Graduate thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

Emil Granqvist, Jonas Krøvel

Prevalence of fractures among women in rural Nepal and association with diabetes

Graduate thesis in Programme of Profesional Study, Medicine
Supervisor: Unni Syversen
Co-supervisor: Miriam K. Gustafsson, Astrid Kamilla Stunes, Mats Peder Mosti

June 2021

NTNU
Norwegian University of Science and Technology

Emil Granqvist, Jonas Krøvel

Prevalence of fractures among women in rural Nepal and association with diabetes

Graduate thesis in Programme of Profesional Study, Medicine
Supervisor: Unni Syversen
Co-supervisor: Miriam K. Gustafsson, Astrid Kamilla Stunes, Mats Peder Mosti
June 2021

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

NTNU
Norwegian University of Science and Technology

Prevalence of fractures among women in rural Nepal and association with diabetes

Supervisor: Prof. Unni Syversen (IKOM, NTNU and Dep. of Endocrinology, St. Olav's Hospital)
Assistant supervisor: Associate Professor MD, PhD Miriam K. Gustafsson at the Department of Clinical and Molecular Medicine (IKOM), Astrid Kamilla Stunes at the Department of Clinical and Molecular Medicine (IKOM) and Mats Peder Mosti (IKOM)
Medical students: Emil Granqvist and Jonas Krøvel

Acknowledgements
We want to thank our main supervisor Dr. Unni Syversen for making the process of writing a paper enjoyable and fulfilling. We could not have done this without Unni.

We also want to thank our assistant supervisor Miriam K. Gustafsson for helping us. Mats Peder Mosti and Astrid Kammila Stunes assisted us greatly with statistical analyses and general advise, and we are very grateful for everything they have done for us.

Lastly, we would like to offer special thanks to Chandra Yogal for collecting the vast amount of data for the project. Chandra has done a very important job with the data collection and administering the data collection process in Nepal. We also want to thank him for helping us understand the data and helping us a lot with statistical analyses.

Abstract

Background

Osteoporosis and diabetes are diseases with alarmingly high prevalence worldwide and are both diseases where treatment exists, and preventive measures can be made. There are few data on the prevalence of osteoporosis and osteoporotic fractures in Nepal. A recent study from Kathmandu including 169 women >50 years found high prevalence of osteoporosis and osteopenia. Little data exist from rural parts of Nepal. Taking into account the fairly recent realization that osteoporosis is a prevalent complication of diabetes, and that South Asians have a predisposition for developing type 2 diabetes (T2D), addressing this association in Nepal is of significance.

Objectives

The aim of this study was to estimate the prevalence of osteoporosis and fractures among the female population in the rural parts of the Kavre district in Nepal. Additionally, the study aimed to assess the relationship between osteoporosis and diabetes and identify other risk factors for osteoporosis.

Methods and materials

The study is a cross-sectional sub-study of a larger project investigating a female population in rural Nepal. Inclusion criteria were non-pregnant, married women aged 21-80 years. Anthropometrics were measured, blood samples were collected, and a comprehensive questionnaire, including previous fractures, was filled in by the participants. Due to limited access to dual x-ray absorptiometry for measurement of bone mineral density, we had to use other criteria for osteoporosis. Accordingly, osteoporosis was defined as having experienced at least one fracture and/or receiving osteoporosis medication. HbA1c ≥6.5% and 5.7-6.4% were used to diagnose diabetes and prediabetes, respectively.

Results

In total, 769 women with a mean age of 48.5 years were included in the study. The prevalence of osteoporosis in the total population was 10.5%, and the osteoporotic women had a mean age of 52.9 years. Previous fractures were reported by 4%. Altogether 46.4% were postmenopausal, and 13.6% of them had osteoporosis. The prevalence of diabetes and prediabetes was 4.4% and 38.2%, respectively. Of the women with diabetes, 15.2% were

osteoporotic, while 10.5% of the non-diabetic women were osteoporotic. Among the women with hyperglycemia (HbA1c ≥5.7%), 14.6% were osteoporotic, while 8.3% of women with normoglycemia women were osteoporotic. Mean HbA1c was slightly higher among women with osteoporosis than without osteoporosis (5.76% compared to 5.55%). Mean HbA1c level was substantially higher in subjects with diabetes and concomitant osteoporosis than in those with diabetes without osteoporosis (8.7% compared to 7.7%). A significant correlation was found between hyperglycemia and osteoporosis (r_s=0.1, p=0.006). Hyperglycemia was identified as a strong predictor for osteoporosis (OR=1.9, p=0.015). Menopause was also a significant risk factor showing strong positive prediction for osteoporosis (OR=2.1, p=0.006 when correcting for diabetes, OR=1.8, p=0.032 when correcting for hyperglycemia).

Conclusion

In this large study, we observed a fracture prevalence of 4.4%. When including women reporting use of medication against osteoporosis, the prevalence increased to 10%. The prevalence increased with age and menopausal status. The prevalence of fractures and osteoporosis in general is surely underestimated. A clear relationship between osteoporosis and diabetes was found. Thus, prevention of diabetes could be a good action for preventing osteoporosis and other diseases, in addition to general long-term complications and death. Many Nepalese women live with undiagnosed and untreated osteoporosis.

Abbreviations

T1D	Diabetes type 1
T2D	Diabetes type 2
PTH	Parathyroid hormone
fT4	Free t4 (thyroxine)
BMD	Bone Mineral Density
BMS	Bone Material Strength
DXA	Dual X-ray absorptiometry
AGE	Advanced glycation end products
BMI	Body mass index
SD	Standard deviation
WHO	World Health Organization
WC	Waist circumference
FPG	Fasting plasma glucose
OGTT	Oral glucose tolerance test

Introduction

Osteoporosis and diabetes are diseases with alarmingly high prevalence worldwide and are both diseases where treatment exists, and preventive measures can be made. In WHO's "Global report on diabetes" from 2016, an estimated 422 million adults were reported to live with DM, compared to 108 million in 1980.[1] Additionally, 212 million adults are thought to be living with undiagnosed diabetes, preferentially type 2 diabetes (T2D).[2] Osteoporosis is also heavily prevalent, affecting 75 million people only in the United States, Europe and Japan.[3] Despite its significance, few estimates of the global burden of osteoporosis exist.[3] Consequently, research regarding osteoporosis in all areas of the world gains importance. Taking into account the fairly recent realization that osteoporosis is a prevalent complication of diabetes, the association between the two summons the need for investigation.[4]

