Prevalence of Polycystic Ovary Syndrome in Trinidad

Abstract

According to World Health Organization (WHO), in 2010, Polycystic Ovarian Syndrome (PCOS) affected approximately 116 million women worldwide (3.4% of the population). It has been considered one of the most common causes of female infertility and the most common endocrine disorder. The standard diagnosis for the syndrome dates back to international conferences organized by the National Institutes of Health (NIH) in 1990 and the Rotterdam European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine (ESHRE/ASRM) sponsored PCOS consensus workshop group in 2003 and 2004. Clinical manifestations of the disease may include menstrual irregularities, amenorrhea, ovulation-related infertility, polycystic ovaries, and signs of androgen excess such as acne and hirsutism. This condition may also lead to chronic diseases such as obesity, type 2 diabetes (T2D), dyslipidaemia, and cardiovascular events. Despite the increasing knowledge concerning PCOS, the global picture of the disorder is deficient in a number of geographic regions. Understanding the global prevalence will help to better assess the public health and economic implications of PCOS in Trinidad, allow for improved screening methods, help elucidate the underlying factors and foster improved understanding of the molecular mechanisms in improving the evolutionary process.

Aims and Objectives

Statement of Problem

Despite the increasing knowledge concerning PCOS, the global picture of the disorder is deficient in a number of geographic regions. Understanding the global prevalence and phenotypic presentation of any common disorder, including PCOS, allows for:

1. A determination of the prevalence and associated morbidities of the disorder, to better assess the public health and economic implications of PCOS in this region.
2. A determination of the phenotype of PCOS in this region, allowing for improved screening methods.
3. The elucidation of underlying environmental or ethnic factors that may affect the prevalence, severity or complications of the disorder, via comparison of data between countries.
4. The elucidation of genetic variants underlying the disorder in this region, fostering and improved understanding of the molecular mechanisms underlying the disorder and a better understanding of the evolutionary selection processes that have paradoxically resulted in the current high prevalence of the disorder in the face of obvious reproductive deficits.

Benefit

These data would not only lead to improved understanding of the public health implications of the disorder in Trinidad, but also potentially highlighting novel etiologic avenues and therapeutic targets.

Aims and objectives

Primary:

1. To determine the prevalence of PCOS among women of reproductive age (a selected population (18 to 45)) in Trinidad.

Secondary

2. To determine the distribution of phenotypes among women diagnosed with PCOS in the above objective overall and by ethnicity. PCOS sub phenotypes are as follows: Phenotype A – clinical and/or biochemical hyperandrogenism (HA) and oligi-/anovulation (OA), and polycystic ovarian morphology (PCOM); B – HA and OA; C – HA and PCOM; and D – OA and PCOM;
3. To determine the risks for, metabolic syndrome, depression, obstructive sleep apnea symptoms, fibroids, and general health issues for PCOS women versus women without PCOS in the age groups 18 – 45 years.
4. To determine the genotype of PCOS in Trinidad.

Outcome measures and variables

Outcome measures (dependent variables)

a) Primary outcome
    i) Prevalence of PCOS and its symptoms
b) Secondary outcome
    i) Phenotype of PCOS
    ii) Genotype of PCOS
    iii) Prevalence and prevalence ration for PCOS subphenotypes in Trinidad, odds ratio for metabolic syndrome, depression, obstructive sleep apnea symptoms, fibroids, and general patient health issues, adjusted for age, ethnicity, BMI, treatment (OCP), and socioeconomic parameters.

Background

According to World Health Organization (WHO), in 2010, Polycystic Ovarian Syndrome (PCOS) affected approximately 116 million women worldwide (3.4% of the population). This condition caused symptoms in about 5-10% of women of reproductive age (12 – 45 years). It has been considered one of the most common causes of female infertility and the most common endocrine disorder (Boomsma, Fauser, and Macklon 2008; Teede, Deeks, and Moran 2010). The standard diagnosis for the syndrome dates back to international conferences organized by the National Institutes of Health (NIH) in 1990 (Zawadzki and Dunaif 1992) and the Rotterdam European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine (ESHRE/ASRM) sponsored PCOS consensus workshop group in 2003 and 2004 (Rotterdam ESHER/ASRM-sponsored PCOS consensus workshop group 2004). Clinical manifestations of the disease may include menstrual irregularities, amenorrhea, ovulation-related infertility, polycystic ovaries, and signs of androgen excess such as acne and hirsutism. This condition may also lead to chronic diseases such as obesity, type 2 diabetes (T2D), dyslipidaemia, and cardiovascular events (Palomba et al. 2015; Mohammed and Nayak 2017).

