PREVALENCE AND CORRELATES OF LOW SERUM VITAMIN D LEVELS AMONG WOMEN WITH RECURRENT PREGNANCY LOSS AT THE KENYATTA NATIONAL HOSPITAL: A CROSS SECTIONAL STUDY

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DECLARATION

This research was undertaken in partial fulfilment of the Master of Medicine in Obstetrics and Gynecology from the University of Nairobi and was my original work and has not been undertaken and presented for a degree in any other University.

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DEDICATION

The dissertation is dedicated to my son Nolan Jabali.

LIST OF ABBREVIATIONS

ANC	Antenatal Clinic
ART	Assisted Reproductive Technologies
ESHRE	European Society of Human Reproduction and Embryology
GOPC	Gynecology Outpatient Clinic
KNH	Kenyatta National Hospital
PCOS	Polycystic Ovarian Syndrome
PL	Pregnancy Loss
PNC	Post Natal Clinic
RPL	Recurrent Pregnancy Loss
UoN	University of Nairobi
WCRPL	World Congress on Recurrent Pregnancy Loss
WHO	World Health Organization

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ABSTRACT

Introduction: Recurrent Pregnancy Loss (RPL) affects approximately 1% of couples and is defined by WCRPL as the loss of two or more pregnancies after spontaneous conception or following ART excluding molar and ectopic pregnancies. In nearly 50% of the cases, the cause is unknown. However, there are many associated factors leading to RPL which include nutritional factors such as Vitamin D deficiency, antiphospholipid syndrome, genetic and uterine anomalies, endocrine disorders, and infections among others. Deficiency in Vitamin D emerged as an important factor in RPL as shown in recent studies and a significant majority of pregnant women have low levels of Vitamin D. There is an observed deficiency of Vitamin D in the Kenyan population and its implication in RPL has not been determined. In addition, the prevalence of RPL in Kenya has not been documented despite having an increasing number of patients with RPL attending ANC in major referral hospitals such as KNH.

Broad Objective: To determine the prevalence and correlates of low serum levels of Vitamin D in women with history of recurrent pregnancy loss at the Kenyatta National Hospital.

Methodology: This was a comparative cross-sectional study with 124 study participants. The serum levels of Vitamin D of 62 women with a history of RPL were compared with 62 women who delivered 2 or more viable fetus at **the Kenyatta National Hospital.** Simple random sampling technique was used to **obtain participants**. Women who gave a written informed consent and fit the inclusion criteria were recruited for the study. Structured questionnaires were used to collect data and any missing sociodemographic information was obtained from the patient's file. Serum Vitamin D levels were assayed at Pathologists Lancet Kenya.

Data management and analysis: Statistical analysis was performed using R software. The mean levels of vitamin D for each group were calculated and comparison done T-test. A correlation of the socio demographic and clinical characteristics and low vitamin D levels was analyzed, and binary logistic regression model performed, controlling for possible confounders. A p value of 0.05 was statistically significant.

Results: Women with RPL had low serum Vitamin D levels with a prevalence of 50.8% compared to women without RPL at 35% (p=0.17). The mean age of women in comparative group was higher compared to women with RPL (32.2 vs 31.3) and BMI was significantly higher among participants with RPL (28.8±4.6) compared to those without RPL (26.4±4.7) before and after adjusting for confounding (P<0.01) Marital status, education level, alcohol consumption, cigarette smoking, and having a history of chronic medical conditions such as diabetes and hypertension were not associated with RPL.

Conclusion: Women with RPL had low serum Vitamin D levels compared to women who had normal viable pregnancies.

Recommendations: Assessment of serum Vitamin D levels in women with a history of RPL as a component of their preconception care and consider including it in protocol. These will help in further categorizing of patients in need of supplementation hence improve on pregnancy outcomes.

CHAPTER ONE

1 INTRODUCTION

1.1 Background of the Study

Pregnancy loss is the spontaneous loss of a pregnancy before the fetus reaches viability, from conception till 24 weeks gestation or the death of a fetus between 20 and 22 complete weeks gestation weighing approximately ≤ 500g (WHO). The losses are further categorized into 3: pre-embryonic as losses less than 5 weeks gestation; embryonic as losses between 5-10 weeks' gestation and fetal as losses from 10 weeks and above (1).

RPL is the loss of 2 or more pregnancies either after spontaneous conception or ART excluding molar, ectopic pregnancies and implantation failure or two or more failed clinical pregnancies documented by an ultrasound scan or histopathology (2). It affects 1-2% of couples (3) and approximately 1%-2% of women in the reproductive age (4) with an incidence of approximately 1 in 300 pregnancies (5). Thirty percent of pregnancies are said to be lost between implantation and the sixth week of gestation (3) and the risk of subsequent miscarriage is increased with a history of a previous miscarriage and advanced maternal age. RPL is commoner in women aged 40 years and above. The risk is lowest in women aged between 20-35 years and higher after the age of 40 (3) so age is a key factor in RPL.

RPL is becoming a common problem among women in the reproductive age in KNH. However, its prevalence has not been documented hence the need to study some of its likely causative factor such as low vitamin D levels.

1.2 Sources and Metabolism of Vitamin D

Vitamin D, is a fat-soluble vitamin that acts as a steroid hormone(6).It is the natural form of Vitamin D which is synthesized from the skin when ultraviolet B light from the sun hits the skin (2). Ninety percent of Vitamin D is from Sunlight, the main source of Vitamin D_3(2). About 10% of vitamin D is from foods that provide vitamin D_3 such as oil-rich fish like salmon, tuna, swordfish, mackerel and herring, egg yolk, red meat, fortified dairy, cod liver oil and mushroom (3). Vitamin D_2 is synthesized when a beam of light strikes yeast.

Vitamin D is metabolized when Vitamin D2 and Vitamin D3 precursors bind to a binding protein (VDBP) which are carried in blood and reach the liver where they undergo metabolization to form calcidiol (25-OH cholecalciferol) then to calcitriol or 1,25 dihydroxycholecalciferol, the most potent vitamin D metabolite (3). Calcidiol metabolism to calcitriol takes place in the kidney (3). Deficiency of vitamin D is described as serum vitamin D levels less than 20 ng/mL (7) which been associated with a higher risk of RPL (4).Vitamin D deficiency results from low intake of foods with adequate vitamin D during the preconception period and during pregnancy, in chronic kidney disease due to impaired vitamin D metabolism (8) and inadequate exposure to sunlight. Social and demographic factors such as age, marital status, educational level and family income also have been associated with low levels of vitamin D levels (8) with people with a low income suffering from vitamin D deficiency due to inability to acquire foods rich in vitamin D.

Sufficient amounts of vitamin D promotes decidualization for a pregnancy to establish and regulates the HOXA10 gene responsible for development of the endometrium during implantation (7). Other genes are also involved in trophoblast invasion and angiogenesis that are vital during implantation and development of the placenta and its functions thus promoting fetal growth and development. A deficiency would therefore increases the chances of pregnancy loss (7). Vitamin D modulates the immune system by inducing production of Th 2 cells that counter inflammation and induces decidualization and inhibits proliferation of Th 1 cells. Th1 cells produce tumor necrosis factor-alpha, gamma interferon and IL-2 which inhibit placental trophoblastic cell growth and metabolic activity through an inflammatory process(9).Low serum vitamin D dysregulates the immune activities at the fetal-placental interface and interferes with regulation of genes taking part in endometrial development therefore increasing the risk of miscarriage (10).

The recommended normal levels of serum vitamin D in pregnancy be 20 ng/ml, while the Endocrine Society recommends 30 ng/ml or more (11). Vitamin D deficiency in adults is treated by administering 50,000 IU of vitamin D every week for approximately 6-12 weeks. However, few patients may be put on high vitamin D doses for a longer duration of time to be able to achieve and maintain blood levels of vitamin D within the normal ranges.

