Original Articles

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Decreased Serum Anti-Müllerian Hormone Level Is Associated with Vitamin D Deficiency in Healthy Japanese Women

YUKA HONDA*1), MITSUYOSHI SUZUKI*1), YUICHI SATO*2), KEIJI KURODA*3), HIROMICHI SHOJI*1), TOSHIAKI SHIMIZU*1)

*1)Department of Pediatrics, Juntendo University Graduate School of Medicine, Tokyo, Japan, *2)Department of Obstetrics and Gynecology, Tatedebari Sato Hospital, Gunma, Japan, *3) Department of Obstetrics and Gynecology, Juntendo University Graduate School of Medicine, Tokyo, Japan

Objective: The aim of this study was to investigate the associations of anti-Müllerian hormone (AMH) levels with physique and 25-hydroxyvitamin D (25OH-D) levels in healthy women of reproductive age based on measurements of nutritional status and physical constitution.

Materials and Methods: Subjects comprised 108 non-obese women (age range, 21–39 years) who underwent examination of their physique, blood biochemistry and nutritional state. For data analysis, subjects were first divided by age. AMH levels were grouped by serum 25OH-D concentration using Holick's classification: deficiency, <30 ng/m*l*; and sufficiency, ≥30 ng/m*l*.

Results: Mean levels were 25.2 ± 8.4 ng/m*l* for serum 25OH-D and 4.9 ± 2.4 ng/m*l* for AMH. Overall, 76 women (70.4%) were diagnosed with 25OH-D deficiency. Serum AMH levels were significantly lower in subjects with 25OH-D deficiency (4.5 ± 2.5 ng/m*l*) than in those with 25OH-D sufficiency (5.7 ± 1.9 ng/m*l*; $p<0.01$).

Significant differences were seen in the frequency of subjects with 25OH-D deficiency and sufficiency between low AMH (< 2.2 ng/m*l*) status and normal AMH (≥2.2 ng/m*l*) (16/17 [94.1%] vs. 1/17 [5.9%] for low AMH status; 60/91 [65.9%] vs. 31/91 [34.1%] for normal AMH status, respectively; $p<0.05$). Independent predictors of serum AMH levels≥2.2 ng/m*l* were serum 25OH-D level ($p<0.05$) and age ($p<0.05$) according to binary logistic regression analysis.

Conclusions: Decreased serum AMH level is associated with vitamin D deficiency, but is unrelated to physique state in this population.

Key words: anti-müllerian hormone, 25-hydroxyvitamin D, body fat (%), body mass index

Abbreviations:
25OH-D: 25-hydroxyvitamin D, AMH: anti-Müllerian hormone, BIA: bioelectrical impedance analysis, BMI: body mass index, DEXA: dual-energy X-ray absorptiometry, BDHQ: brief-type self-administered diet history questionnaire, PCOS: polycystic ovary syndrome

Objective

Anti-Müllerian hormone (AMH) is one of the most reliable markers of ovarian reserve[1]. This hormone is produced in prenatal follicles with little influence from the estradiol cycle, and is affected by the number of growing eggs. AMH is commonly used in useful marker to predict ovarian reserve and to adjust controlled ovarian stimulation[2]. Previous reports have indicated that nutritional status may influence circulating AMH levels. Lower serum AMH levels have been found in obese women compared with normal-weight non-PCOS women, and a strong association between AMH levels and body mass index (BMI) has been

Corresponding author: Mitsuyoshi Suzuki
Department of Pediatrics, Juntendo University Graduate School of Medicine
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
TEL: +81-3-3813-3111 (ext. 3640) FAX: +81-3-5800-1580 E-mail: msuzuki@juntendo.ac.jp
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shown[3) 4)]. It has been also reported that AMH level increases with increasing serum 25-hydroxyvitamin D (25OH-D) level in women ≥40 years of age[5) 6)]. In addition to serum vitamin D levels, premenopausal women show seasonal variations in serum AMH levels, showing an 18% decrease in serum AMH levels in winter compared to summer[5)]. Vitamin D has also been shown to affect AMH gene expression *in vitro*[7)]. However, few reports have examined nutritional status and serum levels of AMH and vitamin D in the younger population of women before pregnancy.

