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Research Article
Lifestyle Differences in Rural and Urban Areas Affected the Level of Vitamin D in Women with Single Nucleotide Polymorphism in North Sumatera

[1]Dina Keumala Sari, [1]Zaimah Zulkarnaini Tala, [2]Sri Lestari, [3]Sunna Vyatra Hutagalung and [4]Ratna Akbari Ganie

[1]Department of Nutrition, Faculty of Medicine, University of Sumatera Utara, Jl. Dr. Mansur Kampus, 20155 Medan, Indonesia
[2]Department of Public Health, Faculty of Medicine, University of Sumatera Utara, Jl. Dr. Mansur Kampus, 20155 Medan, Indonesia
[3]Department of Parasitology, Faculty of Medicine, University of Sumatera Utara, Jl. Dr. Mansur Kampus, 20155 Medan, Indonesia
[4]Department of Clinical Pathology, Faculty of Medicine, University of Sumatera Utara, Jl. Dr. Mansur Kampus, 20155 Medan, Indonesia

Abstract
Background: A large proportion of women living in tropical regions experience vitamin D deficiency, with lifestyle being one of the predisposing factors. Women with single nucleotide polymorphisms of the vitamin D receptor gene living in rural and urban areas have different lifestyles. **Objective:** This study aimed to determine the differences in serum levels of 25-hydroxyvitamin D [25(OH)D] in women with single nucleotide polymorphisms of the vitamin D receptor gene living in rural and urban areas. **Methodology:** The present study used a cross-sectional design involving 100 healthy women, who were evaluated for several parameters including serum levels of 25(OH)D, body mass index, body fat, abdominal circumference and lifestyle, which included types of work, duration of exposure to sunlight, sunscreen use, vitamin D intake and physical activity. Statistical analysis included chi-square and independent t-test. **Results:** From the study population, the prevalence of vitamin D deficiency was 70%, with insufficiency at 29%, sufficiency at 1% and none of the subjects having normal results. Genotype examination showed that homozygote mutant and heterozygote types were found in both groups and only one subject had a homozygote wild type. The average serum level of 25(OH)D was 20.24 ± 4.43 ng mL^{-1} for the rural group and 14.9 ± 3.64 ng mL^{-1} for the urban group, with the difference between the two groups significant ($p = 0.001$). **Conclusion:** The results show that lifestyle differences between rural and urban areas may affect vitamin D levels given that vitamin D deficiency is still found in both settings.

Key words: 25(OH)D, deficiency, vitamin D, TaqI, BsmI, single nucleotide polymorphism, vitamin D receptor gene

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Corresponding Author: Dina Keumala Sari, Jl. Setia Budi, Kompleks TASBIH I Blok G, No. 29, Tanjung Sari, 22132 Medan, Indonesia Tel: (+61) 8212296/+628174894768/+6281397177693

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Women are susceptible to vitamin D deficiency, not only in countries with four seasons, but also in tropical countries where exposure to sunlight is adequate[1,2]. A large proportion of vitamin D deficiency occurs following a lifestyle that involves sunlight avoidance[3,4]. A woman's lifestyle depends on several daily life activities, such as nutrition and dietary habits, sunscreen application, vitamin D intake and physical activity[5]. A desirable lifestyle contributes to levels of vitamin D within the normal range, while a lifestyle avoiding sunlight and low vitamin D intake has adverse effects on health[1,5-7].

Women in rural and urban areas generally have different occupations. In rural areas, a large proportion of women work as farmers; in contrast, women living in urban areas generally work in closed spaces and probably do not get much exposure to sunlight. Many studies have shown that avoiding sunlight may hinder the conversion of cholesterol into 7-dehydrocholesterol in the skin of women, which may lead to low levels of serum 25-hydroxyvitamin D [25(OH)D][8-10]. Activities that avoid sunlight include seeking protection from sunlight and use of sunscreen. By avoiding sunlight, vitamin D conversion is decreased up to 50%; sunscreen use may also reduce vitamin D levels[9,11,12].

