CORRELATIVE ANALYSIS BETWEEN SERUM ANTI-MULLERIAN HORMONE CONCENTRATION AND LH:FSH RATIO AS WOMEN'S AGE ADVANCES: A HOSPITAL-BASED STUDY

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ABSTRACT

Introduction

A term 'ovarian reserve' is described as quantity of ovarian follicular cohort and quality of oocytes. The assessment of an individual's ovarian reserve comprises evaluation of certain variables like age, follicle stimulating hormone, leutinizing hormone, LH:FSH ratio and prolactin as main task. Recently, scientists have reported anti-mullerian hormone as potential test for ovarian reserve. Recently, it is observed that higher levels of AMH correlate positively with greater number of oocytes with then the improved embryo morphology in *in vitro* fertilization (IVF) cycles. However, controversy still exists between correlation of AMH and other hormonal tests.

Objectives:

To investigate correlation of AMH, FSH, LH, LH:FSH ratio and PRL in women who attended the local hospital.

Methodology

Venipuncture was performed to collect venous blood samples (*n=110*) under informed written consents. Following separation of sera, circulating levels of AMH was carried out by ELISA and assessment of FSH, LH and PRL were done using Dry chemistry immunoassay analyzer. Data were analyzed using SPSS software version 16.

Results

Our data demonstrated for existence of significant negative correlation between serum LH:FSH ratio and age. Here, the pattern of changes was in accordance to that of serum AMH concentration. So when we compared serum AMH concentrations with values of LH:FSH ratio in relation to age, the existence of a significant correlation was observed. Further, our findings demonstrate a degree of variations among other variables (such as LH, FSH and Prolactin) and are imperative in analytical point of view.

Conclusion

Our findings behind significant correlation between serum LH:FSH ratio and AMH level suggest that LH:FSH ratio can be a marker for ovarian reserve and applied to clinical evaluation with AMH for diagnostic purpose.

KEYWORDS

AMH, FSH, LH, LH:FSH ratio

INTRODUCTION

Infertility exists as clinical state where a couple at child-bearing age becomes unable to conceive baby even after one-year of unprotected sexual intercourse.[1] It has emerged these days as a social problem and exhibits communal effect on affected couples.[2] Clinicians therefore consider it as an area of interest. A male-dominant society persists in South Asian countries including a developing country like Nepal.[3] So, females hesitate to express their reproductive health concern despite the fact that such negligence may lead them to suffer from devastating consequences. These facts are worried to local socialism.

Biologically, a term called 'ovarian reserve' exists with respect to female's reproductive health concern. Human biologists describe it as a quantity of ovarian follicular cohort and quality of oocytes.[4] Assessing an individual's ovarian reserve comprise with evaluation of certain variables like age, follicle stimulating hormone (FSH), leutinizing hormone (LH), LH:FSH ratio and prolactin (PRL) as main task.[5] Recently, scientists have studied serum anti-mullerian hormone (AMH) as potential test for ovarian reserve.[6] Small antral follicles of reproductive-aged women secrete and granulose cells of ovary express AMH. Recently, it is observed that higher levels of AMH positively correlate with greater number of oocytes with then the improved embryo morphology in *in vitro* fertilization (IVF) cycles.[7-9] However, there has been a controversy between correlation of AMH and other reserve tests such as FSH, LH, LH:FSH ratio and PRL.[10-11] We, therefore, aimed to investigate the possibility of any correlation among AMH, FSH, LH, LH:FSH ratio and PRL in selected population of women who were normal ovulating and attended the hospital at eastern part of Nepal. Interestingly, among these various parameters, we found that serum AMH concentrations significantly correlate with LH:FSH ratio. Based on our findings, the other variables had also demonstrated a degree of changes and are imperative in analytical point of view. In earier study carried out by Lee JE *et al.*[5], circulating levels of AMH had correlated with LH:FSH ratio but, in our study, mean values of the variables of Nepalese women differed and was interesting to be elaborated in future study.

METHODOLOGY

Study Design and enrolment criteria:

This was a hospital-based study carried out in the Departments of Diagnostic Laboratory and Biochemistry at Birat Medical College Teaching Hospital, Tankisinuwari in conjuction with Nilkantha Diagnostic Laboratory, Kanchanbari, Biratnagar, Morang, Nepal. We analyzed Day-3 LH, FSH, PRL & AMH levels in 110 patients at duration between January and December, 2018. The age-range of these attending women was 25 to 45 years and they had regular menstruating cycles (interval: 25-40 days). The women who came for assessment of infertility had been registered as participants. 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.[12]

Evaluation of serum AMH levels stands as age-specific marker for ovarian reserve. So, based on age of the attendants, we arbitrarily categorized them into four groups (as Group 'A' = 25-30 years [*n* = 35], Group 'B' = 31-35 years [*n* = 28], Group 'C' = 36-40 years [*n* = 19] and Group 'D' = 40-45 years [*n* = 28]).