The aim of the present study was to investigate the prevalence of vitamin D deficiency and the relationship between serum AMH levels and 25-hydroxyvitamin D (25OH-D) in healthy, Japanese, non-obese women between 21 and 39 years old based on measurements of nutritional status and physical constitution.

Materials and methods

1. Subjects

A total of 136 healthy Japanese female volunteers between 20 and 39 years old were recruited through the internet from October to November 2012 in Tokyo, Japan (located at 35.4°N, 139.4°E). AMH is usually elevated in women with polycystic ovary syndrome (PCOS), and correlates with the severity of this syndrome[3) 8)]. It is also known that the prevalence of obesity in patients with PCOS is nearly 30%[9)].Of these, 28 subjects were excluded based on the following criteria that influences ovarian AMH synthesis: history of POCS (n=3); endometriosis or gynecological disease (n=2); history or ovarian insufficiency or failure (n=2); during pregnancy (n=4); diabetes (n=1); intake of vitamin D supplements (n=3); and obesity (BMI ≥25 kg/m^2 or %Fat ≥35%) (n=13). Study approval was obtained from the institutional review board at Juntendo University (No. 26-368). Each participant was provided with detailed information about the study protocol, and written informed consent was obtained from all participants prior to enrollment.

To determine serum AMH and 25OH-D levels, blood samples were collected from a cubital vein after 6 h of fasting. Body weight was noted, and body composition was measured via bioelectrical impedance analysis (BIA)[10) 11)]. Nutritional information on energy intake, vitamin D and gynopathy was obtained from a questionnaire that was distributed to each subject[12)].

2. Serum analysis

Within 6 h of collection, blood samples were centrifuged for 10 min at 3,000 rpm to separate the serum, then stored at -80℃ until measurement. Recipient vitamin D status was measured by assessing circulating levels of 25OH-D in serum samples that had been stored frozen, having never been previously thawed, using radioimmunoassay (25-hydroxyvitamin D 125I RIA Kit; DiaSorin, Saluggia, Italy)[13) 14)]. Subjects were also divided into two groups according to clinically accepted ranges for vitamin D deficiency (<20 ng/m*l*) and insufficiency (20-29.9 ng/m*l*) into a Deficient group (<30 ng/m*l*), and a Sufficient group (≥30 ng/m*l*)[15)].

AMH was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (AMH Gen II ELISA; Beckman Coulter and R&D Systems, Fullerton, CA)[16)]. The resulting measurements of 25OH-D and AMH were expressed in nanograms per milliliter. Following the age-related normative model[17)], we used the value for 40-year-olds (2.2 ng/m*l*) as the criterion to categorize AMH results into 2 groups: low AMH (<2.2 ng/m*l*) and normal AMH (≥2.2 ng/m*l*).

3. Measurement of body weight and body composition

Body weight and body composition were measured by the 8-electrode BIA method using a multifrequency body composition analyzer (MC-190EM; Tanita, Tokyo, Japan). BIA is a significantly more cost-effective method of measuring body composition than dual-energy X-ray absorptiometry (DEXA). BIA is also portable, and easier to use than other technologies[10) 11)]. The BIA method offers a high correlation with mean regional lean soft tissue and whole-body skeletal muscle mass estimates using the reference method of DEXA for non-pregnant women[11)]. According to the World Health Organization guideline for the measurement of BMI, all subjects were divided into two groups: underweight (BMI<18.5 kg/m^2), and normal weight (18.5≤BMI<25 kg/m^2).

