RESEARCH PROTOCOL

EFFECTS OF METFORMIN AND COMBINATION OF METFORMIN AND PIOGLITAZONE ON plasma IL-6 AND IL-8 LEVELS IN POLYCYSTIC OVARIAN SYNDROME

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Sponsor:

Office of Research, Innovation and Commercialization

Khyber Medical University, Peshawar, Pakistan

Hayatabad, Phase 5.

Collaborations:

This study is a collaborative project between Khyber Medical University Peshawar; Mardan Medical Complex, Mardan.

Coordinating Centre:

Clinical Trial Unit, Khyber Medical University, Peshawar

Pakistan

Email: CTU@kmu.edu.pk

Study Chair

Dr Mohsin Shah

Collaborative Group Chairs

Dr Farhat Rehman

CTU KMU Staff:

Clinical Lead: Dr Asif Ali

Statistician: Dr Omar Malik

Project Manager: Dr Mohsin Shah, Dr Dure Shehwar Ali

Emergency contact:

Name: Dr Farhat Rehman; **Phone**: 0301 8192880

TITLE:

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Contents

1. Introduction:

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy affecting women of reproductive age, with a frequency of about 4 to 7% (11). The syndrome is characterized by chronic oligo-anovulation, polycystic ovaries and hyperandrogenism (1). The patient has signs and symptoms of infertility, increased risk of insulin resistance, irregular menstruation (oligo-amenorrhea), hyperinsulinemia, type II diabetes mellitus, insulin resistance, increase hair growth (1).

Metformin, basically a biguanide, is an anti-hyperglycemic agent that improves glucose tolerance, increases the action of insulin at cellular levels without affecting insulin secretion (2). Metformin has been used in the management of insulin resistance, hyperinsulinemia and lipid abnormalities (2). A positive effect of metformin is proved by several studies on both metabolic and reproductive aspects in PCOS women (9). Metformin reduces obesity and decreases serum c-reactive protein levels in PCOS women (10). Pioglitazone is a thiazolidinedione (TZD) with hypoglycemic (anti-hyperglycemic, antidiabetic) action in the management of diabetes. It is also useful for reducing the cardiovascular risks associated with polycystic ovarian syndrome, having both anti-inflammatory and anti-arteriosclerotic properties (3). Pioglitazone was suggested to reduce the incidence of diabetes mellitus by more than 50% with administration of pioglitazone in PCOS (3). Interestingly, the protection from developing diabetes mellitus these patients remained even when pioglitazone was stopped (3).

IL-6, a major pro-inflammatory cytokine, shows an essential part in endocrine system, particularly related to ovarian growth and the course of fertilization and implantation (1). IL-6 plays an important role in facilitating low grade chronic inflammation in patients with PCOS (1). IL-6 has been shown to be closely related to insulin resistance and cardiovascular abnormalities (4). Obesity, a major risk factor for type II diabetes, was reported to be associated with elevated IL-6 levels (4). IL-8 is a chemokine formed via macrophages and other cell types like endothelial cells, airway smooth muscle cells and epithelial cells (6). The levels of interleukin-6 and interleukin-8 were decreased in patients of PCOS after they reduced their insulin resistance and body weight (4). The manifestation of increased markers such as c-reactive protein, interleukin-6, interleukin-8 levels and

raised leukocyte count is indication of low grade inflammation in women with PCOS (8). Elevated levels of interleukin-6 and interleukin-8 were related with an increased risk of atherosclerosis and future myocardial infarction (4).

We designed this study to elucidate the role of metformin and combination of metformin and pioglitazone in reducing the levels of interleukin-6 and interleukin-8 in patients with PCOS. This will help us in deciding a better treatment regimen for patients with PCOS. Interleukin-6 and interleukin-8 may emerge as predictive biomarkers of treatment response.

2. Aims and objectives

OBJECTIVES:

The objectives of this study are

1. Primary

   To investigate the changes in the levels of interleukin-6 and interleukin-8 after 3 months treatment with metformin alone and combination of metformin and pioglitazone in patients with PCOS.
2. Secondary

   To evaluate insulin resistance in all the groups at baseline and after 3 months of treatment.

3. Hypothesis:

Combination of metformin and pioglitazone may decrease the levels of IL-6 and IL-8 in women with PCOS better than metformin.

4. Operational Definitions:

POLYCYSTIC OVARIAN SYNDROME: Polycystic ovarian syndrome, or PCOS, is a condition characterized by chronic oligo-anovulation, polycytic ovaries and hyperandrogenism.