Previous studies also stated that body fat is a factor to be considered[2]. High body fat percentages may lead to low levels of vitamin D, which occurs because of the entrapment of vitamin D in fat cells, leading to low levels detected in blood circulation[13-16]. Other studies found a correlation between physical activity and serum 25(OH)D levels, where an increase in physical activity led to an increase in serum 25(OH)D levels[5].

Aside from lifestyle, several studies showed that vitamin D receptor gene polymorphisms might also account for low levels of vitamin D[5,17,18]. This is important because how to treat women with vitamin D deficiency is not an easy problem. If a women had a polymorphism of vitamin D receptor gene especially with *Taq*I and *Bsm*I, there should be a massive change of lifestyle including: (1) Supplementation of vitamin D and food sources, (2) Sunlight exposure and (3) Increase of physical activity. This is a different treatment to women without polymorphism of vitamin D receptor gene, we could suggest a very simple treatment which was she should increase vitamin D food sources. It maybe help her to cure vitamin D deficiency.

The objective of the present study was to investigate whether the presence of vitamin D receptor gene polymorphisms in women leads to lower levels of serum 25(OH)D and whether differences in lifestyles of women in rural and urban settings can prevent low levels of vitamin D.

MATERIALS AND METHODS

Study design and subjects: The present study used a cross-sectional design and was conducted in North Sumatera, Indonesia, from February to April 2015 in urban and rural areas. The urban area was in Medan City, which is a 10.240 sq mile (265.10 km^2) and densely populated (2,210,624) area in North Sumatera Province. The rural area was the Aman Damai district, 57.3 km away from Medan City. The district has a population of 420,000[19]. The Aman Damai district was selected because the subjects are engaged in the same outdoor occupation as a farmer (rubber and palm kernel plantation). This study expected that a farmer would have more sunlight exposure than women who lived in urban areas who have indoor occupations, such as employees, doctors and nurses and probably less sunlight exposure. The subjects of the present study were 100 healthy adult women who lived in urban and rural areas with various types of occupations, Body Mass Index (BMI) values, body fat percentages and abdominal circumferences; the sample was recruited purposively. Subjects were divided into 2 groups: 50 subjects in the rural group and 50 subjects in the urban group. The inclusion criteria were women within the range of 20-50 years old who either lived in a rural area with an outdoor occupation or who lived in an urban area with an indoor occupation. Exclusion criteria were subjects with a BMI >35 kg m^{-2} or a history of diabetes mellitus, myocardial infarction, or renal or liver dysfunction. In addition to those exclusion criteria, subjects who were pregnant, lactating, or using medications that may alter lipid profiles were also excluded.

Lifestyle factors included living area, sunlight exposure, sunscreen application, physical activity and vitamin D intake. The cumulative sunlight exposure per day was divided into two groups: <60 and $\geq$60 min day^{-1}. The cumulative sunlight exposure questions were about how long subjects were exposed to sunlight throughout the day.

All observations were carried out in the subject's home or workplace, in either rural or urban areas in North Sumatera, Indonesia. The present study included subjects with a variety of occupations (indoors and outdoors). Subjects were included in the study after completing an interview. All subjects were also asked to complete an informed consent approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Sumatera Utara (No. 120/KOMET/FK USU/2015).

Nutrition intake and anthropometric assessments: Nutrition intake was assessed using a 24 h food recall for two days (one on a working day and one on a holiday), including the intake of vitamin D. Calculations were performed using Nutrisurvey 2005, including data from Indonesian cuisine. Assessments of vitamin D intakes included vitamin D obtained from meal sources and supplements.

