Vitamin D supplementation in polycystic ovary syndrome: a randomized open label delayed-start design: study protocol

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Method Article

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Abstract

Abstract: Background: Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder affecting reproductive-aged women, characterized by a constellation of symptoms including irregular menstrual cycles, hyperandrogenism, and insulin resistance. Emerging evidence suggests that Vitamin D (VD) deficiency may play a pivotal role in the pathogenesis of PCOS, with low VD levels associated with exacerbation of PCOS-related symptoms and metabolic disturbances. Recently, VD supplementation has garnered increasing attention as a potential therapeutic intervention for PCOS.

Methods: In this randomized open label delayed-start design the intervention group will be given a VD injection of 600,000 I.U I/M once with 1-gram calcium supplement daily for 12 weeks, followed by standard PCOS care which will include: i) ‘Glucophage XR 750 mg’ (once for 15 days then twice daily), ii) progesterone supplementation (1 capsule Progeffik 100 mg every 3 weeks, then 1 week off), with continued calcium supplements for next 12 weeks. Women in the control group will begin with standard PCOS treatment (as above) for 12 weeks, followed by VD and calcium supplementation for the remaining 12 weeks. Blood tests will be done at baseline, week-12 and week-24 between the two groups that will include assessment of: i) hyperandrogenism by ‘Free Androgen Index’ [Total Testosterone, Steroid Hormone Binding Globulin ii) Insulin resistance (IR) by HOMA-IR (serum Insulin, Fasting Blood Glucose) and iii) oxidative stress by Total Antioxidant Capacity at all three levels for comparative analysis. VD levels after supplementation will be assessed for confirmation of correction and calcium and albumin levels for detection of hypercalcemia.

Introduction

Polycystic Ovarian Syndrome (PCOS) is an endocrine disorder of women of reproductive age, defined by “the presence of any two out of three criteria; oligo and/or anovulation, excess androgen activity and/or polycystic ovarian morphology on ultrasound”[1, 2]. The condition is portrayed by a spectrum of clinical features, including irregular menstrual cycles, hyperandrogenism, hypertension and obesity etc [1, 2] [3]. The prevalence of PCOS varies across diverse populations, with global estimates of 8-13% in women of reproductive age. South Asian women show significant susceptibility to PCOS; and studies have a prevalence of up to 50% among infertile women in Pakistan [4]. PCOS is responsible for anovulation, The recurrent dysregulation of menstrual cycle leads to the formation of multiple cysts within the ovaries [5], disruptions in the menstrual cycle, and ultimately impeding fertilization and conception [6]. Even in instances where conception is achieved, the pervasive hormonal imbalances predispose these women to complications like hypertension and gestational diabetes, which affect the health of the newborn, and increase likelihood of stillbirth and abortion [7].

Literature reports the association of PCOS with Vitamin D deficiency (VDD) [8], and is linked to various metabolic dysfunctions, including insulin resistance, dyslipidemia, and obesity [9] [10]. VD status has been shown to be negatively associated with androgen status in PCOS patients. In addition, PCOS
females and VDD have higher levels of estrogen, ‘luteinizing hormone’ (LH), ‘thyrotropin (TSH), ‘cholesterol’, ‘triacylglycerol’, ‘very-low-density lipoprotein’ (VLDL), and ‘low-density lipoprotein (LDL)’ compared to healthy women. [9]. Treatment of VDD is associated with correction of insulin resistance (IR), improved glucose metabolism, improved lipid markers [10], and ovulation in women with PCOS and infertility [11]. Supplementing with VD has demonstrated the capacity to enhance FSH levels and the LH/FSH ratio in women with PCOS. This suggests that it may serve as a beneficial complement to conventional ovulation induction protocols, ultimately resulting in higher clinical pregnancy rates and improved ovulation. [12]. Furthermore, higher serum levels of VD have been correlated with retrieval of oocytes with greater fertilization potential in PCOS patients undergoing ICSI cycles [13]. Overall, correcting VDD in PCOS females may have beneficial effects on fertility outcomes, insulin resistance, and metabolic parameters.