Despite there being published data on the association of deficiency of vitamin D and recurrent pregnant loss in the developed world (4–7),there is paucity of data about this relationship among patients in the African population. Therefore, assessment of serum vitamin D levels in women with a history of RPL and those with normal pregnancies and comparing the values in both groups will not only help bring out this association but also help in the formulation of standard policy in the definitive assessment and care of patients with RPL.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Prevalence and Burden of Recurrent Pregnancy Loss

Recurrent Pregnancy Loss affects approximately 1-2% of women in the reproductive age with the cause being unknown in nearly 50% of the women. Less than 5% of women have had two pregnancy losses and 1% have had three successive pregnancy losses (4,7,12,13). Auto and cellular immune responses have been shown to be linked with RPL. In China, deficiency in Vitamin D is a common setback in both pregnant and women in childbearing age with low levels of Vitamin D in pregnancy being linked to gestational diabetes, pre-eclampsia, bacterial vaginosis and compromised fetal growth (4).

2.2 Risk Factors for Recurrent Pregnancy Loss

Risk factors associated with RPL include chromosomal abnormalities, endocrine conditions such as untreated hypothyroidism, uncontrolled diabetes mellitus, luteal phase defects, hyperprolactinemia, some anatomic uterine abnormalities, anti-phospholipid syndrome (APS), inherited or acquired thrombophilia's, immunologic factors, infections, and environmental factors (4).About 60% of pregnancy losses due to genetic abnormalities are caused by gene mutations and chromosomal aberrations. Balanced translocation is the most common parental abnormality causing approximately 3-5% of RPL cases. Other abnormalities include reciprocal translocations causing 60% of RPL with Robertsonian translocation causing the rest. Karyotypic abnormalities such as mosaicism, deletion and duplication and ring chromosomes are thought to cause RPL (14). Congenital uterine abnormalities, adhesions in the uterus, fibroids or polyps cause approximately 10%-15% of RPL cases with the septum of the uterus being most closely linked to RPL (15).They disrupt the vasculature of the endometrium reducing its vascular supply.

This causes abnormal and inadequate placentation resulting in a miscarriage and subsequently RPL. Müllerian abnormalities such as unicornuate, didelphis, and bicornuate uteri cause RPL (18). Intrauterine adhesions in Asherman's syndrome affect placenta formation thus causing pregnancy loss in the early weeks of gestation. Intramural and submucosal fibroids more than 5 cm and of any size respectively have been shown to cause RPL(16).

Endocrine abnormalities cause approximately 17-20% of RPL cases in women. For instance Luteal phase defect-due to deficiency of endogenous progesterone- results in impaired placenta formation (15),subclinical hypothyroidism causes a high prevalence of RPL(16) with Polycystic Ovarian Syndrome (PCOS (15) accounting for 40% of the cases. Insulin resistance and the resultant hyperinsulinemia in PCOS cases play a role in RPL Poorly controlled type 1 diabetes mellitus increases risk of spontaneous abortion (15).

Immunological factors such as Antiphospholipid syndrome cause RPL. Antiphospholipid antibodies cause impaired trophoblastic invasion, abnormal formation of spiral arteries, improper secretion of the hormone human chorionic gonadotrophin resulting in. programmed cell death of the syncytiotrophoblast (15). An inflammatory response is then mounted resulting in pregnancy loss and complications such as pre-eclampsia (16).

Several environmental factors such as obesity, excessive alcohol intake, excessive caffeine intake and smoking with specific infections such as mycoplasma, urea plasma, chlamydia trachomatis, Listeria monocytogenes, and HSV(15) are thought to cause RPL.

2.3 Serum Vitamin D and Recurrent Pregnancy Loss

Several studies carried out by different authors have been shown to point out the link between low levels of serum vitamin D resulting in recurrent pregnancy loss. A prospective cohort study carried out in the USA aimed at assessing the association between levels of preconception serum vitamin D among women with proven fertility and the resultant outcomes of pregnancy. Study participants who were women aged between 18–40 years with one or two pregnancy losses were recruited from four clinical institutions between 15th June 2007 to 15th July 2011. Follow up was done for six menstrual cycles while they tried to conceive. Once they conceived, follow up was done throughout their pregnancy (17). Serum vitamin D levels were then measured before conception and at 8weeks gestation; 47% of the women had normal concentrations while 53% had low levels of serum vitamin D with normal serum levels presumed to be > 75nmol/L. Women with normal serum Vitamin D levels before conception had higher chances of getting pregnant and having live births, with an adjusted Risk Ratio of 1:10 than those with low levels (17). Women who conceived, in addition, had normal serum Vitamin D levels before conception thus had reduced risk of pregnancy loss. Normal serum vitamin D levels (≥75 nmol/L) predicted a higher chance of carrying the pregnancy to term and having a live birth hence reduced risk pregnancy loss (18).

A retrospective cross-sectional study was carried out from January 2011 to December 2012 where 133 women with a history of 2 or more pregnancy losses over a 2-year period were recruited (9).Records of 186 women with RPL were reviewed as they were registered. 53 women who had uterine fibroids, endocrine disorders, genetic abnormalities and on Vitamin D supplementation were excluded. A sample of 133 women as study participants was obtained and neither of the participants was pregnant.52.6% of the women with RPL had normal serum vitamin D levels (≥30 ng/ ml) while 47.4% had low serum vitamin D levels (<30ng/ml)

Deficiency in vitamin D was an issue in the United States in reproductive-aged women and has been associated with bad outcomes in pregnancy. An analysis to bring out the association between deficiency of vitamin in the first trimester and the clinical outcomes thereafter was done (5)

Blood was collected from nulliparous women with singleton pregnancies, no history of chronic medical conditions during their first trimester aged between 18-40 years with a mean age of 24.3years. Analysis of levels of vitamin D was done (5). Seventy percent of the participants had low serum vitamin D levels (<30 ng/mL) with a mean serum concentration of 27.6 ng/mL and an adjusted odds ratio 1.01 with logistic regression adjusting for age, participants BMI, race, history of tobacco use, and the time of year study was done. There were associated adverse events such as spontaneous abortion, preeclampsia, preterm delivery, gestational diabetes and growth restriction. (5).

The relation between serum levels of vitamin D and the risk of subsequent miscarriage was analyzed in 1683 pregnant women, recruited for a period of 2 years, whose blood was drawn before 22weeks gestation. The risk of miscarriage in the first trimester was low when serum levels vitamin D were normal with a confidence interval of 95% and higher when serum levels were <50 nmol/L. There was an association between low serum levels of vitamin D and risk of subsequent miscarriages in the trimester with low levels of vitamin D intensifying risk for pregnancy loss in 1st trimester but not in the 2nd trimester (3).

The relationship between vitamin D deficiency in women in childbearing age and those with history of pregnancy loss in the first trimester was through a cross-sectional study using a 4-arm model (19). Blood was drawn from 60 women with singleton pregnancies between 7–9 weeks of gestation, 30 with a viable pregnancy and 30 with history of pregnancy loss and 60 non-gravid childbearing aged women,30 with a history of normal pregnancy, and 30 with one or more history of pregnancy loss in the 1st trimester. Serum vitamin D and vitamin D-1 alpha hydroxylase were analyzed with women with a history of normal pregnancy found to have higher serum vitamin D at 49.32 μg/l and vitamin D-1 alpha hydroxylase at 82.00 pg/ml than women with a history of pregnancy loss 34.49 μg/l and 37.87 pg/ml. both P<0.01; the non-gravid women with a successful pregnancy history also had higher Vitamin D at 39.56 μg/l and vitamin D-1 alpha hydroxylase at 39.04 pg/ml compared to women with a history of pregnancy loss 12.30 μg/l and 12.35 pg/ml, both P<0.01(18). Both gravid and non-gravid women with a history of RPL were shown to have had low serum vitamin D levels (18). Thirteen participants (43.3%) (Gravid with a history of pregnancy loss) and 29 (96.7%) (non-gravid with a history of pregnancy loss) while 1

(3.3%) in the normal pregnant group and 1 (3.3%) in the non-gravid women with normal pregnancy of the study participants had low serum vitamin D levels below 30 μg/l.(18). This brought out a strong correlation between low serum levels of vitamin D likelihood of a pregnancy loss with an odds ratio 1.71, 95% confidence interval 1.2–2.4 and a p value $P<0.001$ with an increased risk of pregnancy losses in future pregnancies (18).

