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■ Original Research Article

Ethnic Differences in Anti-mullerian Hormone and Ovarian Reserve Retrospective Analysis of Indian and Ethiopian Women

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ABSTRACT

Background: There is a growing trend amongst many women of the reproductive age group to delay childbearing, and interest in assisted reproductive technologies (ART). Ethnic variations in anti-Mullerian hormone (AMH) levels may influence fertility and ART outcomes. ***Aims:*** To determine the prevalence of low AMH levels and poor ovarian reserve in Indian and Ethiopian women and identify ethnic differences in AMH in these populations. ***Settings and Design:*** Retrospective analysis of records of patients undergoing In-Vitro fertilization (IVF) at a fertility clinic in North India from January 2018 to March 2020. **Materials and Methods:** This study included 120 Indian and 86 Ethiopian women undergoing IVF due to female-cause infertility. Cases of polycystic ovarian syndrome, endometriosis, previous adnexal surgery or pelvic inflammatory disease were excluded. Serum AMH levels were estimated using a chemiluminescent immunoassay (CLIA). AMH levels = 2ng/ mL were considered to signify poor ovarian reserve.

Statistical Analysis Used: Comparison of groups was done using a Mann-Whitney U test. Spearman correlation coefficients were calculated for two continuous variables. Categorical variables were compared using a Chi-Square test. **Results:** AMH levels were significantly higher amongst Indians compared to Ethiopians. AMH levels declined with age overall, though the negative correlation of AMH with age was stronger in Ethiopians. ***Conclusions:*** This study demonstrates that significant differences exist between Indians and Ethiopians in serum AMH level, an ovarian reserve marker. Ethnicity may play a role in ovarian reserve and should be considered during patient counselling and may be useful for personalizing treatment.

Keywords: Anti-Mullerian Hormone, Ovarian Reserve, Ethnicity, Infertility

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Introduction

Anti-Mullerian hormone (AMH) or Mullerian inhibiting hormone plays a role in the post-pubertal female in the regulation of folliculogenesis. AMH is solely produced in the human ovary by granulosa cells, during the early stages of follicle development.[1] Levels rise progressively following birth, plateauing by early adulthood, followed by a steady decline. Up to 5 years before menopause, as the ovarian stock of follicles gets exhausted, AMH concentrations eventually become undetectable.

Soon after recruitment from the primordial follicle pool, the expression of AMH begins in the primary follicles, with the strongest expression occurring during the antral stage. AMH levels have thus been suggested to indicate the number of early small growing follicles. It acts as an effective indicator of ovarian reserve, by reflecting the size of the pool of resting primordial follicles.[2,3] AMH is now a widely used and useful serum marker for measuring the decline of the ovarian pool with age. It has a well-established role in predicting the reproductive life-span, and the ovarian response of patients in the context of in-vitro fertilization (IVF).

AMH assessment can enable physicians to modify treatment plans by predicting poor ovarian response or hyper response. Recent studies have also demonstrated the role of AMH in several other ovarian pathologies, such as polycystic ovarian syndrome, and also in the diagnosis of oligomenorrhea and amenorrhea.[4] AMH evaluation has also been suggested to have a potential role in monitoring ovarian toxicity due to gonadotoxic treatment or radiation, and for the follow up of patients with granulosa cell tumours.[5,6]

It is becoming increasingly clear that reproductive function in females varies by race. Ethnicity plays a role in the differing prevalence of various gynaecological conditions and may likewise influence ovarian reserve. The burden of infertility is on the rise, in countries with lower socio-demographic indices, leading to an increased demand for assisted reproductive technologies (ART).[7] Ethnicity may, therefore, be of significance in categorizing patients when evaluating ovarian reserve and establishing a prognosis. This study aimed to determine the prevalence of poor ovarian reserve in two populations of Indian and Ethiopian women seeking IVF treatment for infertility. Ethnic differences in AMH levels between these two populations are explored.

Methodology

This study was a retrospective analysis of records, conducted at a metropolitan in-vitro fertilization clinic in Northern India.The study population consisted of Indian and Ethiopian women undergoing IVF treatment, over a two-year period from January 2018 to March 2020. Records of Ethiopian women included in the study were of patients who reported to outreach clinics in Ethiopia and then underwent fertility treatment in India. The study was conducted as per the tenets of the Declaration of Helsinki after obtaining ethical approval. Patient consent was not required for analysis of retrospective data.