Considering PCOS as a pivotal factor contributing to anovulatory infertility, the judicious incorporation of VD within the therapeutic paradigm has the capacity to improve fertility outcomes in women in South Asia.

**Rationale of the Project:** Impaired VD status in females can attenuate IR, which leads to development of PCOS, metabolic and hormonal abnormalities and risk of complications like Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease (CVD) [14]. Insufficient substantial evidence and a dearth of literature necessitate further investigation and experimentation to ascertain the role of VD. Our research group is working to assess role of oral supplementation of VD to improve the follicular size and endometrial thickness [15]. A contentious debate surrounds the choice of treatment strategy for VDD, specifically focusing on whether oral or intramuscular (IM) administration of VD is more suitable. Notably, in the Indian population, the IM route of VD replacement (300,000 IU) demonstrated a sustained increase from baseline, contrasting with oral cholecalciferol administration [16]. Safety and efficacy of VD administration with a single IM dose of (300,000 IU) for improvement in oxidative stress and clinical outcome is also reported [17]. Therefore we have designed RCT as a cost effective intervention in supplementation with standard of care in PCOS patients.

**Hypothesis:**

Correction of VD by I/M injections will improve response to standard PCOS treatment in our population.

**Justification:** The hypotheses has been proposed on the basis of role of VD, trend of VDD in PCOS women in Pakistan, genetic predisposition of VDD in our population, and evidence of maximum improvement by one intramuscular dose of (600,000 IU) [18] [19]. Using an open-label, randomized, delayed-start start design, women of reproductive age with PCOS and VDD (VD < 20ng/ml) [20] will be recruited. Single IM dose of (600,000 IU) before and after standard PCOS will be given and comparative results will enable us to predict best time of VD supplementation for improvement of outcome markers; insulin sensitivity, androgen levels and oxidative stress [21-24]

**Objectives:**
I. To interpret the effect of VD supplementation on hyperandrogenism by FAI results before and after standard PCOS treatment

II. To interpret the effect of VD supplementation on oxidative stress by results of TAC before and after standard PCOS treatment

III. To predict usefulness of VD supplementation before or after standard PCOS care for effective management of PCOS

{VD levels after supplementation will be assessed for confirmation of correction/supplementation and calcium and albumin levels for detection of hypercalcemia.}

Reagents

Equipment

Procedure

Approvals:

1. Trial Registration Number: NCT06045351

2. Clinical trial Unit approval Attached

3. Ethical Review Board (attached- all ERCs)

4. Hospital Management (attached)

Study Design: A multidisciplinary randomized, open-label, delayed-start design trial will be conducted at Aga Khan University, involving Departments of Biological & Biomedical Sciences, Pathology and Laboratory Medicine, Medicine (Endocrinology), and Family Medicine for a period of 2 years (ERC: 2021-6763-20038). The trial has been designed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT; Figure 1).

Sample Size calculation: It was calculated by PASS 11 software. In an equivalence test of means using two one-sided tests on data from two-periods, the minimum sample size that we will require is 142, 71 in each group with an inflation of 20% for loss to follow-up to achieve 80% power at a 5% significance level when the true difference between the means is 0.4, the standard deviation of the paired differences is 2.60.

Eligibility Criteria: Recently diagnosed patients with PCOS who exhibit at least 2 of the following 3 criteria: clinical or biochemical signs of hyperandrogenism, chronic anovulation, polycystic ovaries (as determined by reports from routine transvaginal ultrasounds) [1, 2] will be invited to the study.
Additional inclusion criteria: “Serum vitamin D (VD) levels less than 20 ng/ml”, “age between 18 and 45 years”, patients from “all ethnic backgrounds”

Exclusion criteria:

Patients will be excluded from the study with these criteria: pregnancy, hypercalcemia (plasma calcium concentrations greater than 2.65 mmol/L), tuberculosis or other granulomatous disorders, chronic liver disease or alanine transaminase (ALT) levels three times higher than the normal limit, chronic kidney disease or serum creatinine levels greater than 2.0 mg/dL, recent use of certain medications or therapies, including VD injections within the last 3 months prior to recruitment, oral contraceptives, hormonal replacement therapy, glucocorticoids, calcium supplementation, insulin-sensitizing drugs (incretin mimetic drugs, thiazolidinedione, sulfonylurea), lipid-lowering drugs, or other drugs affecting insulin sensitivity or serum androgens (e.g., niacin, corticosteroids, beta-blockers, calcium channel blockers, thiazide diuretics), anti-epileptics, anti-retroviral drugs, cholestyramine, anti-fungal drugs, statins, H2 blockers, immunosuppressants, chemotherapeutic agents, antimicrobials (e.g., Rifampicin, Isoniazid, Hydroxychloroquine), or any other drug modifying lipid metabolism in the previous 3 months prior to the study.[15]

Recruitment of Subjects: All diagnosed PCOS subjects as per the Rotterdam criteria visiting Endocrine, Family Medicine and/or Gynae Obs clinic qualifying the inclusion criteria with will be invited to participate in the study after acquiring written informed consent.

Randomization: Patients will be brought to the Clinical Trial Unit (CTU) at AKU, which is equipped with complete facilities for clinical consultations, trials, and laboratory work-up. To assign patients to different groups, we will use the permuted block randomization method, creating blocks that ensure an equal distribution of participants across all arms in a 1:1 ratio. The randomization list will be generated using AKUH's proprietary randomization software, accessible at http://www.randomizer. This list will be kept confidential and securely stored within the CTU premises, with limited access. Subjects will be randomized into Group A (Intervention) and B (Control) and will receive the treatment protocol

Intervention Group: For the initial 12 weeks will receive VD supplementation (600,000 IU I/M) once during the study period and elemental calcium 1000 mg/day. However, from 12-24 weeks they will receive standard PCOS treatment, Glucophage XR 750 mg once at dinner for 15 days then twice daily, capsule Progeffik 100 mg once at night every 3 weeks, then 1 week off and calcium 1000 mg/day (Table 1)

Control Group: For the initial 12 weeks the control group will receive the standard treatment for PCOS, Glucophage XR 750 mg once at dinner for 15 days then twice daily and Capsule Progeffik 100 mg once at night every 3 weeks, then 1 week off

However, from 12-24 weeks they will receive VD supplementation (600,000 IUI/M) once during the study period with daily calcium 1000 mg/day and standard PCOS treatment will be continued

Table 1: Description of treatment Protocol in allocated groups
Group A (intervention)

Group B (Control)

Initial 12 weeks

Vitamin:

1. VD supplementation (600,000 IU I/M once)
2. Calcium 1000 mg/day

Standard PCOS:

1. Glucophage XR 750 mg once at dinner for 15 days then twice daily
2. Capsule Progeffik 100 mg once at night every 3 weeks, then 1 week off

At 12 weeks after consultation and biochemical tests treatment protocol will be changed as mentioned

After 12 weeks |

Standard PCOS:

1. Glucophage XR 750 mg once at dinner for 15 days then twice
2. Calcium 1000 mg/day at night every 3 weeks, then 1 week off
3. Capsule Progeffik 100 mg once (contd)

Vitamin D:

1. VD supplementation (500,000 IU I/M) daily
2. Calcium 1000 mg/day
3. Standard PCOS (contd)

**Data Collection:** Following randomization and the distribution of drugs, the research team will conduct a thorough assessment, which includes a comprehensive medical history review, physical examination, and the measurement of various biophysical and biochemical parameters. Data will be gathered on the following variables: Body Mass Index (BMI), Waist Circumference (WC), and Waist-Hip Ratio.