Osteoporosis – etiology, risk factors and prevalence

Osteoporosis is a disease characterized by low bone mass and impaired bone quality, which results in reduced bone strength and increased risk for fractures.[5] The most common osteoporotic fractures occur in the forearm, spine and hip, with the hip fractures considered both most morbid and mortal.[6] Nevertheless, non-hip fractures are more frequent than hip fractures, occur at younger ages, and are also associated with significant morbidity and mortality, especially vertebral fractures.[6] Osteoporosis is a disease with multiple etiologies. Primary osteoporosis is caused either by age-related loss of bone mass, affecting both genders, or by postmenopausal lack of estrogen.[7] Secondary osteoporosis is induced by a concurrent condition or medication. Examples of diseases causing osteoporosis are gastrointestinal (celiac disease), rheumatological diseases, chronic obstructive pulmonary disease, and diabetes. Several medications may lead to osteoporosis, with the predominant culprits being glucocorticoids, aromatase inhibitors and proton pump inhibitors.[5] Several additional risk factors are identified, with smoking, vitamin D/calcium deficiency, prior fragility fractures, low or extremely high BMI, alcohol consumption, familial history of osteoporosis and physical inactivity being among the most prominent.[5,8]

Although affecting more women than men due to i.e. menopausal effects and lower peak bone mass, osteoporosis is a significant burden regardless of gender. Estimated to cause 8,9 million fractures annually worldwide, osteoporotic fractures afflict both men and women,

with the lifetime risk of fractures estimated to be in the order of 30-40% in developed countries. Thus, the risk for osteoporotic fractures is actually very close to that for coronary disease.[6]

Defining and measuring osteoporosis

Osteoporosis is defined on the basis of measurement of bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA), with a BMD 2.5 standard deviations (SD) or more below the young adult mean (T-score of <-2.5 SD) confirming the diagnosis.[5] A T-score between -1 and -2,5 SD is defined as osteopenia.[9] Important to add is that a low-energy fracture also notifies osteoporosis, independent of BMD.[9] Osteoporosis, in other words, describes both the clinical end result and the process leading to fracture.[5] By using BMD measurements alone, many individuals with high risk of fracture are not identified.[5] Following this, alternative modes of measuring bone fragility are needed. While DXA mainly measures bone density, little information is gathered concerning bone microarchitecture and material composition.[10] Microindentation is a novel tool for measuring bone material strength (BMS). This method utilizes a needle for incision into the anterior tibia, measuring BMS by using data from the impact of the needle on the bone.[10]

Osteoporosis - a complication of diabetes

Several studies have shown that diabetes, both type 1 (T1D) and T2D, are independent risk factors for osteoporotic fractures.[4,10] Subjects with T1D have lower BMD than non-diabetics.[4] The increase in fracture risk is, however, much higher than the reduction in BMD would suggest, implying impairment of bone quality, not captured by DXA.[4] Intriguingly, subjects with T2D also have an elevated risk of fracture, in spite of normal or higher BMD compared to non-diabetics.[10,11] In patients with T2D there is consequently need for other measurements than BMD to assess fracture risk. Microarchitecture and bone material properties in terms of bone mineralization degree, collagen content, crystal size among others are determinants of the strength of the bone.[12] One study assessing bone quality in patients with T2D by use of microindentation, concluded that the T2D patients displayed significantly lower BMS than the control group.[10]

Mechanism of bone affection in diabetes

The cellular mechanisms leading to bone fragility have not been precisely determined. Diabetes is a disease characterized by hyperglycemia and hypoinsulinemia, both of which

seem to impair bone quality.[13] Chronic hyperglycemia causes formation of advanced glycation end products (AGEs). AGEs are proteins that are irreversibly modified both extra- and intracellularly by glucose and lipids, and can be generated as a result of exposure to high circulating glucose concentrations, and also with ageing.[14] AGEs act upon different receptors in cells of many tissues, and can lead to change of gene expression and activation of proinflammatory genes via NFkB.[14] AGEs are known to cause changes in multiple tissues, resulting in creation of plaques and other forms of vessel damage and inflammation.[14] Direct cellular effects of AGEs are inhibition of osteoblast proliferation and induction of apoptosis. Cross-linking of AGEs within collagen fibers is proposed to deteriorate the mechanical properties of bone.[15]

Diabetes and osteoporosis in Nepal

The overall disease burden of diabetes is on the rise globally, and, particularly, a significant rise in prevalence of T2D in Asia.[16] Especially South Asians tend to be both younger and less obese than Western populations at the time of diagnosis.[17,18] Since 1996, the prevalence of obesity among women in urban areas of Nepal has increased markedly, as a consequence of changes in lifestyle, indicating a higher risk of developing T2D.[19] The same changes have not been reported in rural areas of Nepal, although this might be a result of poor access to information.

There are few data on the prevalence of osteoporosis and osteoporotic fractures in Nepal. Hitherto, one nationwide cluster-randomized, population-based survey addressing fractures has been carried out. The survey identified 1.5 million (5% of the population) cases with fracture, out of whom a substantial number of individuals were untreated.[20] However, it is not possible to differentiate between osteoporotic and traumatic fractures as the fracture mechanism was not reported. In a recent study of 169 women from Kathmandu >50 years, the prevalence of osteoporosis, osteopenia, and normal BMD was 37.3%, 38.5%, and 24.2%, respectively.[21]

In the present study we will examine the prevalence of fractures among women in a rural district of Nepal. Moreover, we want to compare the prevalence of fractures among women with and without diabetes. Other risk factors will also be assessed. Given the long-term consequences of osteoporosis and the fact that osteoporosis has emerged as a complication of diabetes, we consider this study to be of clinical significance.

Hypothesis

We hypothesize that the prevalence of osteoporosis is underestimated among women in rural Nepal, and that women with diabetes have an increased prevalence of fractures compared to non-diabetics in the same population.

Aims of the study

I. Estimate the prevalence of osteoporosis among women in a rural district of Nepal
II. Compare the prevalence of osteoporosis among women with and without diabetes
III. Assess risk factors for osteoporosis

Material and methods

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

The women included in the study lived in five villages within the Kavre district called Bolde Fediche, Thulopersel, Pokhari-Narayanthan, Saramthali and Sarasyunkharka. Dhulikhel hospital, which is 40-80 km away, has an outreach center in Bolde-Fediche, in the other villages there are small governmental primary health centers. These centers were used as study sites.

Data were collected through an extensive questionnaire, anthropometric measures and blood samples. A small group of health professionals and research assistants administered the questionnaire and did the anthropometric measures. This group was led and coordinated by Chandra Yogal, who also collected the blood samples.

Questionnaire

The participants answered a comprehensive questionnaire, including among others: reproductive history (menopause), tobacco use, nutrition (e.g. calcium intake), physical activity, and diabetes.

Anthropometrics

Waist circumference, weight and height were measured, and BMI calculated. Due to cultural reasons, the participants were not asked to remove all of their clothes. To minimize errors in measurements, they were asked to wear light dresses and to take off their shoes.

