CHAPTER-III

MATERIAL AND METHODS
MATERIAL AND METHODS

3.1 Sample Size and Selection Technique:

A total sample of 198 Injecting Drug Users (IDUs) who had the risk for HIV infection were selected following the random sampling technique from various drug detoxification centres (Table-1) run by different NGOs at Imphal, Oinam of Bishnupur District and Manipur Central Jail, where drug injectors along with the non-injecting drug users are kept as inmates irrespective of their caste, tribe and locations. The size 'n' of the study sample so selected have been statistically decided by using the standard equation (Mahajan, 1991) as given below:

\[ n = \frac{4pq}{L^2} \]

where, \( p \) = prevalence rate of HIV positives among IDUs, 
\( q \) = prevalence rate of HIV sero-negatives among IDUs, 
\( L \) = allowable limit of error (5%) of the positive character.

Being a counsellor and researcher of the ICMR Field Unit, Imphal, the scholar, after building up a good rapport with these selected IDUs through counselling and health care services over a period of time and explaining the concepts of informed consent and ethical issues of confidentiality as described below, they (IDUs) were asked to participate in the present study. In due course of the study, 63 of the 198 IDUs were dropped-out in-between on various grounds such as death during the first six months of study, loss of interest, migration, relapse to drug as well as expulsion from the community, etc. and therefore, excluded them from the present study. The centre-wise distribution and status of the study sample is shown in Table -1.
Material and Methods

Table - 1
Centre - wise Distribution and Status of the Study Sample

<table>
<thead>
<tr>
<th>Status of Sample</th>
<th>Name of Centres*</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NL</td>
<td>p.c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LLF</td>
<td>p.c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DN</td>
<td>p.c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH</td>
<td>p.c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCJ</td>
<td>p.c.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Existing</td>
<td>35</td>
<td>17.7</td>
<td>40</td>
<td>20.2</td>
<td>25</td>
<td>12.6</td>
<td>15</td>
<td>7.6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>68.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropped out</td>
<td>07</td>
<td>3.5</td>
<td>14</td>
<td>7.1</td>
<td>10</td>
<td>5.1</td>
<td>8</td>
<td>4.0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>31.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>21.2</td>
<td>54</td>
<td>27.3</td>
<td>35</td>
<td>17.7</td>
<td>23</td>
<td>11.6</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>198</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* NL = New Life, LLF = Life Line Foundation, DN = Dawn, NH = New Hope, MCJ = Manipur Central Jail

After excluding the dropped out cases during the period of data collection, the remaining 135 IDUs were confirmed to follow-up longitudinally in their respective residence after they were departed from the centres.

The age of the study subjects attained during the first drug initiation have been carefully recorded through recall method. However, the exact age of the subjects at the time of recruitment for the present study was determined by examining the birth documents (Horoscope) which is locally known as Kuthi.

Because appropriately conducted qualitative and quantitative methods are highly invasive of intimate aspects of peoples' lives, great care were taken in the protection of the personal identity of the research participants. To guide the present study, three basic ethical points are laid down following Agar (1980) and American Anthropological Association (1990) as given below:
Material and Methods

a) The purpose of the present study and potential risks were explained explicitly to the participants. In addition, the right to choose whether or not to participate in the study was offered openly. Those participants who could not be followed up three times consecutively, were informed to drop out of the study for the limitations of the present research proposal.

b) Confidentiality of the participant’s identity and private life history was ensured.

c) Assurance was given not to cause harm to the participants due to the present research programme.

To achieve these goals, a verbal informed consent was taken from each of the participants. Sensitive data are agreed upon to protect with great confidentiality. Once ethical issues are considered and guidelines for informed consent are decided upon, data collection were proceeded by qualitative interview, physical measurement and clinical examination techniques.

3.2 Data Collection and Its Components:

Using a pre-tested, structured questionnaire, both qualitative and quantitative data were collected longitudinally since January 1994, upto December 1996, at an interval of every three months' time with inputs of regular health education and counselling to examine if there is any changes in the risky behaviours of the IDUs. For the qualitative data, face to face interview and silent observation techniques were employed, to understand the networking activities of drug use. Furthermore, recall method was also employed to collect retrospective data on the
past history of risk behaviour in an attempt to establish *the natural history of AIDS*. But for the quantitative data, standard techniques as would be describing below were used.