Relevant clinical history and demographic details, along with serum AMH values were analysed. No previous data existed for reference in sample size calculation. Accordingly, sample size for comparison of continuous measures in two independent samples was calculated. Effect size was taken to be 0.5, with á = 0.05 and â = 0.2, and the required sample size was 63 each in the Indian and Ethiopian arms, with a total of 126.This study included 120 Indian and 86 Ethiopian women.

Inclusion and Exclusion Criteria

This study included patients <50 years of age, with both ovaries intact, undergoing IVF for any female-cause infertility. Women who were known cases of polycystic ovarian syndrome or endometriosis were excluded from the analysis. Patients with a history of smoking, pelvic inflammatory disease or previous adnexal surgery were also excluded.

Sample Collection and Analysis

Venous blood samples were collected through venipuncture, centrifuged and assayed on the same day soon after collection. Serum AMH values were

estimated using a paramagnetic particle chemiluminescent immunoassay (CLIA) on a fully automated analyser (Immunotech; Beckman Coulter) as per manufacturer instructions. All samples were estimated using the same assay. Limit of quantitation (LoQ) for the assay was = 0.08 ng/mL. An AMH value of = 2.00 ng/mL was taken to signify poor ovarian reserve.

Statistical Analysis

Statistical analysis was performed on the IBM statistical package for the social sciences (SPSS) for Window, Version 24.0 (Armonk, NY: IBM Corp). Continuous data is expressed as Mean ± Standard Deviation (SD) or Median ± Interquartile range (IQR) as appropriate. Categorical variables are expressed as percentage with 95 % confidence interval (CI). Normality of continuous measures was assessed using a Shapiro-Wilk test. Comparison of groups was done using an independent samples t-test or Mann-Whitney U test as appropriate. Correlation coefficients were calculated for two continuous variables using the Spearman correlation. Categorical variables were compared using a Chi-Square test. A p-value of <0.05 was taken to be statistically significant.

Results

This study consisted of 206 subjects, with 120 Indian and 86 Ethiopian women. The average age of the total study population was 33.6 ± 5.2 years. The average age of Indian women was 32.6 ± 4.3 years and that of Ethiopian women was 34.9 ± 6.0 years. The overall average AMH value was 1.63 ± 2.93 ng/mL (Median ± IQR).

The prevalence of poor ovarian reserve (AMH <2ng/mL) in Indians was 49.1%, (95% CI = 40.3% 58.0%) and in Ethiopians was 63.95%, (95% CI = 53.4% - 73.3%).

A Mann Whitney U test was applied to compare AMH values between the Indian and Ethiopian arms [Table 1]. Indians on average had a significantly higher AMH level than their Ethiopian counterparts.

Table 1: AMH Levels in Indian Vs. Ethiopian Women

Ethnicity	AMH (ng/mL) Median (IQR)	Significance*
Indian	2.03 (2.66)	U=4281.0, p=0.037*
Ethiopian	1.14 (3.51)	

*Mann Whitney U test

Amongst Indians, 59 out of 120 patients had low AMH levels, compared to 55 out of 86 Ethiopian patients. A Chi Square test was applied to compare the proportions. Ethiopians were 1.83 times more likely have low AMH levels as compared to Indians, and this difference was statistically significant [Table 2].

Table 2: Chi-Square Test and Odds Ratio for Ethnicity Vs. AMH Levels

Ethnicity	AMH Levels		Total (n)	X^2	Odds Ratio	95% Confidence Interval		*P**
	≤2ng/mL	>2ng/mL				Lower	Upper	
Ethiopian	55	31	86					
Indian	59	61	120	4.43	1.834	1.04	3.23	0.035*
Total (n)	114	92	206					

*Chi-Square test

A correlation coefficient was computed to demonstrate the relationship between patient age and AMH levels. Overall, AMH levels and patient age showed a statistically significant negative correlation, ($r = -0.494$, $p<0.001$). Similarly, age correlated negatively with AMH levels when Indian ($r = -0.381$, $p <0.001$) and Ethiopian ($r = -0.586$, $p<0.001$) patients were analysed separately [Figure 1].