Biochemical analysis

From each participant, fasting blood samples were collected in vacutainers. EDTA-HbA1c, Hb and gel tubes. HbA1c and Hb were analyzed consecutively in full blood at Dhulikhel hospital. The blood samples were centrifuged locally, before being transported to Bolde health station which works as an outreach station for Dhulikhel hospital. Here they were separated into different cryo vials. All serum samples were transported to Dhulikhel Hospital and stored at -80°C until analyses. Vitamin D was analyzed using chemiluminescence technology (CLIA).

For TSH, PTH, calcium, phosphorus and T4 Dhulikhel hospital reference values were used. See appendix for reference values from the Department of Clinical Biochemistry, Dhulikhel Hospital.

HbA1c-levels $\geq$6.5% ($\geq$48 mmol/mol) were used as indication for diabetes, 5.7-6.4% (39-46 mmol/mol) as prediabetes and <5.7% (<39 mmol/mol) as non-diabetes, in accordance with the guidelines of the American Diabetes Association (ADA).[23] Prediabetes refers to those with increased risk of developing diabetes. Prediabetics do not meet the criteria for diabetes but have higher than normal glucose levels. Including prediabetes with a lower.

Vitamin D will be categorized according to guidelines from The Endocrine Society. Adequate vitamin D level is defined as >75 nmol/L, 50-75 nmol/L as vitamin D insufficiency. and <50 nmol/L defines deficiency of vitamin D.[24]

HbA1c method of analysis

The Dhulikhel Hospital laboratory used a Hb-Vario machine which was based on HPLC (high performance liquid chromatography) to measure HbA1c.

Cut-offs for BMI and waist circumference

In this study we have used BMI cut-offs suggested by WHO for Asian populations as follows: <18.5 kg/m^2 equals underweight; 18.5–22.9 kg/m^2 equals increasing but acceptable risk; 23–27.4 kg/m^2 equals increased risk; and >27.4 kg/m^2 equals high risk.[25] Asian populations have proven to generally have a higher body fat percentage than Europeans of the same sex, gender and BMI. The risk for T2D was also proven to be increased at lower levels of BMI than the European cut off at 25 kg/m^2. WHO expert consultation concluded that the European BMI cut-offs do not provide an adequate basis for taking action on risks related to overweight and obesity in Asia.[25] In this study, a cut-off at 80 cm waist circumference (WC) has been used, with WC >80 cm indicating higher risk of disease.[26]

Statistical analysis

Statistical analyses were done using IBM SPSS Statistics Version 27. P-value for significant findings was set to ≤0.05. Data are presented as mean and SD when normally distributed and as median and range when not normally distributed. We used Spearman's correlation to test for association between HbA1c levels and prevalence of osteoporosis. Independent samples T-tests were done to compare means and SD. In addition, binomial logistic regression was carried out to assess predictors of osteoporosis. To check for outliers we created boxplots. HbA1c was not normally distributed, assessed through the Shapiro-Wilk test and calculating kurtosis and skewness, causing Spearman's correlation to be preferred before a point-biserial correlation test.

Ethical approval

The main project with substudies 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.

Results

Study participants

During October-December 2019, 818 of the previous participants were enrolled, age 21-80 years. Reasons why many women did not participate in the follow-up study were migration, death and not accepting to take part. Forty-nine were excluded due to missing data, denial of blood collection or insufficient amount of blood. Finally, 769 women were included in the study, mean age 48.5 (±11.8) years and median age 48 years. Age range was 21-80 years.

Table 1: Main characteristics of the entire study population

Variable	Mean/percentage
Age	48.5 years (±11.8)
Height in cm	148.6 cm (±6.3)
Weight in kg	54.6 kg (±10.2)
Waist circumference	78.5 cm (±10.1)
BMI (kg/m^2)	24.7 kg/m^2 (±4.3)
Smoking	19.9% (n=153)
Daily smoking	15.6% (n=120)
Menopause	46.0% (n=354)
HbA1c (%)	5.6% (±0.8)
Free t4 (pmol/L)	13.1 (±2.9)
TSH (mIU/L)	3.6 (±6.74)
Calcium (mmol/L)	2.4 (±0.2)
Phosphorus (mmol/L)	1.2 (±0.2)
PTH (ng/L)	24.2 (±10.6)
Vitamin D (nmol/L)	51.7 (±16.0)

Mean WC was 78.5 (±10.1) cm, and the median 78 (54.5-115.0) cm. Altogether 299 (39.4%) of 759 had a WC >80 cm, indicating increased metabolic risk in these individuals.[26] Ten were excluded because of extremely low WC, possible erroneous measurement. Mean BMI was 24.7 (±4.3) kg/m^2, median BMI was 24.3 (14.4-41.7). Two women were excluded because of extremely low height. 100,5 and 115.0 cm. Out of the 769 women, 153 (20%) were smokers, 120 (16%) were smoking daily. A total of 354 (46.4%) were postmenopausal.

Prevalence of osteoporosis

Overall, the prevalence of any type of fracture among the women was 4.1% (n=31). The women were also asked if the fracture was caused by a low-energy trauma or not, and 17 women stated that it was a low-energy fracture. Since it is difficult to differentiate between low- and high-energy fracture, all fractures were included in the analyses. Additionally, 51 (6.8%) women reported that they were receiving medical treatment for osteoporosis, three were using alendronate, the others vitamin D and/or calcium

medication. In total, 79 (10.5%) women were on medication for osteoporosis and/or had experienced a fracture. In this study we defined osteoporosis as having experienced a fracture and/or being on medication for osteoporosis, thus making it the main outcome for our statistical analyses.

Table 2: Osteoporosis distributed by BMI categories (Asian cutoffs)

BMI category	Non-osteoporosis (%)	Osteoporosis (%)	Total	P-value
Underweight (<18.5 kg/m^2)	37 (5.0%)	3 (0.4%)	40 (5.4%)	0.058
Normal (18.5-22.9 kg/m^2)	211 (28.6%)	24 (3.3%)	235 (31.9%)	
Overweight (23.0-27.4 kg/m^2)	262 (35.5%)	23 (3.1%)	285 (38.7%)	
Obesity (>27.4 kg/m^2)	149 (20.2%)	28 (3.8%)	177 (24.0%)	
Total	659 (89.4%)	78 (10.6%)		

Table 3: Osteoporosis (and fractures) distributed by age

Age group	Non-osteoporosis	Osteoporosis (%)	Fractures (%)	Total
20-29	27	1 (3.6%)	0	28
30-39	134	8 (5.6%)	7 (4.9%)	142
40-49	209	20 (8.7%)	7 (3.0%)	229
50-59	177	28 (13.7%)	11 (5.3%)	205
60-69	92	12 (11.5%)	5 (4.7%	104
70-79	30	10 (25.0%)	1 (2.4%)	41
80-89	1	0	0	1
Total	670	79 (10.5%)	31 (4.1%)	749

The mean age among the women with osteoporosis was 53 (±11) years, and in those without osteoporosis 48 (±11). Prevalence of osteoporosis increased with age, peaking at 25.0% within the 70-79 age group. Fractures were, however, most common in the age

group 50-59 years (5.3%). In the age group 20-29 years, no fractures were reported, whereas 7 (4.9%) were reported in age group 30-39 years.

