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Assessment of Vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, 2024.

By: Getachew Wolde Eneyew (BSc)

Advisors

Dr. Mistire Wolde (PhD, Associate Professor)

Mr. Gobena Dedefo (MSc)

Mrs. Mekdes Alem (MSc)

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This is to certify that the thesis prepared by Getachew Wolde Eneyew , entitled: ***Assessment of Vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, 2024*** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Advisor _____Signature _____ Date _____

Advisor _____Signature _____Date _____

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Abbreviation and Acronyms

1,25(OH)2D	1, 25-Dihydroxyvitamin D
25OHD	25-Hydroxyvitamin D
7-DHC	7-Dehydrocholesterol
Ca+2	Calcium
CYP24A1	25-Hydroxyvitamin D-24-Hydroxylase
CYP27B1	25ohd 1α-Hydroxylase
CYP2R1	25-Hydroxalase
DBP	Vitamin D Binding Protein
FGF-23	Fibroblast Growth Factor 23
HPO4 2	Phosphorus (Phosphate)
NG/ML	Nano grams Per Milliliter
NM	Nanometers
NMOL/L	Nano Moles Per Liter
PO_4	Phosphate
PRED3	Pre-vitamin D
PTH	Parathyroid Hormone
RANK	Receptor Activator of Nuclear Factor Kappa-B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
T1DM	Type 1 Diabetes Mellitus
UVB	Ultraviolet B
VD	Vitamin D
VDD	Vitamin D Deficiency
VDR	Vitamin D Receptor

Abstract

Background: Vitamin D deficiency is a global health concern, linked to various pregnancy complications and long-term health risks. It is a typical issue during pregnancy, which can have detrimental effects on the developing fetus, the newborn, and the child. Despite its importance, there is a scarcity of data regarding Vitamin D levels and the factors influencing these levels among pregnant women in Ethiopia.

Objective: This study aimed to assess Vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health centers in Addis Ababa, Ethiopia, 2024.

Methodology: A facility-based cross-sectional study was conducted from April 1 to June 7 2024 among a randomly selected 402 first-trimester pregnant women attending antenatal care services. Sociodemographic, behavioral and clinical data were collected through semi-structured questioners. Five (5) ml of blood sample was collected for Laboratory analysis, and Vitamin-D measurement was done by using Cobas Integra e411 chemistry analyzer. The collected data was entered and analyzed by using SPSS version 26 software, and logistic regression model was used to identify the associated factor and P-value <0.05 was considered as statistically significant.

Result: In the current study prevalence of vitamin D deficiency (<20ng/ml) among the first trimester pregnant women were 43.3%, and from this 4.5% were severely deficient (<10ng/ml). The odds of having vitamin D deficiency was higher among participants with BMI of ≥30 (AOR = 6.9, 95% CI: 2.9-16.3, p<0.001) and BMI of 25-30 (AOR=6.75, 95%CI: 3.4 13.3, p<0.001). Who never ate Fish (AOR = 8.5, 95% CI: 4.5–16.2, p < 0.001) and Egg (AOR = 15.6, 95% CI: 5.1–27.9, p <0.001) and also being Multiparous (AOR=3.2, 95% CI: 1.6 6.5, p<0.001). However, Pregnant women who regularly consumed liver were 69% less likely to develop Vitamin D deficiency (AOR = 0.31; 95% CI: 0.16–0.59, p < 0.001).

Conclusion: The current study found VDD is highly prevalent in the study population. Different factors such as overweight, obesity, and being multiparous increases the risk of vitamin D deficiency and regular consumption of fish, egg and liver and also exposing >30% the total body surface area to sunlight were greatly associated with lower risk of developing Vitamin D deficiency in pregnant women.

Key words: - Vitamin D deficiency, Pregnant women, First trimester, Health Center Addis Ababa, Ethiopia

1. Introduction

1.1. Background

Vitamin D(VD) constitutes a collection of fat-soluble secosteroids that contribute to improved intestinal absorption of calcium, magnesium, and phosphate. Additionally, it is crucial for proper functioning of various physiological systems (1, 2). Its antioxidant, protective of neurons, and anti-inflammatory properties improve the condition of muscles, cells in the brain, and the immune system(3).

Vitamin D is obtained primarily from sun contact with the skin and food supplements are another sources(1). The two nutritional forms of VD that humans can absorb and use are vit-D3 (Cholecalciferol) from the skin and vit-D2 (ergocalciferol) from plants(4). Either cutaneous exposure to UVB (Ultraviolet B) light or food consumption can produce VD. After production, VD (D2 or D3) is transformed to 25 hydroxy D in the liver by the enzyme vitamin D-25-hydroxylase (25-OHase). Following this, 1-OHase in the kidneys converts 25 hydroxy D to 1, 25(OH) 2D (active kind). Once generated, this active form helps the intestines absorb calcium and phosphorus. Additionally, it triggers osteoblasts to express Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), which then connect with preosteoclasts' RANK receptor to stimulate mature bone resorption activity and release of calcium (Ca) and phosphorus (HPO4 2-). Furthermore, 1, 25(OH) 2D promotes production of kidney 25(OH) D-24-OHase and inhibits renal 1-OHase. 1, 25(OH) 2D is broken down to the water-soluble inactive metabolite calcitroic acid upon the stimulation of 24-OHase (5-7).

Vitamin D deficiency (VDD) means the body doesn’t get enough VD which primarily causes issues with bones, muscles and other metabolic activities. Despite the lack of agreement over the ideal blood levels of 25-hydroxyvitamin D and it is commonly described as a serum VD level below 20ng/mL (50nmol/L), while insufficiency is between 20-29ng/mL (50-75nmol/L)(8-10).

Vitamin D deficiency is a typical issue during pregnancy, which can have detrimental effects on the growing fetus, the newborn, and the child. The growth of healthy bones and teeth depends on Calcium (Ca) and phosphorus (PO4) absorption, both of which are made possible by VD(11). It also affects the development of muscles and the immune system(12). Furthermore, it aids in

controlling the mother's blood's calcium and phosphorus levels, results in efficient mineralization and maintaining the structure and integrity of bone (5, 13, 14).

The metabolism of Ca and VD during pregnancy undergoes considerable alterations throughout pregnancy so as to supply the Ca required for buildup of minerals in fetal bone. During the first trimester, the fetus stores 2 to 3 mg/d in the skeleton; however, this rate of deposition increases during the last trimester. (15).The pregnant woman's body a adjusts to the needs of the growing fetus by increasing its absorption of Ca early in the phase of pregnancy, reaching its maximum absorption in the final trimester (16, 17). In addition to transferring Ca to the growing embryo, the enhanced absorption in intestine is counterbalanced by increasing the elimination of Ca in the urine, which maintains stable serum ionized calcium levels throughout pregnancy (18).

PTH-related peptide (PTHrP), is generated in the placental tissues and fetal parathyroid glands which enhances placental production of calcitriol, which is a signal for placental Ca transfer and placental production of calcitriol (19, 20). PTHrP may possibly enter the mother's bloodstream and could cause the elevated 1,25(OH)2D and aid in the regulation of Ca and levels of parathyroid hormone during human pregnancy by acting through the PTH/PTHrP receptor in the renal and bone (21). Osteoprotegerin, estrogen, calcitonin, prolactin, and placental lactogen are additional signals that control active Ca homeostasis and VD production during pregnancy (22).

In nations with scarce resources, like Ethiopia, it is challenging to check for VD for every pregnant woman but at least VD screening once during the first trimester could help identify deficiencies early and guide preventive interventions. Understanding the Vitamin D level during the first trimester of pregnancy is very important because these times mark important turning points in in fetal organogenesis and maternal adaption. Identifying the determinants of Vitamin D deficiency in this population can inform targeted interventions to improve maternal and fetal health outcomes; Therefore, this study aims to determine the prevalence of VDD among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, and identify any associated factors.

1.2. Statement of problem

Vitamin D deficiency is an emerging public health concern worldwide, with catastrophic health outcomes. With varying prevalence across different regions among those in the Eastern Mediterranean region, such as in Kuwait 58.9% of the population aged 10 years has VDD. Similarly, in Oman, 44.3% of the population aged 18-55 years has VDD. In contrast, in the America, only 3% of the population 2 years and older have VDD. In Europe, 18% of the population has VDD, and about 53% have insufficient vitamin D levels. In the Western Pacific and Southeast Asia region, 10% and 22% population have VDD, respectively. In the African region, despite limited studies, VDD is relatively low, with 8.0% of the population having VDD and 18.9% having insufficient vitamin D levels (62).

Studies show that mean VD Women's concentrations were lower than men's, and pregnant women had higher rates of deficiency (30). According to a global systematic study carried out in 2016, 64% of pregnant women in the Americas, 57% in Europe, 46% in the Eastern Mediterranean, 87% in Southeast Asia, and 83% in the Western Pacific had VDD (< 20 ng/ml (50 nmol/L)) (31). According to a different systematic evaluation of the literature that took into account original studies on pregnant women, 76% of women in South Asian countries were found to be deficient overall (23).

Several countries have recognized VDD public health concern. Expectant women are considered group with significant risk population for VDD (4).VDD in pregnant women; it varies from 4% to 60% (24, 25). An estimated 5% to 50% of pregnant women in the US are believed to be VDD (26). In Belgian pregnant women 44.6% had VDD and 74.1% had insufficiency (27). In India, 84% of pregnant women had levels<22.5ng/ml (28). Only 35.8% of pregnant women had levels greater than 20 ng/ml, according to Egyptian research (29).

Vitamin D deficiency has been linked to a higher risk of certain detrimental health consequences for both the mother and the unborn child during pregnancy. Preterm birth, vaginal infections, intrauterine growth restriction, preeclampsia, gestational diabetes, cesarean sections, elevated inflammatory cytokine production in mothers (30),symptoms of postpartum depression (31, 32).and spontaneous abortion are among the pregnancy issues that have all been linked to low levels of VD in the mother (33-35). Maternal VDD has been linked to several reported effects for the offspring, including an increased risk of preterm birth (39), small for gestational age newborns

(39), neonatal hypocalcemia (44), rickets in infancy (45), recurrent wheeze, asthma (44) and increased obesity during childhood (46).

Black women in particular bear the double burden of VDD, which is most common on pregnant women (7). Because of its positive health impacts on lowering mortality and morbidity, it has been receiving a lot of attention worldwide (36).

In Ethiopia Foods rich in VD are infrequently eaten, and there is no program in place for fortification (37, 38). Individuals with darker skin colors are more prone to deficiencies in the lack of sufficient sunlight exposure or food consumption (37). Ethiopia, as part of sub-Saharan Africa, experiences unique geographical and socio-cultural conditions that may influence VD status among its population. And according to the study conducted in southern part of Ethiopia VDD among women, revealing a noteworthy prevalence of 39% with 8.8% being severely deficient (39).

The prevalence of VDD and its associated factors in antenatal care attending pregnant women remains largely unexplored, leaving healthcare providers without sufficient evidence to implement targeted interventions. Understanding the extent of deficiency and its determinants is essential for creating efficient public health tactics to address maternal and child health in this region. Therefore, the proposed study, "Assessment of Vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health center of Addis Ababa, Ethiopia, 2024," aims to fill this critical gap by providing valuable insights into the prevalence and contributing factors of VDD in the specified population.

