Chapter 3

METHODOLOGY

Methodology and plan of action is decided by an investigator on the basis of the objective and hypothesis set for the study. Methodological research studies are investigations of the ways of obtained and organizing data and conducting rigorous research. Methodological studies address the development validation and evaluation of research tools and methods.

The chapter consist the detail of research design, variables, sampling, tools and techniques for data collection selected for the study. Statistical analysis is also given in this study.

General characteristics of the study area

Lucknow is the capital city of the state of Uttar Pradesh in India. This metro city is the administrative headquarters of Lucknow District and Lucknow Division. Lucknow has always been known as a multicultural city and flourished as a cultural and artistic capital of North India in the 18th and 19th centuries and as a seat of power of Nawabs. Today it continues as an important centre of education, commerce, aerospace, finance, pharmaceuticals, technology, design, culture, tourism, music and poetry.

Topography

Lucknow ranked 6th among all the cities in India for fastest job creation. It is the largest city of Uttar Pradesh, and after Delhi the second largest metro of North and Central India, and the 11th largest city of India. Lucknow stands at an elevation of 123.45 meters above sea level and covers an area of 689.1 square km, the seat of the government of Uttar Pradesh, Lucknow is the
site of Vidhan Sabha, the High Court (Allahabad's bench) and numerous government
departments and agencies. Since May 1, 1963, Lucknow is the headquarters of the Central Command of the Indian Army prior to this it was headquarters of Eastern Command. Lucknow has several educational and research organizations like IIM Lucknow, Central Drug Research Institute, Indian Institute of Toxicology Research, National Botanical Research Institute, IET Lucknow, Dr. Ram Manohar Lohia National Law University, Sanjay Gandhi Post Graduate Institute of Medical Sciences and King George Medical College.

**Research Design**

This study was carried among the end stage of renal disease (ESRD) patients who was on maintenance hemodialysis at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. This dialysis centre is one of biggest dialysis centre in the Uttar Pradesh with 50 hemodialysis machines and more than 200 patients.

This hospital was selected purposively, considering regular follow-up and feasibility of visiting this hospital in all session of dialysis.

The Pilot study was carried out in Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow.

In the pilot study, it was observed that the patients who had come from the ward for conservative management in dialysis unit for dialysis had to be excluded. This hospital served the need of the individual belonging to vulnerable group of the society, lower socio-economic status and patients in hospital were drawn from all over Uttar Pradesh, Bihar and other state. The response rate was almost 99% and owing to their low cost there were high uptake of the facilities. Therefore this hospital was included in present study.

**Sampling**

The purposive sampling technique was used to draw representative sample. A total of 150 maintenance hemodialysis patients were selected from Sanjay Gandhi Post
Graduate Institute of Medical Science, Lucknow. During the first year of the study the registration of the patient was carried out until desired size of the sample achieved. The baseline registered patients came after three months for follow-up in hospital diet clinic. Patient who did not turn up after baseline, had chronic illness or not able to continue dialysis therapy because of their financial problem or referred their local centre, or expired were excluded.

**Inclusion criteria for sampling**

1. Consent to participate in this study.
2. Receiving hemodialysis twice and three times per week
3. The patient has been receiving regular hemodialysis at the S.G.P.G.I.M. Dialysis Center for at least 3 months.
4. Anuric.
5. Patients with no acute illness, such as pneumonia, acute myocardial infraction or septicemia.

The approval of the ethics committee in the hospital was obtained. All patients were informed about the nature of the study. They were also informed that their participation in this study is voluntary.

**Exclusion criteria for sampling**

1. Patient not willing to participate.
2. Admitted patients, who were taking dialysis for conservative management only.
3. Patients going for peritoneal dialysis

The approval of the ethics committee in the hospital was obtained. All patients were informed about the nature of the study. They were also informed that their participation in this study is voluntary and they have the right to withdraw at any time without any penalization and their refusal to participate and withdraw will not affect their treatment at the Centre.
Tools and Techniques

The information of the present study was collected with the help of ‘Questionnaire cum Interview Techniques’.