Table 4: Baseline characteristics stratified by osteoporosis

Variables	**Osteoporosis (n=79)**	**Non-osteoporosis (n=670)**	**P value**
Age (mean ± SD)	53 (±11.5)	48 (±11.6)	**<0.05**
Height in cm	148.2 (±6.3)	148.7 (±6.3)	0.503
Weight in kg	56.4 (±11.1)	54.4 (±10.1)	0.099
Body mass index (kg/m^2)	25.6 (±4.2)	24.6 (±4.3)	**0.043**
<18.5 (Underweight)	3 (3.8%)	37 (5.6%)	0.058
18.5-22.9 (Normal)	24 (30.8%)	211 (32.0%)	
23.0-27.4 (Overweight)	23 (29.5%)	262 (39.8%)	
>27.5 (Obesity)	28 (35.9%)	149 (22.6%)	
Waist circumference	80.5 (±11.7)	78.3 (±9.9)	0.070
>80 cm (increased risk)	36 (48.0%)	258 (38.7%)	0.120
<80 cm (not increased risk)	39 (52.0%)	408 (61.3%)	
HbA1c (%)	5.76 (±0.96)	5.56 (±0.74)	**0.029**
Vitamin D (nmol/L)	54.36 (±19.29)	51.57 (±15.67)	0.148
Calcium, total (mmol/L)	2.36 (±0.13)	2.36 (±0.15)	0.736
Phosphorus, inorganic (mmol/L)	1.25 (±0.17)	1.22 (±0.19)	0.118
Diabetes			
Non-diabetes (HbA1c < 6.5%)	73 (93.6%)	617 (95.7%)	0.408

Diabetes (HbA1c > 6.4%)	5 (6.4%)	28 (4.3%)	
Prediabetes			
Normoglycemia (HbA1c <5.7%)	37 (47.4%)	408 (63.3%)	**0.007**
Hyperglycemia (HbA1c > 5.6%)	41 (52.6%)	237 (36.7%)	
Menopause			
No menopause	32 (40.5%)	366 (55.1%)	**0.014**
Postmenopausal	47 (59.5%)	298 (44.9%)	
Daily smoker			
Daily smoker	14 (17.7%)	101 (15.1%)	0.537
Non-daily smoker	65 (82.3%)	569 (84.9%)	
Vigorous activity			
No vigorous activity	35 (44.3%)	317 (47.5%)	0.596
Vigorous activity	44 (55.7%)	351 (52.5%)	
Calcium intake (milk)			
Less than 2-4 times a week	45 (57.0%)	418 (62.4%)	0.348
2-4 times a week or more	34 (43.0%)	252 (37.6%)	

Prevalence of diabetes

The prevalence of diabetes in this population was based on measurement of HbA1c and on self-reported answers to the question “Do you have diabetes?” in the questionnaire. Altogether, 28 (3.6%) women reported that they had diabetes, four reported having T1D, ten T2D and 13 did not know what type they had. The knowledge about diabetes was on a low level in the study population suggesting that the self-reported answers could be inaccurate. Due to this uncertainty, we chose to base the diagnosis of diabetes exclusively on HbA1c-levels. Diabetes was defined as HbA1c ≥6.5%, giving a prevalence of 4.4% (n=33). Prediabetes was defined as HbA1c 5.7-6.4% giving a prevalence of 33.8%

(n=251). Including both prediabetes and diabetes in one group adds up to 38.2% of the population (n=284), this group will from here on be called hyperglycemic.

Association between osteoporosis and diabetes

In those who had experienced fractures, 3/31 women had HbA1c levels concordant with diabetes, while 16/31 women with fractures were hyperglycemic. When examining the women who had experienced a fracture and/or received medication for osteoporosis, 5/78 met the criteria for diabetes and 41/78 were hyperglycemic. Several tests were performed to estimate the association between osteoporosis and diabetes.

Table 5: Prevalence of osteoporosis in women with and without diabetes

	Non-Diabetes	Diabetes	Total
Osteoporosis	73 (10.6%)	5 (15.2%)	78 (10.8%)
Non-Osteoporosis	617 (89.4)	28 (84.8%)	645 (89.2%)
Total	690 (100%)	33 (100%)	723 (100%)

Table 6: Prevalence of osteoporosis in women with hyperglycemia and with normoglycemia

	Normoglycemia HbA1c<5.7%	Hyperglycemia HbA1c>5.7%	Total
Osteoporosis	37 (8.3%)	41 (14.7%)	78 (10.8%)
Non-Osteoporosis	408 (91.7%)	237 (85.3%)	645 (89.2%)
Total	690 (100%)	33 (100%)	723 (100%)

Table 7: Mean HbA1c level in women with and without osteoporosis

	N	Mean HbA1c (%)	Std. Deviation
Osteoporosis	78	5.8%	±0.96
Non-osteoporosis	645	5.6%	±0.74

When comparing mean HbA1c levels between the women with (n=78) and without (n=645) osteoporosis, the women with osteoporosis were found to have slightly (0.2%) higher HbA1c mean levels, but the standard deviation was also slightly larger in this group.

Table 8: Mean HbA1c among women with hyperglycemia (HbA1c ≥5.7%) with and without osteoporosis

	N	Mean HbA1c (%)	Std. Deviation	P-value
Hyperglycemia without osteoporosis	237	6.2	±0.85	0.501
Hyperglycemia with osteoporosis	41	6.3	±1.06	

Table 9: Mean HbA1c among women with diabetes with and without osteoporosis

	N	Mean HbA1c (%)	Std. Deviation	P-value
Diabetes, without osteoporosis	28	7.6	±1.82	0.223
Diabetes with osteoporosis	5	8.7	±1.51	

A Spearman's rank-order correlation was run to assess the relationship between osteoporosis and HbA1c levels (measured on a continuous scale). 723 of the women were included in the test. There was a borderline significant correlation between osteoporosis and HbA1c levels, r_s= 0.070, p = 0.059.

When categorizing the participants according to HbA1c levels ≥5.7% including prediabetes and diabetes, and normoglycemia (HbA1c <5.7%), a significant, positive correlation between osteoporosis and hyperglycemia was observed, r_s=0.101, p=0.006.

