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Prevalence of vitamin D deficiency among women in a rural district of Nepal and association with diabetes: A population-based study

Graduate thesis in Programme of Professional Study, Medicine
Supervisor: Unni Syversen
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Graduate thesis

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Kunnskap for en bedre verden

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Abbreviations

1,25$(OH)_2$D	→	1,25-Diydroxyvitamin D
25(OH)D	→	25-Hydroxyvitamin D
BMI	→	Body Mass Index
CI	→	Confidence Interval
T2D	→	Type 2 Diabetes
FBG	→	Fasting Blood Glucose
FPG	→	Fasting Plasma Glucose
HbA1c	→	Hemoglobin A1c
HPLC	→	High Performance Liquid Chromography
IQR	→	Interquartile Range
OGTT	→	Oral Glucose Tolerance Test
SD	→	Standard Deviation
UV-B	→	Ultraviolet B radiation
WC	→	Waist circumference

Abstract

Background

Vitamin D deficiency and insufficiency affect billions of people across the world. Vitamin D is essential for good bone health, as its main action is stimulation of intestinal calcium absorption and regulation of calcium homeostasis. The vitamin is also postulated to have several extra-skeletal effects, among others a role in the pathogenesis of diabetes.

Objective

We aimed to assess vitamin D status among women in Temal, a rural area in Nepal. Moreover, we wanted to study the prevalence of vitamin D deficiency and insufficiency in women with and without diabetes, as well as examine the association of diabetes and vitamin D status.

Methods

This study is a part of cross-sectional study conducted in the outreach centers in the rural municipality Temal, of Dhulikhel Hospital, Kathmandu University. The participants were recruited from a previous study conducted 2012-13. The inclusion criteria were non-pregnant women >15 years who were married. A total of 813 women, 21-80 years, were enrolled. Waist circumference, weight and height were measured, and BMI calculated. A comprehensive questionnaire was filled in, and fasting blood samples were collected. Vitamin D deficiency was classified as 25(OH)D $\leq$ 50nmol/L, insufficiency < 75 nmol/L, and diabetes and prediabetes defined as HbA1c $\geq$ 48 mmol/mol (6.5 %) and 39-47 mmol/mol (5.7-6.4 %), respectively. SPSS Statistics version 27 was used to analyze the data.

Results

Finally, 769 women were included, mean age among the women was 48.5 ($\pm$11.8) years. They were poorly educated and most of them were working within agriculture. Mean BMI and WC were 24.7 ($\pm$ 4.4) kg/m^2 and 78.5 ($\pm$ 10.1) cm, respectively. Mean 25(OH)D level was 51.7 ($\pm$ 16.1) nmol/l. Altogether, 48.8% and 44.0% of the women, exhibited vitamin D

deficiency and insufficiency, respectively. Mean HbA1c level was 37.4 ($\pm$ 8.4) mmol/mol. The prevalence of diabetes, preferentially type 2, and prediabetes was 4.4%, and 33.7%, respectively. A higher rate of vitamin D deficiency was found in women with diabetes compared to those without diabetes (63.6 versus 45.3%). Mean 25(OH)D levels was borderline statistically significant between women with or without diabetes (p=0.055), whereas a significantly higher level was seen in those without diabetes compared to those with prediabetes (52.9 and 50.4 nmol/l, respectively, p=0.05). Vitamin D deficient women displayed a significantly higher mean HbA1c compared to those with vitamin D insufficiency (38.3 and 36.4 mmol/mol, respectively, p=0.004). No significant correlation was found between HbA1c and 25(OH)D levels when adjusting for age (p=0.344).

Conclusion

We observed a high prevalence of vitamin D deficiency and a low prevalence of diabetes among women in rural Nepal. Vitamin D deficiency was more common in women with diabetes, compared to those without. Women with vitamin D deficiency also exhibited significantly higher HbA1c level than those with insufficiency. Our data support previous studies of a possible association between vitamin D deficiency and type 2 diabetes.

Introduction

Hypovitaminosis D is a major problem across the world, especially in developing countries (1). Almost half of the world's population is affected by vitamin D insufficiency and it is estimated that 1 billion people suffer from vitamin D deficiency (defined as serum 25(OH)D levels < 50 nmol/l) (2). A systematic review from 2013 revealed great differences regarding vitamin D status around the world. In adults, the prevalence of vitamin D deficiency ranged from 1% in Tanzania to 80% in Bangladesh (1). Although we see extreme numbers of vitamin D deficiency outside the Western World, the prevalence is high in Europe as well. A large observational study revealed a prevalence of 40.4% and an even larger prevalence of insufficiency in European countries (3). In 2015, a Nepalese study targeting preschoolers in a rural district, showed vitamin D deficiency in 91% of the participants (4). This is of concern, as vitamin D has an essential role in calcium homeostasis (including intestinal absorption) and bone metabolism. It is also postulated to have important extra-skeletal actions. Rickets in children and osteomalacia in adults are results of a prolonged and severe vitamin D deficiency with levels below 30 nmol/L (5, 6). In the 90s, the prevalence of rickets was very high in Asian countries like Mongolia (70%) and Tibet (66%). Recent studies suggest an association between vitamin D deficiency and diabetes (8-10).

Vitamin D, sources and metabolism

Vitamin D is precursor of a steroid hormone. There are two forms of the vitamin, vitamin D_2 or ergocalciferol, occurring mainly in plants, and vitamin D_3 or cholecalciferol from marine foods such as veal liver, cheese and egg yolks (11). Upon exposure to ultraviolet B radiation (UV-B) through sunlight, the zoosterol 7-dehydrocholesterol (7-DHC) in the skin is converted to previtamin D_3, subsequently to vitamin D_3. There is no risk of vitamin D_3 intoxication (> 375 nmol/l) due to sun exposure, as any excess previtamin D3 or vitamin D3 will be destroyed by sunlight (12). The liver transforms vitamin D_3 to 25-hydroxyvitamin D (25(OH)D) (13). The enzyme 1-α-hydroxylase catalyzes formation of the active vitamin, 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) in the kidney and several other tissues (14). Both ergocalciferol (D_2) and cholecalciferol (D_3) are converted into this active metabolite. The serum level of 25(OH)D is used as an indicator of vitamin D status. (15, 16).