A systematic review by Gonçalves et al done in 2018 included eleven studies (4 –IRAN, 4- China, 1-Egypt and 2-USA) reported a high prevalence of low vitamin D levels in women with RPL (4).This brought out the fact that deficiency in vitamin D or vitamin D insufficiency is as a result of immunological dysregulation such as increased cytotoxicity of NK cells, T helper cells dysregulation with proliferation of Th 1 cells inhibiting trophoblastic cell growth resulting in RPL. Vitamin D exposure was pointed out to be important in women with RPL. Vitamin D supplementation in RPL was then thought to be beneficial(4).

A prospective study done in Basrah Maternity and Child Hospital involved 280 women who were divided into 4 groups: 70 pregnant women with normal pregnancy in the first group, 70 pregnant women with history of early pregnancy loss in the second group, further, 70 non-gravid women with a background of normal pregnancy in the third group and then the fourth group included 70 non-gravid women with history of pregnancy loss (19).Plasma was collected from 280 women and serum vitamin D and calcium levels were analyzed and compared between the groups. Serum levels were 45.73±2 ng/ml in group 1, 20.2±1.4 ng/ml in group 2, 37.6±2.1 ng/ml in group 3 and 11.5 ± 3.1 ng/ml in group 4. Women with history of pregnancy loss had both low serum vitamin D and calcium levels thus there was higher risk of pregnancy loss due to low vitamin D and calcium levels. vitamin D is therefore important in pregnancy from implantation and decidualization phase till term (19).

An assessment of Vitamin D levels using a prospective observational study in pregnant women in a tertiary facility in Kashmir had 50 participants taken randomly for the study after a thorough history and examination (20).Blood drawn was taken for analysis. Serum levels of Vitamin D was analyzed using liquid chromatography with levels below 30ng/ml implying a deficiency. Sixty eight percent of patients had low serum levels of vitamin D (<30ng/ml) while 32% patients had

normal levels of serum vitamin D; low levels of vitamin D were said to be a causative factor in RPL (20).

A cross-sectional study analyzed the prevalence of vitamin D deficiency in women who had delivered. The prevalence of low serum levels of vitamin D was 79.4% with a 95% CI. There was no correlation between the levels of vitamin D, Body Mass Indices of the participants, and number of times they were exposed to sunlight (8).

2.4 Conceptual Framework

2.4.1 Narrative

Recurrent Pregnancy Loss in 50% of women has no identifiable cause (7).However low levels of serum vitamin D is thought to contribute to RPL in addition to antiphospholipid syndrome. Several factors contribute to low serum levels of Vitamin D linked to RPL resulting in vitamin D deficiency. This include low intake of foods with adequate vitamin D such as fish, cod liver oil, dairy products during the preconception period and pregnancy, medical conditions such as chronic kidney disease due to impaired vitamin D metabolism, hyperthyroidism, SLE, Diabetes Mellitus, Hypertension and inadequate exposure to sunlight in cases of women who completely cover themselves because of their religion.Social and demographic factors for instance age, marital status, educational level and family income also have an impact on vitamin D levels. People with a low income are unable to acquire foods rich in vitamin D always hence suffer from vitamin D deficiency. Adverse events include RPL and pre-eclampsia.

2.4.2 Diagrammatic Presentation

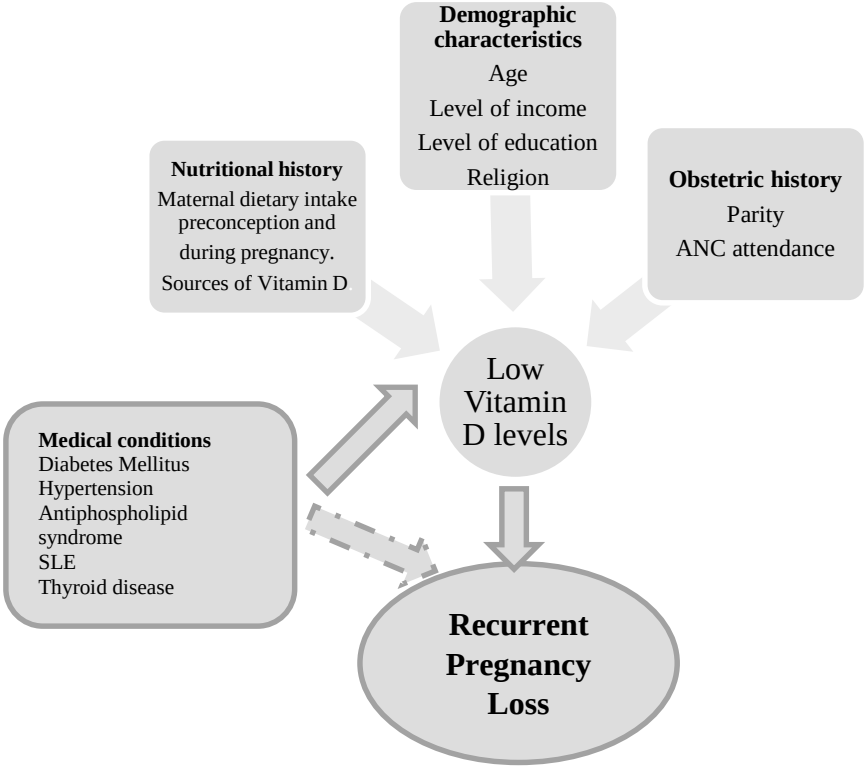

Figure 1: Conceptual Framework

2.5 Study Justification

Vitamin D deficiency is a likely problem in our region and set up as is seen in several facilities (8)among them Kenyatta National Referral hospital, thus resulting in several cases of RPL. However, this has not been documented. Once established as a cause of RPL, it is easily corrected by Vitamin D supplementation prior to conception and this has shown to increases chances of live births according to recent data (4)(18).

Auto- and cellular immunological responses have been associated with RPL with vitamin D playing an anti-inflammatory role in the immune system modulation. Low levels of vitamin D in pregnancy have been linked to spontaneous miscarriages, restricting fetal growth, preeclampsia, gestational diabetes mellitus and preterm labor. Gonçalves et al reviewed several articles whose studies reported a high prevalence of low serum levels of vitamin D in women with RPL and thereby causing immunological dysregulation and consequently with RPL.

Data from this study will contribute to practice and policy changes in management of RPL cases attributed to deficiency in vitamin D. No similar study has been done looking at the prevalence and correlates of low levels of serum vitamin D in women with RPL locally and in Africa hence the need to do the study and bring out the gap as low serum Vitamin D levels being a causative factor for RPL.

2.6 Study Question

What is the prevalence and correlates of low serum vitamin D levels among women with and without recurrent pregnancy loss at the Kenyatta National Hospital?

2.7 Hypothesis

There is no correlation between low serum vitamin D levels and recurrent pregnancy loss among women attending clinic at the Kenyatta National Hospital.

2.8 Study Objectives

2.8.1 Broad Objectives

To determine the prevalence and correlates of low serum vitamin D levels among women with recurrent pregnancy loss at the Kenyatta National Hospital.

2.8.2 Specific Objectives

Among women aged 16-49years:

1. To determine the prevalence of low serum vitamin D among women with and without recurrent pregnancy loss at the Kenyatta National Hospital.
2. To determine the socio demographic and clinical characteristics associated with low serum vitamin D levels among women and without Recurrent Pregnancy Loss.
3. To determine the correlation between low levels of vitamin D and socio demographic and clinical characteristics among patients with recurrent pregnancy loss.

CHAPTER THREE

3 METHODOLOGY

3.1 Study Design

This was a hospital-based comparative cross-sectional study.

