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A HOSPITAL-BASED STUDY ON STATUS OF SERUM ANTI-MÜLLERIAN HORMONE AMONG WOMEN IN EASTERN NEPAL

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ABSTRACT

Anti-müllerian hormone is homodimeric glycoprotein that belongs to the super-family of transforming growth factor-β. Sertoli cells of fetal testes produce anti-müllerian hormone. Production of anti-müllerian hormone by testes and thereby regression of müllerian ducts of female reproductive tract called sex-dimorphic expression pattern, however is lost after birth and granulosa cells of growing follicles in ovary express anti-müllerian hormone. Ovarian reserve constitutes the size of follicle pool and the quality of oocytes therein. The ovarian reserve declines as age advances and so decreases woman's reproductive usefulness. Serum levels of anti-müllerian hormone thus indicate size of the growing follicular pool. Further, anti-müllerian hormone is detectable in serum and, therefore, exist as a promising candidate. In spite of its crucial use in ovarian assessment, status of serum anti-müllerian hormone levels in Nepalese women was still unknown. Thus we determined status of circulating levels of anti-müllerian hormone in local women. In the present study, we found that around 70 % females were < 35-years-old and the bulk of subjects had serum anti-müllerian hormone concentrations within reference range. Moreover, comparative analysis showed that the subjects < 35-years-old had higher serum anti-müllerian hormone levels compared to population > 35, and a significant difference was present therein.

KEYWORDS: anti-müllerian hormone, ovary, follicles, women.

INTRODUCTION

Anti-müllerian hormone (AMH) is homodimeric glycoprotein with molecular mass of 140 kDa and linked by disulfide bonds.[1, 2] AMH belongs to the super-family of transforming growth factor-β. Gene that encodes for AMH is located in short arm of chromosome 19.[2] AMH regulates male sex differentiation.[3] Sertoli cells of fetal testes produce AMH that induces regression of Müllerian ducts, the anal-gen of female reproductive tract.[4, 5] Production of AMH by testes and thereby regression of Müllerian ducts of female reproductive tract is called sex-dimorphic expression pattern in developing fetus. The sex-dimorphic expression pattern, however, is lost after birth and granulosa cells of growing follicles in ovary express AMH.[6]

Ovarian reserve constitutes the size of follicle pool and the quality of oocytes therein.[7] The ovarian reserve declines as age advances and so decreases woman's reproductive usefulness.[8] Germ cells populate the ovary, get surrounded by somatic cells and form the so-called primordial follicles during fetal life.[9] The size of follicle pool is thus established at an early phase. Approximately one million oocytes are present at birth. The amount decreases during childhood and reaches primordial follicle pool of 300000 to 500000 at menarche.[10] Follicles leave primordial reserve account throughout the life and enter into growing pool. Majority of the growing follicles are lost as a result of atresia unless follicle stimulating hormone (FSH) rescue them.[11] The rescue starts after onset of puberty when pituitary-gonadal endocrine axis is excited. Among cohort of rescued follicles, the only one selectively becomes dominant and ovulates under influence of Luteinizing hormone (LH). Ovulation continues throughout the life until primordial follicle is exhausted and, as a consequence, growing follicles are no longer present in ovary resulting into menopause.[12] In contrast, growing non-selected follicles specifically express AMH up to their degree of selection for ovulation. Serum levels of AMH thus indicate size of the growing follicular pool.[13, 14, 15]

Further AMH is detectable in serum and, therefore, exist as a promising candidate. Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women during reproductive age. PCOS is characterized by anovulation, elevated levels of androgens and polycystic ovaries as visualized under ultrasound. Common denominator in PCOS lies at disturbance in selection of growing follicles to become dominant, thus resulting into

anovulation. As non-selected growing follicles specifically express AMH, its levels also serve as a marker of PCOS.[16]

In spite of its crucial use in ovarian assessment, status of serum AMH levels in Nepalese women was still unknown. Thus we determined circulating levels of AMH in women who belonged to eastern region of Nepal. The purpose of our study was to describe status of serum AMH levels among local females in this area. In the present study, we observed that the majority of females (around 70 %) were < 35-years-old and the bulk of subjects had serum AMH concentrations within reference range i.e., 1.0 to 2.4 ng/ml. Further, comparative analysis revealed that subjects < 35-years-old had higher levels of serum AMH compared to population > 35, and a significant difference was present therein.

MATERIALS AND METHODS

Study design and enrolment criteria

This research was hospital-based study carried out in the Department of Diagnostic Laboratory at Birat Medical College and Teaching Hospital (BMCTH), Biratnagar, Morang, Nepal. The local Nepalese women who attended BMCTH for investigation of AMH were enrolled as subjects. Duplication of a similar participant and known patients under treatment were excluded. The inclusion criteria were made in accordance with the guidelines for assessment of AMH, as described earlier.[17] The women with polycystic ovarian syndrome (PCOS), suffering from premature ovarian failure (POF) and having problems related to reproduction were enrolled. Registration of 50 subjects was aimed to meet target population. Our study, therefore, established normal serum AMH values among different age groups in fertile women. We did not find undetectable AMH values among the participants. Thus it omits the practitioners in locality to be cautious when counselling patients would present very low or undetectable serum AMH concentrations because spontaneous pregnancy is still possible at the age of fertility.