Factors affecting 25(OH)D concentration

Low intake, malabsorption and increased metabolization are all factors that may contribute to vitamin D deficiency. According to several studies, sunlight is the most important source of vitamin D (17-20). In the northern hemisphere at latitudes above 40°N, production of dermal vitamin D is absent for at least half the year (21, 22). Therefore, vitamin D supplementation is a great necessity. The amount of UV-B radiation reaching the earth's surface is reduced when the solar zenith angle is elevated (23, 24). Altitude, air pollution, sunscreen use, clothing habits, aging and exposure to sun rays, dependent on time of the day and period of time, are all factors that affect the vitamin D synthesis induced by UV-B radiation as well. (24, 25). Many will need a sufficient dietary or supplementary intake of vitamin D, as meeting a satisfactory vitamin D level through skin synthesis alone has proven to be difficult. Studies have shown that obesity can cause decreased bioavailability of vitamin D due to sequestration in adipose tissue, leading to a decrease in 25(OH)D concentration (23, 26-28). Independent of age and latitude, vitamin D deficiency has been found to be more frequent in obese people (29). Vitamin D deficiency may also be attributed to increased catabolism of 25(OH)D caused by medications, decreased synthesis of 25(OH)D due to hepatic failure, urinary loss of 25(OH)D or decreased synthesis of 1,25-dihydroxyvitamin D due to renal failure (30).

The effects of vitamin D

The main action of vitamin D is stimulation of intestinal calcium absorption and regulation of calcium homeostasis. Studies show that low maternal 25(OH)D levels may have a negative effect on the fetal bone growth (31). During pregnancy, the mother's calcium homeostasis must adapt. Sufficient vitamin D is of importance, as the fetus demands approximately 30 g of calcium for mineralization of the skeleton. Zhu et al. showed an association between maternal levels of vitamin D during pregnancy and peak bone mass in the offspring at the age of 20 years (32). In conjunction with the well-known facts that vitamin D promotes calcium absorption and has an important role in the normal mineralization of the bone, an adequate vitamin D status is important in slowing bone loss and reducing fracture risk (33). In addition to beneficially affecting the skeleton, vitamin D is proposed to have several other actions and benefits. Numerous studies have found that vitamin D affects both muscle strength and function. Low levels of vitamin D have been associated with reduced muscle mass, strength and performance, thereby increased risk of falling (34, 35). Vitamin D has immune modulating effects, and has been shown to suppress the inflammatory response in diseases such as rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus (36-38). Likewise, an association between metabolic syndrome, including hypertension, has been reported (9, 39, 40). A meta-analysis by Parker et al showed a 43% reduction in cardiometabolic disorders in those with the highest levels of serum 25(OH)D (41). Inadequate levels of vitamin D are also associated with various types of cancers, such as: colon, prostate and breast cancer (42-46). A decrease in the proliferation of malignant cells expressing vitamin D receptors or 1-α-hydroxylase has been demonstrated as an effect of $1,25(OH)_2D_3$ and its analogs. Accordingly, a deficit of vitamin D appeared to increase the proliferation (47).

Categorization of vitamin D status

There is some disagreement in categorization of vitamin D status. The Institute of Medicine defines 25(OH)D levels below 30 nmol/L (12 ng/ml) as deficiency and levels below 50 nmol/l (20 ng/ml) as insufficiency. The Endocrine Society defines levels below 50 nmol/L as deficiency, and below 75 nmol/L as insufficiency. The latter is based on the observation that an increase in PTH levels occurred when serum levels of 25(OH)D fell under approximately 75 nmol/L (30 ng/ml) (48, 49). In this thesis, the definition of Endocrine Society is applied.

Diabetes

A global diabetes epidemic is of concern including increasing morbidity and a growing economic burden of society. An increasing prevalence of diabetes in the world is endangering the health of the Earth’s population. A pooled analyze from 2016, including 751 studies, found that the prevalence of diabetes among women rose from 5.0% in 1980 to 7.9% in 2014 (50). During the same period, the prevalence increased by 109% amongst men, from 4.3% to 9.0%. South-Asian countries have had a particularly large increase in the prevalence of type 2 diabetes (T2D) the last decades (51-54). This is attributed to decrease in physical activity and an increase in poor diet consisting of processed or fat food, are associated with urban residency and leading to obesity and development of T2D (55, 56). It has been observed that Asians have higher percentage of body fat at any BMI, compared to Caucasians (57). This correlates with South Asians being younger and less obese at the time of the diagnosis (58, 59).

Diagnostic criteria of diabetes

Most commonly used in the diagnosis is the fasting plasma glucose (FPG) and glucose tolerance test (OGTT), as well as the hemoglobin A1c (HbA1c) criterion (60). The HbA1c has its advantages, such as no need for fasting, higher probability of greater preanalytical stability and no disturbances associated with daily variations related to stress and illness, and is therefore more commonly used compared to FPG (61). In this thesis, we used HbA1c $\geq$ 48 mmol/mol (6.5%) as diagnostic criterion for diabetes and 39-47 mmol/mol (5.7-6.4%) for prediabetes.

Vitamin D and diabetes

There is increasing evidence for an association of vitamin D deficiency and diabetes (62). Maternal vitamin D insufficiency in pregnancy has been shown to be associated with gestational diabetes, as well as low birth weight and type 1 diabetes in the offspring (63-65). Studies suggest that low vitamin D levels are associated with insulin resistance, impaired synthesis and secretion of insulin from the pancreatic ß cells and impaired glucose intolerance. (66-69). Mattila et al. observed a higher prevalence of diabetes and higher BMI in association with low levels of 25(OH)D (70). In a meta-analysis from 2013, a relative risk for T2D at 0.62 (95% CI 0.54-0.70) was found when comparing the groups with the highest and lowest levels of vitamin D (62). Another meta-analysis from 2018 reported that vitamin D supplementation given to deficient T2D patients reduced the insulin resistance effectively (71). However, the same study found no improvement regarding fasting blood glucose (FBG), HbA1c and fasting insulin levels. A significantly reduction of development to T2D was found when giving vitamin D supplementation to subjects with pre-diabetes compared to placebo (72).