Structure of the questionnaire

Initially the information regarding ‘Nutritional Status of hemodialysis’ were collected from library of Sanjay Gandhi post Graduate of Medical Science, Lucknow. The Medline services available in the aforesaid library simplified the tedious job of allocation of related literature. In addition, Internet services also helped a lot in the collection of related review of the literature.

Standardization of the questionnaire

The vague questions which were not responded by the patients were deleted from the questionnaire, whereas other questions which were felt important in the study according to the objectives were added to the questionnaire and finally a standard questionnaire was workout .In this manner questionnaire was standardized.

This standardized questionnaire has the following structure (vide Appendix A). It is divided in five parts (I) General Information (II) Anthropometric Assessment (III) Bio-chemical Assessment (IV) Dietary Assessment (V) Knowledge Assessment

**General information**

A questionnaire was used to collect the following data from each participant: name, hospital registration number, age, gender, dietary habits and medical history including diagnosis, duration and frequency of dialysis.

**Anthropometric Assessment**

Anthropometric measurements are valid and clinically useful indicators of the protein–energy nutritional
status in maintenance dialysis (MH) patients. These measurements include percent usual body weight, percent standard body weight, body mass index and mid upper arm circumference were measured in baseline and again it was repeated after three months in follow-up phase.

**Bio-chemical Assessment:** Hemoglobin, blood urea nitrogen, serum creatinine, serum sodium, potassium, albumin, total protein, and if patient was diabetic then fasting and post prandial (PP) blood sugar.

**Dietary Assessment:** Various food items consumed by the subjects during breakfast, lunch, evening tea and dinner were carefully documented with their actual measurements in this section.

**Knowledge Assessment:** To assess the knowledge 10 questions were asked to the patients regarding dialysis therapy and diet in dialysis protein, sodium and potassium.

Patients were asked to participate in this study when they came to the S.G.P.G.I.M.S for dialysis. The purpose of the study was explained to each patient and then the patient chose to accept or decline to participate. If a patient expressed interest in participating, she was asked his/her age and the length of time on hemodialysis.

Responses to those questions as decided if the patient met the inclusion criteria. Those patients who did not meet the inclusion criteria were thanked for their time and not included in the study.

Data were collected in two phases: Baseline and Follow-up. During the

**Baseline Phase**
This phase is known as beginning phase, in this phase patients were enrolled for the study as per inclusion criteria. Questionnaire was given to the patients (Appendix I) and with their permission, the patient’s file was examined to acquire the necessary biochemical data such as serum hemoglobin, serum creatinine, Blood urea nitrogen (BUN), serum sodium, serum potassium and if patients is diabetic than fasting and post prandial blood sugar was also investigated.

Anthropometrics assessment was also done height, dry weight from the previous dialysis treatment, body mass index (BMI) as well as mid upper arm circumference (MUAC). Lastly, a 24-hour dietary recall was obtained from the patient and recorded on a food data sheet. Diet chart was given to the patients as per their individual requirement and dietary habits (Appendix II).

During awareness program there are so many tips, instructions regarding diet was provided to the patients for maintaining ideal nutritional status. (Appendix II).

### Follow-Up Phase

The second phase known as follow-up phase. In this phase after three months hemodialysis patients were contacted in dialysis unit either in O.P.D or dialysis unit and questionnaire were given to furnish and fill the details of dietary intake of three days, as in phase one. Thereafter anthropometric measurement as in first phase were repeated to see the effect of nutritional intervention as dietary counseling and health awareness and awareness regarding adequacy of dialysis. The anthropometric measurements were obtained after dialysis in order to eliminate water retention from affecting the accuracy of the measurements.

The interview schedule (Appendix-1) was prepared to include all possible questions relevant to the objective of the study. It was prepared in English language and translated into local language. The schedule consisted of the following parts.

### Measurements of Variables
All variables including anthropometric, biochemical and dietary variables which is used for study was given below-

**Dry Weight (Wt.):** Dry weight in dialyzed patient is the weight at the end of dialysis treatment. Electronic weighing machine was used to obtain the weight. Patients are asked to remove their shoes and heavy garments. Weight was measured in all patients and taken to the nearest 0.1 kg using weighing scale.

