Material and Methods:

Present study conducted in Department of pharmacology in collaboration with department of Medicine, MGM Medical College, Aurangabad. Study was conducted during the period of August 2017 to July 2018 after taking approval from MGM MECHS (2017/22). Study was conducted as per ICH-GCP guidelines.

Study Design:

Present study is Comparative, Prospective, randomized, Open-label, Single Center, Parallel group study conducted at MGM Medical college, Aurangabad. Study was conducted in prediabetes patients for assessment of effects of FDC of Metformin with Voglibose and Metformin with Pioglitazone. All patients were evaluated at baseline, 3 months and 6 months for clinical and physical examination and laboratory investigation.

Present Study was conducted in prediabetes Patients for assessment of effect of FDC of Metformin with Voglibose and Metformin with Pioglitazone on Serum Insulin, HOMA-IR, FBS, PPBS, HbA1c and lipid levels attending the outpatient department of Medicine in MGM Hospitals and College, Aurangabad. Details of FDC of Metformin and Voglibose and Metformin with Pioglitazone used in study are as follows:

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of FDC</th>
<th>Brand Used</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tab Metformin500mg + Tab Voglibose 0.2mg BD for 6 months</td>
<td>Tablet Volibo M 0.2mg</td>
<td>Sun Pharmaceutical Industries Limited</td>
</tr>
<tr>
<td>2.</td>
<td>FDC of TabMetformin500mg + Tab Pioglitazone7.5mg BD for 6 months</td>
<td>Tablet Pioz MF 7.5 SR</td>
<td>USV Pharmaceutical Limited</td>
</tr>
</tbody>
</table>
Duration of study:
- Total duration of the study was 1 Year.

Sample size: The sample size for the study was 67 patients.

It is calculated by this formula: 
\[ n = \frac{2 (Z\alpha + Z\beta)^2 \sigma^2}{\delta^2} \]

Whereas: 
- \( Z\alpha = \) level of statistical significance (2.326 for 99% level of confidence),
- \( Z\beta = \) desired power (1.28 for Power= 0.80)
- \( \sigma^2 = \) standard deviation \( \frac{(1.52 + 1.67)}{2} = 1.59 \)
- \( \delta^2 = \) difference of two group mean \( X_2 - X_1 = 9.60 - 8.60 = 1.00 \)

\[ n = \frac{2 (2.36 + 1.28)^2 (1.59)^2}{(1)^2} \]

\[ = 26 \times 2.52 \]

\[ = 65.73 \]

\[ = 67 \]
Inclusion Criteria:

- Patients with prediabetes diagnose according to ADA criteria (IFG: 100 - 125mg/dl, 2hrs post glucose plasma level 140- 199 mg/dl) and with Indian obesity (BMI > 25 kg/m²) as per Indian Endocrine Society.

- Patients of either gender between age group 30 to 60 years.

- HbA1c between levels of 5.7-6.4% %.

- Patient willing to give informed written consent.

Exclusion Criteria:

- Patients with history of Type I DM and Type II DM.

- Patients with history of cardiac, liver and renal disease.

- Patients with history of Gastrointestinal Tract diseases (IBD).

- Patients with history of hypothyroidism and hyperthyroidism

- Patients with history of Alcohol intake & Smoking.

- Patients taking steroid, oral contraceptives & hormone replacement therapy

- Pregnant and lactating females.
Ethics, consent and permissions:

- After approval from Institutional Ethics Committee for Medical Research at MGM Hospital, study was initiated.

- All the prediabetes patients were provided written, vernacular, informed consent to participate in the study.

- Study was conducted as per Declaration of Helsinki, ICH good Clinical Practice (GCP) guidelines and the ICMR guidelines for Biomedical Research on Human Subjects, 2006.