The test was repeated once more, this time categorizing also HbA1c levels into non-diabetics and diabetics, but not including prediabetics (cutoff set at HbA1c ≥6.5%). 723 women were included in the test. Significant correlation between osteoporosis and diabetes (excluding prediabetes) was not found r_s=0.031, p=0.409.

Risk factors assessed by binominal logistic regression

A logistic regression analysis was done to assess which risk factors could contribute to osteoporosis among the participants. The dependent variable was defined as having experienced a fracture and/or being on medication for osteoporosis. Ten risk factors were chosen for the test. Eight were categorical variables, dividing the population into two groups based on respectively diabetes/hyperglycemia (defined by HbA1c levels), vigorous intensity of work, calcium intake via milk intake (defining sufficient milk intake at ≥2-4 times a

week), daily smoking, menopause, normal/abnormal PTH, normal/abnormal TSH and waist circumference (with cut-off at 80 cm). Vitamin D and BMI were assessed as continuous variables. Two separate tests were done, distinguished only by HbA1c cut-offs. For one test, HbA1c was categorized into diabetes or not (HbA1c ≥6.5%), while for the other tests HbA1c was categorized into hyperglycemia or normoglycemia (HbA1c ≥5.7% or <5.7%).

Table 10: Logistic regression analysis for the risk of osteoporosis (HbA1c cutoff at 6.5%)

Variables	OR	Lower	Upper	P-value
Diabetes (HbA1c ≥6.5%)	1.171	0.415	3.305	0.765
Vigorous intensity of work	0.958	0.579	1.585	0.866
Calcium intake (Milk consumption)	0.079	0.426	1.180	0.186
Daily smoking	1.482	0.763	2.879	0.246
Menopause	2.097	1.236	3.558	**0.006**
BMI (continuous scale)	1.070	0.997	1.149	0.061
Waist Circumference (>80 cm)	1.058	0.569	1.970	0.858
TSH (above or below ref. range)	1.160	0.670	2.010	0.596
PTH (hyperparathyroidism)	0.604	0.210	2.010	0.348
Vitamin D (continuous scale)	1.020	0.980	1.062	0.336

In table 10 (above), being postmenopausal (p=0.006) was the only significant risk factor for osteoporosis (OR=2.1). No other risk factors showed a significant association.

Table 11: Logistic regression analysis for the risk of osteoporosis (HbA1c cutoff at 5.7%)

Variables	OR	Lower	Upper	P-value
Hyperglycemia (HbA1c ≥5.7%)	1.896	1.131	3.181	**0.015**
Vigorous intensity of work	0.947	0.572	1.568	0.833
Calcium intake (Milk consumption)	0.706	0.424	1.177	0.182
Daily smoking	1.520	0.781	2.959	0.218
Menopause	1.808	1.054	3.102	**0.032**
BMI (continuous scale)	1.061	0.989	1.139	0.101

Waist Circumference (>80 cm)	1.030	0.550	1.930	0.926
TSH (above or below ref. range)	1.206	0.694	2.096	0.507
PTH (hyperparathyroidism)	0.593	0.205	1.710	0.333
Vitamin D (continuous scale)	1.023	0.983	1.065	0.267

In table 11 (above) it was evident that hyperglycemia was a risk factor for osteoporosis (OR=1,896, p=0,015). Menopause was also identified as a risk factor for osteoporosis (OR=1,808, p=0,032), thus showing significant results in both logistic regression tests.

Menopause

When testing for correlation between menopause and osteoporosis, 743 women were included in the test. There was a significant, positive correlation between osteoporosis and menopause, r_s=0.090, p=0.014. 13.6% (n=47/345) of the postmenopausal women were osteoporotic, while 8.0% (n=32/398) of the premenopausal women were osteoporotic. The mean age among the women with osteoporosis was 52.9 (±11.5) years, compared to the women without osteoporosis whose mean age was 48 (±11.6).

Vitamin D

Mean Vitamin D was 51.7 nmol/L (±16.0), 48.8% had a deficiency. 44.0% had an insufficiency and 7.2% had what is considered adequate levels. Among the participants with osteoporosis, the prevalence of vitamin D deficiency and insufficiency was 45.6% and 45.6% respectively. The corresponding numbers for the participants without osteoporosis was 48.5% and 44.3%. The mean vitamin D level among the participants with osteoporosis was marginally higher, 54.4 (±19.3) compared to the participants without osteoporosis, 51.6 (±15.7). No correlation was found between vitamin D levels and osteoporosis.

Discussion

In this large, comprehensive study including 769 women aged 21-80 years in rural Nepal, we observed a prevalence of osteoporosis of about 10%. In women above 50 years, the prevalence was 14.8%. The diagnosis of osteoporosis was based on history of fracture and/or receiving medication for osteoporosis. Altogether, 6.6% (n=51) were receiving medical treatment for osteoporosis, whereas 4.0% (n=31) reported having experienced fractures. The prevalence of diabetes and prediabetes was 4.3% (n=33), and 32.6% (n=251), respectively. Mean age among women with osteoporosis was 52.9 years, and the majority of women with

osteoporosis were postmenopausal. Among the women with diabetes, 15.2% were osteoporotic, versus 10.5% of those without diabetes. When stratifying the women according to HbA1c levels above or equal to 5.7% (hyperglycemia) and HbA1c <5.7% (normoglycemia), 14.6% of those with hyperglycemia were osteoporotic, versus 8.3% of the women with normoglycemia. Mean HbA1c was slightly higher among the women with osteoporosis than without osteoporosis. A significant correlation between osteoporosis and hyperglycemia was found. Both hyperglycemia and menopause were identified as strong predictors of osteoporosis. To our knowledge this is the first study addressing prevalence of osteoporosis among women in a rural district of Nepal.

As mentioned earlier, there are few data on the prevalence of osteoporosis and fractures in Nepal. The only survey examining fracture prevalence in Nepal was a nationwide cluster-randomized population-based survey, identifying that 5% (n=1.5 million) of the study population had sustained a fracture.[20] The prevalence of fractures in the present study was 4.0% (n=31), which is a similar to the earlier survey, although the study populations are very different when it comes to size. In addition, the present study included only women, whereas the previous did not differentiate between genders. As expected most of the fractures occurred after 40 years of age, the majority of the women being postmenopausal. In developed countries, like Norway and Sweden, 50% of women will experience a fracture after 50 years of age. The corresponding number in Nepal based on our study would be approximately 5%. It is reasonable that the prevalence of fractures is underestimated in our population. Given that vertebral fractures often are “silent” and depend on x-ray to be identified, these fractures will often not be detected. One option could have been inquiring about height loss since young adult age. However, this would be a very unreliable measure as many will not remember or be aware of their previous height. Height loss may also be attributed to other factors than vertebral fractures.