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Outcome measures and variables

Outcome measures (dependent variables)

5. Primary outcome

   ii) Prevalence of PCOS and its symptoms

6. Secondary outcome

   iv) Phenotype of PCOS

   v) Genotype of PCOS

   vi) Prevalence and prevalence ration for PCOS subphenotypes in Trinidad, odds ratio for metabolic syndrome, depression, obstructive sleep apnea symptoms, fibroids, and general patient health issues, adjusted for age, ethnicity, BMI, treatment (OCP), and socioeconomic parameters.

Covariates (independent variables)

a) Socio-demographic variables

b) Baseline characteristics

Methodology

Study design

This is a prospective, cross sectional study. The study will be conducted among females in Trinidad from different geographical locations.

Study period

The study will be conducted over a one-year period, from 1st January 2023 to 31st June 2024.

Study sites

The study will be conducted at The Faculty of Medical Sciences.

Study population

Based on a review of nine studies conducted in the general population, a conservative prevalence estimate for PCOS, using the Rotterdam 2003 definition, is 13.4%. Based on this formula, a sample size of 495 untreated individuals will be required to determine the prevalence with an absolute error of ±3%. Assuming a 50% enrollment rate, 990 women would need to be approached for study inclusion. The study population will be females of reproductive age from Trinidad.

Strategies for Sampling, Recruitment and Retention

Participants will be samples proportionally from all 8 zones as shown above in Trinidad. Approximately 124 women in the reproductive age (18 – 45 years old) will be randomly sampled from each zone. Houses will be numbered and random numbers of these houses will be generated using random number calculator. In each sampled house, females would be prescreened and those that satisfy our inclusion criteria would be selected. Telephone numbers of participants will be obtained and follow-up calls made.

Sample size calculations were based on the following formula (32):

$$n = [(Z_{1-\alpha})^2(P(1-P)/D^2)]$$

where n = individual sample size, $Z_{1-\alpha}$= 1.96 (when α = 0.05), P = assumed prevalence, and D = absolute error.

Sampling units are defined as individual unselected women between the ages of 18 and 45 years, identified in the community. We target power analysis on women who do not use hormonal contraception. We assume a conservative response rate of 50% of sampled women. This response rate includes the following parameters:

use of hormone/contraceptive use (15%), excluded due to hysterectomy or ovariectomy (3%), excluded due to pregnancy (2%), refusal to participate (20%), and study drop-out/lost to follow-up (10%).

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Selection criteria

Inclusion criteria

a) Female, ages 18 to 45 years., all ethnic backgrounds.
b) The participants must have at least one of the following two features:
    i) Dermatological signs or complains of clinical hyperandrogenism such as unwanted facial or body hair, loss of scalp hair (alopecia) or persistence acne (pimple).
    ii) Signs or complains of ovulatory dysfunction such as irregular menses (oligomenorrhoea, amenorrhea or polymenorrhoea), history of anovulation or ultrasonographic findings of polycystic ovarian morphology.

Exclusion criteria

a) Women less than 18 years or older than 45 years
b) Women who are pregnant at the time of evaluation
c) Postmenopausal women
d) Women who had undergone hysterectomy and/or bilateral oophorectomy
e) Anything that would place the individual at increased risk or preclude the individuals compliance with or completion of the study.
f) Unwillingness to participate or difficulty understanding the consent process or the study objectives and requirements.

Study Duration

From when the study opens to enrollment until completion of data analyses – 12 months.

Subject Participation Duration

Minimum duration – 1 visit

Maximum duration – 12 weeks (3 visits)

Estimated Time to Complete Enrollment:

Approximate duration – 9 -12 months.

Sampling technique

A consecutive sampling technique will be used for each phase of the study. Consecutive study participants, who meet the selection criteria and give informed written consent, will be recruited for the study. A unique serial identification number will be given to each study participant until the intended sample is attained.

Study procedure

Potential study participants from selected geographical locations will be invited to participate in the study. A confidential study register (see attached) containing details of potential study participants (name, age, height, weight, blood pressure and contact phone number) will be kept. Potential study participants will be counselled on the objectives of the study and the study protocol. If the potential participant refuses to participate, no further contact will be made with the participant. Inform consent (see attached) will be obtained from each eligible study participant before recruitment for the study. A subject tracking log for longitudinal observation study will be use to track eligible subjects.

