Association between Vitamin D Deficiency and Unexplained Infertility

Thesis

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in Obstetrics and Gynaecology

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بسم الله الرحمن الرحيم
قالوا
سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم
صدق الله العظيم
سورة البقرة الآية: ٣٢

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✍ *Ikran Abdullah Haji Adan Mohamud*

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List of abbreviations

Abbrev.	Full term
1-OHase	**:** Vitamin D-25-hydroxylase
23-FGF	**:** Fibroblast growth factor 23
25(OH)D	**:** 25 hydroxyvitamin D
AIs	**:** Aromatase inhibitors
AMH	**:** Anti-müllerian hormone
ART	**:** Assisted reproductive technology
ASAs	**:** Anti-sperm antibodies
ASRM	**:** American Society of Reproductive Medicine
BMI	**:** Body mass index
Ca2 +	**:** Calcium ions
CC	**:** Clomiphene citrate
CI	**:** Confidence interval
D2	: Cholecalceferol
D3	: Erdocalciferol
DBP	**:** Vitamin d binding protein
ELISA	**:** Enzyme-linked immunoassay
FSH	**:** Follicle-stimulating hormone
GDM	**:** Gestational diabetes mellitus
hCG	**:** Human chorionic gonadotropin
HPO4	: Phosphorus ions
ICSI	**:** Intracytoplasmic sperm injection

OR : Odds ratio
OM : Institute of Medicine
IQR **:** Interquartile range
IUI **:** Intrauterine insemination
IVF **:** In vitro fertilization
LH **:** Luteinizing hormone
MED : Minimal erythema dose
MTI : maximum tolerance intake
PCOS **:** Polycystic ovary syndrome
PTH **:** Parathyroid hormone
PTHr : Parathyroid hormone receptor
RDI : Recommended daily intake
ROC Curve **:** Receiver operating characteristic Curve
SD : Standard Deviation
SE **:** Standard Error
SPSS : Statistical package for social science
TMB **:** Transient monocular blindness
TSH : Thyroid stimulating hormone
UVB **:** Ultraviolet b
VDD **:** Vitamin D deficiency
VDR **:** Vitamin D receptor
ViD : Vitamin D
WHO **:** World Health Organization

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PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRICS AND GYNECOLOGY

Title of the Protocol: The Association between vitamin D deficiency and unexplained infertility

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What is already known on this subject? AND
What does this study add?

The current study will focus on the association of vitamin D deficiency and unexplained infertility.

1. INTRODUCTION

Infertility is a common condition that affects 9%-18% of the general population, representing a complex disorder with medical, psychological, and economic aspects ***(Talmor et al., 2015).***

For healthy young couples, the probability of getting pregnancy per a reproductive cycle is about 20% to 25%. Their cumulative probabilities of conception are 60% within the first 6 months, 84% within the first year, and 92% within the second year of regular fertility-focused sexual activity ***(Talmor et al., 2015).***

According to the Practice Committee of the American Society for Reproductive Medicine, infertility is defined as the inability to conceive a child after 12 months of regular sexual intercourse, without contraception, (***Chighizola et al., 2016).***

The causes of female infertility are different and include genetic and anatomic abnormalities as well as endocrine and autoimmune disorders (ADs), However, unexplained aetiology for female infertility accounts for almost 30% of the infertile conditions ***(Dal Lago et al., 2011).***

It is believed that a significant proportion of unexplained reproductive failure can be either directly or indirectly related to autoimmunity. ***(*Dal Lago *et al., 2011).***

Vitamin D is inactive when it is first synthesized in the body via sun exposure or diet in the form of D2 or D3. The inactive form of vitamin D is then metabolized by the liver to 25 hydroxyvitamin D, or 25(OH) D, which

is the level most often used to assess vitamin D status. 25-hydroxyvitamin D is metabolized by the kidneys into its active form, 1, 25-dihydroxyvitamin D, also known as calcitriol. The calcitriol then binds to and activates the vitamin D receptor (VDR) in the target cells, which have been found in many reproductive organs and tissues throughout the body. In addition to VDR expression, vitamin D activating and inactiving enzymes are necessary in regulating the cellular response to vitamin D. Calcitriol is tightly regulated through negative feedback controls determined by parathyroid hormone, calcium, and phosphorus level cells ***(Orbach et al., 2007).***

Vitamin D is a fat-soluble steroid hormone involved in many functions of the body including calcium and phosphorous homeostasis, bone mineralization, cellular growth, and decreasing the risk for chronic illnesses such as diabetes, cardiovascular disease, cancer, obesity, and autoimmune diseases، Vitamin D receptors (VDR) facilitate the biological activity of vitamin D and are found in many cells throughout the body. VDR have been identified in reproductive tissues such as human testis, sperm, epididymis, seminal vesicle, prostate, ovaries, uterus, placenta, cervix, breast tissue, the pituitary, and hypothalamus ***(Ozkan et al, 2010)***

vitamin D might influence steroidogenesis of both estradiol and progesterone in healthy women where low levels of 25(OH)D levels might be associated with infertility ***(Perricone et al., 2013)***