3.2.1 Qualitative Data:

i) Demographic and Socio-economic Variables:

a) Demographic information for the study included age, place of birth, ethnicity, type and family pedigree (1st & 2nd line generation as counted up from the proband).

b) Information regarding personal and family economic variables such as income, expenditure, occupation and sociological factors like religion, education, marital status, parental response, reactions from the health care providers in terms of giving treatment and care, etc. were also investigated.

ii) The Causes of Drug Abuse and Risk for HIV Infection:

a) Information such as probable influencing factors for drug initiation, type of drugs, mode of taking drugs were collected to describe the causes of drug abuse,

b) Data on drug sharing habit, number of sharing partners, cleaning practice of needle and syringe, drug treatment profiles and relapse episodes and other risk behaviours like sexual liaison during drug using period and after, are investigated and observed longitudinally for a period of three years in order to assess the risk for HIV transmission.

iii) Clinical Parameters Indicating the Symptomatic HIV Infection:

a) The data on clinical manifestations as indicated by the external signs and symptoms were recorded in a pre-designed structured proforma.
b) Available secondary data on TB, Diarrhoea, etc. were also recorded as much as possible from the investigative reports done by the expert physician.

### 3.2.2 Quantitative Data:

i) **Anthropometric Parameters**:  
   a) Physical parameters which is visibly and easily affected in different stages of HIV infection may be expressed as a pattern to describe the disease conditions among IDUs in Manipur. So, a set of four anthropometric measurements such as **Body Weight** (Wt), **Height** (Ht), **Mid-upper Arm Circumference** (MUAC), Skinfolds over **Triceps** (TSF) and **Biceps** (BSF) have been selected for the present study.

b) For assessing the nutritional status and energy intake, data on **Height** (Ht.) and **Weight** (Wt.) are still used along with personal age of the IDUs.

ii) **Biochemical Parameters**:

For examining the association of anaemic conditions in four stages of HIV infection the haemoglobin percentage (Hb gm%) level was estimated by using **Sahli’s method** at field level.

### 3.3 Measurement and Diagnostic Criteria:

#### 3.3.1 Anthropometric measurements:

The selected anthropometric variables such as

i) **Body weight**,  
ii) **Height Vertex**,  
iii) **Mid-upper arm circumference** and  
iv) **Skinfolds over Triceps and Biceps**

have been measured following the standard techniques as given by Weiner and Lourie (1969). Standard instruments such as Anthropometric rod, portable spring
Material and Methods

balance Weighing machine (Libra type), Steel Tape and Skinfold Calliper are used for the measurements.

3.3.2 Identification Procedure for HIV Status:

Of the 135 IDUs in the study sample, 92 of them were identified as HIV sero-positives while the remaining 43 were detected to have never been tested. Self-disclosure of the past HIV test result supported with documentary evidence while seeking treatment care and counselling supports has been the main criteria for identifying the HIV infection status among the selected IDUs. Later course of investigation could be conducted rather successfully after establishment of full confidence and good rapport with the subjects.

3.3.3 Clinical Examination Procedure:

Clinical examination of the signs and symptoms associated to the HIV infected individuals were done following the guidelines provided by NAPCP-Zambia, WHO and USAID in 1990 and WHO in 1991 (Annexure -I, II) which is supplemented by the one given by Melnick, et al. (1993) on the oral manifestations of HIV infection. Records of investigations involving stool examination, tissue culture, biopsy, etc. with documentary evidences were noted for supportive diagnosis of the opportunistic infections. Chest X-rays on postero-anterior view for suspected cases of TB were taken from the IDUs. For confirmed cases of pulmonary tuberculosis, the findings are recorded with slide formation of radiographic film. Association of the nutritional anaemia was also ruled out by estimation of haemoglobin level. Photographic plates based on African and American studies (Parkin and Peters, 1991; Ansary, et al. 1989; Farthing et al. 1987) were used to compare suspected cases of clinical man-
Material and Methods

Furthermore, in-depth investigation was carried out with the help of an expert physician. Photographs are also taken in certain cases with the consent of the patients and his parties with the implication for doubtful diagnosis, comparison, documentation, etc. For confirmation of diagnosis and general treatment of opportunistic infections, experienced physician in the field has been consulted with the consent of the subjects and his party. No attempt were made to diagnose other types of cardinal features such as *Pneumocystis Carinii Pneumonia*, *Toxoplasma encephalitis*, *Cytomegalovirus retinitis*, etc. as there is no sophisticated and expensive laboratory investigation facilities in the present study.