Sample collection and serum preparation:

Venipucture was performed to collect blood samples from January to December, 2018 under universal attentiveness as mentioned earlier.[13] Antecubital venous blood samples were collected from patients in plain vials with informed written consents, strictly as per the norms and approval of the Institutional Ethical Committee. Blood samples were allowed to clot for five minutes followed by centrifugation at 3000 rpm 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, as described earlier.[14] The kit was provided with micro-titre plate having 96 wells coated by monoclonal antibody against 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-raddish peroxidise 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.

Determination of serum LH, FSH and PRL levels using Dry-chemistry immunoassay analyzer:

LH, FSH and PRL were analyzed using reagent packs of ST AIA-PACK LH, FSH and PRL, respectively, in a fully-automated dry-chemistry immunoassay analyzer (AIA-360 Model, TOSOH, JAPAN). Each reagent pack of LH, FSH and PRL were provided with monoclonal antibodies against LH, FSH and PRL, respectively that were immobilized on magnetic solid phase enzyme-labelled monoclonal antibody. Based on manufacturer's protocol, the magnetic beads were washed to remove unbound enzyme-labelled monoclonal antibody followed by incubation with a fluorogenic substrate (4-methylumbelliferyl phosphate (4-MUP)). So, the amount of enzyme-labelled monoclonal antibody that binds to beads is directly proportional to concentration of analytes in test sample.

Prior to performing the test, a standard curve was constructed using a set of calibrators (provided from manufacturer). As stated, the instrument employed 50 µl serum for every assessment and results were then displayed after 10 minutes of incubation time. Based on standards set by precision of an instrument, the reference range of LH, FSH and PRL for ovulating females (during follicular phase) are 1.7-13.3 mIU/ml, 4.5-11.0 mIU/ml and 4.1-28.9 ng/ml, respectively.

Data interpretation:

The validity and reliability of test results were determined using standards supplied with a kit. Data were presented as mean±SD, analyzed under Software Package for Social Sciences version 16 (SPSS 16) and considered statistically significant with p<0.05 with implication of student's T-Test.

RESULTS

Following the collection of antecubital venous blood, we separated sera and performed immunoassay to assess distributive prominence of serum AMH, FSH, LH, LH:FSH ratio and PRL concentrations in local female attendants. In total of 110 participants, mean value for serum AMH, FSH, LH, LH:FSH ratio and PRL were 3.06±2.98 ng/ml, 10.18±3.35 mIU/ml, 7.60±3.09 mIU/ml, 0.75±0.11 and 13.14±1.73 ng/ml, respectively (Figure-1); indicating that subjects, in majority, had normal limits of such variables, except AMH - the level of which was higher than its reference range.

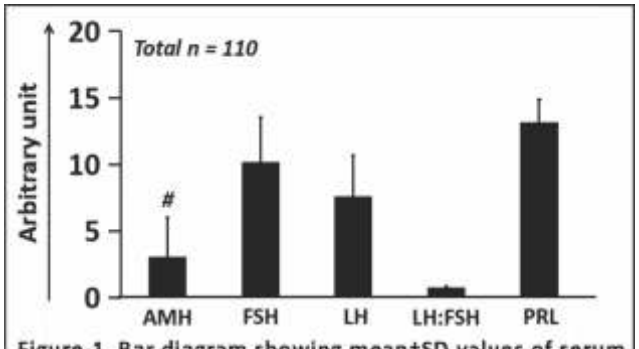

Figure-1. Bar diagram showing mean±SD values of serum AMH, FSH, LH, LH:FSH & PRL. Abbreviations 'n' and '#' denote for number of attendants and specific analyte-the level of which was higher than its normal range, respectively.

We observed that age of most attendants was 25-45 years. So they were separated into four sets as 'A', 'B', 'C' and 'D' having 25-30, 31-35, 36-40 and 41-45 years-old subjects, respectively.