Body Mass Index (BMI) was assessed using a body composition monitor and scale (HBF-362, Karada Scan-Omron, Japan). The BMI categories were based on Asia-Pacific criteria: underweight (<18.5 kg m^{-2}), normal weight (18.5-22.9 kg m^{-2}), overweight (23-24.9 kg m^{-2}), obese I (25-29.9 kg m^{-2}), or obese II ($\geq$30 kg m^{-2}) (20). Fat percentage based on body fat mass represents the amount of body fat mass to total body weight. Categories for body fat calculations were low (5-19.9%), normal (20.0-29.9%), or high (35.0-50.0%). Abdominal circumference was measured using a non-plastic measuring tape and the results were categorized as normal (<80 cm) or at risk of metabolic syndrome (>90 cm) for Asians[20]. Physical activity was assessed with a questionnaire and the results were categorized as low ($\leq$6.2), moderate (6.3-7.1), or high ($\geq$7.2)[21].

Biochemistry analysis: In this study, serum 25(OH)D levels were measured using a chemiluminescent immunoassay (Diasorin, Stillwater, MN). This measurement can detect levels ranging from 4.0 and 150 ng mL^{-1}, based on 3.90% CV inter-assay precision. Serum 25(OH)D levels were categorized into deficient (<20 ng mL^{-1}), insufficient (20-32 ng mL^{-1}), sufficient (32-54 ng mL^{-1}), normal in sunny countries (54-90 ng mL^{-1}), excessive (>100 ng mL^{-1}) and toxic (>150 ng mL^{-1})[22].

Analysis of single nucleotide polymorphisms in VDR genes: Analysis of Single Nucleotide Polymorphisms (SNP) in Vitamin D Receptor (VDR) genes was conducted in three steps. In the first step, DNA was isolated using the 'salting out method'. The second step involved checking the purity of the DNA isolation and the third step was SNP genotyping using an Applied Biosystems StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA)[5].

Statistical analysis: Numerical variables are expressed as Means$\pm$Standard Deviations, while categorical variables are expressed as the percentage values of proportion. Chi-square tests were used to examine differences between the two groups (p<0.05). Unpaired t-test was used to compare numerical data (p<0.05). This study used SPSS (version 11.5; SPSS Inc, Chicago, IL) for data analysis.

RESULTS

The study screened 100 subjects, all of whom completed the assessments; the age categories of the subjects are shown in Table 1. Similar numbers of participants resided in urban and rural regions, women who lived in rural areas significantly older than urban areas (p<0.05). Just over 90% were 40-50 years of age or older in the rural group, with a greater proportion of those at younger ages in the urban group (60%, 30-39 years).

There was a significant difference (p = 0.001; p<0.05) between groups, as shown in Table 1. There were also

Table 1: Characteristics of the study population living in rural and urban areas

	All subjects		Rural group		Urban group		
Parameters	n	%	n	%	n	%	p-value
Age group (years)							
20-29 years	-		-		-		0.001*c
30-39 years	33	33	3	6	30	60	
40-50 years	67	67	47	94	20	40	
Mean±Standard deviation	37.7±9.9		44.1±7.5		31.4±7.9		0.001*t
BMI category (kg m⁻²)							
Underweight	-		-		-		0.001*c
Normal	12	12	2	4	10	20	
Overweight	83	83	44	88	39	78	
Obese I	5	5	4	8	1	2	
Obese II	-		-		-		
Mean±Standard deviation	24.2±3.9		25.8±3.9		22.7±3.5		0.001*t
Body fat percentage category							
Low			-		-		0.001*c
Normal			2	4	35	70	
High			48	96	15	30	
Mean±Standard deviation	31.9±4.3		35.9±3.3		28.1±2.4		0.001*t
Abdominal circumference category (cm)							
Less than 80 cm			45	90	11	22	0.001*c
More than equal to 80 cm			5	10	39	78	
Mean±Standard deviation	82.5±7.6		90.3±8.5		75.2±6.4		0.001*t

BMI: Body mass index, Normally distributed data: Mean±Standard Deviation, *p: Significance value (p<0.05), t: Independent t-test, c: Chi-squared test significant differences in anthropometric measures between groups (p = 0.01; p<0.05), rural women had higher BMI than urban women. Over 80% of the subjects were overweight, while only 5% were obese. The mean BMI was 25.8 kg m^{-2} in the rural group and 22.7 kg m^{-2} in the urban group. Overall, most of the subjects were overweight in both groups. Body fat percentages and abdominal circumferences were significantly differ between the urban and rural groups (p<0.05).

