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Bone Density Status and Vitamin D and Calcium Concentrations in Pregnant and Non-Pregnant Women

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Abstract

Background: The aim of this study was to analyse differences in bone density and vitamin D and calcium concentrations between pregnant and non-pregnant women. **Methods:** This was an analytical, cross-sectional study of women aged 30 years or over, residing in the Puskesmas Bangkalan district. The sample population was chosen at random and included 10 pregnant women in their third trimester and 10 non-pregnant women. This observational study consisted of an interview, a blood test, and a 24-hour recall. The concentration of serum vitamin D (25(OH)D) was measured by Gas Chromatography and a Photometric Test measured the concentration of serum calcium. Participant's bone density was measured by bone ultra sonometer. The data was analysed using the Mann-Whitney test and assessed ultraviolet-B exposure, bone density values, and consumption of vitamin D, calcium, energy, and protein. The Student *t*-test was used to analyse serum vitamin D and calcium concentrations. **Results:** There were no differences between the groups' serum vitamin D concentrations ($p = 0.946$). However, there were significant differences between the groups' serum calcium concentrations ($p = 0.047$) and bone density values ($p = 0.019$). **Conclusions:** A high prevalence of vitamin D deficiency (25(OH)D < 50 nmol/L) and insufficiency (25(OH)D < 80 nmol/L) was observed in both groups. The mean serum calcium concentrations and bone density values were lower in pregnant subjects than non-pregnant subjects.

Keywords: bone density, calcium, vitamin D

Introduction

Vitamin D deficiency is a widespread health concern facing many communities. Previous studies conducted in Indonesia have shown that the incidence of vitamin D deficiency amongst elderly women may be as high as 35%.[1] Furthermore, studies suggests that 60% of non-pregnant women aged 18 to 40 years and living in Jakarta may experience vitamin D deficiency.[2] Vitamin D deficiencies (concentrations <25 nmol/L) in pregnant women are commonly reported throughout the world. Studies suggest that rates are as high as 42% in Northern India, 61% in New Zealand, 89.5% in Japan, and 60% in The Netherlands.[3-6]

Vitamin D is a fat-soluble vitamin that is common in many foods. However, the human body also produces Vitamin D when it is exposed to Ultraviolet-B (UV-B) rays from the sun and this is our main source of Vitamin D. UV-B rays aid the precursor form of vitamin D found in the skin change into Vitamin D3. This active vitamin D plays an important role in calcium and phosphorus absorption in the intestines. Calcium and phosphorus are integral to bone mineralisation and their absorption is directly affected by a vitamin D deficiency, thus leading to decreased bone mineralization and formation.[7]

Pregnant woman are at a higher risk of vitamin D and calcium deficiencies. This is due to changes in a woman's nutritional needs and increases in her metabolism during the pregnancy. These increases are required for foetal growth and development and can change a female's body composition and metabolism. If nutritional needs are not met, it can lead to lasting complications for both the mother and child.[8]

Studies have shown a correlation between vitamin D deficiencies and osteoporosis. Osteoporosis is characterised by bone loss and reduced bone density due deficiencies of calcium and vitamin D. A previous study reported that the risk of osteoporosis in three provinces of Indonesia (North Sulawesi, Yogyakarta and West Java) was 22.3%.[9] It has also been shown that women with a calcium intake of less than 500 mg/day are more likely to have low bone mineral density when compared with women whose calcium intake was adequate.[10]

It was these previous studies that prompted us to investigate the nutritional needs of pregnant women regarding vitamin D and calcium, and their impact on bone mineralisation for both mother and foetus. By understanding the role of vitamin D and calcium in the bone density of pregnant women it is expected that adverse impacts, in the event of deficiency, can be minimised through nutritional interventions.

Methods

This was an analytical observational study, with a cross-sectional design. Research was conducted in the Puskesmas Bangkalan working area of East Java. The study population comprised of 20 women, 10 pregnant and 10 non-pregnant. The inclusion criteria for pregnant participants were (1) gestational age ≥27 weeks (third trimester) and (2) maternal age of ≥30 years. For non-pregnant participants the inclusion criteria included (1) use of a 3 monthly injectable contraceptive or IUD, and (2) aged ≥30 years. All participants were numbered and selected using a simple random sampling technique. All participants were subject to the following exclusion criteria: (1) skin pigmentation disorders, (2) obesity, (3) smoking, and (4) alcohol consumption.

Blood samples were taken on selected participants and these subjects were reviewed again at 24 hours. This recall was used to assess their average intake of nutrients and analyse this relationship with their overall health.