The scale was calibrated at the beginning and end of each examining day. The scale is checked using the standardized weights and calibration is corrected if the error is greater than 0.1 kg. The results of the checking and the recalibrations are recorded in a log book.

**Height (Ht.):** In adult MD patients, height is not a valid method for measuring protein or energy nutritional status. However, it must be measured because it is used in height-adjusted reference tables for weight (including SBW and BMI). Height was measured in all patients, with the patients bare footed and head upright. The height is measured with the measuring rod attached to the balanced beam scale. The floor surface next to the height rule was hard. The height was reported to the nearest 0.5 cm.

**Body Mass Index (BMI):** BMI was used to assess the degree of malnutrition.. The BMI was calculated according to the patient’s post-dialysis weight (kg) divided by height in (meter) squared.

\[
\text{Body Mass Index (BMI) = Weight (Kg) / Height (Meter)}^2
\]

**Table 3.1: The International Classification of adult underweight, overweight and obesity according to BMI.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI(kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Principal cut-off points</td>
</tr>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
</tr>
</tbody>
</table>
Severe thinness  |  <16.00  |  <16.00  
Moderate thinness  |  16.00 - 16.99  |  16.00 - 16.99  
Mild thinness  |  17.00 - 18.49  |  17.00 - 18.49  
Normal range  |  18.50 - 24.99  |  18.50 - 22.99  
  |  23.00 - 24.99  |  
Overweight  |  ≥25.00  |  ≥25.00  
Pre-obese  |  25.00 - 29.99  |  25.00 - 27.49  
  |  27.50 - 29.99  |  
Obese  |  ≥30.00  |  ≥30.00  
Obese class I  |  30.00 - 34.99  |  30.00 - 32.49  
  |  32.50 - 34.99  |  
Obese class II  |  35.00 - 39.99  |  35.00 - 37.49  
  |  37.50 - 39.99  |  
Obese class III  |  ≥40.00  |  ≥40.00  


**Mid upper arm circumference (MUAC):** A narrow flexible, non-elastic tape was used to measure the mid upper arm circumference (MUAC). The measurements were made to nearest 0.1 cm. At the dry weight, patient's right or non-access arm was bent at the elbow at 90° angle palm up, to locate the arm's midpoint on posterior side of the arm. With the same arm hanging loosely by side, the tape was positioned at previously marked midpoint of upper arm and the circumference was observed.

**Bio-Chemical Parameters:** Blood was routinely drawn monthly. It was including hemoglobin, blood urea nitrogen, serum creatinine, sodium, potassium, albumin, total protein, and if patient was diabetic then fasting and post prandial blood sugar.

For the analysis all bio-chemical variables was classified as below.

**Table 3.2 Grading of used biochemical variables as per self-design tools.**
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Bio Chemical Assessment</th>
<th>Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Hb</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 10 gm/ dl</td>
<td>Group A</td>
</tr>
<tr>
<td></td>
<td>10 - 12 gm/dl</td>
<td>Group B</td>
</tr>
<tr>
<td></td>
<td>&gt;12gm/dl</td>
<td>Group C</td>
</tr>
<tr>
<td>2</td>
<td><strong>BUN</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 - 25miligram/dl</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>&gt;25miligram/dl</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td><strong>Creatine</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 to 6 mg/dl</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>6 to 12 mg/dl</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>12 to 18 mg/dl</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>18 to 24 mg/dl</td>
<td>D</td>
</tr>
<tr>
<td>4</td>
<td><strong>Serum sodium (Na)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 120mmol/l</td>
<td>Sever hyponatremia</td>
</tr>
<tr>
<td></td>
<td>120 - 133mmol/l</td>
<td>Moderate Hyponatremia</td>
</tr>
<tr>
<td></td>
<td>133 - 146mmol/l</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>&gt;146mmol/l</td>
<td>Hypernatremia</td>
</tr>
<tr>
<td>5</td>
<td><strong>Serum potassium (K)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;3.8mmol/l</td>
<td>Hypokalemia</td>
</tr>
<tr>
<td></td>
<td>3.8 - 5mmol/l</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>&gt;5mmol/l</td>
<td>Hyper kalemia</td>
</tr>
<tr>
<td>6</td>
<td><strong>Serum albumin</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;3.5gm/dl</td>
<td>Hypoalbuminemia</td>
</tr>
<tr>
<td></td>
<td>3.5 - 5.5gm/dl</td>
<td>Normal albumin</td>
</tr>
<tr>
<td>7</td>
<td><strong>Total protein</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;6gm/dl</td>
<td>Hypoproteinemia</td>
</tr>
<tr>
<td></td>
<td>6 - 8.4gm/dl</td>
<td>Normal protein</td>
</tr>
<tr>
<td>8</td>
<td><strong>Blood sugar- fasting</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;90mg/dl</td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td>Blood sugar- PP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;130 mg/dl</td>
<td>below controlled</td>
<td></td>
</tr>
<tr>
<td>130-180 mg/dl</td>
<td>Controlled</td>
<td></td>
</tr>
<tr>
<td>&gt; 180mg/dl</td>
<td>Uncontrolled</td>
<td></td>
</tr>
</tbody>
</table>