1.3. Significance of the study

The data produced from this research will show the burden and determinants of VDD in the pregnant women attending antenatal care in selected health center of Addis Ababa, Ethiopia. Therefore, the findings will provide critical insights for clinicians, policymakers, and researchers to generate evidence-based strategies for improving maternal nutrition and mitigating pregnancy complications associated with VD status. Furthermore, the results of this study offer background information for upcoming studies involving a larger sample size in this demographic. The study contributed to a comprehensive understanding of the overall prevalence and associated factors, fostering improved healthcare practices and reducing the overall burden of VDD in both local and broader healthcare contexts.

2. Literature review

2.1. Chemical structure of vitamin D

Vitamin D is a 27-carbon molecule produced from the cyclopentanoperhydrophenanthrene ring and has a structure similar to sterol (40, 41). There are four rings on Vitamin D. The side chains of the saturated A, C, and D rings have eight or nine carbons each. There is a double bond in the compound's B ring between decarbonates 5, 6, 7 and 8. The bond between the carbons has been decoupled between 9 and 10.

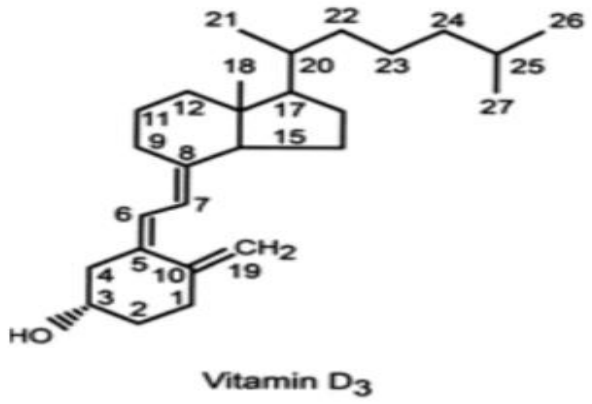

Figure 1:- Structure of vitamin D and numbering of carbon molecules(42).

Due to a double bond between carbon 22 (C22) and carbon 24 (C24) and the presence of a methyl group at carbon 24, D2 is decoupled from D3 (41).

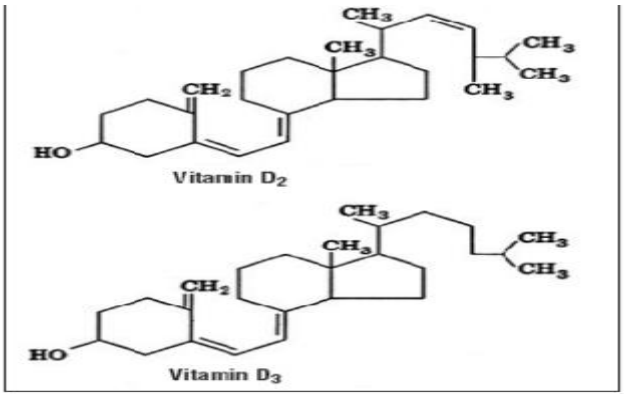

Figure 2:- Molecular structure of vitamins D2 and D3

Cholecalciferol (D3) contains three double bonds. Its melting point is between 84 and 85 degrees Celsius, and it doesn't dissolve in water. C27H44O3 is its chemical formula (40). Ergocalciferol (D2) is derived from plants, particularly yeasts and mushrooms. The bond between C22=C23 and the methyl group at C24 make vitamin D2 structurally distinct from vitamin D3 (43). The breakdown of vitamin D3 differs from that of vitamins C22 and C23. The chemical formula for ergocholesterol is C28H44O3 (40).There are 37 known metabolites of vitamin D, most of which are inactive. Metabolites such 1, 24, 25, (OH) 3D, 1, 25, 26(OH) 2D, and 25(OH)-26, 23 lactones are among them. Biolithic activities have been identified. 25 hydroxyvitamin D (25OHD) and the hormone form 1, 25 dihydroxyvitamin D (1,25(OH)2 D) are produced by the metabolism of 23 VD (43).

2.2. Synthesis and processing of vitamin D

Human skin is subjected to UVB radiation of sunlight, which has a wave length of 290–315 nm. (44) This spectrum enables transformation of 7-dehydrocholesterol (7-DHC) in to pre-vitamin D, which is then thermally isomerized and form VD in the skin (13, 44, 45).

Vitamin D from dietary source is incorporated into chylomicrons and delivered to the venous circulation via the lymphatic system. Dietary VD, which is produced in the skin, can be kept in fat cells and expelled from them. When VD expelled from the plasma membrane into the extracellular space, it binds with VD binding protein (DBP) (46).

25-hydroxalase (CYP2R1) converts VD to 25-hydroxyvitamin D (25OHD) in the liver (45). 25-OHD is the primary circulating type of VD utilized to determine VD status (47). The kidney's mitochondrial 25OHD 1α-hydroxylase (CYP27B1) enzyme converts the immediate precursor metabolite to the active form of VD (1, 25-dihydroxyvitamin D (1, 25(OH) 2 D))(45). Outside of the kidney, several organs express 25OHD 1α-hydroxylase, which produces 1,25(OH)2D. This active form of VD operates locally and has an autocrine or paracrine effect (43). Several variables control the renal production of 1, 25(OH) 2 D, including serum PO4, Ca, fibroblast growth factor 23 (FGF-23), PTH, and VD itself (48). By quickly triggering the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), 1,25(OH)2D causes its own breakdown. This results in the multistep catabolism of both 25(OH)D and 1,25(OH)2D into a water-soluble, biologically inert metabolite. (47). Target tissues expressing VDR are affected genomically by VD, while tissues expressing membrane-bound proteins are affected non-genomically. An essential nutritional component for

the mother's and her child's health is VD. More than thousands of genes in humans are regulated by VD, and several human tissues and cells contain VDRs (49).

In addition to non-classical tissues throughout the body which express its receptor, such as muscles and neurons, target tissues include classical tissues like the gut, bone, kidneys, and parathyroid gland (43).

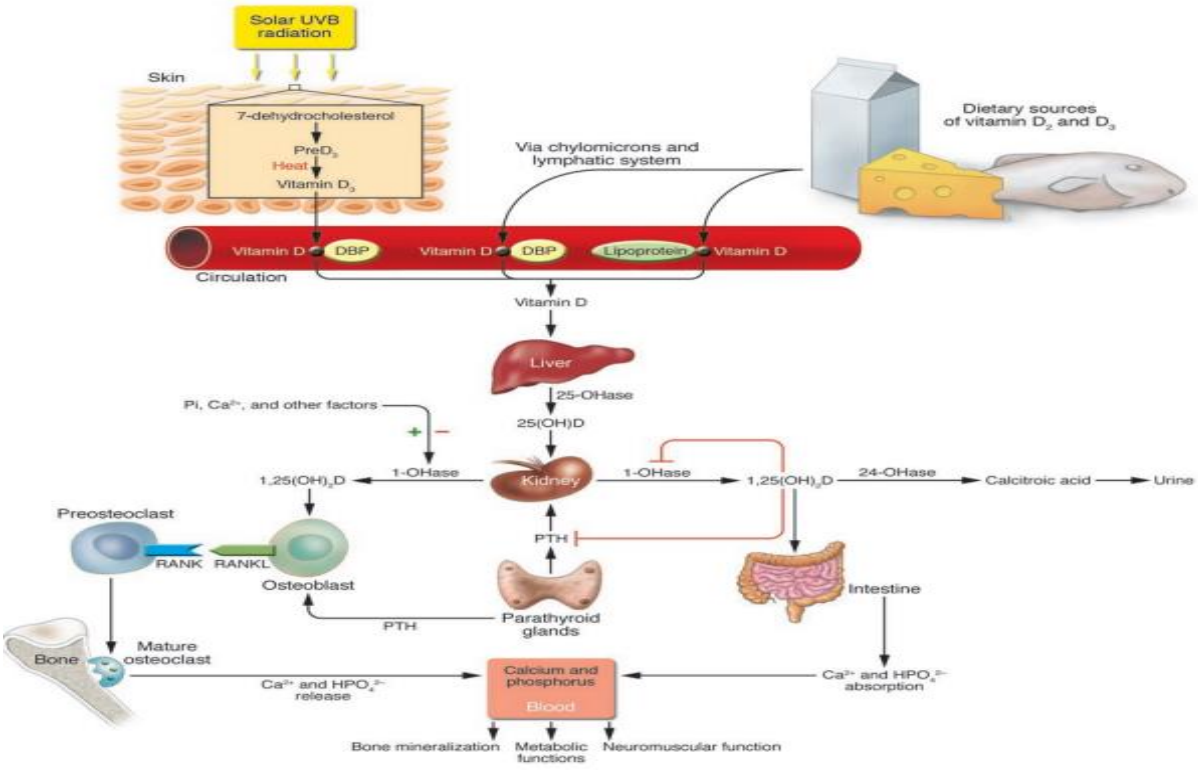

Figure 3 :- Synthesis and processing of VD as well as the many biological effects of 1, 25(OH) 2D on the metabolism of Ca, PO4, and bone. Either cutaneous exposure to UVB light or food consumption can produce VD.

By improving calcium absorption in the kidneys and intestines and controlling PTH levels, VD avoids low calcium levels. VD's function in the metabolism of Ca2+ and PO4 results in a sufficient Ca2+-PO4 product (Ca2+ × HPO42−), which effectively mineralizes bone and maintains its integrity and structure (5, 13, 14).

1 25(OH) 2 D controls gene transcription and quick non-genomic processes in muscles which impact Ca handling and muscle cell regeneration (50).

2.3. Vitamin D in immune system

Beyond its widely recognized impact on homeostasis of bone and calcium, VD plays a number of vital roles. Given that immune cells (Antigen-presenting cells, T cells, and B cells) express the VDR and can all synthesize the active form of the VD metabolite, VD can work as autocrine way within a local immunological milieu. Both the specific and nonspecific immune responses can be modulated by VD. An increased sensitivity to infections and an enhanced autoimmune is linked to VDD. Since VD has a mitigating influence on immune cells in autoimmune disorders, VD supplementation may provide advantages for VDD people with autoimmune diseases that go beyond bone and Ca hemostasis (51).

Immune Cell Regulation: T cells, B cells, and macrophages are just a few of the immune cells that VD helps control in terms of development and activity. It affects how they mature, get activated, and react to infections. Effects against Microbes: VD possesses direct antibacterial qualities. It can increase the synthesis of proteins and antimicrobial peptides, which aid in the fight against invasive microbes. Immunological Balance: By preventing overly strong or inadequate reactions, VD supports a balanced immunological response. It lowers the risk of autoimmune diseases and chronic inflammation by modifying the production of pro-inflammatory cytokines. Respiratory Health: sufficient levels of VD have been linked to a lower risk of contracting respiratory diseases, such as pneumonia and influenza. It functions as a barrier against pathogens, supporting the respiratory epithelium's protective role. General Health: Retaining adequate amounts of VD has been associated with improved general health and wellbeing. It supports cardiovascular health, bone health, and muscular strength all of which tangentially support immune system resilience(52, 53).

VD has an antioxidant effect by reducing inflammation through immune system modulation and during chronic inflammation it act by reducing oxidative stress and on the other hand VD increase the activity of antioxidant enzyme like that of superoxide dismutase and catalase, which has crucial role in neutralizing reactive oxygen species and prevent cells from oxidative damage. And also, VD has indirect effect on antioxidant status by regulating various cellular processes. Furthermore it protect the structure of cells from oxidative damage which can lead to damage protein, lipids and DNA within cells by decreasing oxidative stress (54).