The Health Research Ethics Committee of the Faculty of Public Health, Airlangga University approved this study with regards to the protection of human rights and welfare in medical research. Prior to data collection the objectives, benefits, and impacts of this study were comprehensively explained to each participant. Subjects were then asked to sign an informed consent form as evidence of their willingness to participate in the research.

Each participants UV-B exposure was assessed via interview questions about their use of sunscreen, dress style, and outdoor activities. If the respondent met one or more of the following criteria; using sunscreen, wearing covered clothing and/or no outdoor activities, it was assumed that they experienced insufficient exposure to UV-B. Measurement of vitamin D, calcium, energy, and protein intake via diet was conducted using blood tests and was reassessed after 24-hours. Data was compared to the recommended daily allowance (RDA) 2013 and was expressed in the following units; µg for vitamin D, mg for calcium, kcal for energy, and g for protein.[12]

Serum vitamin D concentrations were measured by the Gas Chromatography (GC) method, which is expressed in units of nmol/L, and classified into adequate if ≥80 nmol/L, insufficient if between 50-79 nmol/L, and deficient if <50 nmol/L. Serum calcium concentrations were measured by Photometric Test and normal levels are considered to be between 8.1-10.4 mg/dL. Bone density was measured by bone ultra sonometer (Achilles Express 2) and bone density ranges from normal if $T > -1$, osteopenia if $-2.5 \leq T \leq -1$ and osteoporosis if $T < -2.5$. The Achilles Express 2 is built on advanced quantitative ultrasound (QUS) technology, which is being used increasingly for the measurement of bone density. Some major advantages of QUS devices are that they do not use ionising radiation, they are relatively inexpensive, and portable systems are available.

To analyse the differences of each variable between the two groups we utilised the *t*-test for ratio scale data, the Mann-Whitney test for ordinal scale data and the Chi-Square test for nominal scale data. Normality of data distribution was tested using the Kolmogorov-Smirnov test.

Results

This study consisted of 20 female participants categorised into 2 groups, 10 pregnant and in their 3rd trimester, and 10 non-pregnant. Pregnant participants were aged between 30 and 37 years old, whilst the non-pregnant subjects were aged between 30 and 45 years old. The majority of subjects identified as Madurese, 80% in the pregnant group and 90% in the non-pregnant group, and the remainder of participants were Javanese.

Humans produce the majority of their vitamin D through sun exposure. It takes just 15 minutes of exposure to UV-B light to the face, hands, arms, and back of neck each day to produce an adequate amount of vitamin D. The amount of UV-B exposure participants experienced was measured based on their use of sunscreen, dress styles, and outdoor activities (Table 1).

A 24-hour recall was conducted to determine vitamin D, calcium, energy, and protein intake to identify any deficiencies and results are laid out in Table 2. Results from blood tests regarding the concentrations of vitamin D and calcium and bone density levels assessed by bone ultra sonometer are shown in Table 3 and Table 4.

Discussion

There was a significant difference between the adequacy of UV-B exposure in pregnant and non-pregnant participants (p = 0.022). This is likely due to the significant difference in outdoor activities between the 2 groups (p = 0.007). A higher proportion of pregnant participants fell into the insufficient range of UV-B exposure (90%) when compared to the non-pregnant participants (40%). This may be due to pregnant women being less likely to engage in outdoor activities resulting in less UV-B exposure, leading to decreased vitamin D formation and calcium absorption.

Spending time outdoors often leads to physical activities such as walking or riding a bike. Physical activity, especially weight-bearing exercises, can increase bone density in the proximal femur.[13] Furthermore, studies have shown that those who engage in high levels of

Table 1. Distribution of UV-B Exposure of Participants

	Pregnant	Non Pregnant	*p* value
	%	%	
Sunscreen Use			0.653
Using	50	60	
Not using	50	40	
Dress style			0.068
Covered on arms and neck	80	40	
Open on arms and neck	20	60	
Outdoor activity			0.007
Not practiced	80	20	
Practiced	20	80	
Adequacy of UV-B Exposure			
Insufficient	90	40	0.022
Sufficient	10	60	

Table 2. Mean Distribution of Vitamin D, Calcium, Energy, and Protein Intake of Participants

Amount of	Mean	SD	*p* value
Vitamin D intake (μg/day)			0.257
pregnant women	10.39	10.36	
non-pregnant women	5.14	5.98	
Calcium intake (mg/day)			0.650
pregnant women	515.08	469.38	
non-pregnant women	269.16	91.05	
Energy intake (kcal/day)			0.363
pregnant women	1838.10	427.88	
non-pregnant women	1675.90	344.94	
Protein intake (g/day)			0.975
pregnant women	56.38	13.36	
non-pregnant women	56.56	11.89	