About 50% of subjects' occupations were indoors (non-government employee, nurse and doctor) and 50% were outdoors (farmer). Another factor that could lower vitamin D levels was sunscreen application, with 87% of all subjects using sunscreen routinely to protect their skin from sunlight exposure. The remainder of the subjects (13%) did not use sunscreen (Table 2). When the subjects asked about physical activity using a questionnaire, over 60% were categorized into the low physical activity group, even if they were farmers; most did not report much routine daily activity (Table 2). Intake of vitamin D food sources in all subjects showed an 83% reduction in intake because vitamin D food sources were not usual foods consumed based on food recall reports for these subjects. For vitamin D intake, subjects had lower vitamin D intake from food sources or supplements than the daily recommendation (10 mg day^{-1}), with most consuming only about 5.24 mg day^{-1} (Table 2).

Regarding genotype distribution in women who lived in rural and urban areas, single nucleotide polymorphisms in *Taq*I and *Bsm*I were mostly homozygote mutant and heterozygote (Table 3). Only one subject in the rural group had the homozygote wild type. *Bsm*I was 44% and *Taq*I 38% homozygote mutant. In the urban group, heterozygote SNP for *Bsm*I was 16% and heterozygote for *Taq*I in all subjects was 50%.

The prevalence of vitamin D deficiency was 0%, insufficiency 29% and sufficiency 1% for all subjects. However, none of the subjects reached normal 25(OH)D serum levels, though both groups showed significant differences (p<0.05). Mean 25(OH)D serum levels in urban women were 14.9±3.6 and 20.2±4.4 ng dL^{-1} in rural women (Table 4).

Table 2: Lifestyle characteristics of the study population living in rural and urban areas

Characteristics	Rural group# n	%	Urban group# n	%	p-value
Occupation					
Non-government employee	-	-	39	78	0.001*c
Nurse	-	-	10	20	
Doctor	-	-	1	2	
Farmer	50	100	-	-	
Sunscreen application					
Yes	39	78	48	96	0.007*c
No	11	22	2	4	
Physical activity					
Low	22	44	42	84	0.001*c
Moderate	28	56	8	16	
High	-	-	-	-	
Vitamin D intake					
Deficient/insufficient	37	74	46	92	0.001*c
Sufficient	10	26	7	8	

Normally distributed data: Mean±Standard Deviation, *p: Significance value (p<0.05), t: Independent t-test, c:= Chi-squared test, #n = 50

DISCUSSION

The results of the present study show that a significant difference (p<0.05) exists in terms of age between locations, with women in the rural group older compared with those in the urban group. Increasing age may cause lower levels of

Table 3: Distribution of the study population based on allele frequency and vitamin D receptor gene *Bsm*I and *Taq*I single nucleotide polymorphism genotype

Allele/single nucleotide polymorphism	Rural n	%	Urban n	%
rs1544410 (*Bsm*I)				
A	6	6	16	16
G	94	94	84	84
AA (homozygote wild type)	-		-	
GG (homozygote mutant)	44	88	34	68
AG (heterozygote)	6	12	16	32
rs731236 (*Taq*I)				
C	13	13	50	50
T	87	87	50	50
CC (Homozygote wild type)	1	2	-	
TT (Homozygote mutant)	38	76	-	
CT (Heterozygote)	11	22	50	100

Table 4: Average values and categories of serum 25(OH)D levels of the study population living in rural and urban areas