METFORMIN: An oral hypoglycemic drug that decreases glucose production by the liver

and increases peripheral glucose uptake, used to treat type 2 diabetes.

PIOGLITAZONE: is a thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action to treat diabetes.

INTERLEUKIN-6 is a cytokine that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. It is in humans encoded by the IL6 gene (5).

INTERLEUKIN-8: is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells (6) and endothelial cells.

5. Materials and Methods:

a. STUDY DESIGN: Two-Arm Randomized Clinical trial.

b. STUDY SETTING: OPD patients in Mardan Medical Complex, Khyber Medical University

c. STUDY DURATION: Six Months after approval of proposal

d. SAMPLE SIZE:

The sample size was calculated using OpenEpi software. With an expected reduction in interleukin-6 and interleukin-8 values of 15% in the Metformin arm, we would need 102 women with PCOS to demonstrate a 40% reduction in interleukin-6 and interleukin-8 in the Metformin and Pioglitazone arm with a power of at least 80% and a ratio of 1 in the two arms.

Arm#1: 51 women with PCOS will be selected based on the inclusion and exclusion criteria. They will receive metformin according to the body weight with maximum dose of 1000 mg (BD) daily.

Arm#2: 51 women with PCOS will be selected based on the inclusion and exclusion criteria. They will receive metformin and pioglitazone combination according to the body weight with maximum dose of 1000 mg and 30mg (BD) daily.

e. SAMPLING TECHNIQUES: Blood samples will be taken from ante cubital vein after an overnight fast. Samples will be taken from all groups at the initiation and at the end of 3

months. Serum levels LH, FSH, prolactin, testosterone, interleukin-6, interleukin-8 and insulin will be analysed by ELISA method. Fasting glucose will be performed by chemistry analyzer. Evaluation of insulin resistance will be performed by using HOMA-IR Index (Homeostasis model assessment for insulin resistance) with following formula. :

HOMA-IR= fasting plasma insulin (μIU/mL) x fasting plasma glucose (mmol/L) /22.5

6. Subject Population:

6.1 Target population

Women with PCOS visiting the outpatient department of participating hospitals.

6.2 SAMPLE SELECTION:

Inclusion criteria:

PCOS women will have the following inclusion criteria: age group 20 - 40 years, oligo-anovulation, hirsutism, elevated serum testosterone levels, menstrual cycle irregulary. Diagnosis of PCOS on ultrasound will be made on the basis of European Society of Human Reproducion and Embryo/ American Society of Reproductive Medicine (ESHRE/ASRM criteria, 2003) according to which 10 or more follicular cysts ranging in size from 2-9 mm or increased ovarian volume of 10 cm in maximal diameter.

Exclusion criteria:

Women with previous history of cushing syndrome, hyperprolactinemia, late onset congenital adrenal hyperplasia, androgen-producing tumors, pregnancy or those taking medications that alters the biochemical or hormonal profile will be excluded by clinical and medical examination.

6.3 Screening:

Before the study-specific screening and investigations, patient will sign a written informed consent and the investigator will also sign the consent with date.

6.4 Registration:

Subjects will be registered before starting study treatment and a registration number will be allotted. Requests for registration will only be accepted from authorized investigators at sites that have received ethical approval. Treatment would be planned to start after registration. Registration would be done according to the instructions in the protocol only after all screening assessments have been performed and the responsible investigator has both verified the subject's eligibility and signed the completed registration form.