Table 3. Mean Distribution of Vitamin D and Calcium Concentrations, and Bone Density of Participants

	Mean	SD	*p* value
Vitamin D concentration (nmol/L)			0.946
pregnant women	48.15	17.66	
non-pregnant women	48.65	14.48	
Calcium concentration (mg/dL)			0.047
pregnant women	8.94	1.03	
non-pregnant women	9.74	0.58	
Bone density value (T score)			0.019
pregnant women	-0.78	0.69	
non-pregnant women	0.28	1.07	

Table 4. Distribution of Vitamin D and Calcium Status, and Bone Density of Participants

	Pregnant	Non Pregnant	*p* value
	%	%	
Vitamin D status			0.661
Deficient (<50 nmol/L)	40	50	
Insufficient (50-79 nmol/L)	60	50	
Calcium status			0.146
Insufficient (<8.1 mg/dL)	20	0	
Sufficient (8.1-10.4 mg/dL)	80	100	
Bone density level			0.131
Osteopenia ($-2.5 < T < -1$)	40	10	
Normal ($T \geq -1$)	60	90	

physical activity are 8 times less likely to experience bone loss than those who engage in medium levels of physical activity.[14] Therefore, a healthy lifestyle of physical activity with weight-bearing exercises and adequate sun exposure is recommended to maintain bone health.

Although pregnant participants were more likely than non-pregnant participants to wear covered clothes, 80% versus 40%, there was minimal difference in the *p* value ($p = 0.068$) Previous studies have shown that dress style has been independently related to low vitamin D status.[15-18] Sunscreen use showed little significant difference ($p = 0.653$). Few adult studies have described the association between sunscreen use and vitamin D concentrations and this is an area that requires further attention. However, one study found that higher use of sunscreen was associated with statistically significantly lower vitamin D concentrations in non-Hispanic, white, adult subjects in the 2000-2004 NHANES.[19]

There were no significant differences between the amounts of vitamin D intake in both groups. However, both groups failed to meet the RDA of vitamin D, which is 20 µg per day for pregnant women and 15 µg for non-pregnant women.[12] Natural food sources that are rich in vitamin D are usually limited to fatty fish and meat. Therefore in many countries, foods such as milk, cereal, margarine, vegetable oil, and bread are often fortified with vitamin D.[20] Several studies have also reported low consumption of vitamin D around the world. A study of adult women in Sao Paulo, Brazil showed the mean intake of vitamin D is 2.72 ± 1.39, this may be due to no mandatory policies for vitamin D food fortification and natural sources of the vitamin are not usually consumed on a regular basis.[21] This condition also occurs in the study site so there needs to be an effort to increase the intake of vitamin D.

The RDA for calcium is 1300 mg per day for pregnant women and 1100 mg per day for non-pregnant women, both groups in this study failed to meet this intake.[12] Furthermore, there was no significant difference in calcium intake between the pregnant and non-pregnant groups. Low calcium intake has also been reported in other populations, a study of females aged between 18 and 65 years in Quebec, –Canada showed a low mean total calcium intake of 895 mg/day due to low milk and supplement comsumption.[22]

Growth, pregnancy, lactation, and calcium deficiencies can increase the demand for calcium within the body. If calcium absorption increases, the amount of calcium in the body is reduced.[20] As such; pregnant women require higher amounts of calcium to sustain foetal growth and their calcium intake should reflect this.

There was no significant difference between the energy intake of the pregnant and non-pregnant groups ($p = 0.363$). The mean intake of energy was 1838.10 kcal/day in the pregnant group and 1675.90 kcal/day in the non-pregnant group. Both groups fell short of the RDA for energy consumption, which are 2450 kcal/day for pregnant women and 2150 kcal/day for non-pregnant women.[12] The majority of respondents in both groups still had deficiencies in the level of energy consumption. 50% of participants in the pregnant group fell into the severely deficient category (<70% RDA). If energy levels are low, the body will use protein stores for energy production and as such, protein may not be available for the metabolism of vitamin D and calcium. Worryingly, 70% of participants in the pregnant group had a low level of protein intake.

Data showed that there was no significant difference in the amount of protein intake between the pregnant and non-pregnant groups. The mean of the protein intake was 56.38 ± 13.36 g/day in the group of pregnant women and 56.56 ± 11.89 g/day in the non-pregnant group. Both groups especially those in the pregnant group, failed to meet the RDA of 77 g of protein per day. Most participants in both groups still had deficiencies in their level of protein consumption. 50% of participants in the group of pregnant women were severely deficient in their protein consumption. Protein plays an important role in the metabolism of vitamin D and calcium in the body and its dietary intake is an important consideration.