Nepal

History, geography and economics

Nepal is mainly located in the Himalayas. It has a diverse geography, consisting of both fertile plains, subalpine forest hills and tall mountains. The highest mountain on Earth, Mount Everest, is located in Nepal. Because of Nepal’s location between India and Central Asia, it is a multi-ethnic country. More than a few various ethnical groups have since the Stone Age immigrated to the area known as Nepal today. Over 100 different ethnic groups having their own traditions and rituals have given rise to dissimilar dynasties in different part of the country. At the end of the 18th century, the King of the Gorkha Kingdom started an epoch of absolute monarchy as he seized control of larger areas, uniting the kingdom. It was not until the 90s that a parliamentary monarchy took shape. It required a civil war before the king abdicated in 2008 and Nepal became a republic. The constitution affirming Nepal as a secular federal parliamentary republic was not adopted until 2015 as there were yet political contradictions after all the prior events. Nepal is one of the poorest countries in the world, and there are big differences within economy and living conditions between classes. With approximately 30 million inhabitants, both electricity and running water are privileges belonging to only half of the population.

Culture, education and health

As previously mentioned, Nepal is a multi-ethnic country, with a broad difference in language and religion throughout the country. Nepali is the mother language of 45% of the population and is appointed as the official language. Hinduism is the most dominating religion in the country, with 81% adherents. The gender equality in Nepal is poor. Women's privileges are limited including reduced inheritance rights and the opportunity to get formal positions. Forced marriage and violence at home are unfortunately not uncommon, and so is the women's' accessibility to health care, especially in poorer rural regions. Dhulikhel Hospital has started projects giving women in these regions the empowerment to be more independent through education and economic aid. For the poorer rural regions, the access to health care has a potential for improvement for both women and men. The government strives to increase this access. When it comes to education in general, 32% of the population above 15 years old cannot read and write, and there is a lack of knowledge about diseases, prevention and treatment. Asthma and chronic obstructive pulmonary disease (COPD) are common disorders as many live in poor ventilated houses where the food is cooked inside over an open fire. The food culture itself is diverse as the different ethnic groups have their own traditions and some forbid certain types of meat. The Nepalese economy also reflects the inhabitants eating habits, as dairy products and meat are too expensive for the majority. Rice, wheat, potatoes and vegetables are cheaper, and are part of the major dietary components for numerous. Because of this, malnutrition and vitamin deficiencies are a challenge.

Vitamin D status in Nepal

Earlier studies have shown high prevalence of vitamin D insufficiency and deficiency in Nepal (4, 73-76), all though the country is located between latitudes 26° and 31° N. Avagyan et al. found that being fed with breast milk was a positive factor associated with vitamin D status (4). When Nepalese women breastfeed, there is a tradition for doing it outdoors under the sun, with the children minimally dressed. Haugen et al. measured and compared the vitamin status among infants and their mothers in a peri-urban community in Nepal (77). They found insufficiency in 3.6% of the infants as opposed to 36.0% among their mothers. 25(OH)D levels <30 nmol/L was observed in 0.6% infants and 14.0% mothers. Another study found that 64% of Nepalese inpatients with disorders related to alcohol-use had s-25(OH)D <50 nmol/L (78). In Nepal there are about 300 sunny days, and the culture does not restrict sun exposure (79).

Diabetes in Nepal

As mentioned, the prevalence of diabetes has increased in South-Asia, Nepal included, during the last decades (54). The risk of developing diabetes seemingly occurs at a younger age and lower BMI levels for ethnic south Asians, compared to other ethnic groups (54). Simultaneously, there has been an increase from 1.6% (1996) to 10% (2006) in the prevalence of obese women in urban Nepal (80). A meta-analysis including ten studies from 2000 to 2014 observed a prevalence of T2D in Nepalese people ranging from 1.4% to 19.0% (81). A more recent meta-analysis found an overall prevalence at 8.5% for diabetes and 9.2% for prediabetes (82). As much as 47.3% of the participants were undiagnosed. FBG and OGTT were the most commonly used as diagnostic methods in these studies. Nepal faces many challenges with diabetes, in addition to unawareness about the disease. High costs of treatment, limited health care facilities and a lack of guidelines regarding treatment and prevention are some of these challenges (83, 84).

Aims

This study aimed to:

1) Assess the vitamin D status among women in a rural district of Nepal.

2) Study the prevalence of vitamin D deficiency in women with and without diabetes.

3) Examine the association of diabetes (HbA1c) and vitamin D status.

Material and methods

Study design and participants

This cross-sectional sub-study is part of a larger project entitled "Early onset and increasing burden of diabetes in Nepalese women. Risk factors, complications, and relation with vitamin A and D. A prospective cohort study in rural Nepal." The participants were recruited from women (n=1498) who originally took part in a study in 2012-13 addressing sexually transmitted diseases and non-communicable diseases (85). All the women are living in Temal rural municipality, situated at a latitude of about 27.5° N, with an average elevation at 1890 meters above sea level and therefore categorized into the subtropical climate zone.

For the original study, the inclusion criteria were: married non-pregnant women >15 years. The same inclusion criteria were applied for the current project. The exclusion criteria were physical and mental conditions that made it difficult to participate.

During October-December 2019, 813 women were enrolled, age 21-80 years. The participants answered a comprehensive questionnaire addressing among others socioeconomic factors, dietary habits, physical activity and smoking. Waist circumference, weight and height were measured without shoes, but with light clothing on. All the data including the blood samples were collected in five villages.

Fasting blood samples were collected and kept on ice under transport, then stored at -80°C until analysis. Vacutainers were used; one EDTA tube for HbA1c and Hb, and two gel tubes for the other biochemical tests. HbA1c was analyzed consecutively at Dhulikhel Hospital in Nepal at a Hb-Vario machine, using a HPLC method. Calcium, phosphate, parathyroid hormone and vitamin D were also analyzed at Dhulikhel Hospital using chemiluminescence technology (CLIA).

Two research assistants from public health professionals were responsible for interviewing the participants, in addition to separating, labeling and transporting the serum samples to Dhulikhel Hospital. Measurement of height, weight, waist and hip circumference, as well as blood sampling were done by health professionals.