Serum concentrations of AMH decline as an individual's age advances.[15] So, we evaluated these subjects to rule out the possible variations in serum concentrations of AMH with respect to age. On comparison, we found that serum AMH levels were 4.19±1.4, 3.54±1.4, 2.71±1.1 and 1.8±0.8 ng/ml among subjects in 'A', 'B', 'C' and 'D' sets, respectively (Figure-2). Our findings, therefore, were evident to the established fact that supports declining pattern of serum AMH concentrations as age advances.[15]

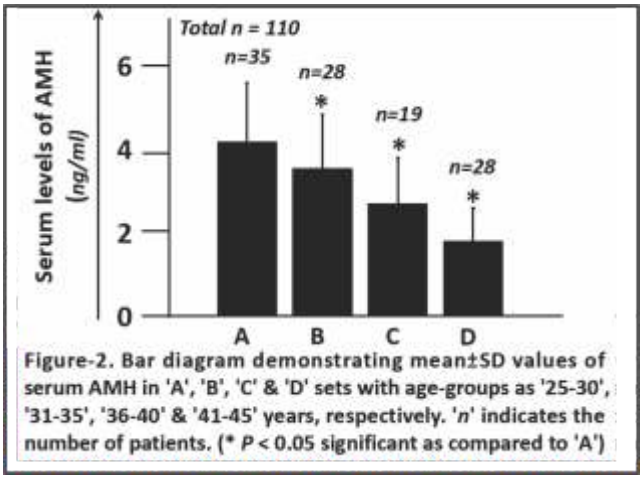

Figure-2. Bar diagram demonstrating mean±SD values of serum AMH in 'A', 'B', 'C' & 'D' sets with age-groups as '25-30', '31-35', '36-40' & '41-45' years, respectively. '*n*' indicates the number of patients. (* *P* < 0.05 significant as compared to 'A')

Figures-3 and 4 demonstrate distribution of FSH and LH, respectively, in relation to age variation. Statistically, a lack of significant difference was observed when we compared serum FSH and LH values of females in group 'B' with 'A'. Fortunately, the attending women in 'C' & 'D' had significantly higher circulating levels of FSH compared to 'A' (Figure-3). We found a lack of significant difference at $p<0.05$ of serum LH when the females under 'C' and 'A' groups were compared. But, our data showed that elder women with age-group of 41-45 years in set 'D' had higher serum LH concentration as compared to 'A'.

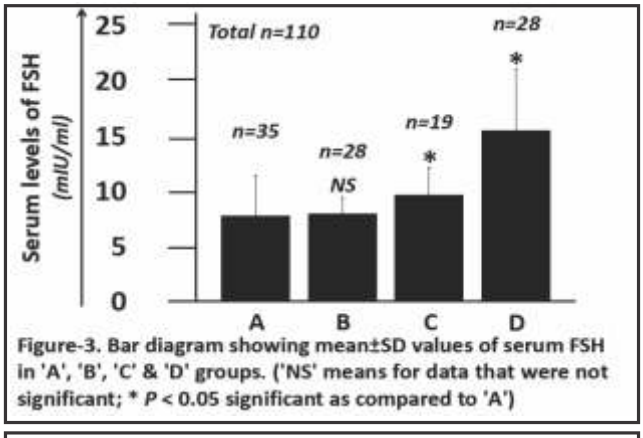

Figure-3. Bar diagram showing mean±SD values of serum FSH in 'A', 'B', 'C' & 'D' groups. ('NS' means for data that were not significant; * *P* < 0.05 significant as compared to 'A')

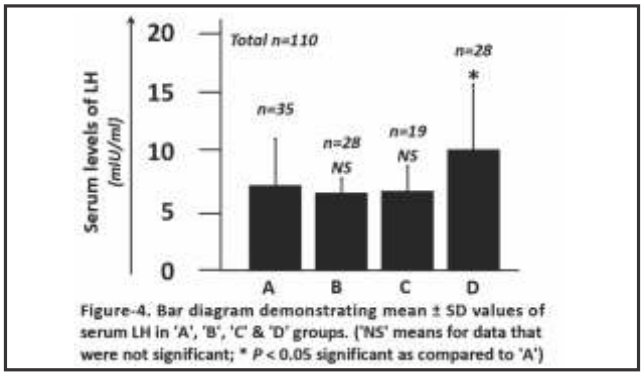

Figure-4. Bar diagram demonstrating mean ± SD values of serum LH in 'A', 'B', 'C' & 'D' groups. ('NS' means for data that were not significant; * *P* < 0.05 significant as compared to 'A')

Figure-5 shows distributive pattern of LH:FSH ratio according to age. We observed significant negative correlation between serum LH:FSH ratio and age. Here, the pattern of changes was in accordance with that of serum AMH concentration. So when we compared serum AMH concentrations with values of LH:FSH ratio in relation to age, the existence of a significant correlation was observed (Figure-7 & 8).