Sample collection and serum preparation

Venipuncture was performed to collect blood samples under universal attentiveness as mentioned earlier.[18] Antecubital venous blood from patients were collected in plain vial with informed written consent, strictly as per the norms and approval of the Institutional Ethical Committee. Blood samples were clotted for 5 min, followed by centrifugation at 3000 r.p.m. for 15 min to separate serum. All steps were carried out under sterile conditions and precautions were taken to prevent blood from hemolysis.

Determination of serum levels of AMH by Enzyme-linked immunosorbant assay (ELISA)

AMH was assayed using human sandwich ELISA method. The kit (E-lab Science) was provided with micro-titre plate having 96 wells coated by monoclonal antibody specific for AMH. Serum (100 µl) was added into unused well of the micro-titre plate and processed according to manufacturer's protocol. The well containing serum was incubated at 37 °C for 90 min, followed by aspiration and washing thrice with provided buffer. Further biotinylated detection antibody (100 µl) was added and incubated at 37 °C for 1 h with similar steps of aspiration and washings to remove free components. Next, horse-radish peroxidase conjugate (50 µl) was added and incubated at 37 °C for 1 h. Substrate (100 µl) was added with following incubation at 37 °C for 30 min. The provided sulphuric acid reagent (50 µl) was then added to stop the reaction and absorbance of yellow colour was read in a spectrophotometer at 450 nm. Results were obtained in ng/ml.

Data interpretation

The validity and reliability of test results were determined using standard supplied with kit. Data were analyzed under Software Package for Social Sciences version 16 (SPSS 16).

RESULTS

Majority of adult women had serum AMH levels within reference range

To assess the status of serum AMH concentrations among local women who attended BMCTH, we collected antecubital venous blood, separated sera and performed immunoassay. The study population were divided into two groups; A (18-35 years) and B (36-45 years) to categorize the stages as before and after 35-years of age. Among total women (*n=60*) who attended the hospital for investigation of AMH, 42 and 18 subjects were enrolled for investigation in groups 'A' and 'B', respectively (Bars 'A' & 'B'; Figure-1), indicating that majority of women (70 %) who came for examination were < 35-years-old and remaining (30 %) > 35. Our data further determined the mean age group of patients to be 30.65 ± 7.14 years (The right-sided bar; Figure-1) and median serum AMH concentration being 2.5 (2.1, 3.1) ng/ml, suggesting that the subjects, in majority, had serum AMH concentrations within reference range i.e., 1.0 to 2.4 ng/ml.

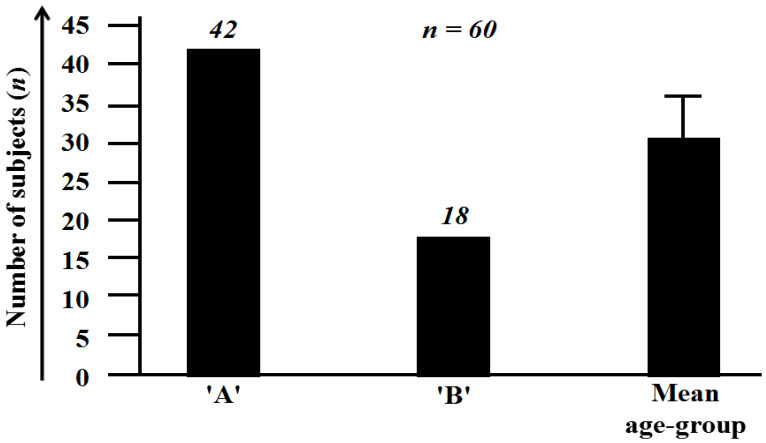

Figure-1. Bar diagram representing number of participants in groups 'A' & 'B'. Group 'A' is indicative to people < 35-years-old and 'B' > 35-years

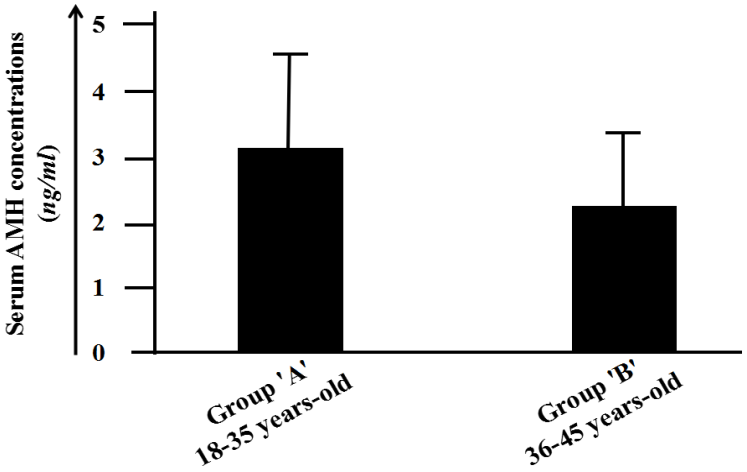

Figure-2. Bar diagram representing serum AMH concentrations among subjects in groups 'A' & 'B'

Women below 35-years had higher levels of serum AMH

Serum concentrations of AMH decline as age advances. We, therefore, evaluated the subjects for existence of any possible variations before and after 35-years-old. On comparing the values between groups 'A' and 'B', we observed that mean serum AMH concentrations among females below 35-years was 3.14 ± 1.5 ng/ml. In second group, the value remained 2.25 ± 1.25 ng/ml. Our finding was, therefore, suggestive of the fact that Group-A holding subjects < 35-years-old had higher levels of serum AMH compared to 'B', and a significant difference was present therein.