After an informed consent, data will be collected from each study participant using a standard format in a clinical report form (CRF) for unform data collection from all participant. Each CRF will contain a unique serial identification number. The CRF will be filled by a suitably trained researcher (including the PI, co-investigators and medically trained research assistants). All CRF will be checked for missing data within 24 hours of completion, and any missing data will be detected and collected immediately.

Each participant will undergo anthropometric measurements (weight, height, waist, circumference and hip circumference) and physical examination for hirsutism, acne, alopecia, acanthosis nigricans, and thyroid enlargement. Participants with an initial hirsutism (mFG) score of 3 or more ($\geq$3) will be re-assessed by a physician.

After overnight fasting (of 8 hours or more), 10 ml of venous blood sample will be collected from each study participant; within the first seven days of spontaneous or induced menstrual cycle. About half (5 ml) of the venous blood will be collected in an EDTA- containing tube for plasma/DNA and the remaining in a plain tube for serum. Aprotinin will be added to plain tubes to prevent breakdown of small proteins that may be useful for measures. Aprotinin is a small protein bovine pancreatic trypsin inhibitor that inhibits trypsin and related proteolytic enzymes. During the mid-luteal phase of the menstrual cycle (between day 20 and 24), 5 ml of venous blood sample will be collected in a plain tube for serum progesterone.

All blood samples will be stored and transported to the laboratory in cooler boxes containing ice packs immediately after collection. At the laboratory, the sample will be separated into serum, plasma and whole blood by centrifugation for 20 minutes at 3000 rpm. The biological specimens will be stored, in small aliquots (of 0.5 ml), in 1.5 to 2.0 ml plastic containers (about 12 cryovials) able to withstand temperatures of -80°C.

Samples for hormonal assay and initial evaluation will be batched at regular interval for analysis to provide study participants with timely results, allow classification of participants, and minimize the impact of inter-assay variability. At the end of the study, a repeat analysis of biological samples for androgen levels and insulin estimation will be performed at a reference laboratory. Each subject will also complete the following standardized data instruments or forms as appropriate: FG Score self-assessment, SF-12, Beck depression inventory, Epworth sleepiness scale, and uterine fibroid assessment (see attached).

We propose to establish collaboration between the University of Lagos and the Laboratory of Dr. Ricardo Azziz, a world renowned scholar on the subject of PCOS in the United States. The genetic analysis will be performed at the Laboratory at the Department of Pre-clinical Sciences. The initial evaluation and classification of study participants will be done using the flow chart/diagnostic tree by Azzizz et al. (Figure 1). [3]

Related or mimicking disorders will be excluded using history, physical examination, and serum TSH, prolactin and 17-OH progesterone. Participants with hypothyroidism on thyroxine replacement therapy will not be recruited for the study until TSH level is normal. Study participants with elevated 17-OH progesterone > 2ng/dl (or 200 ng/dl) will have non-classical congenital adrenal hyperplasia (NCAH).

A summary of initial and follow-up procedures.

Physical Exam

A physical exam will include:

- Baseline medical examination and
- Study specific procedures

Baseline medical examination will include

- Blood pressure
- Height measurement
- Weight measurement

Blood pressure will be determined in the right arm in the sitting position. Large cuff will be used as necessary. Participants with BP > 140/90 would be referred to the resident Physician for management. Height and weight will be recorded to the nearest 0.1 cm and 0.1 kg respectively. Participants will be weighed while dressed in light

clothing, without shoes. A physical exam with standard pelvic and breast exam will be performed on all patients by a study physician.

Study specific medical examination will include:

- Waist and hip circumferences measurement
- Hirsutism assessment
- Acne assessment
- Alopecia assessment
- Acanthosis nigricans assessment

Data Collection:
Types of data:

Independent variables

1. Socio-demographic variables
2. Baseline characteristics

Dependent variables

3. Primary outcome
    I. Prevalence of PCOS
4. Secondary outcome
    II. Phenotype of PCOS
    III. Genotype of PCOS
    IV. Prevalence of obesity
    V. Prevalence of hirsutism
    VI. Prevalence of diabetes

Data collection instrument:
The methods of collecting data for assessment of study objectives include the use of standardized interview-based medical forms; surveys; physical examination.

The CRF developed for this study include; clinical and anthropometric section; radiological report section and laboratory result section. The questionnaire section will seek information on socio-demographic variables, reproductive history with emphasis on menstrual cycle (dating and regularity) and gynecological history, hirsutism, acne, medications, and family history. The anthropometry and clinical section will document the physical findings such as hirsutism, acne, alopecia, acanthosis nigricans, and thyroid enlargement. The pelvic ultrasonographic findings will be recorded in the laboratory result section. Subjects will also complete the

following standardized data instruments or forms as appropriate: FG Score self-assessment, SF-12, Beck depression inventory, Epworth sleepiness scale, and uterine fibroid assessment.