**Hemoglobin:** It is the most important biochemical test carried out in hemodialysis because Anemia is very common in hemodialysis patients. It was estimate to know the severity of anemia. All hemoglobin values were categorized into three stage- A- <10gm/dl   B- 10-12 gm/dl   C-> 12gm/dl.

**Blood Urea nitrogen (BUN):** It was used to estimate net protein degradation or recent protein intake in patients undergoing maintenance hemodialysis.

**Serum Creatinine:** High serum creatinine is an indication of renal failure, during hemodialysis it will be always in higher side.

**Serum Sodium:** Daily dietary sodium intake should be restricted to no more than 5 g of sodium chloride (2.0 g or 85 mmol of sodium). (Clinical Practice Guidelines and Clinical Practice Recommendations 2006 Updates).

**Serum potassium:** Most patients with renal problem do not require aggressive dietary potassium restriction.

Hypokalemia (K+< 3.5 mEq/L).

- ↑ renal losses (diuresis)
- ↑ GI losses (diarrhea, vomiting, fistula)
- K+ wasting meds (thiazide and loop diuretics, etc.)
- Shift into cells (anabolism, re-feeding, correction of glucosuria or DKA)
• Inadequate intake
• Hyperkalemia (K+>5.0 mEq/L)
• Decreased renal excretion as in acute or chronic renal failure
• Medications, e.g. potassium sparing diuretics, beta blockers, ACE inhibitors
• Shift out of cells (acidosis, tissue necrosis, GI hemorrhage, hemolysis)

**Serum albumin**: Serum albumin and total protein was obtained to assess the nutritional status of these hemodialysis patients, as several studies have demonstrated that albumin is a valid indicator of nutritional status in hemodialysis patients. Serum albumin is obtained from all patients in three month. Normal range of serum albumin 3.5-5.5 gm/dl.

**Blood sugar**: A normal fasting blood glucose target range for an individual without diabetes is 70-100 mg/dL. The American Diabetes Association recommends a fasting plasma glucose level of 70–130 mg/dL and after meals less than 180 mg/dL (10 mmol/L).

Normal ranges of all bio-chemical parameters which are used in study according to S.G.P.G.I.M.S. pathology lab are given below:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine</td>
<td>mg/dl</td>
<td>0.5 – 1.6</td>
</tr>
<tr>
<td>Serum BUN</td>
<td>mg/dl</td>
<td>8.0 – 25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Serum Sodium</td>
<td>mmol/L</td>
<td>133 – 146</td>
</tr>
<tr>
<td>Serum Potassium</td>
<td>mmol/L</td>
<td>3.8 – 5.8</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/dl</td>
<td>6.0-8.40</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>g/dl</td>
<td>3.5 – 5.5</td>
</tr>
<tr>
<td>Hemoglobin (Hb)</td>
<td>gm/dl</td>
<td>Male - 12-16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female - 12-14</td>
</tr>
<tr>
<td>Blood Sugar (Fasting)</td>
<td>mg/dl</td>
<td>90-130</td>
</tr>
<tr>
<td>Blood sugar (PP)</td>
<td>mg/dl</td>
<td>130-180</td>
</tr>
</tbody>
</table>

**Dietary Assessment**

Food consumption pattern was assessed on consecutive 3 days dietary recall method. A set of standardized vessels were used to obtain estimates of the amount of raw and cooked food consumed by the patients. The ingredients used in preparing the food and method of preparation was carefully recorded.