Methodology

Subjects:

- All naïve patients with prediabetes according to ADA guidelines were enrolled in the study from department of Medicine.
- Patient fulfilling the criteria enrolled in the study after taking ICF
- Permission from treating consultant was obtained for subjects to participate in the study.
- Patients randomized by chit method into either Group A (FDC of Metformin and Voglibose) and Group B (FDC of Metformin and Pioglitazone)
- Patients were investigated at baseline for list of investigation

  a) Fasting Blood Glucose  
  b) Postprandial Blood Glucose  
  c) HbA1c  
  d) Serum Insulin  
  e) HOMA-IR  
  f) Total cholesterol  
  g) Triglycerides  
  h) HDL  
  i) LDL  
  j) VLDL
In present study, total number of patients screened are around 1636 out of which 150 were randomly allocated into two groups.

**STUDY FLOW CHART:**

1636 patients were screened and 150 Fulfilled criteria

Enrollment ➔ Randomization (n=144)

- 6 Patients were excluded
  - 4 patients were out of Aurangabad
  - 2 patients not interested to give consent

72 patients were allocated in group A (Metformin 500 mg + Voglibose 0.2 mg BD)

- 1 patients lost for follow up and 4 patients were rescue medicine on Pantoprazole.

72 patients were allocated in Group B (Metformin 500 mg + Pioglitazone 7.5 mg BD)

Follow-Up ➔ Analysis

- 2 patients opted out of study due to weight gain
- 3 patients opted out of study due to edema

Analyzed (n=67)

Analyzed (n=67)
**Follow-up Visits:**
Follow-up visits were scheduled at the end of three months and six months for assessment, including measurement of weight and general and systemic examination and laboratory parameters (Serum Insulin, HOMA-IR, HbA1c, Blood glucose and Lipid levels).

**Biochemical Parameters:**
The following laboratory investigation were performed on sample of prediabetes patients before and at the end of 3rd month and 6th month of therapy.

1. Serum Insulin
2. HOMA-IR
3. HbA1c
4. Fasting Blood Glucose
5. Postprandial Blood Glucose
6. Total cholesterol
7. Triglycerides
8. HDL
9. LDL
10. VLDL
Safety parameters:

- The safety was evaluated objectively by recording the ADR in the standard format of ADR reporting form and further reported to PvPi MGM Medical college.

- The patients were explained about possible ADR with drugs during the study.

- Patients with Adverse Events were advised to contact their Principal investigator through telephonic/physically if any adverse events occurred. The nature, time of onset, and severity of the event, the treatment needed, and any relation to the assigned study regimen was recorded.

Statistical Analysis:

- The collected data was compiled in MS Excel sheet for analysis.

- Data analyzed in Statistical Package for the Social Sciences (SPSS) version 25th was applied.

- The qualitative data was represented in the form of frequencies and percentage also represented in visual impression like bar diagram.

- Quantitative data was represented in the form of mean and standard deviation. To check significance difference between baseline and after three months’ effect of FDC of Metformin and Voglibose Versus FDC of Metformin and Pioglitazone in prediabetes patient

- A paired ‘t’ test was applied for same group/within group and also quantitative data was represented in the form of pie diagram and bar diagram.

- An unpaired ‘t’ test was applied for two groups and also quantitative data was represented in the form of pie diagram and bar diagram

- p value <0.05 indicates Statistically significant.
MATERIALS

Biochemical assays: All biochemical assays were carried out with Automated Random access clinical chemistry analyzer ERBA Chem 7 with ERBA TEST REAGENT (Transasia Bio-medicals Ltd., India).

Procedure:

Blood samples were collected by vein puncture under all aseptic precautions from subjects using disposable syringes in fasting condition and Post Meal were collected in tubes containing Sodium fluoride as an anticoagulant.

BLOOD GLUCOSE: \[266\]

Fasting and Postprandial was done on auto analyzer by glucose oxidase /peroxidase [GOD / POD] method.

Principle:

1. Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxidase.