2.4. Prevalence of vitamin d deficiency

According to study conducted in Istanbul, Turkey the magnitude of severe (<10ng) VDD was 45.9% (55). Similar study conducted in Malaysia also reported 90.4% of study participants had less than 50nmol/L serum VD level and 43.9% of participant's had less than 25nmol/L serum VD level. The other finding of this showed that 1.3% of study participants had adequate VD level (56).

Also, the study conducted in Belgium revels that 44.6% of study participants were VDD(<20ng/ml). According to severity assessment of VDD in this study, 12.1% of participants were severely deficient (<10ng/ml) and 74.1% participants had insufficient vitamin D levels (<30ng/ml) (27). a study carried out in Switzerland in 2020 also found high vitamin D deficiency of 73.23% (57).

Another study held at King Fahad Medical City in Riyadh, Saudi Arabia in 2016 to investigate the VD status of pregnant women during the first trimester of 160 study participants 50% of them had VDD (25(OH) D< 50nmol/L) of them 18.1% had severe VDD and 43.8% had VD insufficiency, however only 6.3% of subject diagnosed with VD sufficiency (58).

Among pregnant women in their first trimester 81% had early detection of vitamin D deficiency during pregnancy in a study done in Saudi Arabia and the Gulf region in the year 2018 (59).

The study conducted in south Asia, Lahore, Pakistan among 80 Primigravida women in their first trimester found that 90% of the participants were vitamin D deficient from that about 13.75% were severely deficient and 18.75 were insufficient (60). Another study from south East Asia, Malaysia in 2016 reported higher vitamin D deficiency with prevalence of 90.4% (61).

According to a study conducted in China by Yun C, Chen J, He Y, Mao D, Wang R, Zhang Y, et al. VDD prevalence and risk factors among pregnant Chinese women in 2017 found that 74.9% of pregnant women had VDD, with 25.5% of them having severe VDD levels. The median serum VD level was 15.5ng /ml (IQR of 11.9–20.0, range 3.0–51.5). the prevalence of VDD was higher in younger age group(aged 20-34 years), higher educational level and less sun exposure frequency (62).

On the other hand the study conducted Legos, Nigeria in 2017 on461 pregnant who visited the prenatal clinic between weeks 10 and 28 of their pregnancy had Vitamin D levels tested, and prevalence of vitamin D deficiency were 29% (63).

The study conducted in southern rift valley of Ethiopia in 2013 by Gebreegziabher T, Stoecker BJ to assess VD status of women living in the rural area found that the prevalence of VDD were 84.2%(<50nmol/L) of those 14.8% were severely deficient(<30nmol/L) (37).

Other study investigated in southern Ethiopia, by Haile DT, et al. in the year 2022 for assessing VDD and associated factors among antenatal care attending pregnant women in Sodo town revealing a noteworthy prevalence of 39%, with 8.8% being severely deficient and an average serum level were 24.43ng/ml(39).

2.5. Associated factors of vitamin d deficiency

Vitamin D deficiency during pregnancy remains a significant global health concern, with multiple studies identifying various sociodemographic, environmental, and physiological risk factors.

A consistent finding across diverse populations is the inverse relationship between maternal Body Mass Index (BMI) and serum 25(OH)D levels. In 2016 a study from North American revels that, each 1 kg/m² increase in BMI was associated with a 0.40 ng/ml decrease in maternal serum 25(OH)D levels ($p<0.001$) (64).

The findings of study conducted in Istanbul, Turkey indicated that dressing style, lack of multivitamin intake, gestational age at sampling and the season of blood sampling was as independent factors for VDD (55).

Other study conducted in Belgium in 2012 showed that Women with lower education levels, less sunlight exposure, preference for shadow and smokers had a higher risk of severe VDD, while alcohol consumption and more frequent use of sunscreen lotion was associated with a decreased risk of severe VDD also this study shows that higher BMI was linked to an increased risk of VDD (27). Additionally, a broader review on the study conducted in Switzerland in 2020 highlighted that both higher BMI and darker skin pigmentation were significantly associated with lower serum vitamin D levels ($p<0.0001$) (57).

Another study held at King Fahad Medical City in Riyadh, Saudi Arabia in the year 2016 to find out the VD status of pregnant women during the first trimester found that younger age group, higher academic level, less frequent sun exposure was significantly associated with VD status. Similarly, those exposed to the sun in the evening had a considerably lower prevalence (27.8%) of VDD than individuals exposed to the sun in the morning (67.6%) and midday (55.8%) and also Primigravida and nulliparous (57.1%), Overweight (51.9%) and obese (50.0%) women had a The prevalence of VDD was higher than others women (58).

Environmental and lifestyle factors also contribute significantly. A study from Saudi Arabia identified that vitamin D deficiency was more prevalent among housewives (90%) with insufficient sun exposure (85%), no supplementation (90%), and those residing in urban settings (81%). Multiparity and an age range of 18–45 years further increased the risk (58).

Similarly, a recent study from Singapore held in 2024 reported that higher maternal BMI significantly increased the risk of vitamin D deficiency in first-trimester pregnant women, where an overwhelming 97.8% of participants had serum levels below 30 ng/ml. Despite this, periconceptional multivitamin supplementation appeared to offer a protective effect (65).

Socioeconomic and behavioral factors also play a role. Research from Bangladesh in 2021 revealed that women with lower educational attainment and those who did not travel to sunny climates had a significantly increased risk of vitamin D deficiency. The study suggested that this may be due to greater sequestration of vitamin D in adipose tissue in women with higher BMI, reducing serum levels (66).

According to a study conducted in China (2017) by Yun C, Chen J, He Y, Mao D, Wang R, Zhang Y, et al, Vitamin D deficiency (VDD) was significantly associated with Hui ethnicity (OR=2.52, P=0.016, relative to Han), low (OR=2.52; P<0.001) and medium (OR=1.70; P<0.001) ambient UVB levels, and lack of vitamin D supplement use (OR=1.56; P=0.021). Among participants sampled in autumn, VDD was linked to Hui ethnicity (OR=2.35; P=0.012) and low (OR=2.71; P<0.001) or medium (OR=2.22; P<0.001) UVB exposure. In winter, VDD was associated with younger age (OR=0.96; P=0.050), higher gestational age (OR=1.02; P=0.035), higher pre-pregnancy BMI (OR=1.09; P=0.019), low UVB levels (OR=5.54; P<0.001), and not using vitamin D supplements (OR=1.97; P=0.007) (62).

In China (2017), a nationwide study of pregnant women reported a high prevalence (74.9%) of vitamin D deficiency, with a median serum level of 15.5 ng/ml. Low ambient UVB exposure, especially during winter, along with factors like Hui ethnicity, younger age, later pregnancy stage, and lack of supplementation, were significantly associated with deficiency (67).

Other study investigated in southern Ethiopia, by Haile et al,2022 for assessing VDD and associated factors among antenatal care attending pregnant women in Sodo town found that pregnant women with higher BMI (≥30) and who never consumed egg show significant association with an increased risk of VDD with AOR of 47.31 and 7.48 respectively. Conversely, women exposed to mid-day sunlight demonstrated a decreased risk of deficiency (AOR = 0.30) (39).

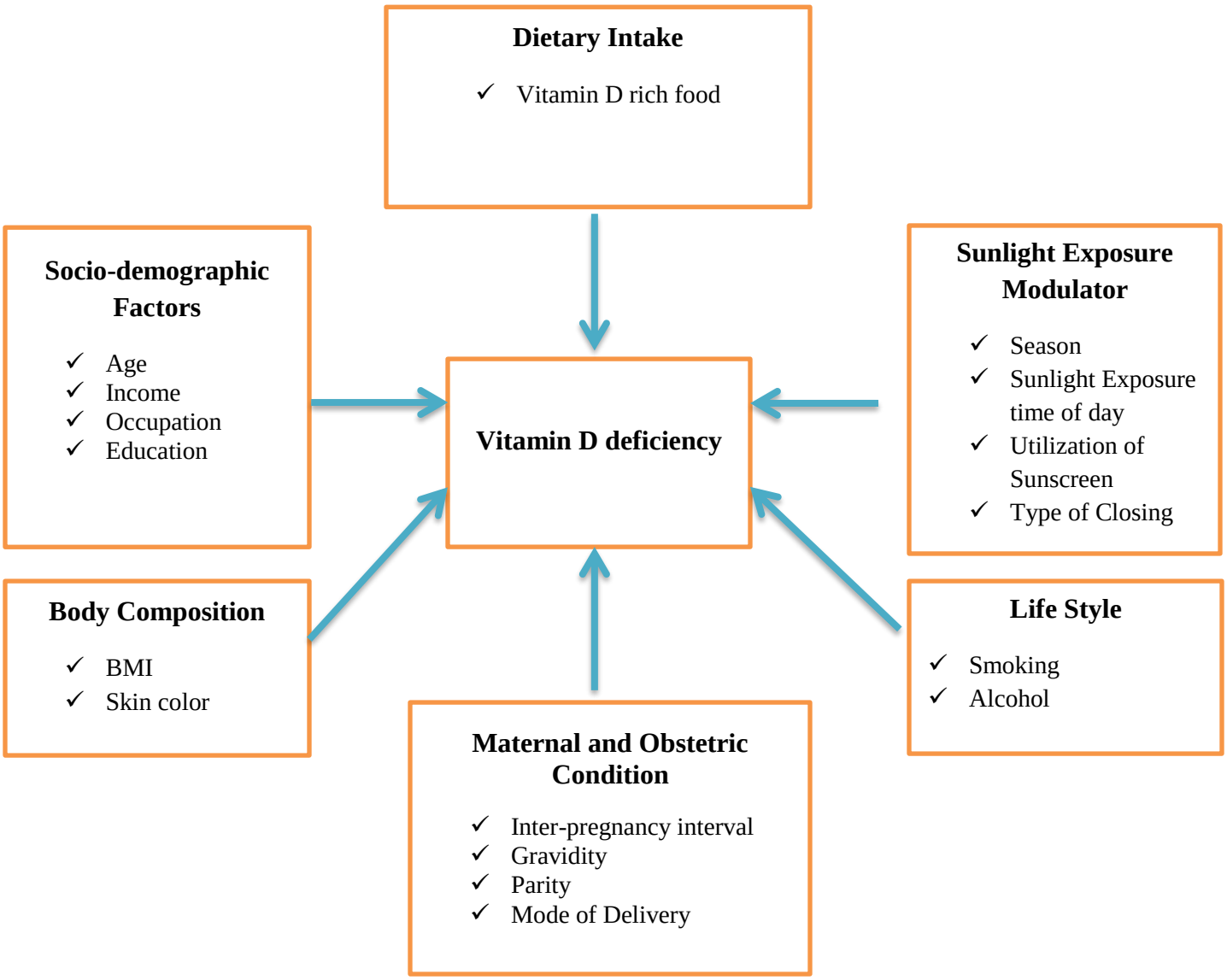

Figure 4: Conceptual framework. (*Haile et al,2022)*

3. Objective

3.1. General objective

- To assess vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, 2024.

3.2. Specific objective

- To determine the prevalence of vitamin D deficiency among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, 2024.
- To identify factors associated to vitamin D deficiency among antenatal care attending pregnant women in selected health center of Addis Ababa, Ethiopia, 2024.