Table-1 Characteristics of subjects categorized by age

Age group	Total	<30 years (n=57)	≥30 years (n=51)	p
Demographics				
Age (years)	29.2 ± 4.2	26.1 ± 2.4	32.7 ± 2.7	<0.01
Weight (kg)	50.5 ± 5.5	50.9 ± 5.1	50.1 ± 5.9	NS
BMI (kg/m^2)	19.9 ± 1.9	19.9 ± 1.8	19.9 ± 1.9	NS
Underweight (<18.5)	23 (21.3)	12 (21.1)	11 (21.6)	NS
Normal (18.5-24.9)	85 (78.7)	45 (78.9)	40 (78.4)	
Body fat (%)	25.2 ± 4.2	25.3 ± 3.7	25.0 ± 4.8	NS
Serum biological parameters				
25OH-D (ng/m*l*)	25.6 ± 8.4	25.7 ± 8.5	25.6 ± 8.4	NS
25OH-D status, n (%)				
Deficient group	76 (70.4)	39 (68.4)	37 (72.5)	NS
Sufficient group	32 (29.6)	18 (31.6)	14 (27.5)	
AMH (ng/m*l*)	4.9 ± 2.4	5.4 ± 2.4	4.3 ± 2.3	<0.01
AMH status, n (%)				
Low (<2.2)	17 (15.7)	5 (8.8)	12 (23.5)	<0.05
Normal (≥2.2)	91 (84.3)	52 (91.2)	39 (47.2)	

Results are given as mean ± SD; percentages are given in parentheses.
n, number; NS, not significant; BMI, body mass index; 25OH-D, 25-hydroxyvitamin D; AMH, anti-Müllerian hormone; SD, standard deviation
Deficient group, 25OH-D<30 ng/m*l*; Sufficient group, 25OH-D≥30 ng/m*l*.

4. Diet history questionnaire

Dietary/nutritional habits from the previous month were reported on the brief-type self-administered diet history questionnaire (BDHQ)[12]. The BDHQ was developed as a scaled-down version of the self-administered diet history questionnaire[18]. In recording food eaten for the questionnaire, examinees tend to underestimate the volume of food eaten[19]. To increase the accuracy of the BDHQ, the present study indicated the vitamin D dietary intake for every 1,000 kcal, in accordance with previous reports.

5. Statistical analysis

Results are reported as means ± standard deviation. Categorical variables are reported as positive percentages. Differences in continuous variables between subjects < 30 and ≥30 years old were assessed using the unpaired Student's t-test. Distributions among serum 25OH-D levels, serum AMH levels, age, and BMI were compared using the χ^2 test in each condition. Binary logistic regression analysis was used to determine factors associated with decreased concentrations of serum AMH levels (≥2.2 ng/m*l*) and to calculate odds ratios (OR) with 95% confidence intervals (95%CIs). All statistical analyses were performed using IBM SPSS Statistics version 21 (Statistical Package for Social Science Japan, Tokyo, Japan). The tests were two-sided, and values of $p<0.05$ were considered statistically significant.

Results

1. Serum AMH levels and 25-hydroxyvitamin D

Table-1 compares participant characteristics by age. Of the 108 participants, 57 were under 30 years old and 51 were ≥30 years old (mean age, 29.2 ± 4.2 years). Mean BMI was 19.9 ± 1.9 kg/m^2, and mean body fat percentage was 25.2 ± 4.2%. Mean serum 25OH-D concentration was 25.6 ± 8.4 ng/m*l*, and mean AMH level was 4.9 ± 2.4 ng/m*l*. In the study population, 70.4% of women were categorized to the Deficient group and 29.6% to the Sufficient group. Regarding AMH, 15.7% of the entire group showed low levels (<2.2 ng/m*l*) and 84.3% had normal levels (≥2.2 ng/m*l*). In age comparisons, subjects ≥30 years old showed significantly lower AMH levels ($p<0.01$) than subjects <30 years old, with a higher frequency of low AMH levels among subjects ≥30 years old ($p<0.01$). No

Table-2 Weight, BMI, body fat %, and nutrition of all participants according to 25OH-D status