Parameters	All subjects n	%	Rural group# n	%	Urban group# n	%	p-value
25(OH)D category (ng dL^{-1})							
Deficiency	70	70	25	50	45	90	0.001*c
Insufficiency	29	29	24	48	5	10	
Sufficiency	1	1	1	2	-	-	
Normal in sunny countries	-	-	-	-	-	-	
Mean±Standard deviation	17.6±4.8		20.2±4.4		14.9±3.6		0.001*t

25(OH)D: 25-hydroxyvitamin D, Normally distributed data: Mean±Standard Deviation, *p: Significance value (p<0.05), t: Independent t-test, c: Chi-squared test, #n = 50 serum 25(OH)D, which occurs because of poorer skin integrity leading to decreased conversion of dehydrocholesterol. However, older women in the rural group tend to have higher serum 25(OH)D levels compared with those in the urban group. A previous study showed that in older age, 25(OH)D serum levels decreased, but increased after supplementation[7]. Compared with this previous study[7], found vitamin D deficiency regardless of age in the urban group. Lower skin integrity as a result of older age was not the problem of occurring vitamin D deficiency; avoiding sunlight exposure could be the reason why vitamin D deficiency was observed. Even younger working women protect their skin from sunlight exposure for many reasons, such as skin cancer risk and lighter or white skin perceived as more interesting than darker or black skin.

Besides avoiding sunlight, previous studies have reported a relationship between obesity and higher fat mass and vitamin D deficiency. Obesity is usually correlated with a higher prevalence of vitamin D deficiency or lower circulating 25(OH)D serum levels in both pediatric and adult populations[16,23,24]. A higher body fat mass will trap vitamin D and lower 25(OH)D serum levels[5]. In the present study, in both rural and urban groups there were significant differences ($p<0.05$) in BMI, body fat percentage and abdominal circumference with lower 25(OH)D serum levels in both groups.

In the present study, rural and urban groups showed significant differences ($p<0.05$) in 25(OH)D serum levels and, interestingly, the rural group, which had higher BMI, body fat mass and abdominal circumferences, also had higher 25(OH)D serum levels compared with the urban group. This result is similar to a previous study that reported that there should be other factors that influence vitamin D deficiency and not only higher fat mass[1,5].

Anthropometric measurements showed significant differences ($p<0.05$) in BMI, where the BMI for the rural group was higher compared with that of the urban group. Previous studies show that a high BMI may lead to lower serum 25(OH)D levels[2]. However, in the present study, despite a higher BMI, the rural group had higher serum 25(OH)D levels compared with the urban group.

Lifestyle factors including occupation, sunscreen application, physical activity and vitamin D intake also showed significant differences ($p<0.05$) between settings. The types of work conducted by women living in rural areas were different than those in urban areas, where non-government employees, nurses and doctors were largely found. The types of work conducted in urban areas were mostly indoors and mostly conducted in buildings that may not have adequate exposure to sunlight. Indoor work generally begins from 7:00 am until 5:00 pm, with no direct sun exposure usually occurring during that period of time. However, women living in rural areas are mostly farmers by occupation. Working on a rubber or palm plantation involves a lot of outdoor activities for long hours. Women in rural areas receive much more exposure to sunlight compared with women in urban areas, but still have low 25(OH)D serum levels.

Outdoor workers such as farmers show sufficient levels of serum 25(OH)D and all eight subjects who achieved this level of 25(OH)D worked outdoors with high activity levels. For instance, working as a street cleaner generally follows the working hours of 7:00-11:00 am (Western Indonesian Time) outdoors, followed by a break and then another outdoor work session from 1:00-4:00 pm. Those particular periods of time provide a radiation contribution of about 1 MED (Minimal Erythemal Dose), whereas the highest is between 11:00 am-1:00 pm, which corresponds to about 2 MED, indicating the period of time when the highest radiation is received from sunlight[22].

Based on the information provided, regardless of the fact that certain lines of work are associated with greater sunlight exposure, people tend to seek protection from sunlight. Sunlight reduces the radiation to the skin by 50% if the subject is protected from it by a shadow, which may account for the fact that none of the subjects had normal serum 25(OH)D levels in tropical countries (50-100 ng mL^{-1})[9,22].