3.2 Study Setting

The study was carried out at clinic 18 and acute gynecological ward (ward 1D) at the Kenyatta National Hospital (KNH). Clinic 18 offers ANC services for all mothers with a minimum of 8 routine ANC visits throughout pregnancy. It also has a High-risk clinic where women with RPL, previous caesarean section deliveries, maternal and fetal conditions among others are seen.

The unit is run by maternal-fetal medicine specialists, obstetricians and gynecologists, graduate school students, nurses, laboratory technicians and nutritionists. There is also an outpatient clinic where such patients are seen and booked for review at clinic 18. A total of 1400 women are seen on average per month at the ANC clinic and receive the services. Even though the prevalence of RPL is unknown, it has been observed to happen in majority of patients seeking ANC services at major referral hospitals like KNH hence the reason was a preferred study site.

3.3 Study Population

The catchment population at the ANC (In clinic 18) includes patients seen previously at the unit when pregnant, patients seen at the reproductive health room (room 5) in accident and emergency and booked to the clinic and referrals from other hospitals for specialized care and follow up. Those with recurrent pregnancy losses are sent to the high-risk clinic, within clinic 18, for proper history taking evaluation of other causes of RPL such as anti-phospholipid syndrome, Hypertension, Uncontrolled Diabetes Mellitus, appropriate examination and evaluation which includes antenatal profile other appropriate investigations. Definitive management is given, the women are then followed up at the unit till delivery.

Study participants were women with a history of recurrent pregnancy losses seen in ANC,GOPC and Ward 1D at KNH aged between 16-49 years, of low to middle income and with medical conditions like hypertension, thyroid disease, Systemic lupus erythematosus and Diabetes Mellitus. The comparable group was age matched women who had normal term pregnancies and delivered viable babies and aged between 16-49 years attending the PNC at the KNH.

3.3.1 Inclusion Criteria

Table 1: Inclusion Criteria for the study group and the comparison group respectively

Women with RPL	Women without a history of RPL
Aged 16 to 49 years	Aged 16 to 49 years

3.3.2 Exclusion Criteria

Table 2: Exclusion Criteria for the study group and comparison group respectively

Women with RPL	**Women without a history of RPL**
On vitamin D supplementation	On vitamin D supplementation Less than 6 weeks post-partum'

3.4 Sampling and Sample Size

Sample size was calculated using the difference in proportions - Fleiss JL formula with CC (Statcalc epi-info^{TM}) as outlined below. The following assumptions, from a similar study by Kashmir were considered during the calculation (20).

n = sample size per arm

r = ratio of unexposed to exposed, 1:1 in this case

P_1= proportion of women in the recurrent pregnancy loss group (exposed group) who had low levels of vitamin D = 68%

P_2= proportion of women in the normal pregnancy group (unexposed group) who had low levels of vitamin D = 32%

Ṕ = measure of variability, taken as 68+32/2= 50

Z_β=Value corresponding to the power of the study, in this case 80% = 0.84

$Z\alpha$ = Value corresponding to the normal standard deviate at 95% C.I in this case = 1.96, with 0.05 level of significance

P_1- P_2 = effect size (difference in proportions) = 68-32=36

N/B: Using the epi info calculation gives us similar results as follows: O.R of 3.0

In total, 124 participants (Fleiss formula with continuity correction) were enrolled to take part in the study, 62 women with a history of RPL and 62 women with a history of normal term pregnancy and attending the post-natal clinic. Consecutive sampling was used to recruit participants for the study.

3.5 Study Flow Chart

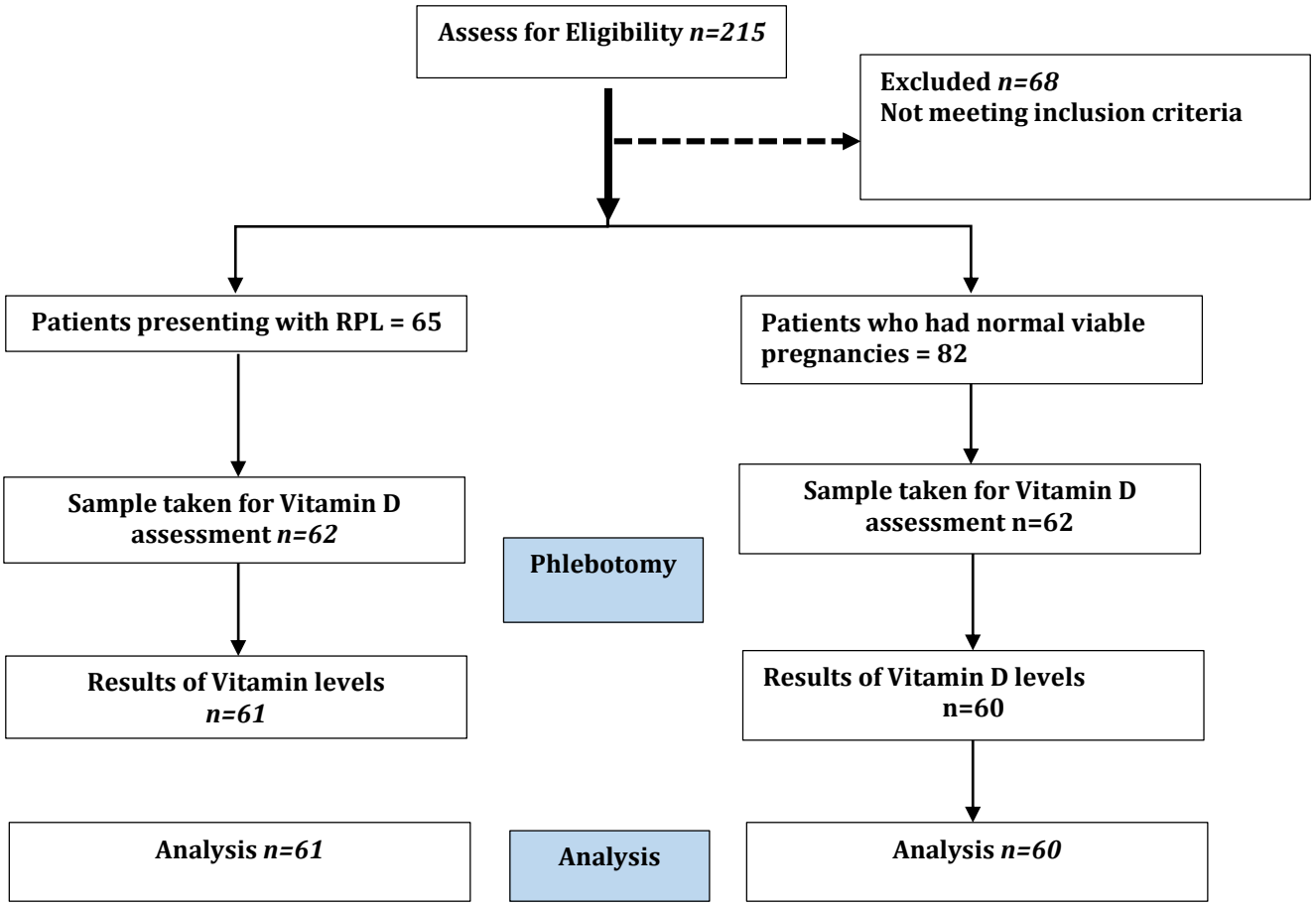

Figure 2: Study Flow Chart

3.6 Recruitment and Consenting

For either of the study groups, health talks were held by the Principal Investigator (PI) or Research Assistant (RA) at the respective clinics to sensitize the patients and health care workers about the study. Patients aged 16 to 49 years old, both with RPL and deliveries of viable babies were identified from the registry. An18-year-old was the only study participant below 20 years of age. The patients' files were then marked with a red sticker for ease of identification on the day when would attend their routine clinics. The PI or RA on a daily basis, for the period of data collection, using computer generated random numbers, randomly selected patients who met the inclusion criteria for enrollment. On average, four participants were enrolled per day until the targeted sample of 62 was arrived at, for each group.