2. The hydrogen peroxide is then oxidatively coupled with 4 aminoantipyrine (4AAP) and phenol in the presence of peroxidase (POD) to yield a red quinonemine dye this measured at 505nm. The absorbance at 505 nm is proportional to concentration of glucose in the sample.

\[
\begin{align*}
\text{Glucose Oxidase} \\
\text{Glucose+ } O_2 & \rightarrow \text{Gluconic acid + H}_2O_2 \\
\text{Peroxidase} \\
2H_2O_2 + \text{phenol} + 4\text{-Aminoantipyrine} & \rightarrow \text{red Quinonemine} + 4H_2O
\end{align*}
\]

Normal range: Random Blood Glucose- 60-120 mg/dl
Glycated Hemoglobin/ Hemoglobin A1c (HbA1c): ²⁶⁷

Principle:
The D-10 utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge. The haemoglobins are separated based on their ionic interactions with the cartridge material. The separated haemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured.
**Serum Insulin:** [268]

**Principle:**
The Insulin (Human) CLIA Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-insulin antibody for solid phase (microtiter wells) immobilization and another anti-insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the Insulin antibody coated microtiter wells. Then anti-insulin antibody labeled with horseradish peroxidase (conjugate) is added. If human Insulin present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 1-hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of Insulin in the sample. By reference to a series of Insulin standards assayed in the same way, the concentration of Insulin in the unknown sample is quantified.
LIPID PROFILE

**CHOLESTEROL** [269]: -

**Method:** - Estimation of serum cholesterol by the end point enzymatic method

**Principle:** - 1. Cholesterol estimation is an enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase.

2. Cholesterol esterase enzymatically hydrolyses cholesterol esters into free cholesterol and fatty acids.

3. Free Cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase,

4. The hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce quinoneimine dye, which has absorbance maximum at 505nm. The intensity of red color is proportional to the amount of cholesterol.

\[
\text{Cholesterol esterase} \\
\text{Cholesterol - ester + H}_2\text{O} \rightarrow \text{Cholesterol + Fatty acid}
\]

\[
\text{Cholesterol oxidase} \\
\text{Cholesterol + O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{Cholesterol-4-en-3-one}
\]

\[
\text{Peroxidase} \\
2\text{H}_2\text{O}_2 + 4 \text{Amino-antipyrine + Phenol} \rightarrow \text{quinoneimine + 4H}_2\text{O}
\]

Normal Cholesterol level: - < 200mg/dl.
**TRIACYLGLYCEROL**<sup>270</sup>

**Method:** - Estimation of serum triacylglycerol by Glycerol phosphate oxidase method.

**Principle:** - 1. Lipoprotein lipase hydrolyzes triacylglycerol to glycerol and free fatty acids.

2. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol-3-phosphate and ADP.

3. Glycerol-3-phosphate which oxidized by glycerol phosphate oxidase to form dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H₂O).

4. The hydrogen peroxide further reacts with phenolic compound and 4-amino antipyrine (4AAP) by the catalytic action of peroxidase to form a colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of triglyceride present in the sample.

Normal reference values – 40-150mg / dl.
**HDL- CHOLESTEROL** [271]

**Method:** - The estimation was carried out by Phosphotungstate Precipitation method.

**Principle:** - 1. The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. 2. LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER).

3. The enzymes selectively react with HDL to produce H$_2$O$_2$ which is detected through a Trinder reaction.

\[
\text{PVS / PEGME} \\
\text{HDL + LDL + VLDL + CM} \rightarrow \text{HDL + (LDL + VLDL + CM) \cdot PVS / PEGME}
\]

\[
\text{CHOD, CHER} \\
\text{HDL} \rightarrow \text{Fatty Acid+ H,0}
\]

\[
\text{Peroxidase} \\
2\text{H}_2\text{O}_2 + 4-\text{AA} + \text{TODB} \rightarrow \text{Quinone + 5 H}_2\text{O}
\]

Normal reference interval for HDL-Cholesterol is 30-70 mg/dl.
**DETERMINATION OF LDL AND VLDL**

LDL and VLDL were calculated from the estimated values of Cholesterol, Triglyceride and HDL-C, using the equation of Frieldwald et al as given below.

\[
[LDL- \text{Cholesterol}] = [\text{Total Cholesterol}] - [\text{HDL-C}] - \text{Triglyceride}/5
\]

All the concentrations are given in mg/dL. The factor [Triglyceride], is an estimation of 5

VLDL-Cholesterol concentrations and is based on average ratio of Triglyceride to Total Cholesterol in VLDL.

*Normal reference range for LDL and VLDL-Cholesterol are 63-130mg/dL and 12-34 mg/dl respectively.*