Expected duration of subject participation is approximately from 1 to 3 cycles or 12 weeks. Approximate time to complete enrollment is 9 to 12 months. Approximate time to complete study is 18 to 24 months.

Laboratory methods and Techniques

Laboratory assay will be conducted under the direct supervision of the investigators in the clinical pathology using facilities at the School of Veterinary Medicine and Department of Pre-clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, Trinidad.

Screening (local clinical) Laboratory

All women will be requested to present fasting for 12 hours at the initial screening visit, and on days 1-10 of their menstrual cycle. Blood work will be sent as described (see Table 1 below) and will be analyzed in the Biochemistry Laboratory at Preclinical Sciences. Research samples will be processed at the sample lab. An aliquot of serum from this visit will be frozen and maintained for subsequent core lab determinations (serum from two 7.5 cc red top tubes at baseline). 1 ml of serum from these visits will be aliquoted into 1.5 cc microfuge tubes and will be stored at –20 to –70 degrees Celsius and batched for periodic shipping to the central core lab facility for eventual assay. The purpose of aliquoting is to preserve basal levels of potential analytes in a frozen state until assay to avoid the deleterious effects of thawing and re-freezing.

TABLE 1. Screening laboratory evaluations*

PCOS Inclusion/Exclusion labs
• TSH**
• PRL**
• 17-OHP***
• P4++
• oGTT**

***** To be determined in local clinical lab (MDS Biochemistry Laboratory) in UWI, Trinidad.

** All subjects.

*** For subjects with oligomenorrhea and/or hirsutism and/or PCOM only, who do not use Hormonal Contraception (HC).

++ For subjects with hirsutism and/or PCOM, who have apparently regular menstrual bleeding a day 22–24 progesterone (P4) level will be obtained.

Central Core Laboratory

Given the variability of assays between labs, analyses for additional outcome measures will be performed in a research laboratory (see **Table 2**)..

TABLE 2. Laboratory evaluations and sample storage in research.

Research lab
• SHBG • Total and Free Testosterone • DHEAS • Blood for DNA extraction for later genetic analysis Extra blood for repository

DNA Core Facility

We will obtain blood for DNA extraction at **Hospital Visit 1**, since all subjects will undergo standard phenotyping. Blood (in EDTA tubes) will be sent to the Biochemistry Laboratory at Preclinical Sciences, UWI where DNA will be extracted and stored for future analyses. Our primary purpose is to serve as a repository to participate in genome wide association studies. In both cases, there will be no release of personal identifiers, and we will obtain a Certificate of Confidentiality, as we did in prior studies. This will be a part of the initial protocol, though subjects will have the option to opt out of this segment of the study or limit the use of their specimen on the consent form. This resource may also prove useful for genome wide association studies or other studies of the genetics of PCOS, oligomenorrhea, and hyperandrogenism.

At this time no genetic analyses are proposed as the number of subjects being collected are insufficient for any significant genetic study. However, as it is our hope that this study will serve as the anchor for subsequent investigations of women's health and PCOS in Trinidad, we intend to isolate and store DNA in the subjects examined in the study. The samples will be stored at The University of the West Indies. If in the future a decision is made to study the DNA samples accumulated, perhaps along with other samples collected, then we will apply for an amendment for genetic analysis.

Study Specific Biospecimens

The study staff will store the specimens with following specifications:

- According to the temperature requirements
- Maintain a written and electronic log of sample receipt

- Maintain an electronic log of sample location
- Maintain a written and electronic log of equipment temperatures (temperatures or conditions will be measured daily at the same time)
- Ensure that the freezers or refrigerators have adequate temperature controls
- Ensure quality assurance of equipment, which includes records of regular maintenance and quality evaluations
- Place the sample in a clearly identified location in the freezer or refrigerator (i.e., bar coded container) Log the sample/s into a database with location of sample for easy retrieval.

Adverse Events (AE)

An adverse event (AE) is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally occurring during the course of the study, whether associated or not with the procedures performed during the study. AEs may include the following types of occurrences:

- Suspected adverse drug reactions.
- Other medical experiences, regardless of their relationship with the study procedures performed, such as injury, surgery, accidents, extensions of symptoms or apparently unrelated illnesses, and significant abnormalities in clinical laboratory values, psychological testing or physical examination findings.
- Reactions due to medication (e.g. solutions administrated for the OGTT and ACTH stimulation test) overdose, abuse, misuse, withdrawal, sensitivity or toxicity.