Definition of vitamin D deficiency and type 2 diabetes

The Endocrine Society Recommendations will be applied for classification of vitamin D status: deficiency < 50 nmol/l (20 ng/ml), insufficiency 25(OH)D < 75 nmol/l (30 ng/ml). The SI unit for vitamin D will be applied. The diagnostic criteria for diabetes and prediabetes will follow the 2020 American Diabetes Association standards: HbA1c blood levels $\geq$ 48 mmol/mol (6.5%) and 39-47 mmol/mol (5.7-6.4%), respectively (60, 86). The cut-points for BMI categories will follow the Asian-Pacific recommendations: Underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5-23 kg/m^2), overweight (23-25 kg/m^2), moderate obesity (BMI 25-30 kg/m^2) and severe obesity (BMI $\geq$ 30 kg/m^2) (87). The cut-points for central obesity are set to waist circumference $\geq$ 80 cm, according to the American Diabetes Associations guidelines for Asian women (88).

Ethical considerations

The main project with sub-studies was approved by REK Midt-Norge (13003), May 2019, The National Health Research Council, Nepal (2715) May 2019, and Kathmandu University School of Medical Sciences (124/19), May 2019. There is no obligation to notify NSD about this project.

Statistical analysis

IBM SPSS Statistics 27 were used for the statistical analyses of the data. Frequencies with proportions are presenting the descriptive statistics. Data are presented as mean values with standard deviations (SD) and medians are stated, or as interquartile range (IQR) when not normally distributed. Pearson's correlation coefficients were used to study correlations. Independent samples t-test was used to compare groups. Linear regression analysis was performed to determine the association of vitamin D levels and HbA1c based on two different models (model 1: crude model; model 2: adjusted for age). Statistically significance was given by a p-value ≤ 0.05.

Results

Of the 813 women enrolled in the study, 44 were excluded due to missing data. Finally, 769 women were included in the study. The mean age was 48.5 (±11.8) years, whereas median age was 48 (21-80) years (n=769). The women were divided into three age groups: 22.8% were between 20-39 years old, 57.7% between 40-59 and 19.5% 60 years and older. The characteristics of the participants are presented in table 1.1 and 1.2.

Table 1.1 – Participants characteristics

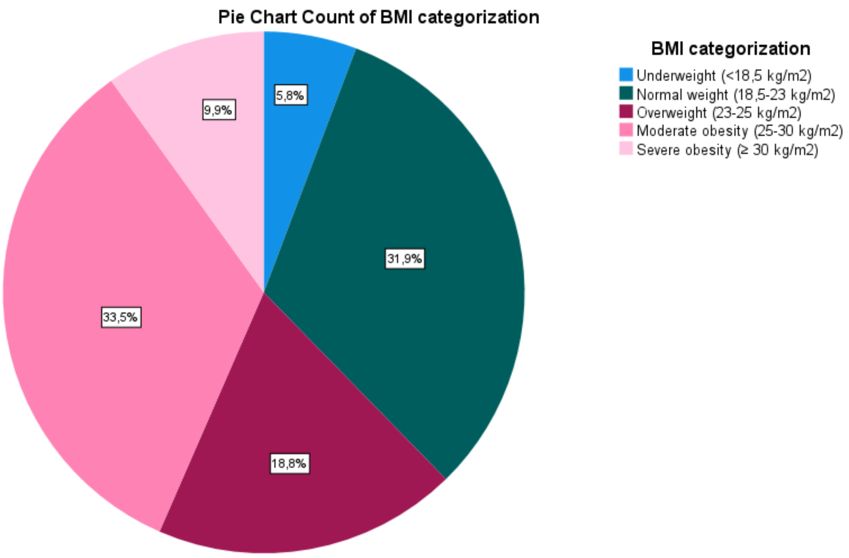
Characteristics	N	%	Mean (±SD)	Range value Min	Max
Age (n=769)			48.5 (± 11.8)	21.0	80.0
20-39 (years)	175	22.8			
40-59 (years)	444	57.7			
60-80 (years)	150	19.5			
Education (n=767)					
No formal education	641	83.6			
Less than primary school	37	4.8			
Primary school completed	29	3.8			
Secondary school completed	20	2.6			
Higher secondary school completed/ Proficiency Certificate Level	20	2.6			
Bachelor's degree	7	0.9			
Refuse to answer	13	1.7			
Work status (n=736)					
Self-employee	580	78.8			
Homemaker	112	15.2			
Government employee	30	4.1			
Non-government employee	8	1.1			
Others	6	0.8			
Smokers (n=768)					
Yes	153	19.9			
No	615	80.1			
Egg consumption (n=709)					
2-4 times a day	10	1.4			
Once a day	64	9.0			
2-4 times a week	343	48.4			
Once a week	292	41.2			
Milk consumption (n=581)					
2-4 times a day	35	6.0			
Once a day	95	16.3			
2-4 times a week	159	27.4			
Once a week	292	50.3			

Vitamin D or calcium supplementation (n=727)	42	5.8			
Yes	685	94.2			
No					
BMI (n=764)			24.7 (± 4.4)	14.3	57.5
Underweight (<18.5 kg/m^2)	44	5.8			
Normal weight (18.5-22.9 kg/m^2)	244	31.9			
Overweight (23.0-24.9 kg/m^2)	144	18.8			
Moderate obesity (25.0-29.9 kg/m^2)	256	33.5			
Severe obesity (≥30.0 kg/m^2)	76	9.9			
WC (n=759)			78.5 (± 16.1)	54.5	115.0
No central obesity (< 80 cm)	417	54.9			
Central obesity (≥ 80 cm)	342	45.1			

Anthropometrics

Mean BMI (n=764) was 24.7 kg/m^2 (± 4.4) with a median at 24.3 kg/m^2 (14.3-57.5). 9.9% of the women had a BMI ≥ 30 kg/m^2 which is classified as severe obesity. A total of 476 (62.2%) women were overweight or obese, having a BMI of 23 kg/m^2 or above. Figure 1 shows the BMI distribution among the women. Two subjects were excluded from the analyses due to very low height (100.5 and 103 cm), probably measurement errors.

Figure 1 – Distribution of BMI in the study population



The mean WC (n=759) for the women were 78.5 cm (± 10.1) with a median at 78 cm (54.5-115.0). 342 of the women (45.1 %) were found to have central obesity (WC ≥ 80 cm). Seven subjects were excluded from the analysis because of extremely low waist circumference (25-42 cm), possibly due to typing or measurement errors.