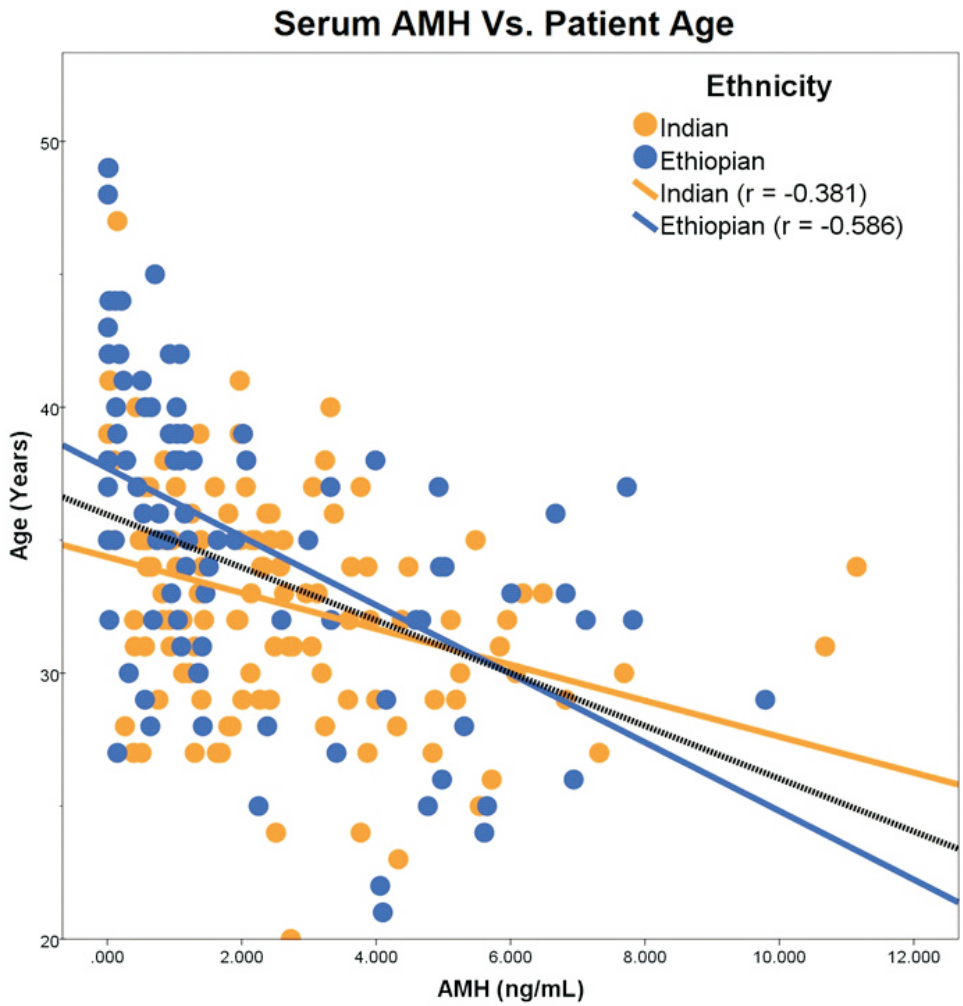

Figure - 1: Scatterplot for AMH Levels Vs. Patient Age for Indians and Ethiopians

All study subjects were stratified as per age into three groups, of 30 years or below (n=56), 31 to 35 years (n=84), and 36 years or above (n=66). A Kruskal Wallis test applied to the three age groups showed that median AMH levels significantly increased with increasing age groups ($H = 38.23$, $p < 0.0001$).

AMH levels were compared between Indian and Ethiopian women after stratifying into three age groups using a Mann-Whitney U test. Average

AMH levels were higher amongst Indians in the 31-35 years, =36 years groups, but lower in the =30 years group. However, statistically significant differences between the two ethinic groups no longer existed after age stratification [Table 3, Figure 2]. Likewise, a Chi-Square test was performed to assess the proportions of patients with low AMH (=2 ng/mL) of either ethnicity across age groups. While overall proportion of Ethiopians with low AMH was significantly greater than that of Indians, significant differences did not exist for the individual age strata [Table 4].