Biochemical estimation; results of routine tests available from desk records (Human Sex Hormone-Binding Globulin (SHBG), Total Testosterone (TT), Plasma Insulin and Glucose) will be included in the profile. For estimation of Total Antioxidant Capacity (TAC), serum calcium and phosphorous levels, phlebotomist will collect blood samples (3 cc) by vein puncture on the first visit.
Calculations;

i. HOMA-IR: The HOMA-IR index, a frequently employed indicator of insulin resistance, will be computed using the following formula: HOMA-IR = fasting glucose levels [mmol/L] \times \text{fasting insulin levels} [\mu\text{U/mL}] / 22.5 [25]

ii. Free Androgen Index (FAI): FAI will be calculated from Total Testosterone (TT) and Sex Hormone-Binding Globulin (SHBG), typically measured in nanomoles per liter. FAI = 100 (TT / SHBG) [24]

Follow-up visits at 12 weeks (Study Mid Point): At this point, fulfillment of instructions will be checked and research team will ask about side effects (if any). Moreover, drugs for the next 12 weeks (as per group allocation) will be given and any other questions from the participants will be responded. Fasting blood sample will be collected for estimation of SHBG, TT, Human Insulin, Glucose, serum calcium and phosphorous and TAC in both groups and VD and serum albumin estimation in Group A.

After blood collection, the serum will be separated, sent to the Multi-Disciplinary Laboratory (MDL), and stored at a temperature of -80°C.

At this point of time, treatment protocol of both groups will be changed (Table 1).

Follow-up visits at 24 weeks (study End Point): After completion of 24 weeks study exposure, subjects will be informed about completion of study. Blood samples in fasting state will be collected for repeat analysis in MDL for all the biochemical parameters (FAI, HOMA IR and TAC) and VD and serum albumin estimation in Group B.

Table 2: Complete cycle of each patient in control and intervention group for a period of 24 weeks

**Step 1**

Identification of PCOS

**Step 2**

Inclusion / Exclusion criteria fulfilled

**Step 3**

Randomization

Family Medicine

+ Diagnosed PCOS

Vitamin D < 20 ng/ml

Group A:
Intervention Group

(Vitamin D (500,000 IU /M) daily Stat + Calcium 1,000mg)

Groups B:

Control Group

(Standard PCOS Care; Glucophage XR + Progeffik capsule 100 mg)

Step 4

Base Line Tests

Step 5

Change of Treatment

Step 6

Repeat all tests at Study End Point

Multipurpose Laboratory: Total Antioxidant Capac (TAC) Calcium & Phosphorous Serum Insulin, Total Testosterone (TT), Steroid Hormone Binding Globulin (SHBG) Fasting Blood Glucose & Insulin

After 12 weeks: Consultation, Counseling, all biochemical tests (VD Calcium and albumin of Group A only) & then Crossover

Group A:

Standard PCOS Care +Calcium C (Contd.)

Group B:

VD & Calcium + Standard PCOS Care (Contd.)

Repeat all tests at Study End Point (VD Calcium and albumin of Group B only)

Creditability of Centre:

AKU Hospital Clinics: These clinics serve patients from the entire city and surrounding areas, equipped with modern facilities and assessment services. Our co-principal investigators at the Family Medicine and
Endocrinology Clinic see more than 10 patients per month, meeting our recruitment requirement of 6 patients per month.

Clinical Trial Unit (CTU) of AKU Karachi: The randomization of study subjects will occur here. We will have access to clinical space, including 4 examination rooms, 4 consultation rooms, a multipurpose area with 3 beds, an infusion bay, storage for investigational products, phlebotomy and lab processing facilities, administrative support space, data entry support, study coordinator workspace, a pharmacy, investigator ports, and a bioequivalence lab, all meeting international clinical trial standards.

Multi-disciplinary Laboratories (MDL): Bench work and ELISA for all experiments will be conducted in MDL. The facility also offers various core facilities and equipment for biomarker analysis, DNA, RNA, and protein work. This includes advanced instrumentation such as spectrophotometers, gel electrophoresis apparatus, centrifuges, conventional and real-time PCR machines, microscopes, tissue culture facilities, Power-lab and Lab-Station, biosafety level 2 and 3 laboratory units, and essential equipment like centrifuges, water baths, hotplates, pH meters, and vortexes. Freezers (-20°C, -40°C, and -80°C) will be available as needed. All labs are equipped with computers with full internet access, secured cabinets, safety hoods connected to a central exhaust system, and uninterrupted electricity supply for data protection. Additionally, the laboratories maintain a safe environment through restricted access, logging, 24/7 monitoring, and surveillance. Effective alarms (hooter, fire bell, smoke/fume detectors) are in place, and all equipment undergoes regular Periodic Preventive Maintenance by a Biomedical Engineer to ensure biosafety and biosecurity.