Vitamin D status

The prevalence of Vitamin D insufficiency and deficiency, based on the Endocrine Society Recommendations, was 44.0% and 48.8% respectively (n=734), 6.3% of the women had 25(OH)D levels < 30 nmol/l. Mean vitamin D in the study population was 51.7 nmol/l (± 16.1) (see Table 2.1), median level was 50.4 nmol/l (13.5-154.5). The vitamin D levels of the study population are shown in a histogram (Figure 2). Vitamin D and calcium supplementation were used by 42 of the women. Data on mean vitamin D levels in different age groups, categories of BMI, WC, smoking status, vitamin D or calcium supplementation and diabetic status are shown in table 2.2.

Figure 2 – Distribution of 25(OH)D serum levels

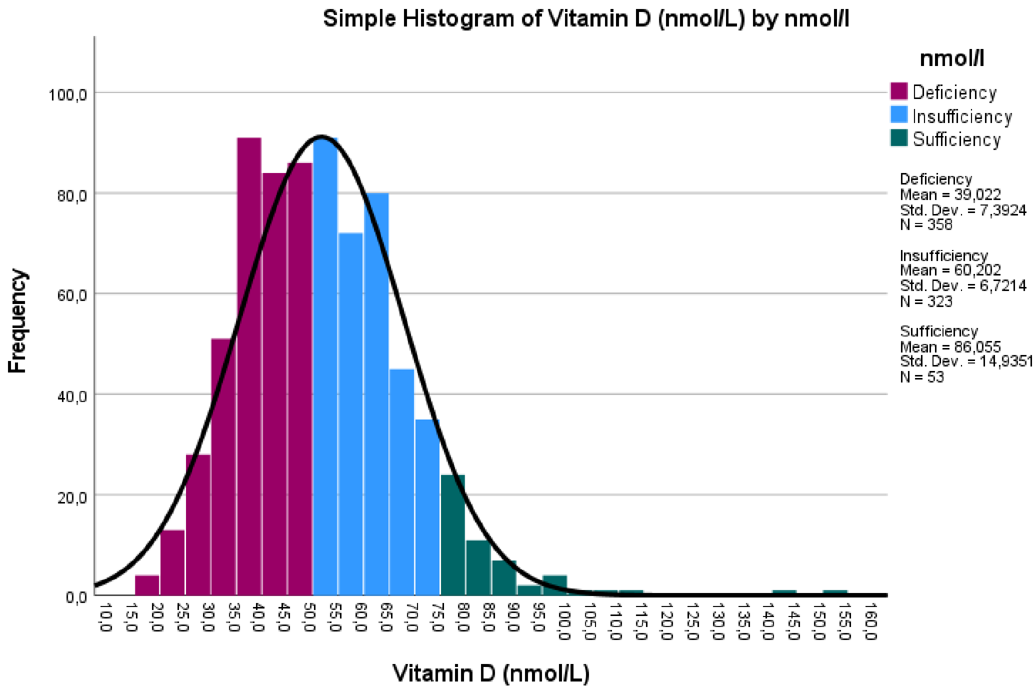


Table 2.1 – 25(OH)D Mean, SD, and Minimal-Maximum Range Value

Characteristics	Subjects (n=734)	Mean	SD	Range Value Min	Max
Vitamin D level		51.7	16.1	13.5	154.5
Deficiency (< 50.0 nmol/l)	358 (48.8%)				
Insufficiency (50.0-74.99 nmol/l)	323 (44.0%)				
Sufficiency (≥ 75.0 nmol/l)	53 (7.2%)				

An inverse correlation between age and vitamin D levels (r=-0.181, p<0.001) (n=731) was found. We observed a higher prevalence of vitamin D deficiency in older participants (Table 2.3). Table 2.4 presents mean 25(OH)D levels in different within BMI categories. No correlation was observed between BMI and 25(OH)D serum levels (r=0.084, p=0.024) (n=731). Women with BMI above 23 kg/m^2 (overweight and obesity) had significantly higher vitamin D concentrations (52.9 $\pm$ 16.1 nmol/l) compared to those with BMI below 23 kg/m^2 (normal weight and underweight) (49.5 $\pm$ 15.7 nmol/l), p=0.005 (Table 2.1). We observed a small positive correlation between WC and 25(OH)D serum levels (r=0.103, p=0.005) (n=724). Mean 25(OH)D concentration for those with central obesity (53.0 nmol/l) was significantly higher compared to those without central obesity (50.5 nmol/l), p=0.032. Smokers had significantly lower mean 25(OH)D level than non-smokers, 49.0 $\pm$ 15.7 nmol/l and 52.4 $\pm$ 16.1 nmol/l, respectively, p=0.025. No significant difference in mean 25(OH)D levels was found between self-employees and those with other occupations.

Table 2.2 – Levels of mean 25(OH)D by age, BMI, WC, smoking status, vitamin D or calcium supplementation and diabetic status

Variable	N (%)	25(OH)D (nmol/l)	P
Age (n=734)			
20-39 (years)	168 (22.9)	54.8 $\pm$ 14.0	*
40-59 (years)	425 (57.7)	57.9 $\pm$ 15.2	0.033
60-80 (years)	142 (19.5)	19.3 $\pm$ 19.6	< 0.001
BMI (n=731)			
Underweight and normal weight (< 23.0 kg/m^2)	274 (37.5)	49.5 $\pm$ 15.7	*
Overweight ($\geq$ 23.0 kg/m^2)	547 (74.8)	52.9 $\pm$ 16.0	0.005
WC (n=724)			
No central obesity (< 80 cm)	397 (54.8)	50.5 $\pm$ 15.7	*
Central obesity ($\geq$ 80 cm)	327 (45.2)	53.0 $\pm$ 15.4	0.032
Smokers (n=733)			
Yes	141 (19.9)	49.0 $\pm$ 16.4	*
No	592 (80.1)	52.4 $\pm$ 16.0	0.025
Vitamin D or calcium supplementation (n=693)			
Yes	42 (6.1)	53.5 $\pm$ 13.7	*
No	651 (93.9)	51.7 $\pm$ 16.3	0.482
Diabetic status (n=723)			
Non-diabetic (HbA1c <39 mmol/mol)	448 (62.0)	52.9 $\pm$ 15.8	*
Pre-diabetic (HbA1c 39-47 mmol/mol	242 (33.5)	50.4 $\pm$ 16.5	0.050
Diabetic (HbA1c $\geq$48 mmol/mol)	33 (4.5)	47.4 $\pm$ 16.1	0.055