4. Hypothesis

H_0: There is no significant prevalence of vitamin D deficiency and no significant association between vitamin D deficiency and various factors in antenatal care attending pregnant women in selected health center of Addis Ababa, Ethiopia in 2024.

H_1: There is significant prevalence of vitamin D deficiency and significant association between vitamin D deficiency and various factors in antenatal care attending pregnant women in selected health center of Addis Ababa, Ethiopia in 2024.

5. Method and materials

5.1. Study area

The current investigation was carried out in the city of Addis Ababa, which is located in central part of Ethiopia and is the largest city in the country. The city has an elevation of 9°00′00.00″ North, longitude 38°45′00.00″ East with an elevation between 2,326 and 3,000 meters above sea level and a population of over four million people in 2021 with the area of 527 Km^2. The city has a diverse population with different ethnic groups and languages. Has a tropical highland climate with an average temperature of 16.5°C. According to Federal Ministry of Health, there are Ten general Hospitals, four specialized hospitals, thirty-six private hospitals, seven hundred clinics and thirty-five non-governmental organizations and about hundred three government health centers eighty-six of them offering antenatal care services to pregnant women in the city and the surrounding community.

There are eleven sub-cities in Addis. Among those we randomly select "Gulele" and "Yeka" sub city for data collection. "Gulele" sub-city located on Northern part of the city covering an area of 30.18 km^2 with a total population of 377,032, from which 180,605 were males and 196,427 were females with 2.3% Annual Population Change from 2007- 2022 (68). A total of 10 health centers serves the community in the sub city, from those Selam health center were selected.

"Yeka" sub-city located in northeastern suburb of the city covering an area of 85.98 km^2 with a total population of 488,537, from which 225,543 were males and 262,994 were females with 2.3% Annual Population Change from 2007- 2022(68).A total of 15 health centers are serving the community in the sub city and from those Gurara health centers were selected.

Those health centers were selected randomly from model health centers in the city and also in the sub city based on the performance on ANC service, number of attending pregnant women and availability of sustainable ANC service and were CS center. Those health centers were selected from deferent sub city (Gulele sub city and Yeka sub city) which makes the data representative.

In Addis Ababa, Vitamin D deficiency and associated factors among antenatal care attending pregnant women are understudies so as to fill this critical gap by providing valuable insights into the prevalence and contributing factors of vitamin D deficiency in the specified population.

5.2. Study design and period

A facility-based cross-sectional study was conducted from April 1 to June 7, 2024 in Addis Ababa, Ethiopia.

5.3. Population

5.3.1. Source population

The source populations for this study were all pregnant women on their first trimester in the selected health facilities in Addis Ababa, Ethiopia.

5.3.2. Study population

The study populations were pregnant women on their first trimester and visit the selected facility during data collection period.

5.3.3. Inclusion and exclusion criteria

5.3.3.1. Inclusion criteria

Pregnant women on the first trimester (< 13 weeks of gestational age) who were attending antenatal care services and residing in Addis Ababa, Ethiopia.

5.3.3.2. Exclusion criteria

- Pregnant women with renal disease, chronic liver disease,
- Pregnant women with known allergies or adverse reactions to components of the study protocol, such as blood sampling.
- Pregnant women having major burn scar.
- Participants using medications that interfere with vitamin D metabolism or absorption including anti-epileptic and anti-TB drugs, glucocorticoids, and weight loss medications were excluded.

5.4. Study variables

5.4.1. Dependent variable

- Vitamin D deficiency levels of first trimester pregnant women (VDD)

5.4.2. Independent variable

- **Sociodemographic and economic factors:** Age, Income, Occupation, Education,

- **Dietary intake:** Vitamin D-rich foods.
- **Body composition:** BMI (Body Mass Index), skin color
- **Maternal obstetric conditions:** Inter-pregnancy interval, Gravidity, Parity, Mode of delivery
- **Lifestyle factors:** Smoking habits, Alcohol consumption
- **Sunlight exposure modulators:** Seasons, Sunlight exposure time of the day, Utilization of sunscreens, Type of clothing

5.4.3. Sample size calculation and sampling method

5.4.4. Sample size calculation

A single population proportion calculation was used to determine the sample size for this investigation, assuming a 95% confidence interval (α=0.05) and a 5% marginal error (d). According to a prior study on pregnant Ethiopian women in Sodo town, the p-value is 39% (39). The following formula is used to get the total initial sample size based on the information given.

$$n = \frac{(Z\frac{\alpha}{2})2P(1-P)}{d^2}$$

Where, n is required initial sample size,

Z α/2 = critical value for normal distribution at 95% confidence interval which equals to 1.96

(Z value at alpha=0.05).

P = the prevalence of vitamin D deficiency in Ethiopian pregnant women in Sodo town=39%

d= 0.05

n= $\frac{(1.96)2\ 0.39(1-0.39)}{(0.05)2}$ n= 365

Taking 10% non- response rate, the total sample size is calculated to be.

N= n+10 % of n

N= 402

5.4.5. Sampling method

Multistage sampling technique followed by systematic random sampling technique was employed to select the study participants. Out of the 11 sub-cities in Addis Ababa, two sub-cities were selected randomly Yeka sub-city and Gulele sub-city. In the selected sub-cities, there were 22 health centers from those only 20 health centers provide ANC service. First, one health centers from Yeka sub-city and one health centers from Gulele sub-city were selected. Those health centers were selected randomly from model health centers in the city and also in the sub city based on the performance on ANC service, number of attending pregnant women and availability of sustainable ANC service and were CS center Then, the required sample size was proportionated to the selected health centers. Every 2^{th} of study participants attending antenatal care services were invited to participate and became part of the research.

Proportional allocation: Retrieve data of three month exactly the same with our study period from 2023 log book and get 452 at Gurara health center and 361 from Selam Health Center. The total pregnant women used as sampling frame is 813, allocate sample size by using this formula, where n=402

P_{Gurara} =452/813=0.556*402=**224**

P_{Selam}=361/813=0.444*402=**178**, then systematic random sampling, calculate K (sampling interval) for each health center K=N/n,

K_{Gurara}=452/224=2.02

K_{Selam} =361/178=2.03

} So, we take sample in every **2^{nd}** pregnant woman.

5.5. Measurement and data collection procedure

5.5.1. Data collection procedure

Data were collected using a pretested structured questionnaire taken and modified from various literatures in English language and translated to local languages by interviewers in order to enhance the understanding of interviewees and limit the bias of data collection. It gathered information on Socio-demographic and economic characteristics, Maternal obstetric characteristics and body composition, Dietary intake of Vitamin D rich or fortified food, Sunlight exposure and life style characteristics of the study participants.

A digital balance connected to a Seca digital weighted scale (Germany) was used to measure the subjects' weight in kilograms (kg) to the nearest tenth of a kilogram. They were barefoot and wearing light clothing. A Stadiometer calibrated by Seca (Germany) was used to measure height (cm) twice and report the results to the closest centimeter. BMI (kg/m2) was calculated using measurements of height and weight. After taking each measurement twice, the average was utilized to determine the final value. Before calculating BMI, a preconception weight adjustment was made and compared to WHO guidelines (69).

The women's gestational age was established using the date of their last normal menstrual period (LNMP), and individuals whose LNMP was unknown, gestational age was determined using ultrasound and fundal height measurements. Skin pigmentation was classified according to the Fitzpatrick's classification of skin types, the study population's skin tones were divided into three categories: light brown, dark brown, and very dark.

Aseptic and standard protocols were used to obtain a 5 ml non fasting venous blood sample from the medial cubital region of each participant's forearm in a dark room. The whole blood sample was then held at room temperature for 10 to 20 minutes until it clotted. Following centrifugation, serum was separated using a pasture pipette, put in a Nunc tube, and refrigerated at the data collection location for fewer than eight hours. Then delivered every day to the laboratory division of St. Paul's Hospital Millennium Medical College, where it kept in a deep freezer (-80 degrees Celsius and higher). Aluminum foil was placed over the sample until it was time for examination. The sample was taken to the EPHI laboratory using a triple ice pack once the data collecting was finished. Although it is advised that samples be kept in the deep freeze for no more than six months, we kept ours for three months prior to analysis (29). Serum Vitamin D measurements were done using the Roche competitive Electrochemiluminescence Immunoassay (ECLIA) method with the Elecsys® Vitamin D Total II assay kit on the Cobas e411 immunoassay analyzer at EPHI National Reference Clinical Chemistry laboratory by the principal investigator and laboratory experts.

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5.5.2. Laboratory analysis

Test principle

Competition principle

The total duration of the assay was 27 minutes. 1st incubation: By incubating the sample (20 µL) with pretreatment reagents 1 and 2, the bound 25-hydroxyvitamin D is released from the vitamin D binding protein (VDBP). 2nd incubation: By incubating the pretreated sample with ruthenium-labeled VDBP, a complex between 25-hydroxyvitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24,25-dihydroxyvitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite. 3rd incubation: After the addition of streptavidin-coated microparticles and 25-hydroxyvitamin D labeled with biotin, unbound ruthenylated labeled VDBP becomes occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25-hydroxyvitamin D is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. The application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier. The results are determined via a calibration curve, which is an instrument specifically generated by 2-point calibration, and a master curve provided via the reagent barcode or e-barcode.

5.6. Data Quality Control and Assurance

Both on the data collection place and the Saint Paulo's Hospital Millennium Medical College MCH department conducted a two-day pretest to test the data collection instruments and train data collectors on the study project's goal, participant rights, consent procedures, interview techniques, and laboratory test protocols. Regular supervision and follow-up were carried out while the data was being collected. Principal investigators cross-checked the data's consistency, completeness, and sample collection process every day.

5.6.1. Pre analytical

Pretests of questionnaires were performed conducted one week prior to the actual data collection on 5% of the participants at the data collection and Saint Paulo's Hospital Millennium Medical College MCH Department to verify the length, logical structure, understanding, and skip patterns of the questions. This questionnaire had been developed in English and translated into Amharic so that study participants could understand it with ease. Skilled lab workers took part in the appropriate sample collection, processing, and transportation. To guarantee labeling with identification numbers, appropriate sample containers, adequate volume, and test processes, SOPs were rigorously followed. The sample that had been collected was left to clot for 10–20 min and was subsequently centrifuged to get the serum for examination. Prior to analysis, the instrument's quality, reagent volume, expiry, room temperature, and sample hemolysis were examined.

5.6.2. Analytical

The laboratory analysis was done following quality control measures derived from feedback reports of the national laboratory. To guarantee the correct operation, validity, and reliability of the instrument, both normal and pathological quality controls were performed prior to the sample analysis. Based on the West Guard rule to accept or reject controls, a LJ chart was created for both controls and interpreted. Following the acceptance of both controls, the samples were examined.

5.6.3. Post Analytical

Based on the sample identification number, the result was appropriately documented, and the principal investigator handled and interpreted the data appropriately. The lab result was noted for future reference in the log book. The investigator received a tidy and unambiguous test result for analysis.