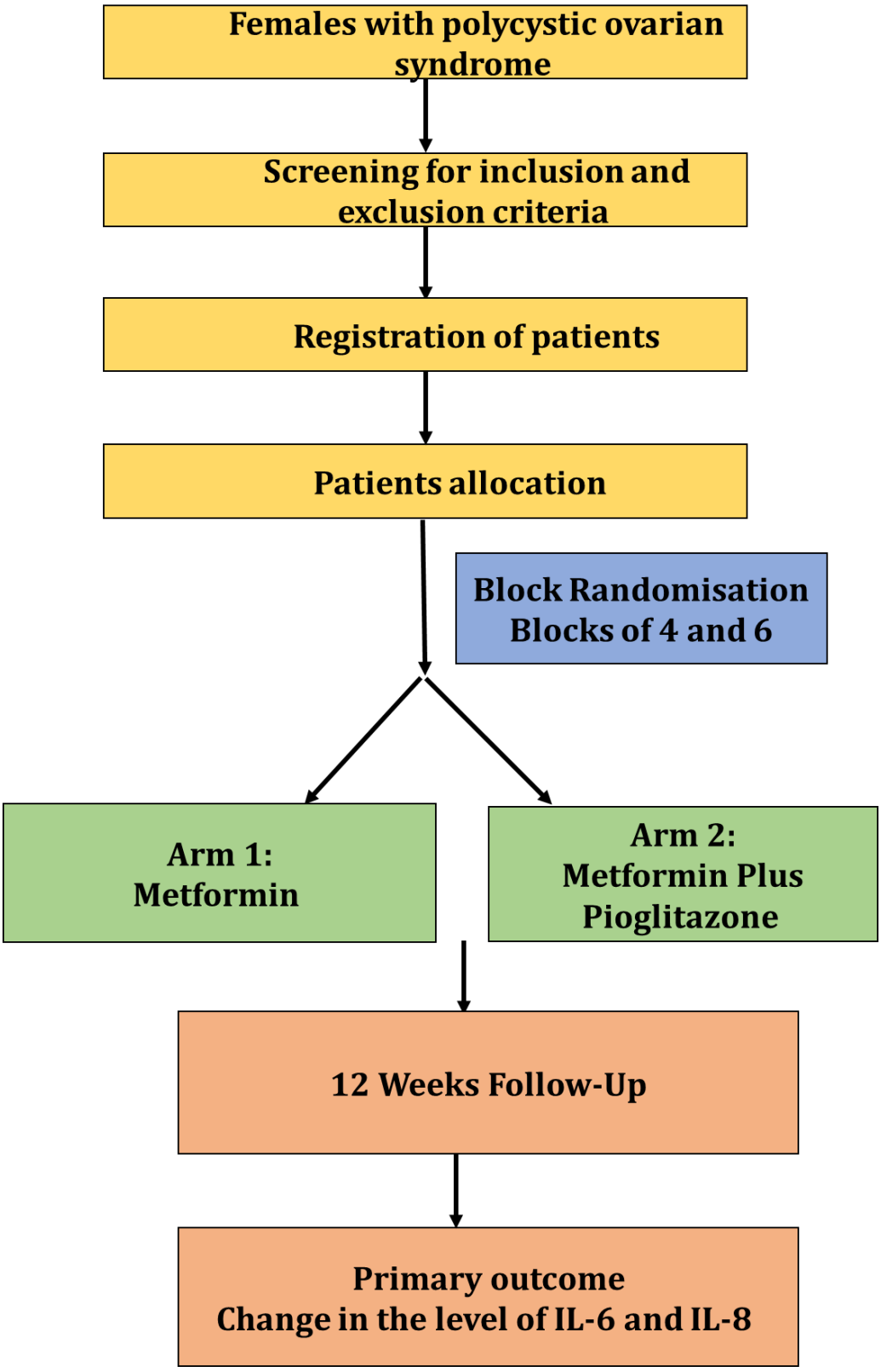
Once the registration process has completed as per the instructions in the protocol, the subject will be assigned a subject, study number, and written confirmation of registration will be provided to the site. Individuals must be registered once in this trial.

6.5 Randomization:

Randomization will be performed using Block Randomization technique using online software Sealed Envelope.

Appendix 1

Study Schema



7. Data Collection Procedure:

7.1 PATIENT RECUIREMENT:

Approval of the study will be obtained from the Ethical Review Committee of the Khyber Medical University Peshawar. Potential participants will be identified in the participatory center. Purpose and components of the study will be explained to each potential participant. Once the participant has agreed to take part, she will be screened for eligibility criteria. All eligible participants will be asked to give a written informed consent. Data regarding age, weight, height, menstural irregularity, hirsutism, past medical and surgical history, serum testosterone and fasting insulin will be taken. Each participant will undergo ultrasound for assessment of ovarian changes. The study participants will be grouped in the following two arms.

Arm#1: 51 women with PCOS will be selected based on the inclusion and exclusion criteria. They will receive metformin according to the body weight with maximum dose of 1000 mg (BD) daily.

Arm#2: 51 women with PCOS will be selected based on the inclusion and exclusion criteria. They will receive metformin and pioglitazone according to the body weight with maximum dose of 1000 mg and 30 mg (BD) daily.

Arm 1	**METFORMIN (1000 mg)**
Arm 2	**METFORMIN (1000 mg)** **AND PIOGLITAZONE (30 mg)**

7.2 Concomitant medication reporting and treatment:

Concomitant medications will not be recorded during the study. The following medications and treatment are recommended during the study

7.2.1 Permitted

The following medications are permitted during the study: NSAIDS, multivitamins, antibiotics and PPIs.

7.2.2 Use with caution

There are no medications that should specifically be used with caution in this study.