Data regarding the frequency of food consumption, choices of foods after the onset of disease and after dialysis were documented. The foods specially included, restricted and avoided for the present condition, reasons and who advised were recorded.

A 3 days dietary record was completed by the patient or one of his relatives. The patient or his relative was instructed on the recording method by using the house hold measurements. Dietary record was then reviewed with patients to check the reliability.

After assessment of daily dietary intake of the individual patients, diet chart was given to the patients as per their requirements. The requirement of energy, fuel nutrients and electrolytes were computed based on the ideal body weight, range of energy prescribed for the patient, associated conditions and age of the patients. The fuel nutrients such as proteins, fats, carbohydrates and electrolytes like sodium and potassium consumed by each group of patient’s viz. baseline and follow-up were compared with the
requirements for each patient. Further, the average nutrients intake of the patients were calculated.

The National Kidney Foundation (Kidney Disease Outcomes Quality Initiatives) (NKF-K/DOQI), adopted to decide the dietary requirement of their maintenance hemodialysis.

**Table 3.4: KDOQI Guidelines for nutrient needs in hemodialysis**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Hemodialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (&gt;/=50% HBV protein)</td>
<td>1.2 gm/kg</td>
</tr>
<tr>
<td>Energy</td>
<td>35 kcal/kg &lt;60 years</td>
</tr>
<tr>
<td></td>
<td>30-35 kcal/kg &gt;60 years</td>
</tr>
<tr>
<td>Sodium</td>
<td>2gm/day</td>
</tr>
<tr>
<td>Potassium</td>
<td>2 – 3gm Monitor serum levels</td>
</tr>
<tr>
<td>Fluid</td>
<td>As per 24 urine output.</td>
</tr>
</tbody>
</table>

**Dietary Protein Intake In maintenance Hemodialysis**

NKF-K/DOQI (2000) recommended dietary protein Intake for clinical stable maintenance hemodialysis patients is 1.2 gm/kg/body weight. At least 50% of the dietary protein should be of high biological value. Carbohydrate requirement was computed by taking 50-60 per cent of total energy.

**Dietary Energy Intake In maintenance Hemodialysis**

The recommended daily energy intake for maintenance hemodialysis patients is 35 Kcal/kg body weight /day for those who are less than 60 years of age and 30-35 Kcal/kg
body weight/day for individuals 60 year or older. Energy expenditure of patients undergoing maintenance hemodialysis is similar to that of normal health individuals.

Total daily energy intake of about 35 Kcal/kg/day induces neutral nitrogen balance and is adequate to maintain the serum albumin and anthropometric indices. Because individual more than 60 year of age tend to be more sedentary a total energy intake of 30-35 Kcal/kg body weight acceptable.

Carbohydrate requirement was computed by taking 50-60 per cent of total energy. Simple sugar was excluded for diabetic patients. For diabetic patients, a total meal was distributed in three meals and two snacks to control the hypoglycemia.

**Dietary Sodium**

Protein of high biological value (HBV), as well as total energy and estimates of intake of sodium and potassium were calculated.

Daily dietary sodium intake should be restricted to no more than 5 g of sodium chloride (2.0 g or 85 mmol of sodium). When observing a low-sodium diet, in addition to refraining from adding salt during cooking and at the dining table, canned, processed, and salty-tasting food should be avoided. Before counseling patients with problematic fluid gains, it is essential to review their serum sodium. Tips to reduce salt (sodium) were given to the patients.

**Dietary Potassium**

Potassium requirement for dialysis were 2-3 g/day. The foods specially included, restricted and avoided for the present condition, reasons and who advised were recorded. Potassium intake to be advised according to pre dialysis potassium levels. For patients with a tendency to high pre dialysis potassium level, we use potassium free dialysate. This practice brings down post dialysis potassium level more and thereby minimizes potassium restriction. Again, it is important to ensure that dietary restriction does not compromise energy and protein intake.
Dietary fluid

In patients on maintenance hemodialysis, fluid and salt intake should be such that interdialytic weight gain does not exceed 1 to 1.5 kg. For patients with good urine output, no restriction may be necessary while anuric patients may need stringent restriction. So fluid intake should be as 24 hour urine output.