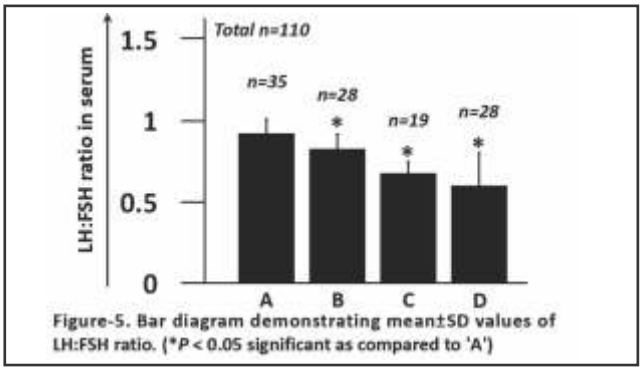

Figure-5. Bar diagram demonstrating mean±SD values of LH:FSH ratio. (*$P < 0.05$ significant as compared to 'A')

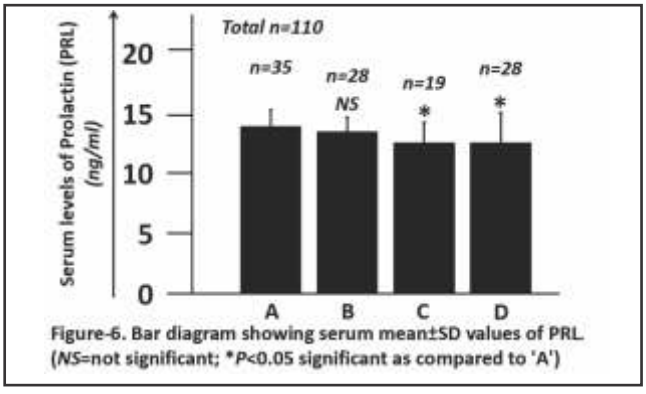

Figure-6. Bar diagram showing serum mean±SD values of PRL (*NS*=not significant; *$P<0.05$ significant as compared to 'A')

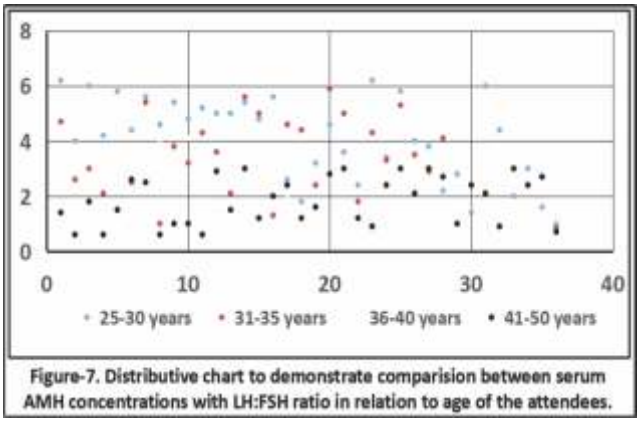

Figure-7. Distributive chart to demonstrate comparision between serum AMH concentrations with LH:FSH ratio in relation to age of the attendees.

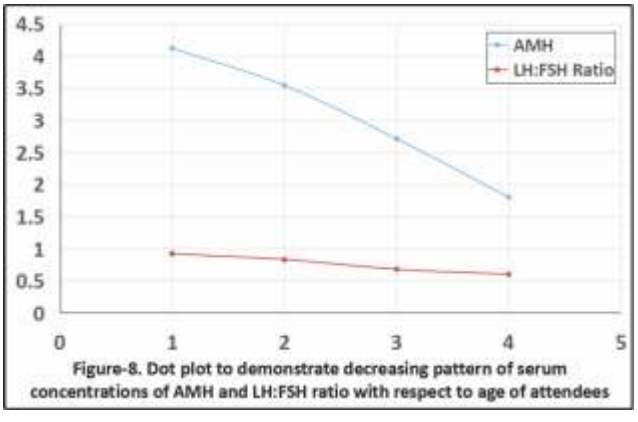

Figure-8. Dot plot to demonstrate decreasing pattern of serum concentrations of AMH and LH:FSH ratio with respect to age of attendees

In next, figures-6 shows distribution of PRL with respect to age. Statistically, we did not find the significant difference at $p<0.05$ when serum PRL concentration of females under group 'B' was compared with 'A'. Providentially, the attending women in 'C' & 'D' had lesser PRL concentration compared to 'A'.