Jumma Lab Facilities: If necessary, bench space in Jumma Lab can be arranged.

Chemical Pathology & Laboratory Medicine: These lab facilities will be used for vitamin D testing and serum sample processing, providing additional support for MDL.

Conference/meeting rooms: These rooms are available for researchers to host collaborators and maintain patient privacy during data collection for periodic meetings. The University Library, which provides access to standard course texts, specialized collections, medical journal subscriptions, and electronic resources, will also be utilized.

Plan of activities in the form of a GANTT chart is given in Figure 2

Plan of analysis: Data will be analyzed using STATA version 15. The primary analysis for each outcome will be conducted at the individual participant level, following the intention-to-treat (ITT) principle. This means that all participants with documented outcomes will be included in the analysis and will be assessed based on the treatment group to which they were initially assigned.

The results will be reported as follows: presented as mean and standard error/ median (IQR) for the outcomes. Pretreatment, 12 weeks and 24 weeks post treatment levels of the outcomes will be assessed by repeated measure ANOVA/ Friedman test as appropriate. Categorical variables will be stated as ‘frequency’ and percentages and will be calculated by chi-square/ fisher exact test. Unadjusted and
adjusted beta coefficient with 95% CI will be reported by using Generalized estimating equation (GEE) to
determine the association of various independent factors with the outcomes. We will adjust for the
independent variables and determine the association of factors causing a greater decline in outcomes by
multivariable GEE. Plausible interactions and confounders will also be assessed. Throughout the study,
statistical significance will be considered; p-value less than 0.05.

**Ethical Considerations:** In this research, we will adhere to ethical principles charted in the Helsinki
Declaration for medical research for human subjects. We have obtained approval from the Aga Khan
Ethical Review Committee. The participants will be informed about the study's purpose and the possibility
of being part of the intervention group based on computer randomization. We will obtain verbal and
written consent from eligible participant. To ensure confidentiality, the entire process from participant
enrollment to testing and outcome assessment will be handled discreetly. Consent forms and completed
questionnaires will be assigned unique codes and securely stored in locked cabinets accessible only to
the Principal Investigator. Participant identifiers will be removed, and only codes will be used in
subsequent steps. Serum samples will be collected and coded without mentioning names. These
samples will be processed at MDL-AKU and disposed of following AKU clinical lab's institutional policies
for biological material disposal, which involves sealing them in red bags and incineration. Afterward,
information will be deleted from computers, and the forms and questionnaires will be securely shredded.
Results and data will be appropriately coded, decoded, and documented.

Figure 2: GANTT chart of activities in an open label delayed start trial on vitamin D

**Discussion:**

The administration of VD in women with PCOS can be considered a safe approach to alleviate clinical
features without any adverse effects [26]. However, the mechanisms underlying the potential benefits of
VD in PCOS are not completely understood. It is uncertain whether VD primarily acts as an independent
treatment option or if its effects are mediated through its influence on insulin sensitivity, inflammation, or
hormonal regulation. Furthermore, the impact of VD treatment on long-term clinical outcomes, such as
fertility and cardiovascular risk, remains an area of ongoing investigation. The interplay between genetic
factors, geographical location, and individual patient characteristics in determining the response to VD
supplementation in PCOS also necessitates further research. Significant gaps in knowledge still exist
regarding VD precise therapeutic utility and the optimal dosage and duration of supplementation.
Addressing these knowledge gaps is crucial to advancing our understanding of the prospective role of VD
as a curative option in the management of PCOS and optimizing its clinical use.