* Reference group for p-value

Table 2.3 – Vitamin D status within age groups (n=734)

Age groups Years	Deficiency % (N)	Insufficiency % (N)	Sufficiency % (N)	Total % (N)
20-39	36.9 (62)	56.0 (94)	7.1 (23)	100 (168)
40-59	48.3 (205)	44.1 (187)	7.5 (32)	100 (424)
60-80	64.1 (91)	29.6 (42)	6.3 (9)	100 (142)
	48.8 (358)	44.0 (323)	7.2 (53)	(734)

Table 2.4 – Mean 25(OH)D levels (nmol/l) within categories of BMI (n=731)

BMI	% (N)	Mean (+ SD)	95% confidence interval for mean Lower bound	Upper bound
Underweight (< 18.5 kg/m^2)	5.6 (41)	48.4 (+ 16.0)	43.3	53.4
Normal weight (18.5-23.0 kg/m^2)	31.9 (233)	49.7 (+ 15.7)	47.7	51.8
Overweight ($<$ 23.0-25.0 kg/m^2)	18.7 (137)	51.8 (+ 16.9)	49.0	54.7
Moderate obesity ($<$ 25.0-30.0 kg/m^2)	33.4 (244)	53.5 (+ 16.0)	51.5	55.6
Severe obesity ($\geq$ 30.0 kg/m^2)	10.4 (76)	53.1 (+ 14.9)	49.7	56.5

Prevalence of diabetes and pre-diabetes and relation with anthropometrics

Mentioned abroad we used HbA1c in the diagnosis of diabetes. In the present study, those with HbA1c < 39 mmol/mol (5.7%) were classified as non-diabetes. The prevalence of diabetes and pre-diabetes was 4.4% and 33.7% respectively (n=742), based on the HbA1c-criteria (see Table 3.1). The mean HbA1c level in the study populations was 37.4 mmol/mol (+ 8.4). The median was 36.6 mmol/mol (19-115). Altogether, 33 women had diabetes, mean age was 54.5 years, mean Hb1Ac 62 mmol/mol (IQR 23) and the median 51 mmol/mol (48-115), mean WC was 83.8 cm, and mean BMI 27.8 kg/m^2. Fifteen of the participants had HbA1c $\geq$ 53 mmol/mol. Six reported being diagnosed with diabetes previously. The prevalence of diabetes and pre-diabetes increased with age (see Table 3.2). A significant, positive correlation was observed between diabetes and age, r=0.264, $p < 0.001$ (n=742). A significant correlation was also seen between HbA1c and waist circumference before and after adjustment for age, r=0.161, p<0.001 and r=0.191, p<0.001, respectively.

Table 3.1 – HbA1c levels in women without diabetes and with pre-diabetes and diabetes

Characteristics	% (N)	Mean	SD	Range Value Min	 Max
HbA1c level (n=742)		37.4	8.4	19.0	115.0
Diabetes (HbA1c ≥48 mmol/mol)	4.4 (33)				
Pre-diabetes (HbA1c 39-47 mmol/mol)	33.7 (250)				
Non-diabetes (HbA1c <39 mmol/mol)	61.9 (459)				

Table 3.2 - Distribution of diabetes within age groups (n=742)

Age groups Years	**Non-diabetes** % (N)	**Pre-diabetes** % (N)	**Diabetes** % (N)	**Total** % (N)
20-39	80.5 (140)	18.4 (32)	1.1 (2)	100 (174)
40-59	60.6 (257)	34.7(147)	4.7 (20)	100 (424)
60-80	43.1 (62)	49.3 (71)	7.6 (11)	100 (144)
	61.9 (459)	33.7 (250)	4.4 (33)	(742)

The diabetes status in the participants were stratified as: non-diabetes (HbA1c < 39 mmol/mol), pre-diabetes (HbA1c 39-47 mmol/mol) and diabetes (HbA1c ≥ 48 mmol/mol). The mean age in those with non-diabetes, pre-diabetes and diabetes was significantly different: 46 years (± 11.4), 52.2 years (± 11.4), and 54.5 years (± 11.0), respectively. The age in the diabetic and non-diabetic, and the pre- and non-diabetic groups were significantly different (p=0.0004 and p<0.0001, respectively). Only 14.4 % of the participants (n=757) reported that their blood sugar had been measured in the past, and 25 (75.8 %) of the women with diabetes had not been diagnosed. Twenty-eight women reported having diabetes, however HbA1c levels were below 48 mmol/mol in 20 of these women. Since information on medication was lacking and they did not fulfill the diagnostic criterion, they were not classified as having diabetes.

Vitamin D status and diabetes

Mean level of 25(OH)D was significantly lower in those with pre-diabetes compared to those with non-diabetes (p=0.05). A higher rate of vitamin D deficiency was found in those with diabetes (63.6%) compared to those with non-diabetes (45.3%). The mean HbA1c levels among those with vitamin D deficiency, insufficiency and deficiency are shown in Table 4.1. A significant difference in HbA1c means was found between those with deficiency and insufficiency, p=0.004. Table 4.2 presents mean 25(OH)D levels among those with diabetes, pre-diabetes and non-diabetes. Mean level of 25(OH)D was lower in those with diabetes compared to those with non-diabetes (p=0.055), although borderline significant, and significantly lower among those with pre-diabetes compared to those with non-diabetes (p=0.05) significant. No significant difference was found between those with diabetes and pre-diabetes (p=0.332). The association of vitamin D and diabetes is shown in table 4.3. After adjustment for age, no significant association of vitamin D and HbA1c levels was observed (p=0.344). Figure 3 shows the relationship between the serum levels of HbA1c and 25(OH)D.