Correlation of serum AMH concentrations with age

As a significant change occurred among participants in groups 'A' and 'B', we correlated the serum AMH concentrations with varying age-groups of females. The serum AMH concentration that we observed at uppermost was 9.7 ng/ml and at lowest being 0.33. The peak age when serum AMH concentrations were at maximum and minimum were 21 and 39-

year-old, respectively. On correlating serum AMH concentrations with age, we found that serum AMH concentration declined with increasing the age. The serum AMH concentration, therefore, correlated negatively with age, and association was statistically significant.

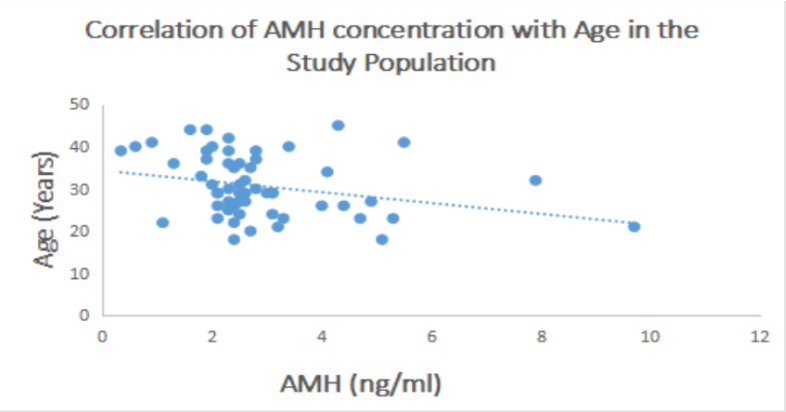

Figure-3. Dot plot demonstrating correlation of serum AMH concentration with age in study populatioin

DISCUSSION

AMH is the most sensitive hormonal parameter to detect the changes in ovarian reserve.[19] In the present study, we prospectively investigated the status of serum AMH concentrations among eastern Nepalese women. As this study aimed to establish serum AMH values in local women in eastern Nepal, we conducted the study exclusively among females at age of pregnancy progressing from 18-years-old. The study population were divided into two groups; A (18-35 years) and B (36-45 years) to categorize the subjects as before and after 35-years of age. Among total women (*n=60*), the majority (around 70 %) of women who attended the hospital for investigation of AMH were < 35-years-old and remaining (30 %) > 35 (Bars 'A' & 'B'; Figure-1). The high loss of AMH occurs in particular after abortion.[20] We, therefore, ignored to measure serum AMH values in population who had undergone for abortion. In our study, the mean age group of patients was 30.65 ± 7.14 years (Right-sided bar; Figure-1) and median serum AMH concentration was 2.5 (2.1, 3.1) ng/ml. Our data thus suggested that the participants, in majority, had serum AMH concentrations within reference range.

Next we compared the serum AMH values between two groups; 'A' and 'B'. In this aspect, we observed that mean concentrations among females < 35-years (Group 'A') was 3.14 ± 1.5 ng/ml. In second group 'B' into which the females > 35-years-old were enrolled, the value remained 2.25 ± 1.25 ng/ml. Our finding, therefore, suggested the fact that subjects < 35-

years had higher levels of serum AMH compared to the second group of subjects > 35, and a significant difference was present therein. We, therefore, found a progressive rise in serum AMH from age < 35 years to that > 35. The present study thus shows a declining correlation of AMH as local women's age advances. Studies in mice and women had progressively shown that mean AMH levels remain constant at young age.[21] The researchers have explained this fact recently. It was suggested that compensatory mechanisms maintain the number of growing follicles at a constant level so that the mean serum AMH levels remain at young age.[10, 22, 23] Serum AMH then shows progressive decline.[24] Several studies have already established the fact that gradual decline of fertility starts at age of 30 years, and our results are concordant with this information.

According to results seen in our present study, there was a significant negative linear correlation between serum AMH concentration and basal mass index (BMI) (Data not shown). Women usually have higher BMI and several studies have explained this correlation.[22, 23] AMH thus tends to decline as a result of ageing and not to owing to the increase in BMI.

CONCLUSION

Our study established normal serum AMH values among different age groups in fertile women. Our findings are in agreement with data in the literature as serum AMH values decreased with age. We did not find undetectable AMH values among the participants. Thus it omits the practitioners in locality to be cautious when counselling patients will present very low or undetectable serum AMH concentrations because spontaneous pregnancy is still possible at the age of fertility. Studies including larger cohorts should be performed among fertile women in order to confirm the values found in our study.

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