3.3.4 Estimation of Haemoglobin Level (gm%) :

With the deterioration of health condition due to prolonged use of heroin, poor food intake habit and infections, there is always the possibility for developing anaemia which is defined by low level of haemoglobin. So, an attempt to estimate the haemoglobin gm % have been made for the present study following the *Sahli's Method*.

3.4 Analysis :

Both the qualitative and quantitative data were computed for statistical analysis so that the behavioural and biological events associated with the study population may be interpreted in a meaningful way. For the information regarding demography and socio-economic characters as well as the risk behaviours related to injecting practices, all the existing 135 patients were considered collectively irrespective of their HIV status. For the anthropometric and bio-chemical variables along with the information regarding clinical and sexual promiscuity, analysis have been carried out separately in two comparison groups depending upon their HIV serostatus.
3.4.1 Classification of Study sample:

By ethnicity, the sample is divided into three categories viz. i) the Meiteis, ii) the Tribes and iii) the Muslims. Meanwhile, three categories viz. i) No education ii) Matric and below and iii) Above matric groups were grouped educationally to the IDUs depending upon their level of education. The IDUs who did technical courses were considered to be in the above matric group. On the other hand, the study sample was geographically identified as representing from i) Urban, ii) Peri-urban and iii) Rural areas.

A standard classification of income group for the present study was developed with an exponent of Rs. 293 by considering the state per capita income (DES, 1990-91) of Rs. 3502 at the current price in 1989 - 90 as the upper limit of the lower income class. The income range used is shown as follows.

<table>
<thead>
<tr>
<th>Income Group</th>
<th>Ranges of Income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>≤ Rs 293</td>
</tr>
<tr>
<td>Middle</td>
<td>Rs 294 - 587</td>
</tr>
<tr>
<td>Higher</td>
<td>≥ Rs 588</td>
</tr>
</tbody>
</table>

The per capita income per month in each family of the study sample was calculated by dividing the total family income per month by the total number of members in the family. Comparing with the above income classification, the study subjects were also grouped into three income classes.

3.4.2 Calculation for the Date of Sero-conversion:

Indirect method to estimate the date of sero-conversion has been employed in different studies (Wasi, C., et al, 1995). For the present study on IDU population
that has a very high risk of HIV infection through sharing habit of needles and syringes contaminated with infected blood during heroin injection, a new method has been attempted to estimate the date of sero-conversion by considering the following facts that:

i) For IDUs in Manipur, probability of HIV infection through injection of infected blood is very high compared to other risk activities (State AIDS Cell, 1994).

ii) Epidemic of drug abuse (both oral and IV intake) exceptionally in Manipur precedes much before the epidemic of HIV infection.

iii) It is well established that Sero-conversion take place within 3-6 weeks of HIV infection (WHO, 1990b).

Assuming the date of IV drug initiation by sharing unclean needle and syringe as the point of infection for IDUs in Manipur, the date of Sero-conversion was calculated by adding 6 weeks (i.e. 1 and 1/2 months) to the date of IV drug initiation of all individuals including those in the non-tested group. Subsequently, the mean years of the development of characteristic clinical signs and symptoms was estimated.

3.4.3 Trend Analysis for Risky Behaviours of HIV Transmission:

To examine the trend of risky behaviours the entire history of drug abuse and association of HIV infection may be classified broadly into three phases.

a) The period of 1980 - 89 during which drug abuse epidemic with rapid increase in the magnitude of drug addicts among the youths in the productive as group was initiated.
b) The period of 1990 - 93 when the wide spread HIV infection among the IDUs began.

c) The last phase i.e. 1994 - 96 is the period during which the AIDS epidemic was initiated with the death of many HIV patients.

The changing disordered behaviours for risk of HIV transmission in the study population were examined under these major periods and changing trend in the selected behaviours were assessed. The 135 IDUs were also classified as per time slot of their drug abuse initiation and onset of injection habit. Cumulative frequency of on and off injecting habit was also calculated. Beside this, frequency of needle and syringe sharing habit in comparison to non-sharing habit were assessed in all the three phases. Developmental changes of cleaning practices were also observed.

3.4.4 Clinical Staging of HIV infection:

Grouping of signs and symptoms were conducted to assess clinical stages of HIV infection as per WHO criteria (WHO, 1990a) for both the comparison groups (Annexure - III). Comparison of the two groups i.e. HIV positive IDUs and never tested IDUs was done to figure out if there is any difference in the manifestation of clinical features related to HIV infection.