The gold standard in diagnosis of osteoporosis is measurement of BMD by dual x-ray absorptiometry (DXA), with a T-score of <-2.5 signifying osteoporosis. Another criterion for osteoporosis is a low-energy fracture. Unfortunately, the access to DXA is limited in Nepal and it is costly. Hence, in our study we have data on self-reported fractures only, with little information about fracture site and trauma. Additionally, 6.6% (n=51) women reported that they were using medication for osteoporosis. In a recent study in Kathmandu where BMD was measured by DXA in 169 women >50 years, osteoporosis was demonstrated in 38.5% of

the women and osteopenia in 37.3%.[21] Estimates from a systematic review of prevalence of osteoporosis according to DXA in China, showed a similar prevalence of 40% in women ≥50 years.[27] In contrast, we observed that 14.3 % had osteoporosis after the age of 50 years. It is reasonable that this prevalence is underestimated.

In the present study, we also wanted to assess risk factors for osteoporosis. Menopause and aging are well-known risk factors that were evident in our study. Given that osteoporosis is a neglected complication of diabetes, we aimed to address the association of osteoporosis and diabetes. The prevalence of diabetes and prediabetes in our population was 4.3% and 32.6% respectively. Previous studies in Nepal have reported a somewhat higher prevalence of diabetes and lower prevalence of prediabetes.[28-30] A recent meta-analysis including 14 studies of both genders, reported a diabetes and prediabetes prevalence of 8.5% and to 9.2%, respectively.[31]

Individuals with diabetes, both type 1 diabetes (T1D) and T2D, are susceptible to higher risk of fractures.[4] The fracture risk is highest among those with T1D, but a substantial fracture risk is also observed in individuals with T2D, in spite of normal or even high BMD.[10,11] In the current study, 6.4% (n=5/78) of the women with osteoporosis suffered from diabetes, preferentially T2D, and 46.2% (36/78) were in the prediabetic range. Altogether, hyperglycemia (HbA1c ≥5.7%) was observed in 52.6% (n=41/78) of those with osteoporosis. This was reflected in a slightly higher mean HbA1c among osteoporotic women compared to those without osteoporosis. Moreover, osteoporosis was more prevalent in women with diabetes, compared to those without diabetes (15.2% and 10.6%). Women with hyperglycemia also displayed a higher prevalence of osteoporosis than those with normoglycemia (14.7% and 8.3%). In support of these findings, we observed a positive correlation between osteoporosis and hyperglycemia and a significantly higher relative risk for osteoporosis among those with hyperglycemia (OR 1.89).

Deficiency of calcium and vitamin D is associated with increased risk for osteoporosis and osteomalacia.[3] The women were asked about their milk consumption as a proxy of calcium intake. Participants with a low milk consumption had a lower prevalence of osteoporosis than those with a higher milk intake, however, not significantly. We did not have data on vitamin D intake, however, serum analyses showed that vitamin D deficiency was prevalent. However, no association between vitamin D levels and osteoporosis was observed.

Overweight and obesity are risk factors both for diabetes and osteoporosis.[32] BMI and waist circumference are applied to identify individuals with overweight/obesity and central obesity. In the current study, the mean BMI among osteoporotic women was marginally higher than in those without osteoporosis, 25.6 compared to 24.6 **(**p=0.043). Among women with overweight and obesity, 8.1% and 15.8% had osteoporosis, respectively. In accordance with these findings, the prevalence of osteoporosis in those with waist circumference >80 cm was 12.2% versus 8.7% in those with WC <80 cm. The mean waist circumference among the osteoporotic women was marginally higher, 80.5 cm (±11.7), compared to the non-osteoporotic women, 78.3 cm (±9.9) (p=0.070). Studies concerning the skeletal effects of overweight/obesity are diverging. Several studies show that obese individuals have higher BMD.[33] However, there is emerging evidence for impairment of bone quality in those with the highest BMI, resulting in increased susceptibility for fracture.[32] Another study reported a negative correlation between bone mass and fat mass, implying that increased fat mass might have a harmful effect on bone mass.[34] This could explain why the highest prevalence of osteoporosis was found in the obese group in the current study. A study including Korean males and females concluded that there was an inverse association between waist circumference and BMD, suggesting that waist circumference is a potential predictor.[35] The association, however, was more strongly correlated in males than females.[35]

The risk of osteoporosis is also increased in underweight women. A study made in 2006 investigated the connection between BMI and BMD and concluded that women with low BMI had a higher risk for osteoporosis.[33] We were not able to show this in the present study, as only 7.5% of those with underweight had osteoporosis, versus as many as 10.2% with normal BMI had osteoporosis.

Smoking of cigarettes, especially current smoking, is considered a risk factor for osteoporosis.[5] In the study population, 15.6% reported smoking of cigarettes daily. Among the osteoporotic women, 17.7% were smoking daily, which is similar fraction to the non-osteoporotic women (15.1%). No relationship between daily smoking and osteoporosis was found in the current study. Nevertheless, the fact that a considerable number of the study population was smoking daily, may have several health effects. According to a meta-analysis addressing the effects of cigarette smoking on BMD, smoking increases the lifetime risk of

developing a vertebral fracture by 13% in women and 32% in men.[36] The same review reported that smoking appears to have an independent and dose-dependent effect on bone loss, and can be partly reversed by cessation of smoking.[36] Following this, the prevalence of smoking in the current study population is worrisome, and action should be taken to inform the public about health benefits of smoking cessation.

Strengths and limitations of the study

A strength of the study is the large size of the study population. The data are representative for females in this rural district but cannot be generalized to men or to urban districts. A weakness of the study is that the most frail and sickest women might not have been able to participate, due to long walking distances. Women with recent fractures might also not have prioritized participating in the study, thus leading to underestimation of osteoporosis prevalence. Following the earthquake, many of the participants from the original study had to migrate from their homes, making even fewer participants available for inclusion in the present study.

We did not have data on location of fractures and the size of the trauma. For assessment of the prevalence of osteoporosis, measurement of BMD by DXA had been ideal in addition to information on fractures. To measure BMD in a subgroup was initially planned, moreover we aimed to assess bone quality by impact microindentation in the same subgroup. Whereas BMD measures bone quantity, microindentation gives information about bone material properties and strength of the bone. When taking into consideration that subjects with T2D regularly have normal and even high BMD, assessment of bone quality by microindentation would be especially useful. Due to the pandemic caused by COVID-19, it was not possible to travel to Nepal, which made it impossible to collect these data from the participants. This was a big setback for the project, and other ways of defining osteoporosis had to be found. We chose to define osteoporosis as either having had a fracture, taking medication for osteoporosis or the combination of both. The consequence was that the data on osteoporosis prevalence are uncertain, and surely underestimated. The answers from the questionnaire are limited by the participants' language and knowledge about their own health and medication, which also could make the results unreliable.