5.7. Data Analysis and Interpretation

Data were entered into Epi Data version 3.1 and transferred for analysis using SPSS software version 26. Descriptive statistics were used to characterize the data, whereas the Kolmogorov–Smirnov test at a p value >0.05 were used to check the normal distribution of continuous numerical variables. Bivariate logistic regression analyses were used to select exposure variables with a crude association with the outcome. All exposure variables with p values less than 0.25 during bivariate analysis were included in multivariate analyses. Finally, multivariate analyses were performed to control for potential confounders and identify independent predictors of the outcome. Adjusted odds ratios along with 95% CIs were used to estimate the strength of association, and statistical significance was set at a p value less than 0.05.

5.8. Operational definition

- **Serum total Vitamin D level**: "*Vitamin D Deficiency*" - Serum 25(OH)D < 20 ng/mL (<50 nmol/L); "*Vitamin D Insufficiency*" - Serum 25(OH)D between 20–29 ng/mL (50–75 nmol/L); "*Severe Vitamin D Deficiency*"- Serum 25(OH)D < 10 ng/mL (<25 nmol/L) (70-72).
- **BMI:** was considered from weight and height measurement and categorized as < 18.5 kg/m2 (Underweight), 18.5–24.9 kg/m2 (Normal), 25.0–29.9 kg/m2(Overweight), and ≥ 30 kg/m2 (Obese).
- **Skin Color**: The Fitzpatrick scale is used to categorize skin color, with Type IV being light brown, Type V being dark brown, and Type VI being very dark brown.).
- **Sun light exposure time in a day**: The amount of time spent outside in direct sunlight during the day is referred to as the "common sunlight exposure time," and it can help the skin produce more vitamin D. Three time periods will be used to classify the amount of time spent in the sun for this study.: "Sun raise/morning" The time period between 7:00AM– 9:00AM, "Mid-day" The time period between 10:00AM– 3:00PM, and Sunset/evening "The time period between 4:00PM– 6:00PM".
- **Utilization of Sunscreen Lotion**: refers to how often sunscreen is applied to the skin in order to shield it from the damaging effects of UV radiation from the sun. Three levels of sunscreen use will be distinguished among research participants for the purposes of this investigation. Regardless of their dietary practices, participants who frequently apply sunscreen or lotion while exposed to the sunlight are referred to as "sunscreen users." Regardless of their dietary

practices, those who don't apply sunscreen or lotion while exposed to the sunlight are referred to as "sunscreen non-users. " Sunscreen or lotion users" are those who, depending on the weather or activities they are participating in, apply sunscreen or lotion inconsistently when exposed to the sunlight.

- **Utilization of Umbrella**: refers to how often people use umbrellas to shield their skin from the sun's damaging UV rays. Three usage levels will be used to classify umbrella use for the purposes of this study. "Always (Regular) Users of Umbrella " are participants who regularly or everyday use umbrellas to protect themselves from the sun when they're outside. "Sometimes (Occasional) Umbrella Users" are participants who, depending on the activity or weather, use an umbrella for sun protection once a week or once a month. Regardless of the weather or outside activity, participants who never use an umbrella for sun protection, are referred as "Umbrella Non-Users (Never)".
- **Dietary Vitamin D intake quantification**: Participants must fulfill the following requirements in order to be deemed vitamin D consumers based on their food intake: "Liver Consumer": Having eaten more than 150 grams of beef liver in a single meal each day. A "fish consumer" is someone who consumes 85-170 grams of medium-sized fatty fish, such salmon and sardines, each day. Consuming products made from fish oil that contain 0.25–0.5 grams (1-2 teaspoons) of EPA (Eicosapentaenoic acid) and DHA (docosahexaenoic acid) combinations daily is referred to as "fish oil consumption. A "Fortified Cereals Consumer" is someone who consumes 250 grams or more of fortified cereal items daily. A person who consumes at least three eggs per day is referred to as "egg consumer". "Fortified Dairy Products Consumer": Having taken servings of fortified dairy products that contain approximately 100 IU of vitamin D, such as 1.5 ounces of fortified cheese, One cup with about 250 ml fortified milk or one cup of about 250 ml fortified yogurt (73).
- **Gestational Week:** Is the number of weeks that have elapsed since the first day of the pregnant individual's last menstrual period (LMP) is known as the gestational week. It is used to calculate estimated due dates, evaluate pregnancy progression, and predict fetal development (74).
- **Regularity of Menstruation:** refers to the consistency of menstrual cycle length over time, typically occurring within a predictable range. Operationally, it is defined as menstrual cycles

that occur every 24 to 38 days, with variations of no more than 7–9 days between cycles over a period of 6 months (75).

5.9. Ethical considerations

The Declaration of Helsinki's rules and recommendations were followed when conducting the study. The research and ethical committees of Addis Ababa University's Medical Laboratory Science department provided their approval. In addition, Permission to carry out the research was received from Addis Ababa Public Health Research and Emergency Management Directorate, and from the medical director of selected health facilities prior to beginning the collection of data and specimens, following an explanation of the study's goal, the participants provided their informed written consent, Participants were given sufficient information and their free involvement was respected, and the use of distinct codes for the samples and findings ensured the confidentiality of any information collected from participants.

5.10. Result Dissemination

The outcomes of this study will be presented to the Department of Medical Laboratory Science, Addis Ababa University, and could serve as reference material for researchers, experts and policy makers for intervention. To reach those bodies, completed paper will intend to submit the Ethiopian Medical Laboratory Association, Ethiopian Ministry of Health, Addis Ababa city administration health office and Addis Ababa Public Health Research and Emergency Management Directorate. Furthermore, a copy of this content will be provided to selected health facility research offices. Moreover, the findings of the present study will be published in a peer-reviewed journal for dissemination. local and international journals and will also be presented in different scientific seminars, scientific conferences, and workshops.

6. Result

Socio-Demographic Characteristics of the study participant

The current study includes a total of 402 first trimester pregnant women were included. The mean ages for the pregnant women was 24.72(±3.23). Of the study participants, 195/402 (48.5 %) had attended at least a secondary education level or above, and about half of the study participants 200/402 (49.8 %) were housewife, 389/402 (96.8 %) were married (Table1).

Table 1: Socio-demographic characteristics of the Study Participant at selected Health Centers in Addis Ababa, Ethiopia,2024. (N=402, Addis Ababa, 2024)

Variable	Category	Frequency	Percent
Age	18-25	257	63.9
	26-49	145	36.1
Education	No formal education	63	15.7
	Primary	144	35.8
	Secondary	88	21.9
	Above secondary	107	26.6
Occupation	Household chores	200	49.8
	Merchant	95	23.6
	Government employee/NGO	107	26.6
Marital status	Married/living together	389	96.8
	Divorce/separated	5	1.2
	Widowed	4	1.0
	Not married	4	1.0
Husband Education	No formal education	22	5.5
	Primary	135	33.6
	Secondary	104	25.9
	Above secondary	141	35.0
Family size	1 - 4	363	90.3
	≥5	39	9.7
Family income in ETB	≤3000	16	4.0
	>3000	383	96.0

- **Family income**: refers to the total income that each family member receives, regardless of whether they all contribute money or only a portion of it to pay for expenses.
- **ETB**: Ethiopian Birr

Maternal obstetric characteristics and body composition of the study participant

Participants' mean gestational ages were 10.79±2.45 weeks. Moreover, 171/402 (42.5%) of the Women were primigravida with 125/402 (31.1%) were multiparous. Among the study participants who had given birth previously, the interbirth interval of 173/402 (75.2%) were greater than two years. A total of 199/402 (86.15%) of the study participants gave birth through spontaneous vaginal delivery during their previous delivery. Additionally, the majority of the study participants had a BMI of 155/402 (38.6%) categorized as overweight, and 64/402 (15.9%) of them were categorized as obese. Among the participants skin color, 236/402 (58.7%) had Type V (dark brown) skin, whereas only 37/402 (9.2%) had a very dark color of skin (VI- very dark brown), and the remaining participants had 129/402 (32.1%) Type IV (light brown) skin (Table 2).

Table 2:- Maternal obstetric characteristics and body composition of the study participant at selected Health Centers in Addis Ababa, Ethiopia, 2024. (N=402, Addis Ababa, 2024)

Variable	Category	Frequency	Percentage
Gravidity (Number of pregnancy)	Primigravida	171	42.5
	Multigravida	231	57.5
Parity (number of delivery)	Nulliparous	172	42.8
	Primiparous	105	26.1
	Multiparous	125	31.1
Interpregnancy interval	≤ 2 years	57	24.8
	> 2 years	173	75.2
Mode of delivery of previous pregnancy	Vaginal delivery	293	72.9
	Caesarian section	82	20.39
BMI	Under weight (<18.5)	47	11.7
	Normal (18.5–25)	136	33.8
	Over weight (>25–30)	155	38.6
	Obese (>30)	64	15.9
Skin color	Type IV	129	32.1
	Type V	236	58.7
	Type VI	37	9.2

Skin color*: with Type IV being light brown, Type V being dark brown, and Type VI being very dark brown.)*

Dietary intakes of Vitamin D rich or fortified food of the study participant

About 203/402 (50.5%) of the women who participated in this study never consumed fish, and none of the participants consumed fish oil. A total of 393/402 (97.8%) of the participants reported never using VD supplements. Additionally, the study participants never consume liver 190/402 (47.3%) and Vitamin D fortified cereals 242/402(60.2%), on the other hand, 256/402 (63.7%) consume egg regularly (Table 3).

Table 3:- Dietary intakes of Vitamin D rich or fortified food, of the study participants at selected Health Centers in Addis Ababa, Ethiopia, 2024. (N=402, Addis Ababa, 2024)

Variable	Category	Frequency	Percent
Fish	Occasional and rarely user	199	49.5
	Never	203	50.5
Mushroom	Ever consumed	6	1.5
	Never	396	98.5
Fortified milk, oil and fish oil	Ever consumed	1	0.2
	Never	401	99.8
Egg	Regularly	256	63.7
	Rarely	82	20.4
	Never	64	15.9
Liver	Regularly	59	14.7
	Rarely	153	38.0
	Never	190	47.3
Vitamin D fortified cereals	Ever consumed	160	39.8
	Never	242	60.2
Cake and confectionary	Regularly	116	28.9
	Rarely	57	60.2
	Never	229	28.9
Vitamin D supplements	Occasional	9	2.2
	Never	393	97.8

- ***Regular consumers:*** *Participants who say they eat at least one serving of a food high in vitamin D every day or many times a week.*
- ***Occasional consumers:*** *Participants who say they eat foods high in vitamin D less regularly, such once or twice a month or a few times a year.*

Sunlight exposure and life style characteristics of the study participants

About 359/402 (89.3%) participants stated that they never use Sunscreen lotion and 304/402(75.5%) of the participants use umbrella whenever they go outside of home. Almost half of the participants 200/402(49.8%) usually exposed to sunlight throughout the mid-day whereas 110/402(27.4%) were in the Evening. Furthermore about 58/402(14.4%) of the study participants drink alcohol and only 1/402(0.2%) has smoking history and about 340/402 (84.6%) of participants expose >15% of TBSA for sunlight (Table 4).