Statistical Analysis

Statistical analysis done by the computer. Minitab 16 and MS Excel Software were used for data analysis and appropriate tests applied as per objectives.

Variables were summarized by frequency and measures of central tendency and dispersion. Significant tests were used, including Chi square test for measuring difference between discrete variables and t-test for measuring difference between continues variables. Correlation and partial correlation tests were used for measuring association between different variables.

Percentage:

In this study percentage was calculated as follows-

\[
\text{Row } \% = \frac{\text{Frequency} \times 100}{\text{Row Total}}
\]

\[
\text{Column } \% = \frac{\text{Frequency} \times 100}{\text{Column Total}}
\]

Mean :
The sample mean of a variable \( X \) is the sum of the \( X \)-scores for a sample of the population divided by the sample size:

\[
\bar{x} = \frac{\sum x_i}{n} = \text{Sum of } x \text{-values} \quad \text{Sample size}
\]

**Standard Deviation**

The standard deviation of a set of measurements is defined to be the positive square root of the variance etc.

The variance of a set of \( n \) measurements \( y_1, y_2, \ldots, y_n \) with mean \( \bar{y} \) is the sum of the squared deviations divided by \( n - 1 \):

\[
\frac{\sum (y_i - \bar{y})^2}{n - 1}
\]

**Chi-square test**

The chi-square test for association whether the percentage in each outcome category significantly differs for two samples.

**To test:-**

**Ho:** Two attributes are independent vs.

**H1:** Two attributes are not independent. (They are associated)

**Test Statistics:-**

\[
\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{s} \frac{O_{ij}^2}{e_{ij}} - N \quad \rightarrow \quad \chi^2_{(r-1)(s-1),\alpha} \quad \text{distribution}
\]

\( r \): No. of rows
s: No. of columns

Where

\[ e_{ij} = \frac{(Ai)(Bj)}{N} \]

**t-Test: Paired Two Sample for Means**

The paired t-test examines the mean difference between dependent observations.

\[ T = \frac{\bar{x}_1 - \bar{x}_2}{S \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \rightarrow t_{n_1+n_2-2} \]

Where

\[ S^2 = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2} \]

**COVARIANCE AND CORRELATION**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population formula</th>
<th>Sample formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covariance between variables X and Y</td>
<td>( \text{COV}(X,Y) = E(XY) - E(X)E(Y) )</td>
<td>( \sum (x_i - \bar{x})(y_i - \bar{y}) ) [ n-1 ]</td>
</tr>
<tr>
<td>Pearson’s correlation</td>
<td>( \frac{\text{Cov}(X,Y)}{\sigma_x \sigma_y} )</td>
<td>( \sum xy - n \bar{xy} ) [ s_x s_y ]</td>
</tr>
</tbody>
</table>

**ANOVA F test (One-Way Analysis of Variance):**

Comparing Several Means

- Anova F tests whether all of I populations have the same mean, based on independent SRSs from I normal populations with the same \( \sigma \). \( P \)-values come from the F distribution with I – 1 and \( N – 1 \) degrees of freedom, where \( N \) is the total observations in all samples.
- Describes the data using sample means and standard deviations and side by side graphs of the samples.
- The ANOVA F test statistic (use software) is $F = \frac{MSG}{MSE}$, where

\[
MSG = \frac{n_1 (\bar{x}_1 - \bar{x})^2 + \cdots + n_I (\bar{x}_I - \bar{x})^2}{I - 1}
\]

\[
MSE = \frac{(n_1 - 1)s_1^2 + \cdots + (n_I - 1)s_I^2}{N - I}
\]

**Level of Significance:**

Every study was calculated by the two level i.e 0.05 and 0.01. Null hypothesis was suspected when the statistics was significant at 0.05 level and 0.01 level of the statistics was highly significant and a very little risk of type 1 error was there in rejection the null hypothesis.