Conclusion

In this large study, we observed a fracture prevalence of 4.4%. When including women reporting use of medication against osteoporosis, the prevalence increased to 10%. The prevalence increased with age and menopausal status. The prevalence of fractures and osteoporosis in general is surely underestimated. A clear relationship between osteoporosis and diabetes was found. Thus, prevention of diabetes could be a good action for preventing osteoporosis and other diseases, in addition to general long-term complications and death. Many Nepalese women live with undiagnosed and untreated osteoporosis. Studies are warranted to explore the magnitude of burden of osteoporosis in Nepal in general.

Appendix

Department of Clinical Biochemistry, Dhulikhel Hospital

Reference values

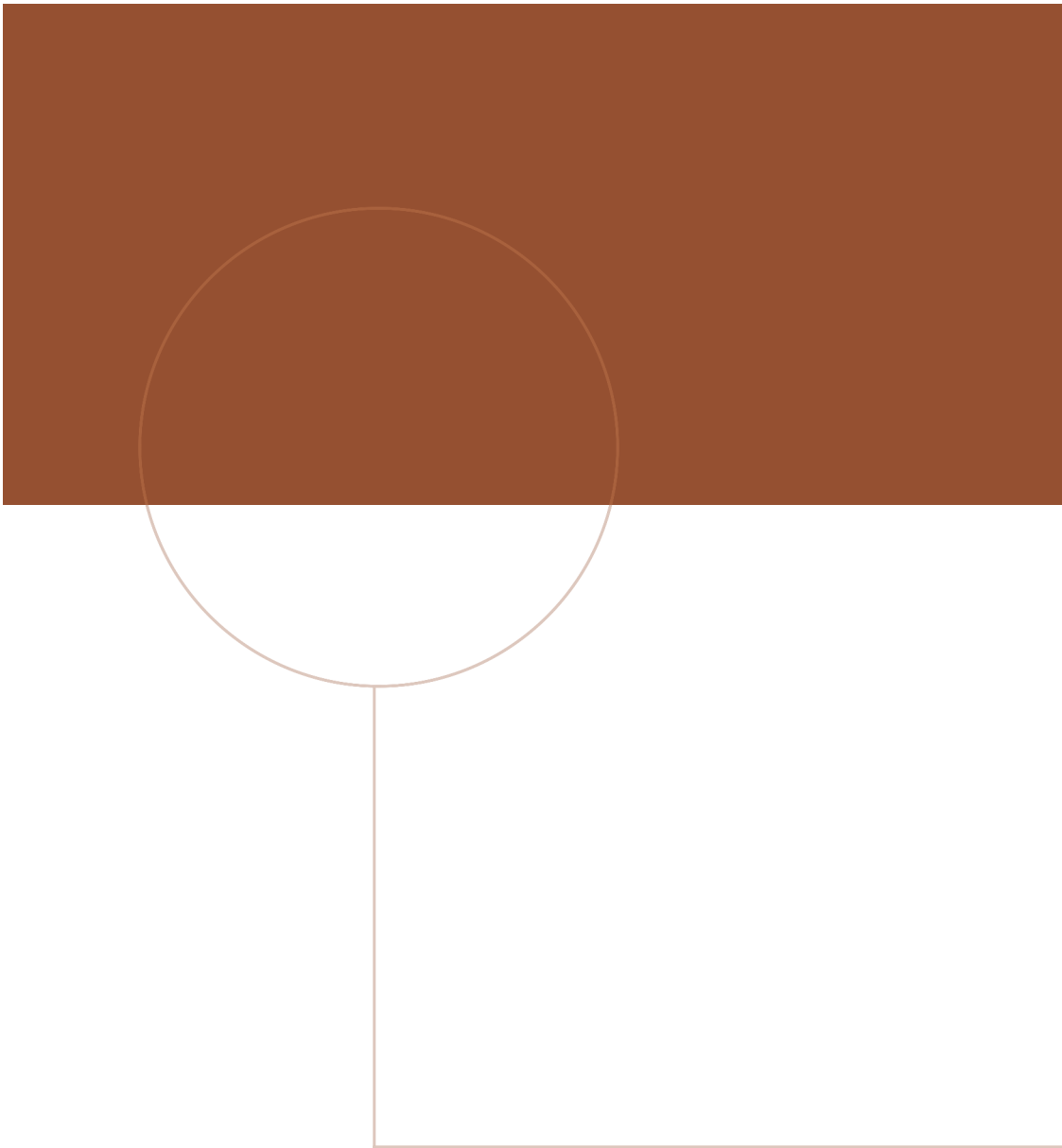
Test	Unit	Reference value	Method
Glycosylated Hb/hbA1c	%	Normal 4.5-5.6 Pre-Diabetes 5.7-6.4 Diabetes > 6.4	HPLC
TSH	µIU/mL	0.3-3.6	LIAISON (CLIA)
Free t4	ng/dL	0.8-1.7	LIAISON (CLIA)
25-OHVit D Total	ng/mL	30-100 Vitamin D excess : > 150.0 Vitamin D sufficiency : > 30.0 Vitamin D insufficiency: 10.0-30.0 Vitamin D deficiency : <10.0	LIAISON (CLIA)
Albumin, Serum	g/dL	3.5 - 5.0	BCG
Calcium-Total	mg/dL	8.4 – 10.2	C
Phosphorus, Inorganic	mg/dL	2.4 – 4.5	C
PTH	pg/ml	6.5 – 36.8	LIASION

1. Organization WH. Global report on diabetes. World Health Organization. (https://www.who.int/publications/i/item/9789241565257).