3.4.5 Anthropometry in Different Stages of HIV:

Following 92 HIV infected IDUs at an interval of every three months, measurement of Body weight, Mid-upper Arm Circumference (MUAC), Skinfolds over Triceps and Biceps were computed. The mean values in each stage of HIV infection as classified during the onset of the present study and last follow-ups were calculated and converted in the nearest first decimal place to observe if any difference has occurred during the two episodes of observations.
Material and Methods

The median of individual mean weights of all the sero-positive IDUs in their respective infection stages as decided in the last observation were also calculated. And corresponding body weight in different percentiles was also estimated from the median weights of individual mean weights.

The skinfold values are also transferred into log units.

3.4.6 Assessment of Nutritional Status:

In the present investigation, the assessment of nutritional status has been attempted primarily on the basis of weight deficit for height, by adopting the Waterlow's classification (Pelletier, 1993). In that, assessment of nutritional status by height deficit for age of standard reference was avoided on three reasons viz.

a) Drug addiction habit and subsequent HIV infection initiated only when the study subjects are fully grown up and there is not much or negligible growth in stature.

b) Short duration effect of malnutrition due to drug use and HIV infection seems to affect less on body stature.

c) Normal reference standard was selected from among HIV infected asymptomatic IDUs of the same study subjects in the same age group.

Using the weight for height ratio for patients in stage-I of HIV infection as reference standard, other patients in stage-II, III and IV were graded into different degree of nutritional impairment. The cut off level used for assessing different grades of nutritional status are given below by following Waterlow's Classification.
Material and Methods

Classification of Nutritional Status

<table>
<thead>
<tr>
<th>Nutritional Status</th>
<th>% level of reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Mildly impaired</td>
<td>80 - 90</td>
</tr>
<tr>
<td>Moderately impaired</td>
<td>70 - 80</td>
</tr>
<tr>
<td>Severely impaired</td>
<td>&lt; 70</td>
</tr>
</tbody>
</table>

3.4.7 Estimation of Total Energy Needs:

Besides the assessment of the nutritional status, total estimated energy needs (TEEN) in all the stages of HIV infection was also estimated by computing basal energy expenditure (BEE) using Haris - Benedict’s Equation (Long, et al, 1979) as the studied subjects are all considered sick of HIV infection and other co-infections.

The equations used are:

\[
BEE = (66.47 + 13.75W + 5.0H - 6.76A) \quad - \quad (1)
\]

where W, H, A are weight in Kg, Height in cm, Age in years of the subjects investigated respectively and,

Total Estimated Energy Needs (TEEN) \n\[
= BEE \times \text{Activity factor} \times \text{Infection factor} \quad - \quad (2)
\]

where activity factor may be 1.2 for subjects confined to bed, 1.3 for subjects whose activities are out of bed. The infection factors may be 1.2 for mild, 1.4 for moderate, 1.8 for severe patients respectively.
3.4.8 Statistics Applied:

Both the qualitative and quantitative data collected during the present study have been computed by using univariate and multivariate analysis where it is applicable. When the frequencies and percent frequencies were calculated for the qualitative data on the socio-economy, demography, behaviours, clinical signs and symptoms of the IDUs, the means, medians, standard deviations and standard error of standard deviations of the quantitative data on bio-anthropological variables have also been worked out by following the Methods in Bio-statistics (Mahajan, 1991). Calculation of odds ratio, chi-square, t-test and coefficient of correlation values have also been achieved in order to examine, if any, the pattern of variability between the comparison group of data and samples.

Some of the important statistical equations used in the present study are described below.

i) **Confidential Interval (CI):**

Using the Standard Error of Mean (S.E.M.), the 95% CI was also calculated for all the quantitative data such as age of the patients, parameters on anthropometry, haemoglobin levels, etc. to know the range of values that is likely to cover the true but unknown value by using the equation,

\[ \bar{X} \pm 2 \text{S.E.M.} = \bar{X} \pm 2 \frac{\text{SD}}{\sqrt{N}} \]

In classic significance testing, the confidence interval is based on the concept of repeated trials or studies i.e. with 95% confidence interval. If the study was repeated 100 times, the confidence interval would be expected to include the true value on 95 occasions. As per normal distribution of samples,
we say that 95% of the sample mean (\( \bar{X} \)) will lie within the confidence limits that is 95% of the sample mean/s will contain within this limit (Bulpitt, 1987).

