MATERIALS AND METHODS:

SOURCE OF DATA:
Study will be conducted in the Department of Biochemistry with the collaboration of
department of General Medicine in RAMA MEDICAL COLLEGE, Kanpur, U P
The study will be approved by the Ethical Committee of Institution. A written
informed consent, in the vernacular language, will be obtained from all the
participants, upon fulfilling the inclusion criteria

SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS:
Sample size will be calculated in order to control type I & type II error. Assuming a
minimum power 80% and 95% significance level sample size will be calculated using
this formula.

\[
n = \frac{2(P)(1-P)(Z\beta + Z\alpha/2)^2}{d^2}
\]

\(p\)- Incidence of the disease (Type 2 Diabetes Mellitus)
\(q=(1-p)\)
\((P1-P2)^2\) or \(d^2\) – Is the difference which we want to detect at a specified power & level
of confidence.
\(Z\beta\) - power of statistical test we want to be minimum 80% for which is \(Z\beta\) is 0.84.
\(Z\alpha/2\) - is the level of confidence we have chosen 95% confidence in this \(Z\alpha/2=1.96\).
When \(P\) indicates the incidence of the clinical condition e.g.: Type 2 Diabetes
Mellitus.
Following the literature the incidence of Type 2 Diabetes Mellitus will be assumed
between 10%.
The calculated minimum sample size for our study is 141.
The calculated minimum sample size for control group is 141.
In order to control loss of follow up and manual errors, for which we rounded the
sample size of 200 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 21\textsuperscript{st} version software.
METHOD OF SAMPLING:
The subjects selected for study will be grouped as follows;

**Group I – control group (n=200)**
This group will consist of age and sex matched non-diabetic healthy subjects. They are free from any major ailment which could affect the parameters under study.

**Group-II – Type 2 Diabetes Mellitus patients without microvascular complications (n=200)**

**Inclusion criteria**
1. Duration of Diabetes Mellitus is 5 years or less
2. They are on hypoglycemic drugs and life style modifications
3. They are free from clinical evidence of any microvascular complications of diabetes mellitus

**Group –III Type 2 Diabetes mellitus patients with Microvascular complication (n=200)**

**Inclusion criteria**
1. Duration of Diabetes Mellitus is minimum 5 years or more
2. They are on life style modifications, oral hypoglycemic drugs, insulin or combinations of all three.
3. Associated with one or more micro vascular complications of diabetes mellitus; either of diabetic nephropathy diabetic retinopathy or diabetic neuropathy

**Exclusion criteria:**
Patients with type I Diabetes Mellitus
- Pregnant and lactating females
- Patients taking diuretics, lipid lowering, and multivitamins drugs.
- Patients with disease unrelated to diabetes which may alter chosen parameters i.e. Renal disease, thyroid disease, tuberculosis, and cancer patients.
Method of examination:

For each case and control group, three ml of venous blood sample in the fasting state will be collected under aseptic precautions. An additional two ml venous blood will be collected in EDTA vials for the estimation of glycated haemoglobin (HbA1c). Urine sample will be collected in universal container for urinary albumin estimation. Serum separated within an hour and stored at -2-4°C temperature.

The following investigations will be done;

1. **Blood glucose (FBS & PP2BS):** Glucose Oxidase-Peroxidase method (GOD-POD).

2. **Serum Insulin Resistance:** HOMA-IR (Homostasis model assessment of Insulin Resistance).

3. **Serum Zinc:** NITRO-PAPS Method (kit supplied by Tulip diagnostics).

4. **Serum Magnesium:** Calmagite indicator method (Kit by Tulip Diagnostics).

5. **Serum Copper:** Di-Br-PAESA method (Kit by Coral Diagnostics).

6. **Serum MDA:** By Thiobarbituric acid reagent (TBA) Method.

7. **Serum HbA1c:** HbA1c levels will be estimated based on latex agglutination inhibition assay. (The kit is from M/s Randox).

8. **Urinary microalbumin:** Pyrogallol red colorimetric end point essay (Kit by Tulip Diagnostics).

9. **Vitamin D:** Chemiluminescence assay (kit by Abbott architect company).