Those performing indoor work did not have serum 25(OH)D levels reaching the sufficient category based on information received from the subjects working indoors with light physical activity. Therefore, sunlight exposure is only received when leaving for work at around 7:00 am-8:00 am and when returning from work at around 4:00 pm-5:00 pm. During those periods of time, sunlight exposure only provides a radiation of 0.5 MED, which may contribute to low serum 25(OH)D levels. A worker is 2.6 times more likely to be working indoors compared with outdoors, but other factors must be considered because normal levels were not met.

Sunscreen use is also a lifestyle factor that may lead to decreased sunlight exposure and eventually decreased serum 25(OH)D levels. In the present study, sunscreen use was more common among women living in urban areas compared with those in rural areas. Based on the observations of women in urban areas, the capability to purchase sunscreen was higher compared with that of women in rural areas.

Vitamin D intake was found to be very low in both groups, with no significant difference between settings. Food sources for vitamin D such as fish oil and mushrooms are not food products routinely consumed in North Sumatera[1,5]. Intake of consumable vitamin D sources such as butter, oil and fortified milk were also found to be low in both groups.

A previous study showed that there are two factors that correlate to serum 25(OH)D levels: sunlight exposure and physical activity. In this case, physical activity may lead to a thermal reaction in the body following increased body metabolism and the generated heat may aid in vitamin D metabolism[1,2,5].

Vitamin D receptor genes have been suggested to be potential key players in the pathophysiologic mechanisms of obesity and lower 25(OH)D serum levels[17,18,25]. Different ethnic gene and allele variations occur in different frequencies; studies have shown that VDR polymorphisms across ethnicities were correlated with different incidences of several diseases, regardless of lifestyle[26-28]. In the current study, the underlying cause of low serum 25(OH)D levels in both groups could be because of SNPs of VDR genes. Single nucleotide polymorphisms were found in most subjects who were heterozygous and homozygous mutant. The VDR gene carried the TC genotype for the polymorphism of *Taq*I and the AG genotype for *Bsm*I. Even though the mutation only occurred in one base (silent mutation), this mutation appeared to affect serum 25(OH)D levels. This study showed that vitamin D levels were affected by genetic mutation based on the examination of SNPs of the VDR genes *Taq*I and *Bsm*I.

The present study showed a significant difference ($p<0.05$) in serum 25(OH)D levels, where women in rural areas had higher 25(OH)D serum levels compared with those living in urban areas. However, none of the subjects achieved normal values, which may be attributed to the presence of VDR gene polymorphisms. The present study assumed that even though lifestyle factors influenced, but yet silent mutation influenced 25(OH)D serum levels.

The present study did not evaluate calcium and parathyroid hormone levels to assess the effect on bone health; however, it is hoped that the results of the study may describe factors that influence serum 25(OH)D levels. This was a limitation of the study; however, a strength was the observation of SNPs in VDR genes probably led to lower 25(OH)D serum levels that occur in rural and urban women.

CONCLUSION

It is concluded that lifestyle factors such as lack of sunlight exposure, use of sunscreen, lack of vitamin D intake and low levels of physical activity may lead to low levels of serum 25(OH)D, regardless of BMI. Additionally, SNPs of VDR genes (*Taq*I and *Bsm*I) alter vitamin D levels, leading to suboptimal values in sunny countries. Lifestyle changes can be among the strategies to increase serum 25(OH)D levels in women with VDR gene polymorphisms.

SIGNIFICANCE STATEMENTS

This study discover the difference of lifestyle betwen rural and urban areas may increased vitamin D levels that can be beneficial for women who lived in different areas to change their lifestyles, but they also have to know whether there is a polymorphism of vitamin D receptor or not (*Taq*I and *Bsm*I). This study will help the researcher to uncover the critical areas of vitamin D receptor gene polymorphism, women lifestyles and how it affects 25(OH)D serum level.

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