Table 4.1 – Mean HbA1c levels according to vitamin D status (n=723)

Vitamin D status	**Mean HbA1c** Mmol/mol	**SD**	**95% CI**	**Total number** N (%)
Deficiency (< 50 nmol/l)	38.3	10.1	37.2-39.4	350 (48.4)
Insufficiency (50-75 nmol/l)	36.4	6.4	35.7-37.1	321 (44.4)
Sufficiency (> 75 nmol/l)	36.9	6.2	35.2-38.6	52 (7.2)

Table 4.2 – Mean vitamin D levels according to diabetes category (n=723)

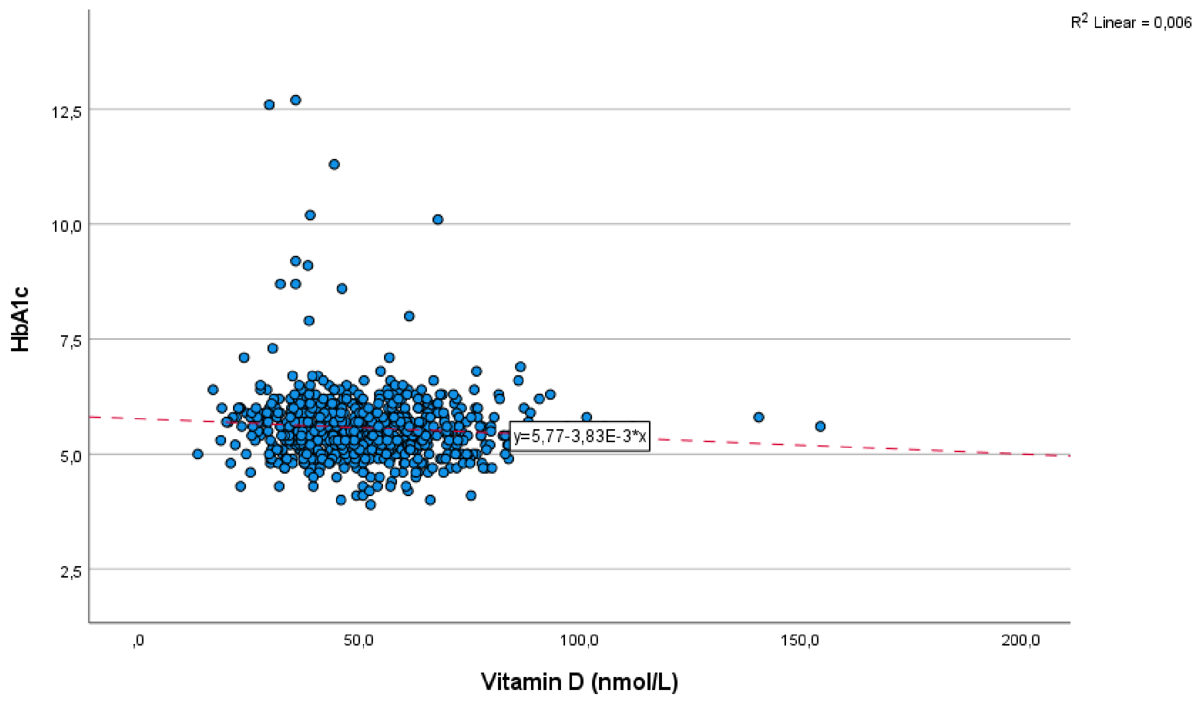
HbA1c categories	**Mean 25(OH)D** Nmol/l	**SD**	**95% CI**	**Total number** N (%)
Diabetes (≥ 48 mmol/mol)	47.4	16.1	41.7-53.1	33 (4.5)
Pre-diabetes (39-47 mmol/mol)	50.4	16.5	48.3-52.5	242 (33.5)
Non-diabetes (< 39 mmol/mol)	52.9	15.8	51.4-54.4	448 (62.0)

Table 4.3 – Linear regression model for prediction of HbA1c in relation with 25(OH)D (n=723)

	B (95% CI)	**Std. Error**	**Beta**	**t**	**P**
Model 1	-0.042 [-0.080, -0.004]	0.019	-0.080	-2.148	0.032
Model 2	-0.018 [-0.056, 0.019]	0.019	-0.035	-0.948	0.344

Notes: Model 1: Crude model; Model 2: adjusted for age.

Figure 3 – HbA1c and vitamin D levels (n=723)



The distribution of diabetes status among participants with vitamin D deficiency, insufficiency and sufficiency are shown in table 4.4.

Table 4.4 – Vitamin D status and the prevalence of diabetes (n=723)

Vitamin D status	**Diabetes status**			**Total number**
	Normal	Pre-diabetic	Diabetic	
Deficiency (< 50 nmol/l)	203 (58.0%)	126 (36.0%)	21 (6.0%)	350 (100%)
Insufficiency (50-75 nmol/l)	211 (65.7%)	101 (31.5%)	9 (2.8%)	321 (100%)
Sufficiency (> 75 nmol/l)	34 (65.4%)	15 (28.8%)	3 (5.8%)	52 (100%)
	448 (62.0%)	242 (33.5%)	33 (4.6%)	723 (100%)

Discussion

To our knowledge, this is the most comprehensive study addressing vitamin D status in Nepal. In this cross-sectional among women living in a rural area in Nepal, we observed a high prevalence of both vitamin D deficiency and insufficiency by 48.8% and 44.0%, respectively. Very low levels were seen in 6.3% of the women, thus being at risk for developing osteomalacia (89). Vitamin D deficiency was more prevalent among the elderly, demonstrated by a significant inverse correlation between age and 25(OH)D levels. The prevalence of pre-diabetes and diabetes was 33.7% and 4.4%, respectively. Higher age was associated with higher prevalence of diabetes and pre-diabetes. The HbA1c levels were significantly higher among women with vitamin D deficiency compared to those with insufficiency. Women with pre-diabetes had significantly higher mean 25(OH)D level compared to non-diabetics. Vitamin D deficiency was more prevalent among diabetics than non-diabetics, 63.6 % and 45.3 %, respectively Altogether, 62.4 % of the study population were obese or overweight according to their BMI ($\geq$ 23 kg/m^2), whereas 45.1% were found to have central obesity (WC $\geq$ 80 cm).