Table 4: - Sunlight exposure and life style characteristics of the study participants at selected Health Centers in Addis Ababa, Ethiopia, 2024. (N=402, Addis Ababa, 2024)

Variable	Category	Frequency	Percent
Sunscreen lotion use	Ever used	43	10.7
	Never	359	89.3
Umbrella	Sometimes	179	44.5
	Often	42	10.4
	Always	83	20.6
Taboos practice	No	289	71.9
	yes	113	28.1
Outdoor activity	No activity	5	1.2
	Walking	100	24.9
	Marketing	207	51.5
	Washing cloth	15	3.7
	Working	75	18.7
Day time sun exposure	Morning	92	22.9
	Mid-day	200	49.8
	Evening	110	27.4

Duration of sunlight exposure	<30 minuet	284	70.6
	30- 1hour	100	24.9
	>1 hour	18	4.5
Total body surface area exposed	<15% of TBSA	62	15.4
	15-20% of TBSA	166	41.3
	20-30% of TBSA	72	17.9
	>30% of TBSA	102	25.4

- ***Day time sunlight exposure:*** *Morning: (7:00AM– 9:00AM) Mid-day (10:00AM– 3:00PM) and Afternoon – Sunset (4:00PM– 6:00PM).*
- ***Types of taboos*** *restricted were Fatty food, Egg, Coca Cola, Milk, Soft drink, tight cloth, Tomato, Pineapple, Linen, Row meat, Undercooked food and Fruit.*

Serum Vitamin D level of the study participants

In the current study the mean serum vitamin D level was 21.76ng/ml ±7.34ng/ml. the prevalence of Vitamin D deficiency (<20ng/ml) among first trimester pregnant women was found to be 43.3% (174/402), On the other hand, the prevalence of Vitamin D insufficiency (20 – 29ng/ml) was found to be 42.5% (171/402). The general prevalence of Vitamin D deficiency and insufficiency among the study participants discovered to be 345(85.8%) (Figure 5).

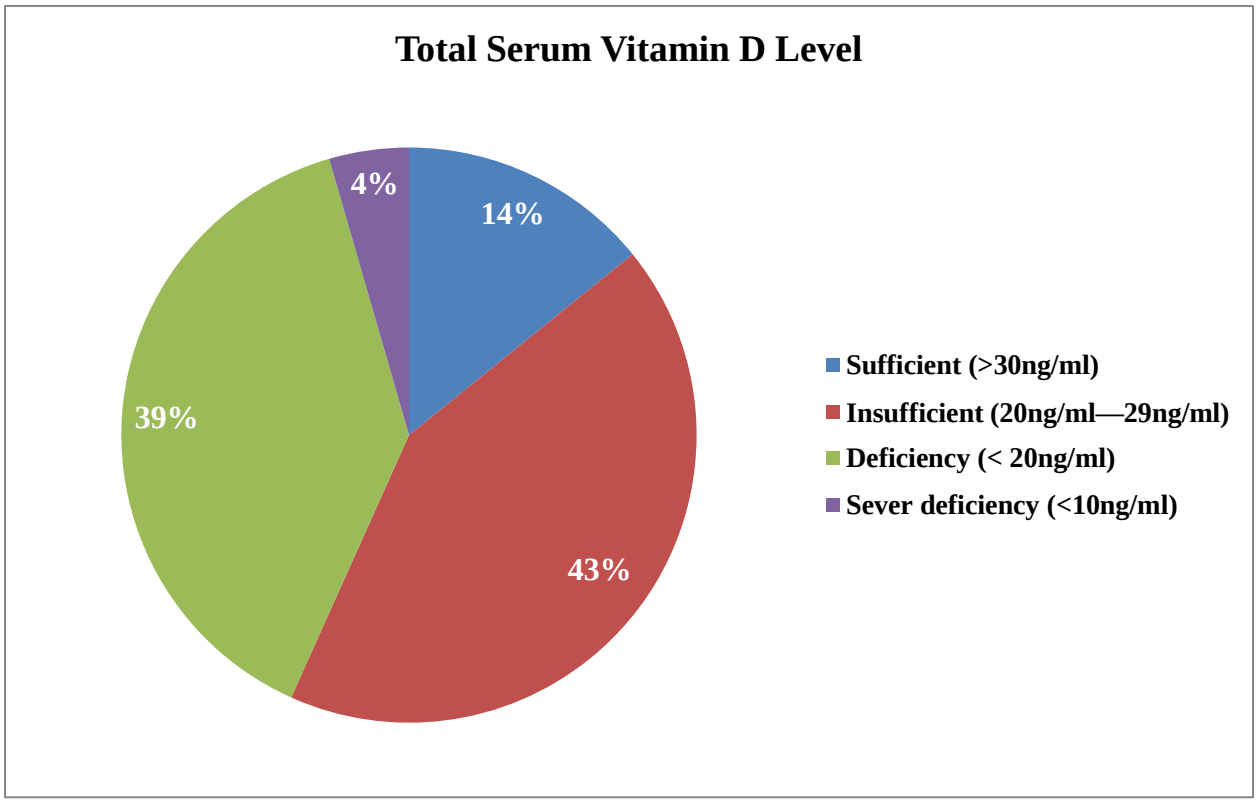

Figure 5: - Serum Vitamin D level of the study participants at selected Health Centers in Addis Ababa, Ethiopia, 2024. (N=402, Addis Ababa, 2024)

Table 5: - Bivariate logistic regression analysis results of the variables associated with Vitamin D deficiency.

Variable	Category	Vit-D status		COR (95% CI)	P-value
		Deficient	Not deficient		
Age	18-25	111	146	1.011(0.670-1.524)	0.960
	26-45	63	82	1*	1*
Education	No formal education	30	33	1.237(0.682-2.242)	0.483
	Primary	61	83	1.313(0684-2.520)	0.413
	Secondary	36	52	1.161(0621-2.167)	0.640
	Above secondary	47	60	1*	1*
Occupation	Household chores	97	103	1*	1*
	Merchant	28	67	1.253(0.238-3.794)	0.272
	Government employee/NGO	49	58	1.115(0.696-1.785)	0.651
Mode of delivery of previous pregnancy	Vaginal delivery	140	153	1.021(0.650-1.924)	0.923
	Caesarian section	47	35	1*	1*
Mushroom	Ever consumed	1	5	1*	1*
	Never	173	223	0.258(0.030-2.227)	0.261
Vitamin D fortified cereals	Ever consumed	70	90	0.951(0.589-1.534)	0.837
	Never	104	138	1*	1*
Cake and confectionary	Regularly	49	67	0.931(0.580-1.493)	0.766
	Rarely	26	31	0.908(0.507-1.627)	0.746
	Never	99	130	1*	1*
Family size	1 - 4	155	208	1*	1*
	≥5	19	20	0.784(0.405-1.520)	0.472
Family income in ETB	≤3000	8	8	1*	1*
	>3000	166	220	1.325(0.487-3.604)	0.581
Gravidity (Number of pregnancy)	Primigravida	71	100	1*	1*
	Multigravida	103	128	0.882(0.592-1.316)	0.539
Sunscreen lotion use	Ever used	20	23	1*	1*
	Never	154	205	1.158(0.614-2.184)	0.651
Umbrella	Sometimes	94	85	0.521(0.312-0.869)	0.333
	Often	16	26	0.944(0.447-1.990)	0.879
	Always	28	55	1.141(0.618-2.105)	0.674
	Never	36	62	1*	1*

Taboos practice	No	127	162	1*	1*
	yes	47	66	1.101(0.709-1.710)	0.669
Outdoor activity	No activity	2	3	1*	1*
	Walking	42	58	1.105(0.682-1.790)	0.686
	Marketing	92	115	1.135(0.665-1.939)	0.642
	Washing cloth	7	8	0.914(0.320-2.615)	0.867
	Working	31	44	1.200(0.196-7.333)	0.843
Duration of sunlight exposure	<30 minuet	138	146	1.058(0.408-2.743)	0.908
	30- 1hour	27	73	1.704(0.271-7.528)	0.357
	>1 hour	9	9	1*	1*
Birth interval	Never	72	100	1*	1*
	<2year	23	34	0.89(0.49–1.61)	0.69
	>2year	79	94	1.14(0.74–1.75)	0.54
Alcohol	Yes	24	34	1.03(0.59–1.80)	0.91
	No	150	194	1*	1*

Note: *: Indicates reference group

Factors associated with Vitamin D deficiency

Vitamin D deficiency was 6.9 times more common in Obese pregnant women with a BMI of ≥30 than in women with a normal BMI (AOR = 6.9; 95% CI: 2.9-16.3, p<0.001) and being overweight had also 6.75 times more likely to become vitamin D deficient with compared to BMI of normal pregnant women (AOR=6.75; 95%CI: 3.4-13.3, p<0.001). The risk of vitamin D deficiency was 8.5 times higher for pregnant women who never ate fish than for those who ate it occasionally or rarely (AOR = 8.5; 95% CI: 4.5–16.2, p < 0.001). On the other hand, the odds of vitamin D deficiency were 15.6 times higher for pregnant women who never ate eggs than for those who did (AOR = 15.6; 95% CI: 5.1–27.9, p <0.001). Pregnant women who regularly consumed liver were 69% less likely to develop Vitamin D deficiency (AOR = 0.31; 95% CI: 0.16–0.59, p < 0.001). Pregnant women with TBSA of >31% had a 69% less risk of developing vitamin D deficiency (AOR = 0.31; 95% CI:0.13–0.74, p=0.014) (Table 6).

Table 6: - Factors associated with Vitamin D deficiency among the study participants at selected Health Centers in Addis Ababa, Ethiopia, 2024.

Variable	Category	Vit-D status		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Deficient	Not deficient				
Skin pigmentation	Light brown	53	76	1*	1*	1*	1*
	Dark Brown	96	140	2.5(1.4 – 6.5)	0.005	2.3(0.83 – 6.4)	0.11
	Very dark	25	12	3.04(1.5 – 6.3)	0.003	2.63(0.91- 7.6)	0.071
BMI	Normal	37	99	1*	1*	1*	1*
	Under weight	17	30	1.52(0.8 – 3.0)	0.25	1.86(0.67 – 5.2)	0.23
	Over weight	81	74	2.93(1.8 – 4.8)	<0.001	6.75(3.4 – 13.3)	**<0.001**
	Obese (>30)	39	25	4.2(2.3 – 7.8)	<0.001	6.9(2.9 -16.3)	**<0.001**
Fish consumer	Never	134	69	7.72(4.91 – 12.1)	<0.001	8.5(4.5– 16.2)	**<0.001**
	Occasional and rarely	40	159	1*	1*	1*	1*
Egg consumer	Regular	103	153	1*	1*	1*	1*
	Rarely	29	53	2.84(1.6 – 5.0)	<0.001	6.96(2.9-16.5)	**<0.001**
	Never	42	22	3.5(1.8 – 6.93)	<0.001	15.6(5.1-27.9)	**<0.001**
Liver consumer	Regular	16	43	0.25(0.13 – 0.47)	<0.001	0.31(0.16-0.59)	**<0.001**
	Occasional	44	109	0.27(0.17 – 0.42)	<0.001	0.5(0.22 – 1.2)	0.12
	Never	114	76	1*	1*	1*	1*
TBSA	<15%	38	24	1*	1*	1*	1*
	15 – 20%	88	78	1.12(0.56 – 2.2)	0.77	1.28(0.55 – 3.0)	0.566
	21 – 30%	19	53	0.35(0.21 – 0.6)	<0.001	0.43(0.22 – 0.85)	**0.009**
	>31%	62	166	0.25(0.13 – 0.5)	<0.001	0.31(0.13 – 0.74)	**0.014**
Parity	Nulliparous	65	60	1*	1*	1*	1*
	Primiparous	37	68	1.6(0.9 – 2.4)	0.084	2.1(0.99 – 4.43)	0.053
	Multiparous	72	100	2.0(1.2 – 3.4)	0.011	3.2(1.6 – 6.5)	**0.001**

Note: *: Indicates reference group

7. Discussion

A total of 402 pregnant women were participated in this study; and the prevalence of vitamin D deficiency was 43.3%. with higher BMI (being Obese and overweight), participants level of Fish and Egg consumption, and TBSA were the key determinants associated with serum Vitamin D deficiency.

