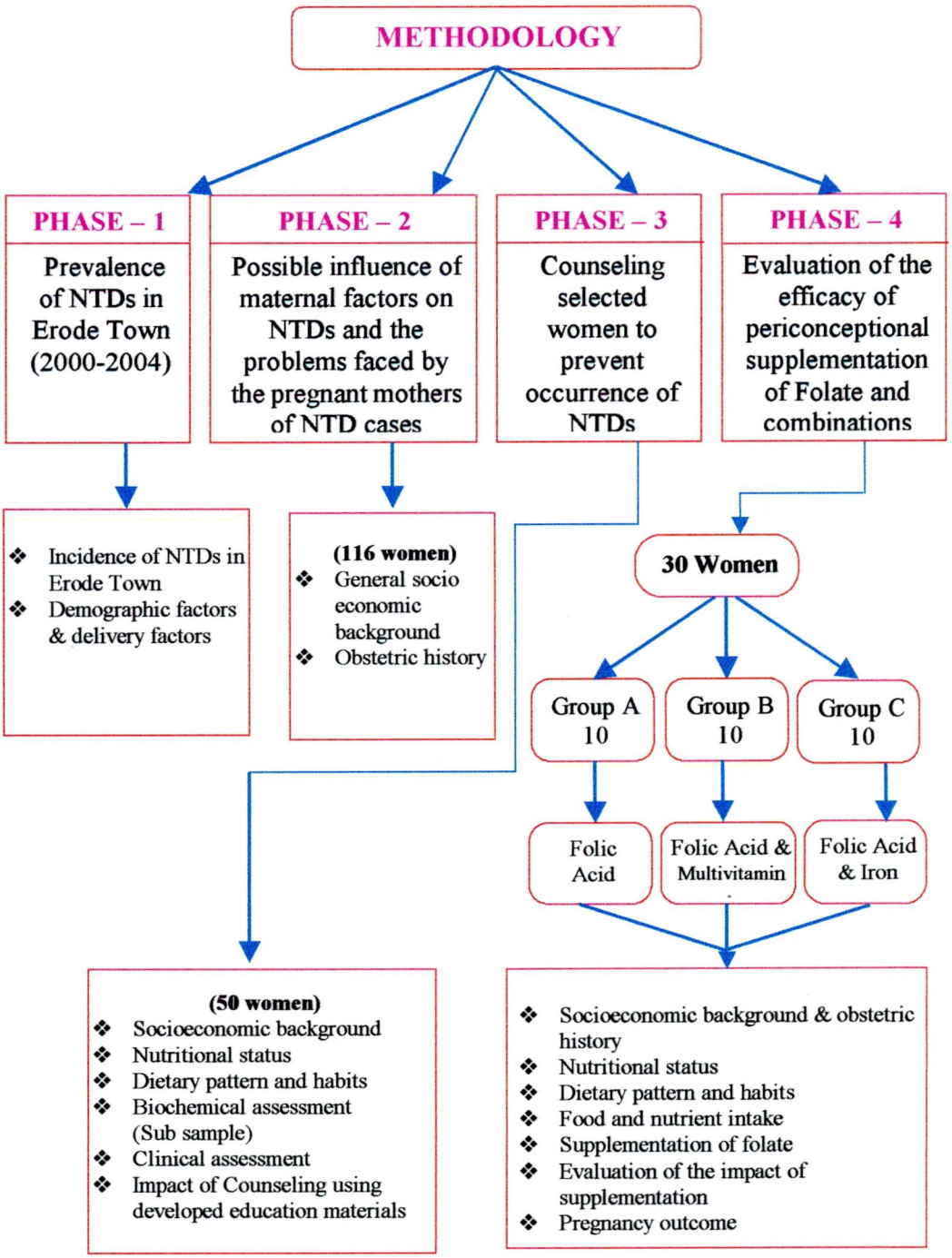
ULTRA SCANNING AND ANTHROPOMETRIC MEASUREMENTS OF THE NEW BORN

PLATE - 8

e) Mid arm circumference

Measurement of mid upper arm circumference of the delivered infants was taken at the mid point between the acromial and olecranon processes of the scapula and the ulna respectively to the nearest 0.1cm with a flexible fibre glass tape which was placed gently, but firmly to avoid compression of the underlying soft tissue (Jelliffe, 1996) (Plate 8).

The outline of the study is presented in Figure 2.



OUTLINE OF THE STUDY

FIGURE 2