The study was explained to the participants individually by the principal investigator and research assistant. This included the procedure of obtaining the sample, any harm and benefits outlined to them including how data shall be disseminated. Written informed consent was obtained from participants willing to take part in the study and who met the inclusion criteria. Questionnaires were administered to the respondents and the files of each participant interviewed was marked with a code to avoid re-interviewing the respondents.

3.7 Sample Collection and Processing Procedure

All participants had their blood drawn by the PI or RA on the day when they gave consent to participate in the study after administration of the study questionnaire. Two mls of venous blood from the cubital vein of the non-dominant hand was drawn from each of the study participants using a 23-gauge needle and put in a red top. The collected blood was taken to the Lancet laboratory for the assay of serum vitamin D levels. The sample processing took an average of 27 minutes and the results were taken back to the PI for analysis.

The Lancet laboratory is an ISO certified laboratory based in Nairobi. The laboratory uses Cobas analyser machine to run vitamin D tests. The minimum reportable concentration of this test is 3.00ng/ml. Testing is monitored using PreciControl Varia and various concentration ranges run every 24hours when test is in use, once per reagent kit or following each calibration for internal quality control.

3.8 Data Variables

The demographic and clinical characteristics of the study participants were analyzed.

Table 3: Data Variables

Variable Type	Variable Definition
Independent variable	Vitamin D levels
Dependent variable	Recurrent pregnancy loss
Intermediate variables	Uterine fibroids are likely to cause recurrent pregnancy losses hence had to be excluded in the study

3.9 Ethical Considerations

The study was submitted to the Kenyatta National Hospital/ University of Nairobi ethics review committee (KNH/UON ERC) for ethical approval before the recruitment commenced. Permission was also sought from the University of Nairobi department of Obstetrics & Gynecology before submissions to ERC and the KNH administration before data collection. The following ethical issues that may arise were considered:

- Consent was obtained from the participants after informing them about the study objectives and benefits.
- Only serum Vitamin D analysis was carried out on the blood sample obtained from the study participant.
- Participants seeking to withdraw from the study were allowed to do so.
- Participants with Low serum vitamin D levels in both groups were informed of their results through a phone call through their provided mobile contacts, booked for review at the ANC, GOPC and PNC clinic and started on vitamin D supplementation.

3.10 Materials

Materials used for data collection and storage included stationery (pens, markers), questionnaires, hard drives, flash drives, data storage files and password protected computers.

3.11 Quality Assurance

The questionnaires were pre- tested and analyzed before a final draft was administered to the study participants. Serum vitamin D levels obtained were within normal.The research assistants were trained on appropriate interview techniques and filling the questionnaires. Recording of clinical findings was entered after thorough scrutiny. Unique identifiers were assigned to all the study participants. If double entries were discovered, one of the questionnaires was withdrawn, discarded and serialization rectified. Information filled on the questionnaires was checked for any errors and corrected.

Interviews were conducted in closed, quiet rooms by the investigator and/or research assistant. Kiswahili, English or both languages were used to interview participants to facilitate understanding and accurate responses. Serum vitamin D levels obtained were communicated to the patient in the next clinic appointment approximately 2 weeks later. Data was stored in password-protected computers, hard drives and flash drives to ensure confidentiality. The data was accessible to only the principal investigator, supervisors and statistician.

3.12 Data Collection and Management

A pre-tested questionnaire was used to collect data. Data on patient demographics, obstetrics and gynecology factors and other risk factors that may have influenced serum vitamin D levels were collected from the patient by the research assistant who was a healthcare worker. The information was corroborated using the patient records. Once collected, the data was entered into a password protected excel computer software and transcribed into SPSS software for data cleaning and analysis.

3.13 Data Analysis

The study was designed to include 62 women per group to detect proportion difference of 0.5 of vitamin D levels between the women who had RPL and those with normal pregnancy, matched for age with an 80% power and a two-sided p value of 0.05. The collected data was uploaded into SPSS version 21 for cleaning and coding before analysis as per the objectives:

1. To determine the socio-demographic status of women with Recurrent Pregnancy Loss and those without Recurrent Pregnancy Loss, the socio demographic characteristics was analyzed and presented in tables of frequencies as shown in the dummy table below:
2. To determine the levels of vitamin D in women with Recurrent Pregnancy Loss and determine the prevalence of low vitamin D levels <30ng/ml.
3. To determine the correlation between low levels of vitamin D and socio demographic and clinical characteristics among patients with recurrent pregnancy loss
4. To determine the difference in mean levels of Vitamin D among women with RPL compared to women with normal pregnancies

Descriptive data was presented using means and standard deviations around the mean for ages, parity, body mass index and Vitamin D levels. Demographic variables in the RPL and normal pregnancy groups was compared using chi square test and logistic regression1 was used to evaluate whether any of the serum Vitamin D levels predicted RPL.

CHAPTER FOUR

4 RESULTS

Data collection was done from October 2020 to November 2020 at Kenyatta National Hospital. All the study participants were identified at clinic 18 and ward 1D and recruited for the study. Two hundred and fifteen patients were assessed for eligibility with 68 study participants excluded. This study participants were either on vitamin D supplementation or had uterine fibroids. 5% (3) of study participants with RPL and 25% (20) with normal pregnancies declined to give consent to take part in the study. 95% (62) of women with RPL and 75% (62) of women without RPL had their samples taken for serum vitamin D analysis. Serum Vitamin D results were availed for analysis with 1 missing result (RPL) and 2 missing results (normal pregnancy) as shown below.