	Deficient group (n=76)	Sufficient group (n=32)	p
Age (years)	29.4 ± 3.8	28.8 ± 5.0	NS
Weight (kg)	50.4 ± 5.3	50.8 ± 5.9	NS
BMI (kg/m^2)	19.9 ± 1.9	20.0 ± 1.8	NS
Body fat (%)	25.6 ± 4.1	24.0 ± 4.4	NS
Vitamin D (μg/day)	10.0 ± 7.2	10.9 ± 5.4	<0.01
(μg/1,000 kcal)	6.3 ± 4.8	8.2 ± 4.0	<0.05
25OH-D (ng/m*l*)	21.5 ± 4.8	35.5 ± 6.6	<0.01

Results are given as mean ± SD; percentages are given in parentheses.
BMI, body mass index; 25OH-D, 25-hydroxyvitamin D; NS, not significant
Deficient group, <30 mg 25OH-D (ng/m*l*); Sufficient group, ≥30 mg 25OH-D (ng/m*l*).

Table-3 AMH status in 25OH-D deficient and sufficient groups

25OH-D levels	Deficient group (n=76)	Sufficient group (n=32)	p
AMH (ng/m*l*)	4.5 ± 2.5	5.7 ± 1.9	<0.01
AMH status, n (%)			
<2.2 ng/m*l* (n=17)	16 (94.1)	1 (5.9)	<0.05
≥2.2 ng/m*l* (n=91)	60 (65.9)	31 (34.1)	

Results are given as mean ± SD; percentages are given in parentheses.
AMH, anti-Müllerian hormone; 25OH-D, 25-hydroxyvitamin D; SD, standard deviation
Deficient group, <30 mg 25OH-D (ng/m*l*); Sufficient group, ≥30 mg 25OH-D (ng/m*l*).

differences in other categories were observed between age levels.

2. Weight, BMI, body fat percentage, and nutrition of all participants separated by 25OH-D status

Table-2 indicates the comparisons of weight, BMI, body fat percentage and dietary intake of vitamin D by serum 25OH-D levels. Although weight and BMI showed no correlations with serum vitamin D, intake of vitamin D per 1,000 kcal was lower in the Deficient group (6.3 ± 4.8 μg/1,000 kcal) than in the Sufficient group (8.2 ± 4.0 μg/1,000 kcal; $p<0.05$).

3. AMH status between 25OH-D Deficient and Sufficient groups

Mean serum AMH level was lower in the Deficient group (4.5 ± 2.5 ng/m*l*) than in the Sufficient group (5.7 ± 1.9 ng/m*l*; $p<0.01$) (Table-3). When distinguishing between AMH status (<2.2 ng/m*l* and ≥2.2 ng/m*l*), significant differences in the frequency of 25OH-D deficient and sufficient subjects were seen between two groups (16/17 [94.1%] vs. 1/17 [5.9%] for low AMH status; 60/91 [65.9%] vs. 31/91 [34.1%] for normal AMH status, respectively; $p<0.05$).

4. Binary logistic regression for AMH

We used binary logistic regression modeling to evaluate independent variables showing strong correlations with normal serum AMH levels (≥2.2 ng/ml) in subjects. The following covariates were included in the model: serum 25OH-D level ≥30 ng/m*l*, age ≥30 years, BMI, and body fat (%). Binary logistic regression analysis revealed serum 25OH-D levels (≥30 ng/m*l*) (odds ratio [95%CI]= 8.270 [1.033-66.199], $p<0.05$) and age (≥30 years) (odds ratio [95%CI]=-1.164 [0.099-0.985], $p<0.05$) as independent predictors of AMH levels (≥2.2 ng/m*l*) (Table-4).