Table 3: AMH Levels in Indian Vs. Ethiopian Women Stratified by Age

Age Group	AMH (ng/ mL)		Significance*
	Indian	Ethiopian	
≤30 Years	2.96(3.21)	3.73 (3.87)	P = 0.758
31-35 Years	2.14 (2.43)	1.51 (3.72)	P = 0.954
≥36 Years	1.25 (2.22)	0.65 (1.01)	P = 0.181

*Mann Whitney U test

Table 4: Chi-Square Test for AMH Levels in Indians Vs. Ethiopians Across Age Groups

Age Group	AMH	Indian	Ethiopian	Total	Significance*
≤30 Years	≤2 ng/mL	12	7	N=56	0.900
	>2 ng/mL	14	13		
31-35 Years	≤2 ng/mL	28	16	N=84	0.385
	>2 ng/mL	29	11		
≥36 Years	≤2 ng/mL	19	32	N=66	0.266
	>2 ng/mL	8	7		
All Ages (Total)	≤2 ng/mL	59	55	N=206	0.035*
	>2 ng/mL	61	31		

*Chi-Square test

Discussion

Major variability exists between individuals in the rate of follicle pool depletion, and consequently the age range at which menopause occurs. Intrinsic variations in AMH levels exist in women even of the same chronological age, making it a predictor for the remaining duration of reproductive life. Each stage of the reproductive period is affected by environmental factors that are influenced by genetics and ethnicity.[8] This study demonstrates that significant differences exist between women of Indian and Ethiopian origin in serum AMH level, an

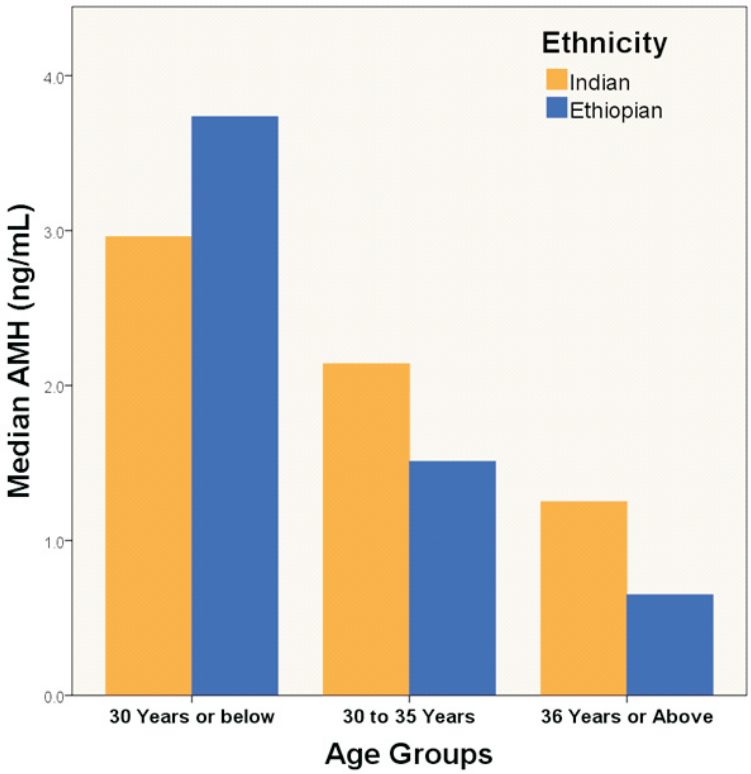

Figure - 2: AMH levels in Indian Vs. Ethiopian Women Across Age Groups

ovarian reserve marker. About 49% of the Indian and 64% of the Ethiopian patients had sub-optimal AMH levels of below 2ng/mL. Indian women on average had significantly higher AMH levels compared to Ethiopian women in this study. Expectedly, AMH levels showed a strong negative correlation with age. AMH levels also declined significantly with age when the Indian and Ethiopian populations were analysed independently, with a stronger negative correlation seen among Ethiopian women. However, on stratifying by age-group, the differences between the two ethnic sub-groups were not very significant. Our data suggests that ethnicity may be an important factor when considering age-related decline in AMH and ovarian function.