ii) **Odds Ratio (OR):**

In the present study, odds ratio as a measure for the strength of the association between the economic and employment status of the IDUs, and the changes of sharing habit as an effect of counselling/health education and fear psychosis, etc. are calculated from the 2 × 2 contingency table (Park, 1994). The general formula used is presented as,

\[
\text{Odds Ratio} = \frac{ad}{bc}
\]

where 'a' and 'd' are positive outcomes while 'b' and 'c' represent the negative outcomes in both risk and riskless conditions.

iii) **Percentiles:**

Percentiles are the values of the variates dividing the total frequency into hundred equal parts respectively. The following formula is applied in the computation of percentiles for a grouped frequency distribution.

\[
P_k = l + \left( \frac{kN}{100} - C \right) \times h
\]

where, 
- \( l \) = lower limit of the class in which the percentile lies,
- \( f \) = frequency of the class,
- \( h \) = width of the class,
- \( C \) = cumulative frequency upto and including the class preceding this class,
N = total frequency, and
k = 1, 2, 3, .... 99.

This equation is applied in the estimation of various percentiles values of the body weights of the present study patients.

iv) t-test:

The t-test is a statistic applied to assess the significance of difference between two populations or groups of data or two means \( \bar{X}_1 \) and \( \bar{X}_2 \) of two independent sample of size \( N_1 \) and \( N_2 \) and SD of \( S_1 \) and \( S_2 \). The main purpose of this is to test the hypothesis that the study samples came from the same normal population,

\[
\text{i.e., } \bar{X}_1 = \bar{X}_2 \\
\text{or, } \bar{X}_1 - \bar{X}_2 = 0.
\]

It is calculated by using the formula,

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{S} \times \sqrt{\frac{N_1 N_2}{N_1 + N_2}}
\]

where, \( \bar{X}_1 \) and \( \bar{X}_2 \) = Means of the two samples,
\( S_1 \) and \( S_2 \) = Standard deviation of the two samples,
\( N_1 \) and \( N_2 \) = Size of the two samples,
\( S \) = Combined S.D.

\[
S = \frac{S_1^2 N_1 + S_2^2 N_2}{N_1 + N_2 - 2}
\]

This statistics is also used to test the significance of differences between the two groups of sample as assessed during the onset of the study and the last follow ups.
v) **Chi-square ($\chi^2$) test**

Chi-square test is the test of significance of overall deviation square in the observed and expected frequencies divided by expected frequencies. It is measured by the formula,

$$\chi^2_{k-1} = \sum_{i}^{k} \frac{(O_i - E_i)^2}{E_i}$$

where, $O_i$ and $E_i$ are the observed and expected frequencies in $k$ classes or cells for an experiment and $k - 1$ is the degree of freedom. In the present study, Chi-square ($\chi^2$) test is used to find the significance of difference in two or more than two proportions. Since the number of frequencies in certain cell/s of the contingency table of the present study is less than five ($5$), Yate’s correction is done by reducing half to the absolute difference between observed and expected frequencies. In this case, the formula used is

$$\chi^2_{k-1} = \sum_{i}^{k} \frac{(|O_i - E_i| - \frac{1}{2})^2}{E_i}$$

Where, $|O_i - E_i|$ is the absolute difference. For the calculation of $\chi^2$ with or without Yate’s correction and probability values Epi Info Version 6 was used.

vi) **Log Transformation**

Readings on skinfolds were usually taken to the nearest 0.1 mm and routinely converted to a logarithmic scale because this brings the distribution of the measurements in the population more nearly in Gaussian form and takes cognizance of the measuring error being systematically greater for larger than for smaller readings (Tanner, 1962).
Material and Methods

\[ Z = 100 \log_{10} \text{(reading in } 1/10 \text{ mm - 18) } \]
where, \( Z \) = skinfolds transformation.

vii) Coefficient of correlation (r):

To examine if there is a correlation between the changes in body weights and haemoglobin levels in the different stages of HIV infection, the "r" value was calculated by using the pearson's product moment method as expressed by the following equation;

\[ r = \frac{\sum xy}{\sqrt{\sum x^2 \cdot y^2}} \]

Where,
\[ r = \text{correlation coefficient} \]
\[ x = \text{deviations of } X \text{ variables} \]
\[ y = \text{deviations of } Y \text{ variables}. \]
\[ \sum xy = \text{sum of multiplication of deviations } x \text{ and } y. \]