Investigators must systematically assess the severity of AEs according to the following definitions:

- ***Mild:*** The subject is aware of the event or symptom, but the event or symptom is easily tolerated.
- ***Moderate:*** The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.
- ***Severe:*** Significant impairment of functioning: the subject is unable to carry out usual activities and/or the subject‘s life is at risk from the event

Serious Adverse Events

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity

- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. In such a case the participant would be referred to the Eric Williams Medical hospital.

Example AE & SAEs in proposed study:

Overall, the likelihood of AEs and SAEs in this study is very small. Examples of possible AEs may include:

- Bruising at the site of blood drawing (mild AE)
- Flushing with the administration of ACTH-1-24 for acute ACTH stimulation test to exclude NCAH (mild AE).
- Discomfort with transvaginal ultrasound (mild AE).
- Emotional distress upon knowing of the diagnosis of PCOS (mild to moderate AE).

Since the protocol does not entail any intervention, it is very unlikely that any SAE will occur.

Reporting Procedures

The current study provides minimum to modest additional patient risk than standard of care. All SAEs throughout study participation from the start of the study will be reported within 24 hours (or 1 business day) of learning of the event to the local IRB. Reporting will be accomplished by completing the Serious Adverse Event Report. Only study number will identify subject and no other identifying information will be included on the form. The investigators will include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB.

- Appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- A detailed description of the adverse event, incident, experience, or outcome;
- An explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

To satisfy the requirement for prompt reporting, unanticipated problems will be reported using the following timeline:

- Unanticipated problems that are serious adverse events will be reported to the IRB within 24 hours of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the IRB within two weeks of the investigator becoming aware of the problem.
- All unanticipated problems should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

Quality assurance

1. Instrument validation – A standardized uniform assessment tool (the CFR) was developed for this study. The CFR will be de-identified and pretested.
2. Data collection – the study register will be used to compare participants with non-participants and this will help exclude self-selection bias. The CRF will be used for the collection of data from all the participants. Each study participant will be given a unique identification number to enable follow-up, retrieval of data including missing information and clarification of information. The questionnaire section of the CRF will be completed by a trained researcher to ensure completeness and accuracy of data. The clinical and anthropometric section will be completed by a medically trained researcher. Participants with an initial hirsutism (mFG) score of 3 or more will be re-assessed by a single physician.

   The pelvic ultrasonography will be performed by experienced Gynecologist trained in pelvic ultrasonography. The antral follicular count (AFC) will be done in the transverse section of the ovary.

   The blood samples and initial laboratory analysis will be performed by suitably trained research/laboratory assistance. The blood specimens will be collected in the appropriate tubes/containers, and Aprotinin will be added to plain tubes to prevent the breakdown of small proteins that many be useful for measures. The initial blood specimen will be labelled with the participants' unique identification (ID) number, last menstrual period (LMP), date and time of sample collection; while the small aliquots in plastic containers will be labelled with the unique ID number and date. There will also be a laboratory system that records how often the sample has been retrieved, thawed and refrozen. A subject tracking log for longitudinal observational study (Appendix IV) will be used to monitor the data collection process/procedure.
3. Staff training – The members of the research team (PI, Co-investigators and Research Assistants) will undergo a few days training on the various aspects of the study procedure and protocol. The training will cover the following areas: responsible to conduct research, the study protocol, education of

potential study participants, recruitment of study participants, completion of the consent form and CRF, physical examination and anthropometric measurements, biological sample collection and labelling, pelvic ultrasonography, and laboratory techniques and procedures for biological specimen handling, processing and storage. The degree of intra-observer accuracy and consistency, and inter-observer consistency will be established for key physical measures such as hirsutism score, clinical examination and anthropometric measurement, volume of blood sample measurement and pelvic ultrasonography.

4. External quality assessment laboratory - A reference laboratory will be used for the batched analysis of stored sample for external quality assurance. The laboratory of Dr. Ricardo Azziz will serve as the reference laboratory.
5. An external advisory group will be established a prior to:
    a. Advise on the study design
    b. Help train local staff in study techniques
    c. Assist in resolving issues that arise during the study
    d. Assist in identifying collaborative units and laboratories.

