Research Article

Correlation between 25-OH Cholecalciferol and Anti Mullerian Hormone in Infertile Women: A Cross-Sectional Study in Indonesia

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Abstract

Infertility is a health problem with a high level of morbidity, social effects, and economic burdens. Vitamin D deficiency is found in many women with infertility but many studies show inconsistent results according to their correlation with Anti Mullerian Hormone (AMH). This study aims to determine the correlation between 25-OH Cholecalciferol (vitamin D) and AMH in women with infertility. The research design used a cross-sectional design, with a total sample of 117 infertility women with varies etiology. The study was conducted at dr. Ciptomangunkusumo General Hospital, Jakarta, Indonesia, in February−June 2020. Vitamin D and AMH levels were obtain from medical record of dr. Cipto mangunkusumo Hospital, Jakarta, Indonesia. Vitamin D levels were measured using the Architect i2000 (Abbott, Illinois, United States) with the chemiluminescent microparticle immunoassay technique. Meanwhile, AMH uses the Ultra-Sensitive AMH/MIS ELISA reagent (AnshLabs, Webster, USA). In this study, the median vitamin D was 10.00 ng/mL with the minimum level of 2.50 ng/mL to 37.40 ng/mL, while AMH had a median value of 3.1 ng/mL with a range of 0.02−19.14 ng/mL. Statistically correlation between vitamin D and AMH is not significant (p=0.671) with r=0.04. The varies etiology of infertility in this study showed no correlation between vitamin D and AMH levels.
***Keywords**: AMH, infertility, vitamin D.*

Korelasi antara 25-OH Cholecalciferol dengan Hormon Anti Mullerian pada Wanita dengan Infertilitas: Studi Potong Lintang di Indonesia

Abstrak

Infertilitas merupakan suatu masalah kesehatan dengan morbiditas, efek sosial, dan ekonomi yang tinggi. Defisiensi vitamin D banyak ditemukan pada wanita dengan infertilitas tetapi penelitian yang ada mengenai hubungan vitamin D dan Anti Mullerian Hormone (AMH), yang digunakan untuk menilai fungsi ovarium, masih menunjukkan hasil yang inkonsisten. Penelitian ini bertujuan untuk menilai korelasi antara 25-OH Cholecalciferol (vitamin D) dan AMH pada wanita dengan infertilitas. Disain penelitian ini adalah cross-sectional, dengan jumlah sampel 117 wanita infertilitas dengan berbagai etiologi. Penelitian dilakukan di Rumah Sakit dr. Cipto Mangunkusumo, Jakarta pada bulan Februari−Juni tahun 2020. Kadar vitamin D dan AMH diperoleh dari data rekam medis, Indonesia. Kadar vitamin D diperiksa menggunakan Architect i2000 (Abbott, Illinois, United States) dengan Rumah Sakit dr. Cipto Mangunkusumo, Jakarta metoda chemiluminescent microparticle immunoassay. Pemeriksaan AMH menggunakan reagen Ultra-Sensitive AMH / MIS ELISA (AnshLabs, Webster, USA). Penelitian ini mendapatkan median vitamin D adalah 10,00 ng/mL dengan rentang nilai 2,50 ng/mL sampai 37,40 ng/mL, dan median AMH adalah 3,1 ng/mL dengan rentang nilai 0,02−19,14 ng/mL. Korelasi antara vitamin D and AMH ditemukan tidak signifikan (p=0,671) dengan r=0.04. Pada wanita infertilitas dengan berbagai etiologi ditemukan kadar vitamin D yang tidak berkorelasi dengan kadar AMH.
***Kata kunci**: AMH, infertilitas, vitamin D.*

Introduction

One of the public health problems with high morbidity, social, and economic is infertility.[1,2] The World Health Organization was stated that the infertility, based on the clinical definition, is the reproductive system disorder in which a female failed to achieve pregnancy after 12 months or more after having routine sexual intercourse without protection. Epidemiologically, infertility is defined as a woman of reproductive age who is likely to become pregnant but is reported to have failed to achieve a pregnancy of ≥2 yr.[3,4] In infertility management Consensus in Indonesia, vitamin D is not part of established treatment. However, in practice, obstetricians usually require vitamin D level measurement as a part of initial assessment in infertility women. Indeed, some of the patients are given vitamin D supplementation instead of controversial evidence.