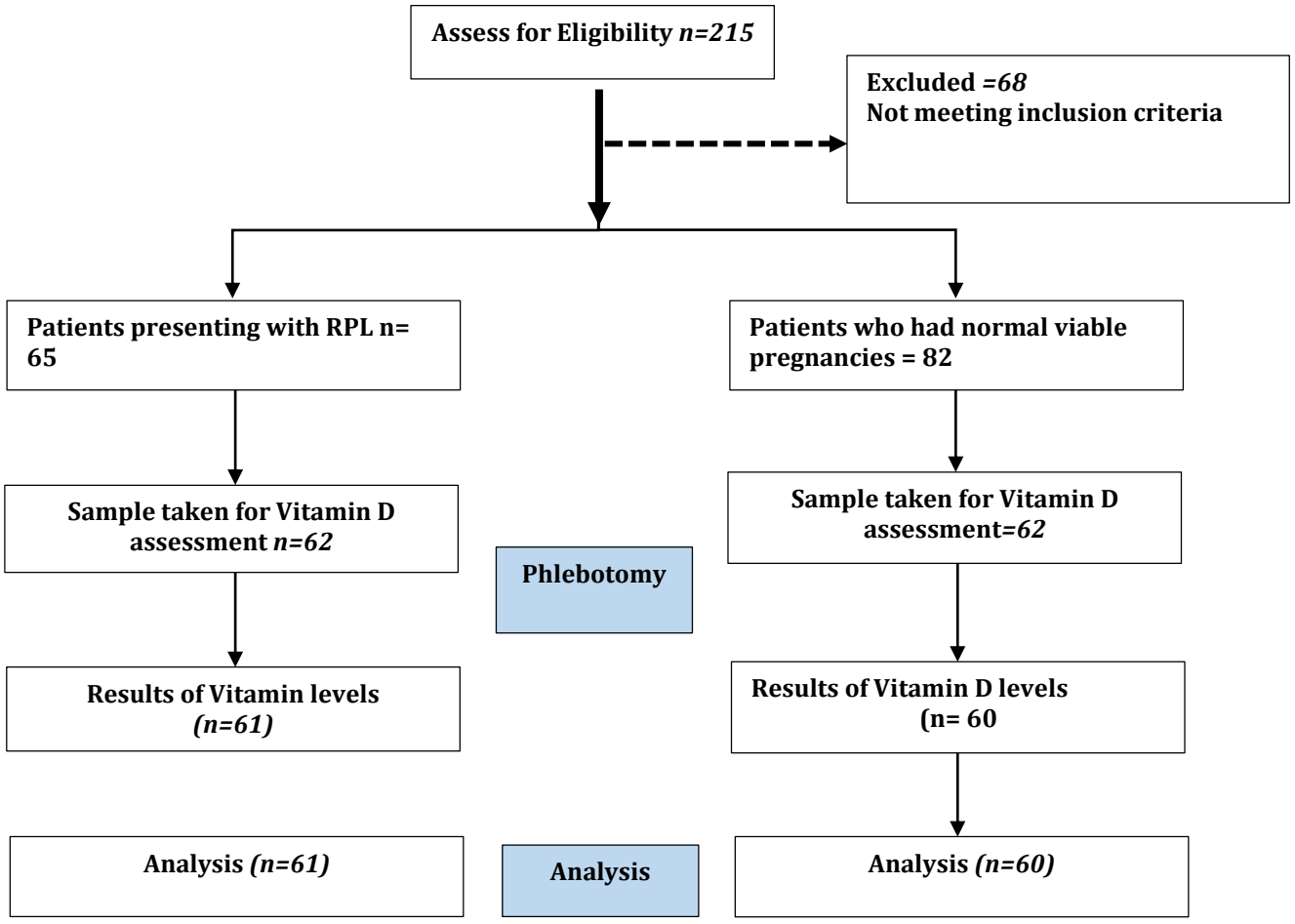

*Figure 3. Study Flow chart*No Sample was rejected by the Pathologists Lancet Kenya: Results for 122 samples were received for Analysis, however we 3 missing results that were reported.*

4.1 Prevalence of low vitamin D levels in women with Recurrent Pregnancy Loss compared to those without recurrent pregnancy loss

The prevalence of low vitamin D levels was 50.8% among participants with RPL and 35.0% among participants without RPL as shown in Table 2. After adjusting for occupation and body mass index of participants, the adjusted odds for having low vitamin D level was 1.69 fold (95% CI, 0.79-3.63) higher among participants with RPL compared to those without RPL, but the difference was not statistically significant (P=0.17).

Table 4. Prevalence of low vitamin D levels in women with Recurrent Pregnancy Loss compared to those without recurrent pregnancy loss at Kenyatta National Hospital

	Vitamin D level					
	Low (52)	Normal (69)	OR (95% CI)*	P value	AOR (95% CI)**	P value
RPL	31 (50.8)	30 (49.2)	1.91 (0.90-3.92)	0.07	1.69 (0.79-3.63)	0.17
No RPL	21 (35.0)	39 (65.0)	Reference			

* Bivariate analysis [chi square test]
** Multivariable analysis [Logistic regression] - adjusted for BMI and occupation
RPL – Recurrent Pregnancy Loss

4.2 Socio-demographic status of women with recurrent pregnancy loss and those without recurrent pregnancy loss

Demographic and reproductive characteristics were associated with recurrent pregnancy loss (RPL) in the studied patients as shown in Table 5. While the age of patients with RPL (31.3±4.2 years) compared to without RPL (32.3±5.1 years) was not statistically different (P=0.14), BMI was significantly higher among participants with RPL (28.8±4.6) compared to without RPL (26.4±4.7) before and after adjusting for confounding (P<0.01). Moreover, the adjusted odds for RPL was 2.88 fold (95% CI=1.21-6.81) higher among participants who were employed compared to

unemployed (P=0.01). Marital status, education, alcohol consumption, cigarette smoking, and history of chronic complication such as diabetes and hypertension were not associated with RPL.

Table 5. Socio-demographic status of women with recurrent pregnancy loss and those without recurrent pregnancy loss

		RPL (61)	No RPL (60)	OR (95% CI)*	P value	AOR(95% CI)**	P value
Age [Mean±SD]		31.3±4.2	32.3±5.1	-	0.33	-	0.14
Body Mass Index [Mean±SD]		28.8±4.6	26.4±4.7	-	<0.01	-	<0.01
Marital status	Single	8 (13.1)	6 (10.0)	Reference			
	Married	48 (78.7)	54 (90.0)	0.66 (0.20-2.19)	0.47	0.43 (0.12-1.51)	0.18
	Divorce/separated	5 (8.2)	0 (0.0)	-	0.07	-	0.99
Education level	Primary	14 (23.0)	9 (15.0)	Reference			
	Secondary	38 (62.3)	45 (75.0)	0.54 (0.22-1.33)	0.20	0.57 (0.20-1.63)	0.29
	Tertiary	9 (14.8)	6 (10.0)	0.96 (0.24-3.24)	0.96	1.01 (0.23-4.45)	0.98
Occupation	Employed	27 (44.3)	17 (28.3)	2.00 (0.97-4.39)	0.07	2.88 (1.21-6.81)	0.01
	Unemployed	34 (55.70	43 (71.7)	Reference			
Alcohol consumption	Yes	3 (4.9)	4 (6.7)	0.72 (0.17-2.80)	0.68	0.52 (0.07-3.47)	0.50
	No	58 (95.1)	56 (93.3)	Reference			
Cigarette smoking	Yes	1 (1.6)	0 (0.0)	-	0.31	-	1.00
	No	60 (98.4)	60 (100)	Reference			
Chronic medical conditions	Yes	9 (14.8)	5 (8.5)	1.90 (0.59-5.31)	0.26	1.95 (0.57-6.74)	0.28
	No	52 (85.3)	55 (91.7)	Reference			

* Bivariate analysis [chi square test]
** Multivariable analysis [Logistic regression] - adjusted for other demographic and reproductive factors

4.3 Correlation between low levels of vitamin D and socio demographic and clinical characteristics among patients with recurrent pregnancy loss at Kenyatta National Hospital

Socio demographic characteristics of patients with RPL and without RPL were correlated with low levels of vitamin D as shown in Table 6. The adjusted odds for having low vitamin D level was 2.73 fold (95% CI = 1.17-6.34) higher among participants who were employed compared to those who were unemployed (P=0.01). Age, BMI, marital status, education level, alcohol consumption, cigarette smoking, and having a history of chronic medical conditions were not associated with RPL statistically significant.

Table 6. Correlation between low levels of vitamin D and socio demographic and clinical characteristics among patients with recurrent pregnancy loss at Kenyatta National Hospital

		Vitamin D level					
		Low (52)	Normal (69)	OR*	P value	AOR**	P value
Age [Mean ± SD]		31.9±5.3	31.6±4.3	-	0.97	-	0.45
Body Mass Index [Mean ± SD]		28.2±4.9	27.2±4.7	-	0.35	-	0.47
Marital status	Single	7 (50.0)	7 (50.0)	Reference			
	Married	42 (41.2)	60 (58.8)	0.70 (0.24-1.97)	0.53	0.52 (0.15-1.81)	0.31
	Divorce/ separate	3 (60.0)	2 (40.0)	0.05 (0.01-0.22)	<0.01	1.65 (0.17-15.7)	0.66
Education level	Primary	10 (43.5)	13 (56.5)	Reference			
	Secondary	39 (47.0)	44 (53.0)	1.15 (0.47-3.07)	0.76	1.06 (0.39-2.87)	0.89
	Tertiary	3 (20.0)	12 (80.0)	0.32 (0.08-1.50)	0.14	0.22 (0.04-1.12)	0.06
Occupation	Employed	24 (54.5)	20 (45.5)	2.10 (1.01-4.49)	0.05	2.73 (1.17-6.34)	0.01
	Unemployed	28 (36.4)	49 (63.6)	Reference	0.38		
Alcohol consumption	Yes	4 (57.1)	3 (42.9)	1.83 (0.47-7.50)	0.43	1.59 (0.24-10.30)	0.62
	No	48 (42.1)	66 (57.9)	Reference			
Cigarette smoking	Yes	0 (0.0)	1 (100)	-	0.38	-	0.14
	No	52 (43.3)	68 (56.7)	Reference			
Chronic medical conditions	Yes	4 (28.6)	10 (71.4)	0.53 (0.17-1.77)	0.31	0.37 (0.09-1.40)	1.00
	No	48 (44.9)	59 (55.1)	Reference			

* Bivariate analysis [chi square test]

** Multivariable analysis [Logistic regression] - adjusted for other demographic and reproductive factors

4.4 Difference in mean levels of Vitamin D among women with recurrent pregnancy loss compared to women with normal pregnancies

The average level of vitamin D was lower among patients with recurrent pregnancy loss (29.4±10.5) compared to those without pregnancy loss (32.9±9.9) but the difference was not statistically significant before adjusting (P=0.058) and after adjusting for occupation (P=0.109).