7.2.3 Prohibited

There are no medications and treatments that are specifically prohibited in this study.

7.2.4 Compliance

Subject medication compliance will be determined at each visit after 1 month and the patient will be counseled appropriately if significant non-compliance is determined.

7.2.5 Treatment discontinuation

The treatment of the patient or study on the subject will be discontinued in case of pregnancy or any disease given in exclusion criteria and in patient with hypersensitivity to drugs. There is no serious adverse effect observed after treatment discontinuation.

8. Assessment Plan:

HISTORY RELATED QUESTIONS AND CLINICAL ASSESSMENT

Name: Age: Date:

Address: Marital status: Phone No:

- Menstrual irregularity: _____
- Age of menarche: _____
- Hirsutism: _____
- Acne: _____
- Daily physical exercise: _____
- Infertility: _____
- Height (m) _____
- Weight (kg) _____
- BODY MASS INDEX (kg/m2): _____
- Drug history (current and past medications): _____
- Surgical history: _____
- BP: _____
- Pulse: _____

Schedule of assessments

	Screening	**Baseline investigations**	**On treatment**	**End of treatment**
	14-21 days prior to registration	Within 7 days prior to registration	Within 3 days prior to first dose	After 3 months
Informed consent				
Clinical assessment (mentioned above)				
Pelvic ultrasound right ovary and left ovary (10 or more follicles. 2 – 9 mm in diameter)				
Other imaging				
FBS RBS				
LH FSH Testosterone				
IL-6 IL-8				
HOME. IR				
Concomitant medications				
Adverse events				
Outcome events				

ASSESSMENT PHASE DEFINITONS AND SPECIAL CIRCUMTANCES

SCREENING

All screening will be done before registration of the patients.

BASELINE

All baseline investigations including (but not limited to) levels of interleukin-6 and interleukin-8, insulin resistance and pelvic ultrasound will be performed before giving treatment to the patient.

ON TREATMENT

Assessment during the treatment will be performed.

END OF TREATMENT

All baseline investigations including (but not limited to) levels of interleukin-6 and interleukin-8, insulin resistance and pelvic ultrasound will be performed 12 weeks after treatment.

FOLLOW UP AFTER TREATMENT

No follow up will be performed.

9. Outcomes and Endpoints:

The outcomes of the study is the change in the levels of interleukin-6 and interleukin-8 with the use of antidiabetic drugs (metformin alone and combination of metformin and pioglitazone). Other symptoms like hirsutism, polycystic ovaries and serum testosterone levels may decrease and menstrual cycles might become become regular.

10. Safety Reporting:

PREGNANCY

Follow up in the treatment will be discontinued with pregnancy.

REPORTING OF SERIOUS ADVERSE EVENTS

Any adverse effects that occur during the treatment will be reported.

11. Treatment Information:

Metformin with the trade name of Glucophage is manufactured by Merck International Pharmaceutical Company Limited. Metformin plus Pioglitazone combination with the trade name of Zolid plus is manufactured by Getz Pharmaceutical Company Limited. We would consistently use these two brands. The dosage will be according to according to body weight.

Cost bearing: The cost of the medicines and investigations will be provided to the study participants by the project managers (Dr Durre Shehwar and Dr Mohsin Shah)

12. Data Analysis Procedure:

Mean ± SD will be used for numeric data generated from our work. In each arm the mean difference in the interleukin-6 and interleukin-8 values will be calculated using paired sample t test. For between arms analysis, mean difference in the interleukin-6 and interleukin-8 values will be calculated using independent sample t test. Non-parametric statistics will be used if the data is skewed. A cut-off will be calculated for interleukin-6 and interleukin-8 separately using ROC curve analysis. Based on the cut-off, a binary logistic regression analysis will be performed to evaluate the predictive significance of the two treatment arms in reducing the levels of inflammatory markers interleukin-6 and interleukin-8. P-value of < 0.05 will be considered significant. Results will be presented in form of tables & figures. SPSS version 21 will be used for all the statistical analysis.

13. Study committees:

The study will be managed with the help of Graduate Study Committee, Clinical Trial Unit and ORIC of Khyber Medical University, Peshawar, Pakistan.

14. Administrative aspects:

The administrative aspects will be managed with the help of Office of Research, Innovation and Commercialisation (ORIC) of Khyber Medical University, Peshawar, Pakistan.

15. References:

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STEPS/months	1st	2nd	3rd	4th	5th	6th
Sample collection and recruitment						
Experimental and data tabulation						
Thesis writing						
Submission of thesis						