Theoretically, the role of Vitamin D in the reproductive system is enormous, both in men and women, and is believed to be linked to infertility. The frequency of vitamin D deficiency varies between 20-90% in several countries. Vitamin D has a role in assisting the implantation process. In fact, in some studies, giving vitamin D can increase the success of in vitro fertilization.[5-7] One of the necessary tests in assessing female infertility is an anti-Mullerian hormone (AMH), which is useful for assessing ovarian reserve function. AMH is a glycoprotein in gonads formed by granulosa cells through a follicular phase. AMH is considered to regulating early ovarian development. Because AMH represent the oocyte pool so when the oocyte pool decrease AMH will decrease too. AMH levels are relatively stable throughout the menstrual cycle so that AMH is believed to be useful as a predictive marker of reproductive technology.[8]

However, some studies show some factors can alter AMH expression and serum concentration, such as vitamin D levels.[5,6] Therefore, we must consider vitamin D levels in cases of low AMH.[9] Some studies have showen a possitive assosiation between vitamin D and AMH in women of reproductive age. However, several other studies in different populations have shown inconsistent results.[8-11] Our hyphotesis is vitamin D level correlate with AMH level. So, this study purpose to determine the correlation between 25-OH Cholecalciferol (vitamin D) levels and AMH levels in women with infertility in Indonesia.

Methods

The design of this study was cross-sectional with descriptive and analytical data presentation. The study was conducted at dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, in February−June 2020. The inclusion criteria subjects were women with infertility aged 18-45 years old who obtained an AMH test in laboratory and first time consult to infertility clinic. Infertility diagnosed based on obstetrician and fertility consultant consideration from the hospital information system. The subjects who received vitamin D supplementation, the samples that icteric or hemolysis were excluded from this study. The independent variable in this study was serum 25-OH cholecalciferol level and the dependent variable was AMH. Serum 25-OH cholecalciferol levels were measured using the Architect i2000 (Abbott, Illinois, United States) instrument that uses chemiluminescent microparticle immunoassay technique. AMH assay was examined using an Ultra-Sensitive AMH/MIS ELISA reagent (AnshLabs, Webster, USA). Blinding was not carried out in this study because the results of serum 25-OH cholecalciferol and AMH tests were objective data obtained from a measurement.

The minimum sample size of 51 was calculated using the sample size formula for the numerical- numerical correlative analysis test, with Za=1.64, Z_{β}=1.28, and the minimum correlation coefficient which was considered significant was set at 0.4. The subjects were taken by consecutive sampling until the minimum number of samples was met. First, data of the patients who examined AMH in our laboratory were collected from medical records and laboratory information system hospital. Then, if the patients fufilled inclusion criteria, the samples were collected and stored in -80^{o}C until the minimum number of sample size was met. After that, the samples were run for 25-OH cholecalciferol level. The subjects were divided into three categories based on vitamin D level (deficiency:level <20 ng/mL; insufficiency: 21−29 ng/mL), and sufficiency: ≥30 ng/mL). However, the sufficient vitamin D only in one subject, so the subject divided into two group for analysis, with vitamin D <20 ng/mL and >20 ng/mL.

Data processing was performed using IBM SPSS Statistics for Windows, version 22.0. Armonk, NY. Normality tests were performed to determine data distribution. Mean and standard deviation was presented if normal distribution, median and maximum-minimum levels if abnormal distribution. Vitamin D was divided into 3 categories, deficiency (<20 ng/mL), insufficiency (21−29 ng/mL), and sufficiency (≥30 ng/mL). Pearson correlation test was used for data with normal distribution and Spearman statistical test if the data were abnormally distributed. The level of significant result were

determined if p<0.05 with 95% CI. This study was approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia (KET.99/UN2.F1/ETIK/PPM.00.02/2020).

Results

This study has 117 total subjects with a mean age of 33±5.8 yr and the majority of the subject have primary infertility (64.1%). Ovulation disorders (36.8%) were the most common etiology of infertility after unknown etiology (43.6%) (Table 1). In all subjects, the median vitamin D was 10.00 ng/mL, ranging from 2.50 ng/mL to 37.40 ng/mL. Most of the subjects had vitamin D deficiency (91.5%), and only a few were insufficient (7.7%) and sufficient (0.8%). The median AMH value in the overall subjects was 3.1 ng/mL with a range of 0.02−19.14 ng/mL. There was a difference in the mean AMH levels in the group with the vitamin D category <20ng / mL (4.47 ng / mL) and> 20 ng / mL (5.66 ng / mL) but it was not statistically significant (p = 0.469) (Figure 1).