Table 7. Difference in mean levels of Vitamin D among women with recurrent pregnancy loss compared to women with normal pregnancies

		Vitamin D level		
	N	Mean±SD	P value*	P value**
Recurrent pregnancy loss	61	29.4±10.5	0.058	0.109
No pregnancy loss	60	32.9±9.9		

* Bivariate analysis [independent samples t-test]

** Multivariable analysis [Analysis of Covariance] - adjusted by occupation

CHAPTER FIVE: DISCUSSION

RPL is defined by WCRPL as the loss of two or more pregnancies, before viability, after spontaneous conception or ART excluding molar and ectopic pregnancies. Several studies have linked Vitamin D deficiency to RPL in developed countries.

The prevalence of low serum Vitamin D levels among women with RPL was seen to be higher (50.8%) compared to the comparative group (35%) in this study (odds ratio 1.91;95% confidence interval :0.90-3.92, p value 0.07) in women aged (16-49years) and found no significant difference between the two study groups with a p value 0.14. However, there was an association between BMI and occupation and recurrent pregnancy loss in the studied population. Women with RPL had a higher BMI (28.8%) compared to women who had normal term pregnancies (26.6%) p value <0.01 with employed women having higher chances of getting RPL (44.3%) compared to women with normal pregnancies (28.3%). There was a strong association between employment and RPL (adjusted OR 2.88; 95% CI:1.21-6.81, p value 0.01). There was no association between other factors such as chronic conditions, marital status, education level, alcohol consumption, cigarette smoking with RPL.

This study had similar findings with W.Hou et al who carried out a cross sectional study in Shaanxi Province, China where 29 participants (96.7%.) with history of RPL had low serum Vitamin D levels while women with normal pregnancies had normal serum Vitamin D levels and a p value< 0.01(18). It was then reported that both gravid and non-gravid women with RPL had low serum Vitamin D levels. There was a strong association between low vitamin D levels and RPL (odds ratio 1.71; 95% confidence interval: 1.2-2.4, P<0.001)(18). Kunioka et al also reported a similar finding where 52.6% of the women with RPL had normal serum vitamin D levels while 47.4% had low serum vitamin D levels (9). It was then thought that to achieve a successful clinical pregnancy, sufficient serum Vitamin D levels are key. Gonçalves et al in the systematic review also reported a high prevalence of low serum vitamin D levels among women with RPL (4). This study looked at the prevalence of low serum Vitamin D levels, adopted a similar study design with other studies and had a similar eligibility criterion hence bringing out prevalence of serum vitamin D as low in women with RPL.

A Cochrane collaborative systematic review(21)(22)(23) demonstrated a statistically significant association between BMI and RPL with women with excess weight and obesity having 34% and 75% higher odds of having RPL respectively compared to women with a normal BMI. These also revealed a higher risk of future pregnancy losses in women with a history of RPL(21). Another study in Nepal (22) reported a 45% increase in the odds of RPL with a high BMI, while Lashen et al. reported a 3.5 fold increase in the odds of having RPL in the first trimester when women were obese in a 2004 age-matched case control study in the United Kingdom. These findings were similar to this study where women with RPL had a higher BMI (28.8%) compared to women who had normal term pregnancies (26.6%) p value <0.01. There was an association between high BMI and risk of RPL. During ANC, obese or overweight women should be urged to maintain an optimal weight, as the odds of RPL seemed to increase with increasing BMI.A study done by Kunioka et al in the USA showed no association of socio demographic characteristics of women with RPL and low serum vitamin D levels with p values greater than 0.005 (9).This was quite different from this study findings since we had associations brought out. This would probably be due to a different study design used and a larger study population sampled and had serum vitamin D levels analyzed. There was an association between occupation and the odds of having low serum vitamin D levels with employed patients being 2.73 times more likely to have low vitamin D level compared to unemployed – a common finding .Low level of vitamin D3 was seen in indoor and shift workers (23) who were more at risk of vitamin D deficiency than unemployed. This was due to poor exposure to sunlight predisposing employed women to low vitamin D levels. Moreover, available evidence suggest that prolonged working hours, exposure to antineoplastic agents in workplaces (24), and exposure to ionizing radiations or sterilizing agents in workplaces predispose the employed to low vitamin D levels.

In conclusion, this study brings out the association between low levels of Vitamin D levels and RPL. Women with RPL noted to have low serum vitamin D levels compared to those who had normal pregnancies with women with a higher BMI and those employed being at higher risk of RPL.

STRENGTHS.

This was the first study to look at the prevalence of low serum Vitamin D levels in women with RPL and bring out an association between the two in this region. Previous study done showed a high prevalence of low serum vitamin among pregnant women (8)

WEAKNESSES.

1. However, this being the first study to look at prevalence of low serum vitamin D levels in RPL in the region and setup, it could not bring out causality.
2. It was difficult to assess and bring out with abnormal maternal and paternal karyotypes causing RPL,
3. It was difficult to get baseline serum vitamin D levels due to the costs since this was a self sponsored study
4. BMI at baseline and factors such as prenatal supplementation could help affect the levels of serum vitamin D and most patients couldn't remember their weights prior to carrying their pregnancies.
5. This study had a small sample size to bring out the association between low serum Vitamin D levels and RPL

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the findings of this study found demonstrated the following:

1. Women with RPL have low serum Vitamin D levels compared to women with history of viable pregnancies
2. Employment status is associated with low serum Vitamin D levels and RPL.

6.2 Recommendations

It is recommended that:

1. Institutions should consider assessing serum vitamin D levels in women with RPL and this be included in the protocol
2. Vitamin D supplementation be done in women with low serum levels both with and without RPL and assess their subsequent pregnancy outcomes. A larger study will be required to do so.

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ANNEXES

Annex 1: Consent Form

Study Title: PREVALENCE AND CORRELATES OF LOW SERUM VITAMIN D LEVELS IN WOMEN WITH RECURRENT PREGNANCY LOSS AT KENYATTA NATIONAL HOSPITAL: A CROSS SECTIONAL STUDY

Principal investigator: Doreen Nekesa Wekesa.

Introduction:

I Dr. Doreen Wekesa, a postgraduate student at the Department of Obstetrics & Gynecology, University of Nairobi, is conducting a study on assessing the Vitamin D levels in women aged 16-49 years with RPL compared with women with viable pregnancies. You are hereby requested to participate in the study.

Information provided below will help you decide whether to participate in the study or not. You may ask any questions, clarification about the study or anything in this form that is not clear to either the principle investigator or research assistants.

Purpose of the study:

Recurrent pregnancy loss has been a major concern in our study setup with 50% of causes unknown. With the rising number of cases and paucity of data, Vitamin D levels as a cause has not been evaluated hence the purpose of the study.

Benefits:

Your participation in the study will help us obtain information that will be used to manage recurrent pregnancy losses due to Vitamin D levels among other causes. This will benefit your household, Kenya and women globally.

Possible risks:

You will be required to answer a few questions; no invasive procedures shall be performed on you.

Voluntarism:

This is a voluntary exercise and you only decide to take part in it if you wish to. You can withdraw at any point during the study with no repercussions. The management you receive at the hospital will be standard and not influenced by your decision.

Compensation:

No compensation will be offered for participation in the study.

Procedure:

As a study participant, the researcher and research assistant will obtain some information from you by conducting a short interview with you and your responses filled in a questionnaire. Blood will then be withdrawn at our lab to ascertain your vitamin D levels.