2. (IDF) IDF. IDF Diabetes Atlas 8th edition. International Diabetes Federation (IDF).
3. Group KJobotWHOS. Assessment of osteoporosis at the primary health-care level. Technical Report. University of Sheffield. (https://www.sheffield.ac.uk/FRAX/pdfs/WHO_Technical_Report.pdf).
4. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes--a meta-analysis. Osteoporos Int 2007;18(4):427-44. (In eng). DOI: 10.1007/s00198-006-0253-4.
5. Qaseem A, Forciea MA, McLean RM, Denberg TD. Treatment of Low Bone Density or Osteoporosis to Prevent Fractures in Men and Women: A Clinical Practice Guideline Update From the American College of Physicians. Ann Intern Med 2017;166(11):818-839. (In eng). DOI: 10.7326/m15-1361.
6. Organization WH. WHO Scientific Group on the assessment of osteoporosis at primary health care level. Summary Meeting Report, Brussels, Belgium. World Health Organization. (https://www.who.int/chp/topics/Osteoporosis.pdf).
7. Manolagas SC. Pathogenesis of osteoporosis. In: Post TW, ed. UpToDate. Waltham, MA: UpToDate; 2021.
8. Health NIo. Osteoporosis Overview. National Institutes of Health. (https://www.bones.nih.gov/health-info/bone/osteoporosis/overview).
9. Organization WH. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis : report of a WHO study group [meeting held in Rome from 22 to 25 June 1992]. World Health Organization. (https://apps.who.int/iris/handle/10665/39142).
10. Farr JN, Drake MT, Amin S, Melton LJ, 3rd, McCready LK, Khosla S. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. J Bone Miner Res 2014;29(4):787-95. (In eng). DOI: 10.1002/jbmr.2106.
11. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL. Mechanisms of diabetes mellitus-induced bone fragility. Nat Rev Endocrinol 2017;13(4):208-219. (In eng). DOI: 10.1038/nrendo.2016.153.
12. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. N Engl J Med 2006;354(21):2250-61. (In eng). DOI: 10.1056/NEJMra053077.
13. Inzucchi SEL, B. Clinical presentation, diagnosis, and initial evaluation of diabetes mellitus in adults. In: Post TW, ed. UpToDate. Waltham, MA: UpToDate; 2021.
14. Omsland TK, Bangstad HJ, Berg TJ, Kolset SO. [Advanced glycation end products and hyperglycaemia]. Tidsskr Nor Laegeforen 2006;126(2):155-8. (https://www.ncbi.nlm.nih.gov/pubmed/16415936).
15. Farlay D, Armas LA, Gineyts E, Akhter MP, Recker RR, Boivin G. Nonenzymatic Glycation and Degree of Mineralization Are Higher in Bone From Fractured Patients With Type 1 Diabetes Mellitus. J Bone Miner Res 2016;31(1):190-5. (In eng). DOI: 10.1002/jbmr.2607.
16. Park Y. Why is type 1 diabetes uncommon in Asia? Ann N Y Acad Sci 2006;1079:31-40. (In eng). DOI: 10.1196/annals.1375.005.
17. Jayawardena R, Ranasinghe P, Byrne NM, Soares MJ, Katulanda P, Hills AP. Prevalence and trends of the diabetes epidemic in South Asia: a systematic review and meta-analysis. BMC Public Health 2012;12:380. (In eng). DOI: 10.1186/1471-2458-12-380.

18. Shai I, Jiang R, Manson JE, et al. Ethnicity, obesity, and risk of type 2 diabetes in women: a 20-year follow-up study. Diabetes Care 2006;29(7):1585-90. (In eng). DOI: 10.2337/dc06-0057.
19. Vaidya A, Shakya S, Krettek A. Obesity prevalence in Nepal: public health challenges in a low-income nation during an alarming worldwide trend. Int J Environ Res Public Health 2010;7(6):2726-44. (In eng). DOI: 10.3390/ijerph7062726.
20. Stewart BT, Kushner AL, Kamara TB, et al. Backlog and burden of fractures in Sierra Leone and Nepal: Results from nationwide cluster randomized, population-based surveys. Int J Surg 2016;33 Pt A:49-54. (In eng). DOI: 10.1016/j.ijsu.2016.07.009.
21. Chaudhary NK, Timilsena MN, Sunuwar DR, Pradhan PMS, Sangroula RK. Association of Lifestyle and Food Consumption with Bone Mineral Density among People Aged 50 Years and Above Attending the Hospitals of Kathmandu, Nepal. J Osteoporos 2019;2019:1536394. (In eng). DOI: 10.1155/2019/1536394.
22. Shakya S, Syversen U, Åsvold BO, et al. Prevalence of human papillomavirus infection among women in rural Nepal. Acta Obstet Gynecol Scand 2017;96(1):29-38. (In eng). DOI: 10.1111/aogs.13036.
23. American Diabetes Association (ADA). Understanding A1C: Diagnosis. American Diabetes Association (ADA). (https://www.diabetes.org/a1c/diagnosis).
24. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(7):1911-30. (In eng). DOI: 10.1210/jc.2011-0385.
25. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363(9403):157-63. DOI: 10.1016/S0140-6736(03)15268-3.
26. Organization WH. Waist circumference and waist-hip ratio: report of a WHO expert consultation. World Health Organization. (https://www.who.int/publications/i/item/9789241501491).
27. Wang Y, Tao Y, Hyman ME, Li J, Chen Y. Osteoporosis in china. Osteoporos Int 2009;20(10):1651-62. (In eng). DOI: 10.1007/s00198-009-0925-y.
28. Ono K, Limbu YR, Rai SK, et al. The prevalence of type 2 diabetes mellitus and impaired fasting glucose in semi-urban population of Nepal. Nepal Med Coll J 2007;9(3):154-6. (In eng).
29. Singh DL, Bhattarai MD. High prevalence of diabetes and impaired fasting glycaemia in urban Nepal. Diabet Med 2003;20(2):170-1. (In eng). DOI: 10.1046/j.1464-5491.2003.00829_4.x.
30. Mehta KD, Karki P, Lamsal M, et al. Hyperglycemia, glucose intolerance, hypertension and socioeconomic position in eastern Nepal. Southeast Asian J Trop Med Public Health 2011;42(1):197-207. (In eng).
31. Shrestha N, Mishra SR, Ghimire S, Gyawali B, Mehata S. Burden of Diabetes and Prediabetes in Nepal: A Systematic Review and Meta-Analysis. Diabetes Ther 2020;11(9):1935-1946. (In eng). DOI: 10.1007/s13300-020-00884-0.
32. Fassio A, Idolazzi L, Rossini M, et al. The obesity paradox and osteoporosis. Eat Weight Disord 2018;23(3):293-302. (In eng). DOI: 10.1007/s40519-018-0505-2.
33. Asomaning K, Bertone-Johnson ER, Nasca PC, Hooven F, Pekow PS. The association between body mass index and osteoporosis in patients referred for a bone mineral density examination. J Womens Health (Larchmt) 2006;15(9):1028-34. (In eng). DOI: 10.1089/jwh.2006.15.1028.

34. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab 2007;92(5):1640-6. (In eng). DOI: 10.1210/jc.2006-0572.
35. Cui LH, Shin MH, Kweon SS, et al. Sex-related differences in the association between waist circumference and bone mineral density in a Korean population. BMC Musculoskelet Disord 2014;15:326. (In eng). DOI: 10.1186/1471-2474-15-326.
36. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif Tissue Int 2001;68(5):259-70. (In eng). DOI: 10.1007/bf02390832.



NTNU
Norwegian University of
Science and Technology