Table 1. Characteristic Subject

Characteristic	Overall	Vit D <20 ng/mL	Vit D >20 ng/mL
Subject total[a]	117 (100)	107 (91.5)	10 (8.5)
Age (yr)[b]	33.42±5.8	33.70±5.9 [d]	30.78±4.1[d]
Infertility type[a]			
Primary, n(%)	75 (64.1)	69 (64.5)	6 (60)
Secondary, n(%)	42 (35.9)	38 (35.5)	4 (40)
Etiology[a]			
PCOS	15 (12.8)	13 (86.7)	2 (13.3)
Ovulation disorders	43 (36.8)	40 (93.0)	3 (7.0)
Tubal disorders	8 (6.8)	6 (75.0)	2 (25.0)
Unknown	51 (43.6)	48 (94.1)	3 (5.9)
AMH (ng/mL)[c]	3.10 (0.02−19.14)	3.10 (0.02−19.14)	2.41 (0.27−12.49)

Data preseted as [a] n(%), [b] mean±SD, [c] median (min-max). PCOS=polycystic ovarian syndrome; AMH=anti Mullerian hormone. [d] *p*>0.05 shows no significant differences between two groups.

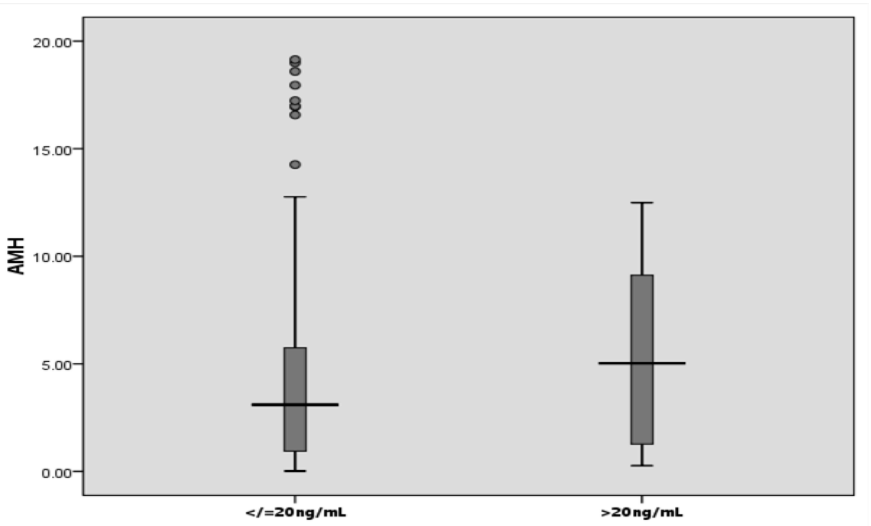

Figure 1. Distribution of Serum AMH Levels in Subjects based on Vitamin D Levels
The bar diagram of serum AMH levels in subject with vitamin D level </ = 20 ng/mL (left side) and vitamin D level > 20 ng/mL (right side). The *p* value between two group >0.05 (independent t-test).

There was no statistically significant correlation in the correlation test between vitamin D and AMH (r=0.04, p =0.671), as shown in Figure 2. However, there was a correlation between the variable age and AMH levels (r=-0.32; p≤0.001), and age variables with vitamin D levels (r=-0.18, p=0.049). For this reason, the age variable was considered a confounding factor. Furthermore, adjustments were made for the age variable, but there was still no correlation between vitamin D levels and AMH (r=-0.11, p=0.080).

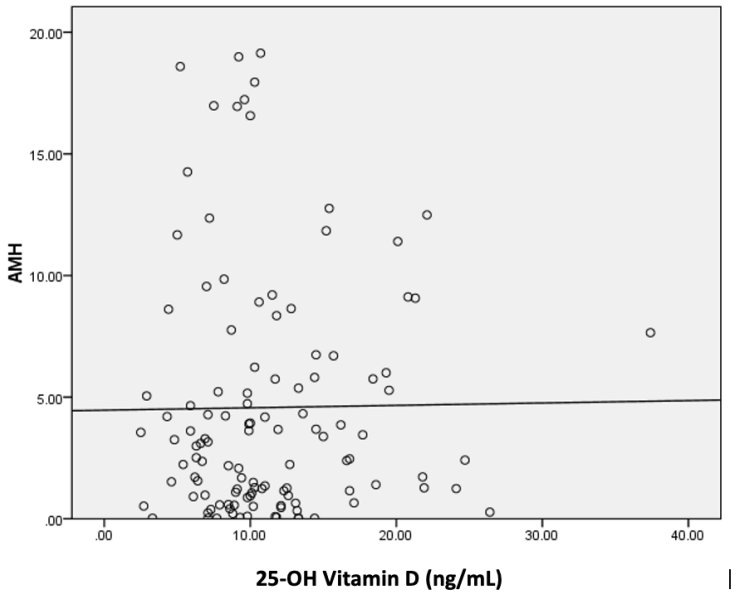

Figure 2. Correlation between Serum Vitamin D and AMH Level
The dot represents the subject in this study. The scattered dot in the graphic shows there is no correlation between serum vitamin D and AMH level (r = 0.04, p = 0.671) based on Pearson correlation test.