Confidentiality:

The information from you will be confidential. No names or any information identifying you will be included in the questionnaires and the final report. You will be issued with a unique participants code.

Right to withdraw from the study

Kindly note that you have right to withdraw from the study at any point; the services for your care at the KNH will however continue being provided irrespective.

Contact information:

If you have any questions regarding the study, you can contact

Dr. Doreen N Wekesa

Mobile No;0726472241

or

Prof Omondi Ogutu
Department of Obstetrics & Gynaecology
University of Nairobi
P.O BOX 30197-00100 Nairobi.
Mobile: +254722510215

You may also contact the KNH/UoN/ERC Commitee-0735-274288/0721-665077.

Or

The Chairperson,

KNH/UON Ethics and Research Committee

P.O. Box 20723-00202, Nairobi.

Telephone number: (254-020) 2726300-9 Ext 44355

Email:uonknh_erc@uonbi.ac.ke

Your participation in the study will be highly appreciated

Consent:

I…… hereby voluntarily consent to participate in the study. I acknowledge that a thorough explanation of the nature of the study has been given to me by Dr./Mr./Mrs……. I clearly understand that my participation is completely voluntary.

Signature of Participant _____ Date _____

Signature of Researcher/ Assistant _____ Date _____

Annex 2. Consent Form – Swahili

Ridhaa ya mafunzo

Sehemu ya kwanza: maelezo

Utangulizi

DaktariDoreen Wekesa ni mwanafunzi wa Chuo Kikuu cha Nairobi. Anaangazia maswala ya uzazina afya ya wanawake kwa jumla. Ninafanya uchunguzikuhusu: kiwango cha Vitamini D kwa akina mama waja wazito ambao wamepoteza mimba mbili au Zaidi ikilinganishwa na wale ambao wamebeba mimba mbili au Zaidi hadi kujifungua.

Lengo la utafiti

Matokeo ya stadi hii yatatusaidia kutatua hii shida ya upotezaji mimba inayosababishwa na ukosefu wa kiwango wa kutosha wa Vitamini D mwilini kwa wamama wajawazito nchini Kenya na duniani nzima.

Namna

Ukiamua kushiriki katika utafiti huu utatia sahihi na tarehe katika fomu ya makubaliano. Utabaki na nakala moja ya makubaliano haya. Utahitajika kujibu maswali utakayopatiwa, na kutakuwa na msaidizii kuwapa maelezo Zaidi yatahitajika.

Hasarainayotarajiwa

Hakuna hasarai nayotaraji wakati kauchunguzi huu.

Faidainayotarajiwa

Matokeo ya uchunguzi huu yanalengo la kutoa matibabu bora kwa akina mama wanaojifungua katika hospitali yetu pamoja na watoto wao.

Usiri

Matokeo ya uchunguzi huu yatawekwa siri.Hakuna majina yatatumika,kila muhusika atapewa nambari halisi.Matokeo ya uchunguzi yatakabidhiwa kwa wanaohusika.

Hakiyakukataa

Kushiriki katika uchunguzi huu, nikwakujitolea kwa hiari yako. Unahaki ya kujitoa kwa uchunguzi wakati wowote bila ya madhara yoyote,na matibabu yako bado yataendelea kwa njia mwafaka bila matatizo yoyote. Kutoshiriki ni haki yako, na haki hiiitaheshimiwa.

SEHEMU YA PILI: MAKUBALIANO

Ukiwa na swali lolote tafadhali wasiliana nasi Dr. Doreen Wekesa nambari ya simu 0726472241 ama Prof Ogutu kwa nambari 0722510215. Ama KNH/UoN/ERC Commitee-0735-274288/0721-665077.

SLP 20723-00202, Nairobi.

Nambari ya simu: (254-020) 2726300-9 Ext 44355

Email:uonknh_erc@uonbi.ac.ke

Nimesoma na nikaelewa ujumbe ulioko hapa juu.Nimeelezewa kwa makini kuhusu uchunguzi huuna nilipata nafasi ya kuuliza maswali yaliyojibiwa kamili.

Nimekubali kushiriki katika uchunguzi huu bila kulazimishwa amakupewa hongo.

Jina la Muhusika: ……

Saini ya Muhusika:……Au Alama ya Kidole…….

Tarehe: ……..

Saini ya Shahidi: ……..…. Tarehe: ……

Annex 3: Study Questionnaire

A: SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Date:

Participant's code:

B. MATERNAL DEMOGRAPHICS

Date of birth	_ _/ _ _/ _ _ _ _ (dd/mm/yyyy)
Weight (kg)	_ _ kg
Height (cm)	_ _ _ cm
BMI (kg/m^2)	_ _ _. _ kg/m^2
To be calculated	

C: SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS

1.Age (years):

2. Marital status:

☐ Single ☐ Married ☐ Divorced ☐ Separated

3.Educational level:

☐ Primary ☐ Secondary ☐ Tertiary ☐ None

4.Occupation:

☐ Employed ☐ Unemployed

5.Alcohol consumption

☐ Yes ☐ No

6.Cigarette smoking

☐ Yes ☐ No

7.History of chronic maternal medical conditions

☐ Yes ☐ No If yes, specify……..

8.Parity: (using the format Para…..+……)…….

9.Gestation at which previous pregnancy loss(es) occurred

_ _. _ _

10. Serum Vitamin D level in current pregnancy

_ _. _ _

Annex 4: ERC Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
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Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/323

18th September 2020

Dr. Doreen Nekesa Wekesa
Reg. No. H58/7093/2017
Dept. of Obstetrics and Gynaecology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Wekesa

RESEARCH PROPOSAL – PREVALENCE AND CORRELATES OF LOW SERUM VITAMIN D LEVELS AMONG WOMEN WITH RECURRENT PREGNANCY LOSS AT THE KENYATTA NATIONAL HOSPITAL; A CROSS SECTIONAL STUDY (P285/05/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 18th September 2020 – 17th September 2021.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Obstetrics and Gynaecology, UoN
Supervisors: Prof. Omondi Ogutu, Dept .of Obstetrics and Gynaecology, UoN
Dr. Anne Pulei, Dept.of Obstetrics and Gynaecology, UoN

Annex 5: KNH Approval

KENYATTA NATIONAL HOSPITAL
P.O. BOX 20723, 00202 Nairobi

Tel.: 2726300/2726450/2726550
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OFFICE OF HEAD OF DEPARTMENT, OBSTETRICS $ GYNAECOLOGY
EXT.43370

KNH/HOD-OBS&GYN/07/VOL.1 **DATE: 1st October, 2020**

Doreen Nekesa Wekesa
Reg.No.H58/7093/2017
School of Nursing Sciences
College of Health Sciences
University of Nairobi

RE: PREVALENCE OF CORRELATES OF LOW SERUM VITAMIN D LEVELS AMONG WOMEN WITH RECURRENT PREGNANCY LOSS AT KNH; A CROSS SECTIONAL STUDY (P285/05/2020)

This is to inform you that the department has given you permission to conduct the above study which has been approved by ERC.

Liaise with Senior Assistant Chief Nurse - Incharge Clinic 18 - Room 5, A&E and Ward 1D Obstetrics and Gynaecology to facilitate your study.

You will be expected to disseminate your results to the department upon completion of your study.

Dr. Maureen Owiti
HEAD OF DEPARTMENT
OBSTETRICS & GYNAECOLOGY

Cc.
SACN
I/c Clinic 18
I/C A&E
I/c Ward 1D

Annex 6: Turnitin Report

PREVALENCE AND CORRELATES OF LOW SERUM VITAMIN D LEVELS AMONG WOMEN WITH RECURRENT PREGNANCY LOSS AT THE KENYATTA NATIONAL HOSPITAL: A CROSS SECTIONAL STUDY

Professor Omondi Ogutu
17-8-2022

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Prof. Cheseren
17-8-2022

DEPARTMENT OF OBSTETRIC AND GYNAECOLOGY
P.O. Box 19676, NAIROBI
Date
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF NAIROBI