DISCUSSION

In spite of having lower prognostic value, clinicians still use serum FSH and LH concentrations to predict ovarian reserve at early follicular phase.[4, 16-17] Researchers have recently reported that elevated Day-3 FSH:LH ratio correlates with higher rates of cancellation in IVF-embryo transfer cycles.[18-19] Based on few studies, one can use a day-3 FSH:LH or LH:FSH ratio as predictor of ovarian reserve.[20-21] Serum concentration of FSH increases early in reproductive age and a rise of LH occurs at later. So, a decreased LH:FSH ratio might be a sign of diminished ovarian reserve even if female has normal basal FSH level. LH:FSH ratio decreases as ovarian age declines. So, evaluation of LH:FSH ratio can play a significant role in determining appropriate status of ovarian reserve. In our study, we obtained a reduced value of LH:FSH ratio as women's age advances. We found significant negative correlation and so suggest that LH:FSH ratio correlates with age and was in accordance with the reports from previous studies.[8-10]

Serum AMH concentrations diagnostically exist as marker of ovarian reserve.[13] Based on our study, serum concentration of AMH falls as age advances. So, we observed significant correlation between AMH and LH:FSH ratio (Figures-2 & 5).

Clinicians commonly use LH:FSH ratio in assessing the polycystic ovarian syndrome (PCOS) although we could not prove the sufficient utility of it in our study.[22-24] In infertility, researchers have shown the highest correlation of LH:FSH ratio with clinical pregnancy over other measures of ovarian reserve.[5, 25-27] Based on certain studies, a clinician can use high FSH:LH ratio as biomarker of poor ovarian reserve.[28-30] An elevated Day-3 FSH:LH ratio is useful in prediction of IVF outcome among older women, but it does not appear as accurate predictor as scientists have not shown a detailed analysis of it in accordance with age. Based on few studies, combination of LH and FSH at Day-1 of menstrual cycle can be employed to predict reproductive age. We therefore divided a total of 110 attendees into four groups as 'A', 'B', 'C' & 'D' that comprise of 25-30, 31-35, 36-40 and 40-45 years-old aged women, respectively. Investigation was then followed to present age-specific levels and compare LH:FSH ratio with serum concentrations of AMH. To the best of our knowledge, this is first study to report on correlation between LH:FSH ratio and serum AMH concentrations in eastern Nepal.

The clinical usefulness of AMH has been confirmed earlier in numerous studies.[9, 12] In present study, our data support clinically the valuable meaning of LH:FSH ratio as we can consider this fraction along AMH as useful marker for ovarian reserve. As Day-3 serum FSH and LH concentrations are already available biomarkers, their ratio could be used without additional effort. Instead of serum AMH concentrations, LH: FSH ratio could be an age-related reference value for ovarian reserve in women of reproductive age and we can apply it in clinical evaluation for infertility work-up. Most of all, we can predict the ovarian reserve using commonly checked tests with a higher accuracy in women of reproductive age. Especially in women of infertile patients,

the age-specific value of LH:FSH ratio may have a role in determining the appropriate stimulation protocol and assist in patient counselling. LH:FSH ratio may allow physician to stimulate patients in more individualized treatment and will maximize the IVF outcome. Although further active and prospective studies could be necessary to confirm such a role of LH:FSH ratio in clinical setting, our results will be helpful to evaluate the ovarian reserve of women.

CONCLUSION

Based on present study, a significant negative correlation exists between serum LH:FSH ratio with age and, in addition, we found the significant partial correlation between serum LH:FSH ratio & AMH level. These findings, therefore, suggest that LH:FSH ratio can be a marker for ovarian reserve and applied to clinical evaluation with AMH for diagnostic purpose.

RECOMMENDATIONS

There have been few studies about LH:FSH ratio and no age-related reference values were provided in large-scaled population. The strength of our study is that we compared LH: FSH ratio with AMH values in larger size.

LIMITATIONS OF THE STUDY

The limitation could be as we just evaluated the correlation between LH:FSH ratio and age. Further investigations combined with the present correlation results are needed on direct ovarian response to a controlled ovarian stimulation according to LH: FSH ratio. This is necessary to estimate the value of markers of exact ovarian reserve.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCIAL DISCLOSURE

None.

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