In the present study, the prevalence of vitamin D deficiency(<20ng/ml) was 43.3% (38.6% - 48.5%). This result was consistent with previous investigations from Belgium(44.6%) (27), Turkey(45.9%) (55), Saudi Arabia (50%) (58), and southern Ethiopia(39%) (39).During pregnancy, the mother's body requires higher levels of Vitamin D to support fetal development, especially for bone mineralization, immune function, and overall growth. This increases the mother's demand for Vitamin D and prone the body for vitamin D deficiency.

However, it is significantly higher than the 29% (95% CI: 24.8–33.2%) reported among pregnant women in Nigeria (63). This discrepancy most likely reflects Nigeria's distinct advantages, which include traditional meals high in vitamin D and maximum equatorial UV exposure at low elevations. On the other hand, despite stronger UVB in Ethiopia, urban residents, especially in cities like Addis Ababa, people often wear more clothing due to the cooler temperatures, and may spend more time indoors, reducing sun exposure. Disparities in methodology, such as diagnostic thresholds, could potentially be a factor.

In addition to this result a study carried out in Switzerland also found high vitamin D deficiency of 73.23% (57). This might be due to even if access to healthcare has improved; the prevalence of severe vitamin D deficiency is increasing, most likely as a result of less exposure of sunlight since Switzerland's higher latitudes result in less sunlight, especially during the winter lowers the country's natural production of vitamin D. Another study from south East Asia, Malaysia reported higher vitamin D deficiency with prevalence of 90.4% (61) This may be because of study participants' limited outdoor activity and majority of the study participants reported frequent morning sickness that make them more susceptible to vitamin D deficiency. Among pregnant women in their first trimester 81% had early detection of vitamin D deficiency during pregnancy in a study done in Saudi Arabia and the Gulf region (59), this high prevalence rate might be due

to spending of significant amount of time indoor and their clothing preference that covers the face and feet might affect the level of vitamin D deficiency.

Other study conducted in Lahore, Pakistan found that the prevalence of vitamin D deficiency were 90% (60) from a total of 80 primigravida women. The strikingly increased prevalence when compared to our study might be due to the small sample size that only included Primigravida. Furthermore, a diet deficient in vitamin D, women's clothing styles that cover their faces and feet outside might shield them from sunlight exposure. which all can contribute to vitamin D deficiency. Another study from Shanghai, China also report higher prevalence of vit D deficiency 74.9%(62). This high prevalence rate in industrialized Chinese cities like shanghai might be attributed to the presence numerous Skyscraper and high levels of pollution that can block UV rays and reduce the production of vitamin D. Research carried out in central Ethiopia also revealed a higher prevalence of vitamin D deficiency 81% (76) among pregnant women, which was relatively higher when compared to the result in this study. This high prevalence might be explained by variations in sample size, the sampling strategy used, and the fact that data collection took place during the rainy season, which probably reduced exposure to sunshine. Another research carried out in South Ethiopia among women of reproductive age revealed an even greater incidence rate of 84.2% (37). This increment might be due to high fluoride concentration of the Rift Valley region's lakes and groundwater, which is connected to rickets and hypocalcemia, such environmental factors, may make vitamin D deficiency worse in certain populations (77, 78).

This study revealed a strong relationship between vitamin D deficiency and an increase in BMI, specifically being overweight (AOR = 6.75; 95% CI: 3.4–13.3) and obese (AOR = 6.9; 95% CI: 2.9–16.3). These results consistent with research that found a clear correlation between lower vitamin D levels and greater BMI in North America (64), Belgium (27), Switzerland (79) , Saudi Arabia (58), China (62), Singapore (80) and Ethiopia (39). These result supports the global trend that vitamin D deficiency is more common in overweight and obese people, most likely due to lifestyle factors associated with increased BMI and sequestration of adipose tissue, which reduces the bioavailability of vitamin D and Obese patients generally poor vitamin D status is entirely explained by the dilution of ingested or continuously produced vitamin D in their considerable fat mass (81). Unlike the finding, the study conducted in Turkey (55) and Morocco (82), has revealed that BMI were not significantly related to vitamin D deficiency.

In this study, pregnant women who never ate fish had an 8.5-fold higher risk of vitamin D deficiency in comparison to those who ate it occasionally or infrequently (AOR = 8.5; 95% CI: 4.5–16.2, $p < 0.001$). this finding is consistent with the well-documented role of fish as a key dietary source of Vitamin D , particularly fatty fish such as salmon, tuna, and mackerel (83, 84). A substantial source of this vital vitamin is eliminated when fish is not consumed, which raises the risk of deficiency. however, the odds of vitamin D deficiency were also 15.6 times higher for pregnant women who never ate eggs than for those who did regularly (AOR = 15.6; 95% CI: 5.1–27.9, $p <0.001$). This result in lined with study from Italy, North America and Ethiopia (37, 45, 85-87),and contrary to this result the study conducted in Bangladesh revealed that the prevalence of vitamin D deficiency or insufficiency showed no significant variation among groups based on how frequently they consumed eggs, dairy products, meat, or large fish (88). Alongside fish and egg, liver is one of significant source of vitamin D (84), and this study showed that the odds of vitamin D deficiency was 69% lower for pregnant women who routinely consumed liver than for those who never did (AOR = 0.31; 95% CI: 0.16–0.59, $p < 0.001$).

The risk of vitamin D deficiency increases with each extra birth, with an AOR of 3.2, multiparous women had a significantly increased risk of being deficient than nulliparous women, with the likelihood being over three times higher. This outcome was consistent with a research conducted in Saudi Arabia among first trimester pregnant women (59) A contrary finding was observed in Bangladesh that showed being nulliparous had a greater risk of being vitamin D deficient (88) Also study conducted in turkey in 2015 (89) and America north Carolina (90) revealed that being nulliparous and Primigravida significantly associated with VDD than multiparous women and This could be due to a shortage of vitamin D supplements program in our study area that may cause depletion of stored vitamin d due to repeated pregnancy which worsen the deficiency when there is multiple pregnancy.

8. Strength and limitation of the study

The research project includes 402 individuals, which enhances the generalizability of the results and the use of systematic random sampling may also minimize selection bias. And also considers socio demographic, dietary, life style and sunlight exposure as well as maternal and obstetric characteristics in the analysis. This study was limited to health facilities in scope and used a cross-sectional data that didn't include long-term impacts of VDD on maternal and newborn outcomes. Furthermore: it didn't indicate the prevalence among women without ANC follow-up, some of the information including the usage of sunscreen, fish intake, egg intake, hours of exposure to the sun, smoking practices, and consumption of alcohol were subjectively acquired from patients. Additionally, we did not include a quantified measurement of the study participants UVB sunlight exposure.

Conclusion

The current study found VDD is highly prevalent (43.3%, of which 4.5% were severely deficient) in the study population. Different factors, such as overweight, obesity, and multiparity, increase the risk of vitamin D deficiency, whereas the regular consumption of fish, egg, liver and exposure of >30% of the total body surface area to sunlight are strongly associated with a lower risk of developing vitamin D deficiency in pregnant women.

Recommendation

- Promoting outdoor activities and tackling lifestyle choices like overusing umbrellas.
- Promote the Consumption of vitamin D-rich foods, including fish, liver, and eggs. Use culturally competent nutrition advice to dispel food taboos and misconceptions.
- Prenatal care programs should include regular vitamin D assessment and management, especially for high-risk populations such women with high body mass indexes (BMI) or restricted food intake.
- Considering supplementation for pregnant women at risk of deficiency.
- Investigate the long-term impacts of VDD on maternal and newborn outcomes, conduct longitudinal research.

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9. Annex

9.1. Annex: 1 Laboratory SOPs

9.1.1. Blood sample collection

Venipuncture is the collection of blood from a vein which is usually done for laboratory testing. The blood is normally drawn from a vein on the top of the hand or from the inside of the elbow. Venipuncture requires good skills in order to perform the procedure not only correctly, but also painlessly. There are some slight risks associated with venipuncture which may include excessive bleeding, feeling light-headed, fainting, nerve damage, hematoma (accumulation of blood under the skin), and infection. The area where the blood is to be drawn from is first cleaned with a 70% alcohol.

Materials the equipment used during the venipuncture test can vary, but the following are most commonly used for routine venipuncture:

- Collection tubes
- Needles
- Tourniquet
- Wipes/Swabs
- Gauze
- Bandages
- Gloves
- Disposal unit

Procedure

1. Drawn 5 ml whole blood into serum separator tube containing no anticoagulant.

2. Kept in upright position at room temperature for 10-20 min to allow clotting.

3. Centrifuged for 5 min at manufacturer’s recommended speed.

4. Carefully aspirate the serum at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use clean pipette for each tube.

5. Inspected serum for turbidity. Centrifuge turbid samples and aspirate again to remove remaining insoluble matter.

6. Separate serum sample

7. Aliquot into Nunc tubes and stored at –80 °C. Label Nunc tubes with patient identification number.

9.1.2. SOP for Vitamin D determination

Purpose

The measurement of 25-OH-D (referred to as the vitamin D assay) is becoming increasingly important in the management of patients with various disorders of calcium metabolism associated with rickets, neonatal hypocalcemia, pregnancy, nutritional and renal osteodystrophy, hypoparathyroidism, and postmenopausal osteoporosis

Principle of the test

Competition principle, Total duration of assay: 27 minutes. 1st incubation: By incubating the sample (20 µL) with pretreatment reagent 1 and 2, bound 25-hydroxyvitamin D is released from the VDBP. 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the 25-hydroxyvitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24, 25-dihydroxyvitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite. 3rd incubation: After addition of streptavidin-coated micro particles and 25-hydroxyvitamin D labeled with biotin, unbound ruthenylated labeled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated 25-hydroxyvitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Specimen: serum or plasma

Sample preparation: Although a fasting specimen is recommended, it is not required. No special instructions such as special diets are required. Diurnal variation is not a major consideration. Specimens for vitamin D analysis should be fresh or frozen serum. Serum specimens may be collected by using regular red-top or serum-separator Vacutainers. Serum specimens should be stored at < -20 o C. A sample volume of 50µL is required for the assay; 150µL will permit repeat analysis and adequate pipetting volume as well. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample. Because vitamin D is very stable, serum samples may be frozen at -20 o C to -70 o C for years before analysis. After thawing frozen sample were homogenized by vortex mixer before testing. Samples with fibrin clot or erythrocyte stroma were centrifuged before testing.

Limitation of the assay: interference may be encountered with certain sera containing antibodies directed against reagent components.

Range of expected values: vitamin D deficient (<20ng/ml), vitamin D insufficient (20-29ng/ml), vitamin D sufficient (30-100ng/ml) and vitamin D potential toxicity (>100ng/ml).