The mean levels of serum vitamin D were 48.15 nmol/L in the pregnant group and 48.65 nmol/L in the non-pregnant group (Table 3). Both values fall into the category of vitamin D deficiency, which is defined as <50 nmol/L. There was no significant difference in serum vitamin D levels between the pregnant and non-pregnant groups, however all participants had serum vitamin D levels below the optimal amount of ≥80 nmol/L (Table 4).

Vitamin D deficiencies are not uncommon around the world and are often due to lifestyle choices. Skin pigmentation, location, and climate can play a role but the most important factor is exposure to sunlight.[23] Pregnant women with vitamin D deficiencies (concentrations <25 nmol/L) are also common throughout the world with studies reporting rates as high as 42% in northern India, 61% in New Zealand, 89.5% in Japan, and 60% in The Netherlands.[3-6] Furthermore, the prevalence of serum vitamin D levels <50 nmol/L among pregnant women have been reported to be 96.3% in India, 96.8% in Beijing, and 75% in the UK.[24-26] Vitamin D deficiency has also been reported among non-pregnant women, a study in Jordan found that 60.3% of women experienced vitamin D deficiencies (<30 nmol/L) and 95.7% had insufficient vitamin D levels (<50 nmol/L).[27] This shows that whilst pregnant women may have a higher risk of vitamin D deficiency, due to greater demand from the foetus, non-pregnant women are still susceptible.

Vitamin D deficiency can be due to a multitude of factors including insufficient production of vitamin D, inadequate consumption of food, or impaired absorption of fat.[7] Severe vitamin D deficiencies have also been linked to the use of sunscreen lotions in women in Belgium. The same study also reported that the prevalence of vitamin D deficiency amongst pregnant, Belgian women was 44.6%, with as many as 62% taking multivitamins containing vitamin D.[11]

The mean levels of serum calcium were 8.94 mg/dL in the group of pregnant women and 9.74 mg/dL in the non-pregnant group (Table 3). These values are within the normal range of 8.1-10.4 mg/dL. There was a significant difference in serum calcium levels between the pregnant and non-pregnant groups (p = 0.047) and the average value of serum calcium levels was lower in the pregnant group (Table 3). This is most likely due to the pregnant group being in their 3rd trimester, where the foetus absorbs a large amount of calcium for bone growth and teeth formation. In the final trimester, foetal calcium requirements are about 30 g; this high amount of calcium is usually obtained from the mother's skeleton. This leads to decreased storage of maternal calcium, despite an increase in the efficiency of calcium absorption.[28]

20% of participants in the pregnant group were diagnosed with insufficient calcium as shown in Table 4. Low levels of total serum calcium can occur after prolonged calcium deficiency followed by impaired absorption of calcium.[20]

Vitamin D aids calcium absorption in the intestines and together these two minerals play an important role in bone mineralisation. Vitamin D and calcium needs increase during pregnancy and, in addition to phosphorus, both micronutrients play a vital role in the formation of bones and teeth of the foetus. When nutrient intake is not sufficient the foetus will absorb the majority of it's required vitamin D, calcium, and phosphorus from the mother, via the placenta, for the formation of bones and teeth.

Bone density test results showed that there were no participants who had osteoporosis (bone density rate <-2.5). However, there was a significant difference between the values of bone density in pregnant and non-pregnant groups; the mean value of bone density in pregnant women was lower (-0.78 ± 0.69) when compared with non-pregnant women (0.28 ± 1.07). Insufficient intake of calcium during pregnancy combined with an increasing need from the foetus for growth will lead to increased loss of maternal bone calcium levels. It is likely that these factors were the cause of the differences in bone densities between the two groups.

When a woman is pregnant, the need for vitamin D and calcium for bone health of the mother and foetus will increase. However, if the production of vitamin D or dietary intake of vitamin D, calcium, and protein is insufficient this will lead to a decrease in maternal bone density, as the foetus will absorb the required nutrients from the mother's skeleton. This bone turnover, if not accompanied by simultaneous bone formation due to insufficient production of vitamin D, vitamin D and calcium intake, will continue to reduce maternal bone density, which in turn can lead to osteoporosis.

Conclusions

A high prevalence of vitamin D deficiency (25(OH)D < 50 nmol/L) and insufficiency (25(OH)D < 80 nmol/L) was observed in both groups. The mean serum calcium concentration and bone density values were lower in the pregnant group than the non-pregnant group. This study highlights the importance of improving exposure to ultraviolet-B rays and increasing vitamin D, calcium, energy, and protein intake of women living within the study area. Expectant mothers should be targeted through community-based nutrition and health education programmes because optimal nutrition can have the greatest impact on bone mineralization for themselves and their children.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest. The findings in this article represent the conclusions of the authors.

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