    Dr. Ricardo Azziz and the other collaborators form USA will form the external advisory group.
6. Data management - The CRF will be used to collect data from each study participant. All CRF will be checked for missing data within 24 hours of completion, and any missing data will be detected and collected immediately. The sociodemographic and baseline characteristics, and the clinical findings and anthropometric measurements will be documented in hard copy (CRF) and then imputed into the appropriate software (such as Microsoft Access and SPSS) at regular intervals. The radiological and laboratory data will be documented in the hard copy CRF of each study participant and subsequently imputed to the software. The study register and the CFRs will be kept confidential to avoid identification of the participants beyond the purposes described above. Data entered into the software will only bear the unique ID number. All data and records will be linked with the participants' unique ID number. The electronic database will be password protected. Quality control staff will perform periodic checks for accuracy of data entry (by comparing the hard copy and the electronic chart) and for data accuracy (by re-interviewing the participants). Statistical analysis will be done using SPSS.

Data management and Statistical analysis:

Data entry, data processing, and statistical analysis will be done using IBM Statistical

Software for Social Sciences (SPSS, Inc., Chicago, IL) version 20.0. Continuous variables (such as age, anthropometric measurements, laboratory values of assay) will be checked for normality using one-sample

Kolmogorov-Smirnoff test, and expressed as means ± standard deviation or median ± interquartile range (for data that are not normally distributed).

Categorical variables (such as race, socioeconomic characteristics) will be expressed as frequencies with accompanying percentages. Differences between groups will be compared using the Pearson's Chi-square test or Fisher's exact test for categorical variables. Odds ratio and the corresponding 95% confidence intervals (CI) will be presented. The student t test and ANOVA will be used to compare difference between groups for normally distributed continuous variables. Comparison of continuous variables that were not normally distributed will be done using non- parametric (Mann-Whitney U test or Kruskal-Wallis test) inferential statistical test.

Linear and logistic regression analysis (univariate and multivariate) will be performed to evaluate the relationship between dependent and independent variables. Test of statistical significance will be set at p-value less than 0.05.

Ethical considerations:

This study will be conducted according to the ethical guidelines and principles of The International Declaration of Helsinki, the Guidelines for Good Clinical Practice and the National Code of Health Research Ethics (NCHRE). The Ethical Committee for The University of the West Indies will issue approval. All researchers involved in this research project have received training in Responsible Conduct of Research.

No participant recruitment will commence until the approval is received. Written inform consent will be obtained from all participant before recruitment. Material transfer agreement will be signed with collaborators. In designing this study, the following ethical issues were put into consideration.

Consent to participate and withdraw from study:

The purpose of the study will be explained to all the potential participants. An informed written consent will be obtained from each study participant before recruitment into the study. Study participants will be informed of their freedom to withdraw or refuse to take part in the study without prejudice to their usually expected standard of care. No financial or material incentive will be given to participants.

An investigator may terminate a study's participant in the study if:

1. Any medical condition, event or situation arises or is recognized such that continued participation in the study would not be in the best interest of the subject.
2. The subject is found to meet an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

Handling of subject withdrawals:

When a subject chooses to withdraw, stating so directly or passively (i.e., does not return as scheduled) an attempt will be made to reach that individual in follow-up to ascertain that she is not suffering an untoward event that required evaluation or follow-up. Whatever data/tissue has collected until the time the subject withdraws will be available for analysis, unless the subject specifically states that such data/tissue specimens should be discarded and not used further, in which cause the samples will be discarded and the data deleted from the database. Withdrawn subjects will not be replaced.

Confidentiality of data:

All information about the participants will be obtained using anonymous questionnaires and shall be kept strictly confidential. The participants will be assured that their identity will be kept in confidence by the investigators and that the results obtained will be presented in aggregate manner.

Beneficence to participants:

No participant in this study will be made to pay for any of the procedures. The study participants will be informed of the OGTT results and pelvic ultrasonographic findings. All study participants with abnormal findings will be notified of the results of their evaluation, and those with abnormal physical, historical or biochemical findings will be encouraged to undergo further investigation or treatment.

Non Maleficence to the participants:

All precautions will be taken to reduce the discomfort that may result from the venipuncture or during ultrasonography.

Job description

The physicians and nurses will be responsible for identification of potential study participant, education and counselling, and filling the questionnaires. The medically trained Research Assistants will be responsible for the anthropometric measurements and initial physical examination. The physicians will be responsible for the physical examination. The Gynecologist will be responsible for the pelvic ultrasonography. The Research Technician and Assistant will be responsible for the blood sample collection and processing under the supervision of the pathologist.

References:

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