9.2. Annex 2: Information sheet

9.2.1. Information sheet (English version)

Title of the Study: Assessment of Vitamin D deficiency and associated factors among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, 2024.

Principal Investigator: Getachew Wolde (MSc student from Addis Ababa University).

Your permission is being sought to have you participate in this study. Please read the following information carefully before you decide whether or not to give your permission.

Introduction: You are invited to participate in a research study in Addis Ababa, Ethiopia. The study aims to determine the prevalence of Vitamin D deficiency among antenatal care attending pregnant women in selected health centers of Addis Ababa, Ethiopia, and identify any associated factors, Addis Ababa, Ethiopia, 2024.

Before you decide whether to participate, it is important that you understand the purpose of the study, its procedures, potential risks, and benefits. Please take your time to read this form carefully and feel free to ask any questions.

Purpose of the study: the purpose of this research is to determine the prevalence of Vitamin D deficiency among antenatal care attending pregnant women in Addis Ababa, Ethiopia, and identify any associated factors.

Study Description: The study involves monitoring antenatal care pregnant women. We will collect biochemical data, such as Vitamin D to determine the prevalence and associated factors of regarding vitamin d deficiency. Your blood samples will be collected after the questionnaires will be carried out in accordance with established medical standards.

Risks and Benefits: The risks associated with this study are minimal and may include discomfort or inconvenience related to blood sampling. The benefits include contributing to the understanding of the prevalence and associated factors regarding vitamin d deficiency among pregnant women, which may understand to identify pregnant who can benefit from vitamin D supplementation therapy and it will alert clinicians and health policy makers to give attention to this problem.

Incentives: There is no any payment to be gained by taking part in this research.

Confidentiality: All information and data collected during the study will be kept confidential. No personal information will be disclosed in any research reports or publications.

Voluntary Participation: your consent to participate in this study is entirely voluntary. You may choose not to participate or withdraw at any time without any consequences or loss of benefits.

Informed Consent: By signing this form, you acknowledge that you have read and understood the information provided in this document. Your participation in the study is entirely voluntary, and you also understand that the study may involve blood sampling.

Contact persons: If you want to know more information, have any question, you can contact through the researcher and advisor address below.

Principal investigator

- Phone No- +251-939626805
- Email- getachewwolde5483@gmail.com

Advisors:

1. Dr. Mistire Wolde (PhD, Associate Professor)
    - Tel: +251-911699710
2. Mr. Gobena Dedefo (MSc)
    - Tel: +251-913983634
3. Ms. Mekdes Alem, (MSc)
    - Tel: +251-913601036

You will be provided with a copy of this informed consent form for your records. Thank you for your consideration in participating in this study. Your contribution is greatly appreciated

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9.3. Annex 3: Consent and assent forms for adults (≥18)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study. To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Phone number

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

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9.4. Annex 4: Questionnaire (English version)

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences

Questionnaire to Asses prevalence of vitamin D deficiency among pregnant women and to identify the main risk factors associated with vitamin D deficiency (English Version)

Good morning/good afternoon. I am ----------; I am working with an investigator, Getachew Wolde, who is doing his thesis for the partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences in Clinical Chemistry. He is conducting research on vitamin D deficiency among first trimester pregnant women to identify prevalence of vitamin D deficiency among pregnant women in Addis Ababa. You are randomly selected from pregnant women with less than of 14 weak gestational ages. If you are willing to participate, I would like to ask some questions, measure your weight and height as well as take 5ml blood sample from your hand to test vitamin D level by experienced health professional. You may feel mild pain while taking blood sample. Your name will not be written in this format and never be used in connection with any of the information you are going to give me. You are not obliged to answer any question that you do not want to answer and you can discontinue this interview at any time you want to stop. Your participation should be voluntary bases; however, your honest answers to these questions will help us to identify the main risk factors associated with vitamin D deficiency, helps to government and other concerned bodies to plan and solve the identified problems in the future to control and prevent vitamin D deficiency. Beside; if you have vitamin D deficiency it helps you to know your status and take supplement as early as possible though it is not free. If you have any question related to the study you can ask. We would like to appreciate your help in responding to these questions, and the interview will not take more than 30 minutes.

Informed consent

I am the individual asked to be a study participant. Based on the information provided by the interviewer, i understand that it is not necessary to write my name, the information that i am going to give her/him will not to be used for other purpose and the information obtained from me will help to identify prevalence of vitamin D deficiency among pregnant women to solve the identified problem in the future as well as it helps me to be treated if I am vitamin D deficient. So, I agree to be a study participant. Understanding all the provided information very well, I fully agree and confirm to participate in the study by putting my signature in the space provided here below.

Date----------

Participant's signature----------

Questioner code/Mother ID ----------

Name of data collector----------

Signature of data collector ----------

Address of principal investigator

- Phone No. +251-939626805
- Email=getachewwolde5483@gmail.com

- **Advisors:**

1. Dr. Mistire Wolde (PhD, Associate Professor)

- Email= mistire.wolde@aau.edu.et

2. Mr. Gobena Dedefo (MSc)

- Email= gobenadedefo@gmail.com

3. Ms. Mekdes Alem, (MSc)

- Email=alem12@gmail.com

Eligibility criteria for selection of pregnant women

1. All questionnaires are administered to mothers in the first trimester who attend ANC clinic for ANC follow up purpose only.

2. Is the pregnant mother gestational age less than 14weeks? If yes continue. If No stop interview

3. Currently do you have any of listed medically proven illness like **liver disease, chronic kidney disease** or having on **medication of anti TB drug, anti-epileptic drug**? If yes stop interview. If no continue

4. Questionnaire identification number:

Sub-city name……. Woreda …….

ID……..... Phone number _____

Note: please encircle or write the appropriate answer on the space provided

Part 1: Socio-demographic and economic characteristics of the study participants

No.	Items	Options	Skip pattern
101.	ID		
102	Age of the women	……in Year	
103.	What is your educational Status?	1. Able to write and read 2. Primary (1-8) 3. Secondary (9-10) 4. Diploma 5. First degree and above	
104.	What is Occupation of the women (main Occupation)?	1.Housewife 2.Merchant 3.Government employee 4.NGO	

		5.Daily laborer 6.Others specify_____	
105.	What is marital status of the women?	1.Never married 2.Married/living together 3.Divorce/Separated 4.Widowed	If your answer is never married skip to Q107
106.	What is husband education	1. Able to write and read 2. Primary (1-8) 3. Secondary (9-10) 4. Diploma 5. First degree and above	
107.	Family size in number	_____	

Part 2: Maternal obstetric characteristics and body composition of the study participants

201.	Number of previous Pregnancies	_____			
202.	When was the last time you gave Birth?	1.Never gave birth 2.Before one year 3.Before two year 4.Before three year 5. Four year and above			
203.	Is your menstrual period come in the same pattern?	1. Yes 2. No			
204.	First day of last menstrual period (LMP)	_____			
205.	Number of Deliveries	_____			
	Mode of delivery	1st child	2nd child	3rd child	4th child

206.	i. Vaginal delivery				
	ii. Cesarean Section				
	iii. Abortion or ectopic				

Part 3: Food frequency questions (FFQ). If you have you eaten any of listed foods below answer accordingly

No.	Food types	Type/Brand name	Per day	Per week	Per month	Occasional/in holy days	Never
301.	Fish						
302.	Fish oil						
303.	Mushroom						
304.	Fortified milk and milk Product with vitamin D						
305.	Egg						
306.	Fortified oil with vitamin D						
307.	Liver						
308.	Vitamin D fortified cereals						
309.	Cakes and confectionery						
310.	Other vitamin D fortified foods						
311.	Vitamin D supplements						

Part 4: Anthropometric Measurements

401.	Weight measurement in Kg	W1_____ W2_____
402.	Height measurement in meter	H1_____ H2_____
403	BMI	----------
404.	Skin pigmentation	1. Light Brown (IV) 2. Dark Brown(V) 3. Very Dark (VI)

Part 5: Sun exposure and life style

No	Items	Options	Skip pattern
501	Do you use sunscreen?	1. Yes 2. No	If your answer is 2 skip to Q504
502	If yes how often do you use sunscreen lotion?	1. Rarely 2. Sometimes 3. Often 4. Always	
503	Have you ever used any kind of shade or umbrella for sun screen purpose?	1. Yes 2. No	If your answer is 2 skip to Q506
504	How often do you stay in the shade or under umbrella?	1. Rarely 2. Sometimes 3. Often 4. Always 5. Rarely	
505	Is there any taboos related with diet or clothing for pregnant mother in your culture	1. Yes 2. No	If your answer is 2 skip to Q509
506	If yes what kind of it?	_____	

507	Have you apply this culture			1.Yes 2.No		
508	Do you have smoking history in the last two month			1.Yes 2.No		
509	Do you have alcohol drinking history in the last two month			1.Yes 2.No		
510.	What activities have you done outdoor?			_____		
511.	How much time do you spent in outdoor activity?			a. _____minuet b. _____hour		
512.	How frequent do you act on outdoor activity?			a. _____per day b. _____per week c._____per month		
513.	**Duration of sun exposure**	**Per day**	**Per week**	**Per month**	**514.Time of exposure (in local time)**	
	<15 minuet				Morning (1-3)	
	15-30 minuet				Mid-day (4-9)	
	>30-60minuet				Evening (10-12)	
	>1 -2 hour					

Sunlight exposure time *Morning*: The time period between 7:00AM– 9:00AM, *Mid-day*: The time period between 10:00AM– 3:00PM, *Evening*: The time period between 4:00PM– 6:00PM

515. Shade the box to calculate body part exposed to sun light for most of the time (How do you dress when you do outdoor activity)?

			Head		Top			Bottom	
	Rule of nines	Adapted Value	No Head scarf	No neck scarf	No sleeve	Short Sleeve	Long Sleeve	Long bottom	Short bottom
Anterior head									
Posterior head									
Anterior neck									
Posterior neck									
Anterior upper arm (Both)									
Posterior upper arm (Both)									
Anterior lower arm (Both)									
Posterior lower arm (Both)									
Anterior hand (Both)									
Posterior hand (Both)									
Anterior upper leg (Both)									
Posterior upper leg (Both)									
Anterior lower leg (Both)									
Posterior lower leg (Both)									
Foot									
Calculated percent of body surface area exposed to sunlight----------									
516. Do you have major skin burn in your body? 1. Yes 2. No									

[illegible]

[illegible]

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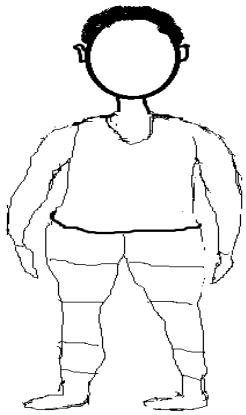

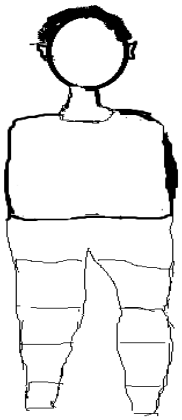

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9.5. Annex 5: Data collection sheet

Check list for laboratory result

Laboratory parameter	**No**	**Serum vitamin D level in(**ng/ml)	**Remark**
Serum Vitamin D	1		
	2		
	3		
	4		
	5		
	6		

Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. Candidate: Getachew Wolde (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Mistire Wolde (PhD, Associate professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Gobena Dedefo (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mrs. Mekdes Alem (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.