MATERIALS AND METHODS

SOURCE OF DATA:

- Study will be conducted in the Department of Biochemistry with the collaboration of Department of Gynaecology Rama Medical college, Hospital And Research Institute, Kanpur, U.P.
- The study will be approved by the Ethical Committee of the institution and Professors and Head of the Department of Gynaecology.
- A written informed consent, in the vernacular language will be obtained from all the participants, upon fulfilling the inclusion criteria

SAMPLE SIZE CALCULATIONS AND STATISTICAL ANALYSIS

Sample size has been calculated in order to control type I & type II error. Assuming a minimum power 80% and 95% significance level.

Sample size has been calculated using this formula:

\[ n \geq \frac{Z^2 \cdot \alpha}{d^2} \cdot P(1-P) \]

\[ Z = 1.96 \]

- \( p \) - Incidence of the disease (polycystic ovary syndrome)
- \( Z\alpha/2 \) - is the level of confidence we have chosen 95% confidence in this \( Z\alpha/2 = 1.96 \).
- \( d \) = Allowable error=5%
- When \( P \) indicates the incidence of the clinical condition e.g. Polycystic ovary syndrome
- Following the literature the incidence of Polycystic ovary syndrome has been assumed between 5 to 10%
- The calculated minimum size for our study is 85.
- The calculated minimum size for our control is 85.
- In order to control loss of follow up and manual error we take the sample size of 100 for each group.
- Data will be collected and entered in MS excel worksheets and results will be analysed with appropriate statistical tools like, tests of significance, logistic regression analysis etc using SPSS version software.
**STUDY GROUP:**

The subjects selected for study will be grouped as follows:

**Group I:** [Control group]

- This group will consist of normal reproductive and fertile women.
- Age group between 20 to 40 years

**Group II:** [PCOS patients]

**INCLUSION CRITERIA:**

- Diagnosed clinically having PCOS as a case of infertility.
- Subjects of age group 20 to 40 years.

**EXCLUSION CRITERIA**

- Female with any other reproductive disorder.
- Female below 20 and above 40 year group.
METHOD OF EXAMINATION:

5ml venous blood sample will be collected under aseptic precaution then serum is separated within an hour and store it at -2 to -4°C.

The following investigation has been done:

ESTIMATION OF ADIPONECTIN LEVEL:

Method: Elisa method

Principle: Sandwich enzyme immunosorbent assay principle is applied in this kit. In this kit microtiter plate is being given which is precoated with an antibody specific to Adiponectin. Microtiter plate wells with a biotin-conjugated antibody which is specific to Adiponectin (ADP) samples are added in it. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, the well that consist of adiponectin, biotin-conjugated antibody and enzyme-conjugated Avidin have a colour change. As soon as sulphuric acid solution is added the enzyme-substrate reaction is stopped change in colour is spectrophotometrically evaluated at a wavelength of 450nm ± 10nm. The concentration of Adiponectin (ADP) in the samples is determined by comparing the O.D. of the samples to the standard curve.

ESTIMATION OF LEPTIN LEVEL:

Method: Elisa method

Principle:

Sandwich enzyme immunosorbent assay principle applied in this kit. The microtiter well had a coating of a monoclonal antibody which is directed towards a unique antigenic site on leptin molecule. The sample of a patient kept in an aliquot is incubated in the coated well with a specific rabbit antileptin antibody and there is a formation of sandwich complex. When the incubation is completed the unbound material is being washed out. Then an antirabbit peroxidase conjugate is added for the detection of bound leptin. The intensity of colour which is being developed is proportional to the concentration of leptin in the sample of patient.
**Oxidative stress Biomarker MDA (Malonaldehyde) level:**

Serum - A serum separator tube is used and samples are allowed to clot for two hours at room temperature or overnight at 4 oC before centrifugation for 20 minutes at approximately 1,000xg.

Assay freshly prepared serum immediately or store samples in aliquot at -20oC or -80oC for later use.

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**Estimation of MDA level:**

**Method: Elisa method:**

**Principle:** The quantitative measurement of Malondialdehyde in serum in vitro analysis is being done by the kit which provides a test principle of competitive inhibition enzyme immunoassay technique.

In this technique microplate is being precoated with a monoclonal antibody which is specific to malondialdehyde. There is a competitive inhibition reaction between biotin labeled malondialdehyde and unlabeled malondialdehyde with the precoated antibody which is specific to malondialdehyde. When the incubation is over the conjugate which is not bound is washed off.

Then we add avidin which is being conjugated to Horseradish peroxidise(HRP) to each microplate well and then incubation is being performed. The amount of HRP conjugates which is bounded is inversely proportional to the concentration of malondialdehyde in the sample. After addition of substrate solution the intensity of colour developed is reverse proportional to the concentration of malondialdehyde in the sample.
Oxidative Stress Biomarker SOD(superoxide dismutase) level:

Estimation of SOD level:

Method: Elisa method

Principle:

The quantitative measurement of superoxide dismutase level in vitro is applied by the Elisa kit method. The principle behind this measurement is sandwich enzyme immunoassay technique.

The coating of monoclonal antibody is done on microtiter plate. The sample is being added to the microtiter plate wells and if there is a presence of SOD it will bind to the antibody pre-coated wells.

Now the amount of SOD present had to be determined quantitatively in the sample.

We take a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody which is being specified for SOD are added to each well inorder to "sandwich" the SOD which is being immobilized on the plate.

Then incubation of microtiter plate is being done and the wells are washed nicely to remove the components which is not being bounded.

Then a substrate solutions are added to each well.

The substrate and the enzyme (HRP) are allowed to react for a very short incubation period. The wells that contain SOD and enzyme-conjugated antibody will able to change the color. The enzyme-substrate reaction is cut out as soon as sulphuric acid solution is added and the change in colour is measured spectrophotometrically at a wavelength of 450 nm.

Taking in account the intensity of the colour (O.D) to the concentration of standards a curve is being plotted.