Discussion

Vitamin D has a significant role in the female reproductive system. Several studies show that vitamin D deficiency is related to decreased fertility in females through various complex mechanisms.[7,10] In this study, almost all subjects had vitamin D deficiency (91.5%). This phenomenon is consistent with the previous studies that show a high frequency of vitamin D deficiency in women with infertility.[11-14] In fact, the proportion of vitamin D deficient patients in this study is higher than in studies in other countries, that may be associated with seasonal and demographic factor. High prevalence of Vitamin D deficiency in infertile woman cause increased awareness that vitamin D plays an important role in reproduction. Supporting this hypothese, several clinical studies suggest a correlation between adequate vitamin D levels and successful fertility treatments in women with infertility. Therefore, vitamin D examination is recommended for infertility women.[5,7] In this study, only one subject had sufficient vitamin D levels because the subject had vitamin D supplementation several months before the time of the pregnancy planning program (in vitro fertilization).

At present, an inconsistency has been observed regarding whether vitamin D has ability to influence ovarian reserve, such as AMH as the predictor. Studies in the literature showed conflicting results, with several studies finding a positive correlation between vitamin D level and ovarian reserve markers[9-13] while other studies reported largely negative findings.[14-19] In this study, there was no correlation between vitamin D levels and AMH levels, this result in line with several other studies with observational designs that did not have a relationship between vitamin D levels and AMH levels that were observed at the same time

in the observational study. One of the underlying hypotheses is that current AMH levels result from vitamin D regulation that occurs previously. Therefore, interventional studies with vitamin D supplementation have more consistently shown an positive correlation between vitamin D and AMH levels[9-13], although some studies only shows increasing trend without significant statistical correlation.[17,19]

Adequate vitamin D supplementation is believed will improve patients' fertility status through various mechanisms, one of which is through AMH regulation. In the ivitro studies, vitamin D shows relation to AMH levels produced by granulosa cells, which can occur because vitamin D can regulate AMH levels by affecting the promoter and the number of granulosa cells.[5] AMH function to inhibit transition between the primordial to primary follicle. This AMH inhibitory effect on granulose cell differentiation process is mediated by the very specific type II AMH receptors (AMHR-II). So the higher AMH will make the greater effect on suppresing follicles maturation. The role of vitamin D is to inhibit AMHR- II.[5-7] Vitamin D supplementation has been suggested to reduce excessive AMH production, thereby enhancing follicular sensitivity to follicle-stimulating hormone (FSH) and restoring normal ovulation.[9-11] In young women without infertility problems who were given high doses of vitamin D supplementation and had average AMH levels, serum AMH levels increased progressively within seven days with a mean increase of 12.9%. This supports the theory that the AMH levels regulation is influenced by vitamin D.[11] In contrast, in this study, almost all subjects had low AMH levels.

The significant association of vitamin D with ovarian reserve markers and depression has been revealed in numerous basic or clinical studies, but some observational and interventional clinical studies have shown inconsistent results.[8,13] It was found that there was a complicated relationship between AMH and Vitamin D in the human body.[13]

The observed causal relationship between vitamin D and AMH levels can have different directions depending on ovulatory status. AMH serum levels will decrease in women with polycystic ovary syndrome who receive vitamin D supplementation but increase in women with another etiology.[10] In this study, the subjects were infertile women with various etiologies. Therefore, the response of AMH to a patient's vitamin D levels can vary. It may be the possibility of why there is no correlation between vitamin D and AMH in this study.

This study shows that age is associated with AMH and vitamin D. Age has a negative correlation with AMH, following the theory that AMH levels will decrease along with the decrease in the number of granulosa cells in women ages.[13] The relationship between vitamin D and age has a negative and weak correlation. This is consistent with several studies that state that lower vitamin D levels are more often found in the older age group.[20]

The weakness of this study was not take the genetic factors on vitamin D metabolism as consideration. Subsequent studies should be performed to investigate the effect of increased vit D3 level after intervention on AMH changes in different reproductive ages. For further study, a case-control or randomized controlled trial design with vitamin D supplementation is needed so that changes in AMH levels in the subject can be observed. It is also necessary to uniform the etiology of infertility in subjects considering the response to changes in AMH levels with varying vitamin D administration. Because there is many heterogeneity in study subjects in this area which includes study design, intervention, ovarian reserve marker measurement methods, vitamin D status of study subjects.

Conclusion

This study was finding high prevalence of vitamin D deficiency in women with infertility (91.5%), and no significancy in correlation between vitamin D and AMH levels with varies etiology of infertility.

Conflict of Interest

All Authors declare no confilct of interest.

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