There is a growing trend amongst many women of the reproductive age group to delay childbearing. As a result, interest in fertility and ART has been on the rise. Serum AMH levels are used as a predictor for quantitative aspects of controlled ovarian stimulation (COS). Ovarian hyper-responses are known to lead to lower probability of pregnancy, poor-quality embryos and cycle cancellations. Higher basal AMH levels also put the patient at risk for ovarian hyper-stimulation syndrome.[8] Similarly, low AMH levels are useful for predicting a poor ovarian response to gonadotropins in COS, leading to lower numbers of oocytes retrieved.[10] Ethnic and racial influences may also be considered risk factors for diminished ovarian reserve, and AMH levels alongside ethnicity and patient age can be used to assess the probability of conception. Our study findings may help physicians to appropriately counsel women according to their age and ethnic background, as inherent biological variations may warrant intervention at earlier stages.[8,9]

Previous studies have shown ethnicity to considerably affect ART outcomes. Compared to Caucasians, almost all races have been demonstrated to have lower birth rates following ART.[11] Another study showed that women of Indian origin had significantly lower live birth rates compared to white American counterparts.[12] Certain ethnic groups including Asian, African, and Hispanic women have been shown to have lower live birth rates and higher miscarriage rates after ART than whites.[13] Serum AMH levels have also been proven to be lower in African and Hispanic women compared to white women.[14] This study is amongst the first to compare AMH levels in women belong to South-Asian Indian and Ethiopian populations. It is becoming increasingly clear that ethnicity plays an independent role in declining ovarian function and AMH levels with age.

Race dependent differences also exist in the reproductive life cycle and span. Puberty onset is known to play a role in influencing serum AMH levels. Earlier puberty has been observed in African American compared to white children.[15] Genetic regulation of puberty has been reported to account for at least 50% of the variability in puberty onset timing, besides environmental factors.[16] Age at menopause is an extremely significant determinant for the length of the reproductive cycle and fertility. Time to natural menopause was reported to be four times faster in blacks than in whites, with black women entering menopause two years earlier.[17] Studies have also suggested that sunlight exposure and therefore vitamin D status may be a positive regulator of AMH production.[18] However conflicting studies have also shown no relationship with ovarian reserve or ovarian response after stimulation.[19] This study consists of two populations that are categorically located in tropical latitudes with seemingly adequate sunlight exposure. Further studies to elucidate the role of vitamin D levels on fertility may be warranted.

Lastly, dietary factors may also play a role in determining ovarian reserve in two ethinic populations with diverse dietary habits. AMH concentrations have been showed to be positively associated with percentage energy from dietary carbohydrates, and negatively with increasing levels of omega-6 fatty acids.[20] The prevalence of obesity is on the rise worldwide, primarily due to lifestyle changes. Besides cardiovascular co-morbidities, obesity has a considerable impact on reproductive health in women. Excess adiposity has been shown to compromise adiposity in women belonging to the reproductive age group.[21] Further research is needed to elucidate whether ethnic differences in ovarian reserve are determined genetically or if other contributory environmental, nutritional or lifestyle factors play a role. Moreover, studies involving multiple ethnicities are warranted especially in the present day, to enable physicians to make better informed decisions for therapeutics.

Limitations

Retrospective data on anthropometric variables such as weight, height and body mass index were unavailable and could not be included in the analysis in this study. The antral follicular count obtained by transvaginal ultrasonography is also an effective marker to quantify the ovarian response to stimulation, and could not be included in this study. Further studies are needed including multiple ethnicities, especially in under-studied populations worldwide.

Conclusion

AMH levels varied significantly between Indian and Ethiopian women. AMH levels also declined with age independently in both Indian and Ethiopian women, but did not vary significantly between respective age groups of both ethnicities. Ethnicity may play a significant role in determining ovarian reserve. Understanding of ethnic variations can help in patient counselling about fertility options as per age group. Further research is needed especially amongst less-studied populations to explore the role of ethnicity in categorizing patients seeking IVF treatment, and to personalize treatment modalities.

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