There is an increasing trend to explore the potential role of VD for the treatment of PCOS [26]. Studies
have recommended the use of oral VD and our group is also conducting a RCT on effect of oral VD on
fertility outcomes [15, 26]. Clinical studies have employed varying doses at varied durations depending
upon convenience of availability of formulations in local settings. This makes it challenging to establish
standardized guidelines for VD use. Moreover, VD toxicity, though relatively rare, is a growing concern in
Pakistan. While VDD has been widely documented in the country, there is a nascent awareness leading to
an increased use of supplements. Paradoxically, this push for supplementation comes with a potential risk of toxicity, as highlighted by emerging reports from Pakistan[27]. VD is not a benign drug. Not only does its inadvertent use require regulation, but carefully controlled interventional studies are needed to address management in the local set-up.

By using a randomized open label, delayed-start design trial, with controlled conditions and standardized protocols, we will be able to scientifically validate the effectiveness of VD treatment in PCOS. We have used this study design in comparison with standard parallel-group study design as the effects of VD develop overtime and we do not want to deprive the VD deficient PCOS from VD supplementation.

To the best of our knowledge this is the first clinical trial which will confirm not only the impact of VD but also predict the time of its administration. In addition, with randomly assigning participants to intervention and control groups; causality assessment can be effectively conducted for observed effects. All subjects will be given similar doses of VD either before or after standard PCOS care which will help us in determining the optimal dosage and duration of VD supplementation for PCOS management. In addition to the routine parameters of IR and hyperandrogenism, this study will also estimate and document the improvement in OS because of the replacement therapy, thus helping to assess the role of VD in rectification of endocrine and metabolic derangements and the potential holistic benefits of VD supplementation in PCOS. This is expected to provide a strong scientific foundation about whether to incorporate VD into PCOS treatment protocol or not and hence in developing evidence-based treatment guidelines, for effective and safe interventions.

Conclusion:

The contextual problem of VDD with endocrine, metabolic and reproductive profile will be explored for urgent need for targeted treatment strategies that address VDD in women with PCOS. The results of this study will propose the usefulness of VD to ameliorate the symptoms and complications of PCOS and also improve overall health and quality of life for affected individuals. In addition to that researchers will be able to predict the time of VD supplementation; either before or after the standard PCOS care. Furthermore, in a developing country like Pakistan, the cost-effective treatment with one injection of VD in 12 weeks will march towards reducing medical, social, psychological and economic burdens on health and society.

Grant Approval: Project No. PSF/ Res/S-AKU/Med (537): Pakistan Science Foundation

Disclaimer: ERC was applied before grant submission as per AKU policy , grant fund has not been released

Strength & Limitations: The delayed-start design has some methodological issues in comparison with standard parallel-group study design of RCT. Nonetheless the effects of VD develop over time and we do not want to deprive VD deficient PCOS from VD supplementation in both groups, therefore we have selected this study design; all subjects will be given similar doses of VD either before or after standard
PCOS care. To the best of our knowledge this is the first clinical trial which will confirm not only the impact of VD but also predict the time of its administration. Novelty of the study is that in addition to the routine parameters of IR and hyperandrogenism, this study will also estimate and document the improvement in OS as a result of the replacement therapy. These results will inform clinicians of the role of VD in rectification of endocrine and metabolic derangements in women with PCOS.

Conclusion:

Th contextual problem of VDD with endocrine, metabolic and reproductive profile will be explored for urgent need for targeted treatment strategies that address VDD in women with PCOS. The results of this study will propose the usefulness of VD to ameliorate the symptoms and complications of PCOS and also improve overall health and quality of life for affected individuals. In addition to that researchers will be able to predict the time of VD supplementation; either before or after the standard PCOS care. Furthermore, in a developing country like Pakistan, the cost-effective treatment with one injection of VD in 12 weeks will march towards reducing medical, social, psychological and economic burdens on health and society.

References


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**Figures**
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Figure 1

Figure 1.

Template of recommended content for the schedule of enrolment, interventions, and assessments. *