Few studies have examined vitamin D status among Nepalese. We observed a high prevalence of vitamin D deficiency among women in a rural district of Nepal, however, lower than in reported in previous studies. Two studies in Bhaktapur; a peri-urban area, found a prevalence of vitamin D deficiency at 59.8% and 81.0% among lactating and pregnant women, respectively (73, 77). Sedhain et al found a higher prevalence of vitamin D deficiency in the plains compared to the hill districts (76). The most alarming discovery was among preschoolers in rural Nepal, where vitamin D deficiency was found in 91.1% of the children (4). Data from India show a prevalence of vitamin D deficiency ranging from 44% to almost 90% (90). An even higher prevalence was demonstrated in urban parts of India, ranging from 78% to 94%. This difference may be attributed to the agrarian lifestyle and less air pollution. Most women in our survey spent hours outside exposed to sun, as 78.8% of the women were self-employed, most likely within agriculture. Vitamin D levels did not differ between women with different occupations. The majority of the women (93%) had serum 25(OH)D levels < 75 nmol/l. An explanation for this could possibly be the clothing culture which consists of covering garments, as well as a lack of foods containing vitamin D in their diet. A study from 2020 found a concerningly low intake of meat and fish, with only 16.3% of the Nepalese population including it in their diet (91). The data concerning the effect by altitude on vitamin D level are diverging (12, 92). Confounders such as pollution, skin color, latitudinal global position, outdoor activities and clothing traditions, among others, must be considered.

It is challenging to estimate the exact prevalence for the global extent of vitamin D deficiency, partly because of different measurement methods and absence of representative population samples for each country (93). Nevertheless, widespread vitamin D deficiency is suggested (1). The prevalence of vitamin D deficiency and insufficiency seems to be elevated among women and girls in the middle eastern areas overall, despite being exposed to sunlight most of the year (1).

In our study population, the prevalence of diabetes was 4.4%, which is in accordance with previous studies in rural Nepal (102-107). Pre-diabetes was very prevalent in our study population, affecting about one third of the participants. Other prevalence rates reported are 11.1% (semi-urban) and 14.9% (urban and rural) (106, 107). A role of vitamin D deficiency in development of diabetes has been postulated. In support of this, we observed higher levels of HbA1c in subjects with vitamin D deficiency compared to those with insufficiency. Moreover, women with diabetes and pre-diabetes exhibited lower vitamin D levels than those with non-diabetes, although borderline significant for the former. This concords with the higher prevalence of vitamin D deficiency in women with diabetes compared to non-diabetic women in our study, 63.6 and 45.3%, respectively. However, no difference in mean vitamin D levels was revealed between subjects with and without diabetes, and no significant correlation of HbA1c and vitamin D levels was found. Previous studies are conflicting. Studies among postmenopausal and elderly women showed no correlation between vitamin D levels and diabetes (94-96), whereas others have shown a significant relationship between HbA1c and vitamin D levels (70, 97). A meta-analysis indicated a 4% lower risk of T2M for every 10 nmol/l increment in 25(OH)D levels based on 21 prospective studies (62).

We observed an inverse correlation between age and 25(OH)D serum levels. This could be anticipated as the epidermal concentrations of provitamin D decrease by age (98). In contrast to previous studies, we found significantly higher vitamin D levels among those with BMI > 23 kg/m^2 compared to those with BMI < 23 kg/m^2. We expected vitamin D levels to be lower in overweight and obese people as excessive fat has been associated with lower levels due to dilution or sequestration in adipose tissue (23, 26-28). A small positive correlation between WC and serum levels of vitamin D was also found. This findings are in contrast to a study reporting a 35% higher prevalence of vitamin D deficiency in obese people (29). Interestingly, our data are in accordance with results from another study in Nepal (77). We speculate if higher intake of energy-rich food containing vitamin D among those with higher BMI and

WC could be a possible explanation for our observation. A meta-analysis reported a significant inverse correlation between serum 25(OH)D levels and BMI in adult population, except for women living in developing countries (99). In line with other studies (100, 101) , smoking seemed to have a significantly negative effect on vitamin D levels.

Strengths and limitations

The major strength of this study is the large number of participants (n=769). The analysis of serum HbA1c levels were performed with HPLC, indicated as the preferred measuring technique (108). The CLIA method was used to measure 25(OH)D levels. A high inter-assay disagreement for 25(OH)D levels has been found between high-pressure liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS) and CLIA. 25(OH)D levels have been reported to be 30% lower using CLIA in contrast to HPLC-APCI-MS (109). Thus, our findings may overestimate the prevalence of hypovitaminosis D. All the anthropometric measurements were done by one healthcare worker, which made the measurements more standardized. Measurement of WC and weighing were performed without shoes although with thin clothing. This may have led to an overestimation of central obesity as well as overweight and obesity, as no correction on the data of the were done. Seven cases had extreme low WC measurements (< 50 cm) and were hence excluded for the analysis as they were error numbers.

The study population may not be representative for the rural female population as the elderly and least healthy women may not participated due to travel distances. The single site of the study is not necessarily representative for other rural areas in Nepal as factors like culture, access to meat and other sources of vitamin D, as well as altitude also have an impact on the vitamin D levels. There were no collected data regarding sun exposure, including time spent in the sun, skin pigmentation, clothing habits or other sun protecting factors. Nor were there any questions about the women’s meat or fish consumption. Although we know that most women in rural Nepal have a diet poor of meat and fish (91). An approximately age was reported of some of the women as some did not know their exact age. This may have caused some information bias.

Factors such as age, sex, ethnicity, climate, alcoholism, smoking, diseases causing erythrocyte destruction and iron-deficiency anemia can affect the HbA1c levels (110). A smaller proportion of the women had diabetes, and an even smaller percentage had optimal vitamin D levels which may affected the statistical strengths negatively. Finally, the prevalence of diabetes may have been underestimated as elaborated on previously.

Conclusion

We found a high prevalence of vitamin D deficiency among women in rural Nepal, reaching almost 50%. A higher rate of vitamin D deficiency was found in diabetics compared to non-diabetic. Mean level of 25(OH)D tended to be lower in those with diabetes compared to those with non-diabetes, and significantly lower among those with pre-diabetes compared to those with non-diabetes. Moreover, vitamin D deficient women displayed higher HbA1c level than those with vitamin D insufficiency. Our data may suggest an association between hypovitaminosis D and diabetes.

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