Vitamin D supplementation may improve female fertility, since it has been reported that vitamin D receptors are found in various reproduction tissues and the correction of vitamin D deficiency confer positive effects in terms of follicle maturation, menstrual regularity and improvement of hyperandrogenis ***(Triggianese et al., 2016).***

Recent data from retrospective and prospective trials have demonstrated contradictive results concerning the role of vitamin D in female reproduction and in vitro fertilization outcome, In this view, the

supplementation of vitamin D might be useful in women with reproductive failure and it is under investigation ***(Paffoni et al., 2014)***

Serum vitamin D (25[OH] D) level is recognized as the best indicator of vitamin D status. The purpose of this study was to explore whether 25(OH) D levels might be a marker of female subfertile women. **(Al-Jaroudi at al., 2016)**

Normal range of vitamin D

Table 1 Common Vitamin D Reference Ranges	
Vitamin D level	**Vitamin D status**
<20ng/ml	Deficient
20-30ng/ml	Insufficient
>30 ng/mL	Sufficient

2. AIM/ OBJECTIVES

This study aims is to assess vitamin D status in unexplained infertile women.

Research hypothesis:

In women with unexplained infertility vitamin D may be deficient.

Research question:

In women with unexplained infertility does vitamin D deficient?

3. METHODOLOGY:
Patients and Methods

Type of study:

Case –control study

Sample size :

Study will be carried out on 140 women , 70 each group

Study sitting:

This is case-control study will be conducted at infertility outpatient clinic at Ain-Shams University maternity Hospital

Study population:

The patient will be recruited from women attending outpatient infertility clinic and family planning clinic. 140 patients will be recruited to this study.

Inclusion criteria

Cases : Patients with unexplained infertility:-

- Age between 20-35 years
- Normal semen analysis
- Normal hormonal profile (FSH , LH and prolactin ,TSH, AMH)
- Normal transvaginal ultrasound (normal appearance of adnexa)
- No evidence of endocrine disorder
- Normal hysterosalpingography or laparoscopy.

Exclusion criteria:

- Medical condition that can cause infertility such as diabetic mellitus, polycystic ovary, hypothyroidism, hypertension, liver or renal disease.
- Uncorrected Congenital or acquired uterine abnormality
- Patient who refused
- Current use of contraceptive
- Patient less than 20year or more than 35years
- Male factor infertility
- Patient under vitamin D therapy

Group A(Cases) : women with unexplained infertility

Group B(Control) : non pregnant fertile women from family planning clinic as control group

Methods of randomization:

Simple random sampling

Methodology:

all included women after informed consent will be subjected to :

A. Full history taking with especial emphasis to maternal age, maternal weight, as well as presence of any disease.
B. General examination
C. Abdominal and pelvic examination.
D. Revising of criteria for diagnosis of unexplained infertility.
E. Unexplained infertility will be diagnosed according World Health Organization **(WHO)** criteria, all women will undergo hormonal assessment to evaluate their ovulatory cycle, thyroid function test, circulating prolactin levels. The ovarian reserve will be checked by measurement of serum level FSH, AMH and luteinizing hormone. Screening of infertility will also include transvaginal ultrasound and hysterosalpingography to exclude possible uterine malformation or pathology and to assess the potency of the fallopian tube.

Study intervention and Laboratory :

Venous blood sample (5ml) blood will be collected using sterile syringe from every participant at any day of menstrual cycle then vitamin D level will be measure by using enzyme linked immunosorbent assay technique (lot n. vds4203; calbiotech Inc., Austin drive , spring vall , California, USA) the procedure will carried out according to the manufactures instruction as supplied with kit. All biochemical measures will perform in single batch and comparable number of cases and control samples will always assayed simultaneously in same enzyme linked assay plate. The kit recorded the result as deficient if vitamin D is less than 20ng/ml, insufficient 20-30ng/ml and sufficient >30ng/ml.

Primary Outcome Measures :

1. Vitamin D level

Serum vitamin D level in women with unexplained infertility and fertile women from family planning clinic.

Data collection:

Demographic data and laboratory evaluation will be recorded and compared in both groups.

Data or results which are collected after management in suitable manner by a process known as processing of data.

Statistical analysis:

Statistical analysis will be done on personal computer using IBM, SPSS statistical version of 21(IBM , Corp ,Armonk , NY ,USA)

Data will be collected, tabulated then analysed using appropriate statistical test. The D'Agostino-Pearson test will be used to test the normality of numerical data distribution. Numerical data will be presented as mean and standard deviation (if normally distributed) or as median and interquartile range (if skewed). Categorical data will be presented as number and percentage or as ratio.

The student t test will be used to compare normally distributed numerical data. For skewed data, the mannwitney U test will be used, the chi square test or (Fisher's exact test, when appropriate) will be used to compare categorical data p <0.01 will considered statistical significant.

Sample size justification:

Based a relevant study ***(Al-Jaroudy et al., 2016)*** on vitamin D deficiency in subfertile women 60% compared 40% among control group, alpha error 5%, power study 80%, required sample size 140 subjects,70 each group.

Ethical:

The study will be approved from ethical committee of the department of obstetrics and gynaecology faculty of medicine Ain shams' university.

Informed consent will be taken from all participant before recruitment in the study and after explaining the procedure.