Discussion

The present study found that 70.4% of the study population was suffering from either vitamin D insufficiency or deficiency according to Holick's classification[15] and decreased serum AMH level was associated with vitamin D deficiency. Although previous studies have investigated serum 25OH-D

Table-4 Results of binomial logistic regression analyses for categories of serum AMH level (≥2.2 ng/m*l*)

Independent variable	*Standardized partial regression coefficient* (*β*)	Odds ratio (95% confidence interval)	p
25OH-D (≥30)	2.113	8.270 (1.033-66.199)	<0.05
Age (≥30)	-1.164	0.312 (0.099-0.985)	<0.05

The Hosmer-Lemeshow test was used to test the goodness-of-fit of the model (χ^2=2.653, p=0.265).

concentrations in Japanese women by age group, few have studied this topic specifically in women of childbearing age. A study of Japanese women (mean age, 32.9 ± 11.3 years) in Niigata Prefecture (latitude 37° 48 to 59° N), found that women <30 years old had significantly lower mean serum 25OH-D levels compared with women ≥30 years old, and 42.1% of these younger women were vitamin D-deficient[20]. In the present study, although no significant differences in serum 25OH-D levels were evident between women under or over 30 years old, women of childbearing age in Tokyo (located at 35.4° N, 139.4° E) were commonly vitamin D-deficient.

Serum 25OH-D level and body fat percentage have previously been reported as inversely proportional to each other[21,22]. According to a meta-analysis of 21 studies conducted in North America and Europe, when BMI increases by 10%, blood levels of vitamin D decrease by a mean of 4%. For obese individuals, weight loss, exposure to sunlight, and vitamin D intake from diet are recommended[23]. In underweight individuals, when body fat percentage decreases due to body weight loss, the frequency of menstrual abnormalities rises[24]. Decreased body weight is linked to abnormalities in sex hormone secretions from the hypothalamus and pituitary, which can lead to diminished ovarian function[25]. In the present study, multiple regression analysis showed that both BMI and body fat percentage had no significant impact on AMH. The influence of physical constitution on AMH could have been lower among non-obese women in this population.

In the present study, levels of serum 25OH-D depended on the oral intake of vitamin D, which was 6.3 ± 4.8 μg/1,000 kcal in the Deficient group and 8.2 ± 4.0 μg/1,000 kcal in the Sufficient group, showing a significantly lower level in the Deficient group (p<0.01). While we investigated <40-year-old women in the present study, we also found more women with low AMH levels among those who were vitamin D-deficient. Vitamin D deficiency has recently been reported as common among women who have experienced recurrent pregnancy losses, and low serum 25OH-D levels suppress peripheral blood NK cells in recurrent pregnancy loss[6]. This indicates that vitamin D is an extremely important nutrient in preparing for pregnancy. Although the reference intake for vitamin D has been defined as that required to achieve a serum 25OH-D level above 20 ng/m*l* by the Institute of Medicine in the United States[26] and 30 ng/m*l* by the International Society of Endocrinology[27], no reference intake has been established for vitamin D with respect to pregnancy potential. An investigation concerning serum vitamin D and a reference intake for vitamin D with consideration for pregnancy potential represent future research challenges.

Some limitations to this study must be considered when interpreting the present findings. Serum levels of AMH in women are influenced by conditions such as PCOS. Although PCOS patients were screened in the medical interview and obese women were excluded to reduce the risk of including patients with PCOS, the possibility of some patients with this pathology being included could not be completely ruled out, since medical checks using ultrasonography were not performed. Second, seasonal changes are seen in serum vitamin D levels[28] and the promotor of the *AMH* gene contains a vitamin D-response element that is active in cultured cells[29]. Consideration must be given in the next study to the fact that latitudinal and regional differences[30], as well as seasonal variations[28], are seen with respect to serum 25OH-D concentrations.

Conclusions

Vitamin D deficiency or insufficiency were commonly observed in healthy women of reproductive

age in Tokyo area. The current study suggests that pre-pregnancy serum 25OH-D status affects AMH levels. In subjects with decreased serum AMH levels, vitamin D deficiency may need to be considered. Nutritional support that considers the appropriate nutritional status at childbearing age may be warranted.

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Declaration of conflicting interests

All the authors of this study declare that they have nothing to disclose regarding conflict of interest with respect to this manuscript.

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