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Application of machine learning tools for evaluating the impact of premenopausal hysterectomy on serum anti-mullerian hormone levels

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Women who have had premenopausal total hysterectomy at a young age could probably experience partial or total loss of ovarian function. The purpose of this retrospective cross-sectional study is to investigate the ovarian function in women underwent hysterectomy at an early age. A total of 1165 subjects comprised of 685 hysterectomised women and 480 age matched controls were enrolled in the study. We found that there is a steady decline in serum Anti Mullerian Hormone (AMH) levels, a marker of ovarian function after every five years post - hysterectomy in early age groups (20-30 years and 31-40 years) followed by loss of ovarian function in the age group of 40-50 years. The application of multiple linear regression and machine learning tools has revealed that AMH is positively correlated with LH and estradiol and negatively correlated with age, FSH, years since hysterectomy and vitamin D. Serum AMH level of <0.08 ng/mL is associated with the increased of FSH, decreased LH and estradiol. The decreased ovarian function is associated with lower calcium levels, which are likely to influence the bone health. In conclusion, by utilizing multiple linear regression and machine learning tools, we found that serum FSH is the most important in predicting the AMH-mediated ovarian function.

Keywords: Anti-Mullerian Hormone (AMH), Hysterectomy, Pre-menopause, Ovarian function, Oxidative stress

Hysterectomy is a procedure for surgical removal of uterus, one of the common gynecologic procedures in medical practice. Hysterectomy before natural menopause, is one of the major gynecological procedures in reproductive-age women, the hysterectomy rate increases as women get older and peaks between the 40 and 50 years for benign gynecological indications mainly uterine leiomyoma’s, dysfunctional uterine bleeding and endometriosis to improve the quality of life[1,2]. Hysterectomy with oophorectomy in premenopausal women results in an abrupt hormonal imbalance, sudden onset of menopausal symptoms and may lead to cardiovascular risk, neurodegenerative disease and osteoporosis[3-9].

In 2003, over 600000 hysterectomies were performed in the United States alone, of which over 90% were performed for benign conditions[10]. The analysis from UK suggest a hysterectomy rate of 42/100000 population, Canada (108/100000), whereas in Germany reporting rates of 236/100000 and Australia 165/100000[11,12]. Hysterectomy rates in women aged 25 years and over have declined in the first decade of the 21st century. However, in the last 5 years, the rates appear to have stabilized[12-14].

Unlike developed countries, many Indian rural women undergo hysterectomy with or without oophorectomy before 40 years of age for benign gynecological complaints thus experience the early onset of menopause symptoms compared to non-hysterectomised women. The National Family Health Survey-4 in India provided the first nationally representative estimates of hysterectomy among the women aged 15–49 years. The prevalence estimates were highest in four states, where the proportion of hysterectomy cases among the women aged 40–49 years was: Andhra Pradesh (22.4%), Bihar (14.5%), Gujarat (12.6%), and Telangana (20.1%). Almost one-half (46.1%; 95% CI 44.8–47.5) of women who reported having undergone hysterectomy had already been previously sterilized, as reported for contraception use[15].

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Abbreviations: AMH, Anti Mullerian Hormone; BMI, body mass index; Chol, cholesterol; E2, estradiol; FSH, Follicle stimulating hormone; Hb, hemoglobin, HDL-Chol, high density lipoprotein cholesterol, LH, luteinizing hormone; MDA, malondialdehyde MLR, multiple linear regression; TGL, triglycerides; WC, waist circumference; YSH, years after hysterectomy

In our preliminary study, we found that women with poor socioeconomic status and less literacy are preferring hysterectomy as final remedy for benign gynecological complaints instead of other alternate medical treatment[16]. Till now, very limited data is available on premenopausal hysterectomy associated effects on ovarian function and health outcome[17-19]. Studies have shown that the determination of serum AMH levels is the direct predictor of menopause and ovarian function[20,21]. In a study, it was demonstrated that the serum AMH levels of < 0.2 ng/mL occur on average 5.99 years preceding to menopause in women aged 45-48 years and 9.94 years in women aged 35-39 years[22]. Hence a retrospective cross-sectional study was carried out in women with premenopausal hysterectomy to assess the serum AMH levels, a marker of ovarian function.

Materials and Methods

The retrospective cross-sectional study was conducted on hysterectomised women from October 2015 to June 2018 to assess the impact of premenopausal hysterectomy on ovarian function and associated health related consequences. This study was approved by Institutional Ethics Committee (IEC), Nizam's Institute of Medical Sciences (EC/NIMS/1478/2014), Hyderabad, India

Hysterectomy Group

Women who have undergone hysterectomy for benign gynecological indications.

Control Group

Age-matched women with intact reproductive organs were considered as control Subjects.

Inclusive criteria

Women, who had completed the family, undergone bilateral tubal sterilization and non-smokers with no history of any type of cancer, were included as the cases.

Exclusion criteria

Women with type 2 diabetes mellitus, hypothyroidism, past history of cancer followed by radiation/ chemotherapy and debilitating illness *etc*.

A total of 1165 women were enrolled into this study. Hysterectomised women were recruited through field survey in rural areas of Nalgonda and Yadadri districts of Telangana state, India by snowball technique and age-matched control subjects were enrolled from their neighbour houses or associates in same geographical area. Blood samples from control subjects were collected on day 2 - 4 of their menstrual cycle.

All the participants were interviewed with the support of female health worker (Like Asha worker/village level volunteers) and informed consent was obtained prior to enrolment. As part of study, participants' demographic information was collected with the help of IEC approved questionnaire. From each participant, 8 mL of fasting blood sample was collected for assessing biochemical parameters (*i.e*. fasting blood glucose, total cholesterol, HDL cholesterol, triglycerides, calcium and hemoglobin) and hormones (*i.e*. FSH, LH, TSH, estradiol and AMH). Following centrifugation at 3000 × g for 15 min, serum samples were separated and two aliquots of the serum were stored at −80°C for hormones, 25-OH-Vitamin D3 and MDA (malondialdehyde) estimations. All other biochemical and hematological investigations were performed on the same day as per the standard protocols.

Blood hemoglobin level was estimated by spectrophotometric Drabkin's method and fasting glucose, lipid profile and serum calcium were estimated by using Roche C411 fully automated chemistry analyzer. Follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and thyroid stimulating hormone (TSH) levels estimated were by using commercially available kit (Cal Bio ELISA kit), whereas anti mullerian hormone (AMH) levels were estimated by using ELISA kit (Ansh Labs, USA).

Serum 25-hydroxy vitamin D3 (25-OH Vit D3) levels were estimated by high performance liquid chromatography (HPLC) as per the protocol of Galunska BT *et al*.[23]. Serum malondialdehyde level was determined by HPLC as per the protocol of Ana-Marija Domijan *et al*.[24] and reconfirmed by TBARS spectrophotometric method[25].

Statistical analysis

Student t-test was performed to assess whether the mean difference between the two given groups is statistically significant. Analysis of variance (ANOVA) was used if there were more than two groups. Fisher exact test was used to calculate odds ratio (OR) and 95% confidence interval (CI) based on the distribution of categorical variable in two groups. Multiple linear regression (MLR) equation was deduced by applying the $y = m_1x_1 + m_2x_2 + \ldots\ldots\ldots\ldots + m_nx_n + C$ formula where in 'y' represents a dependent variable (outcome variable) and '$x_1, x_2, \ldots, x_n$' represent independent variables likely to influence the dependent variable (input variables).

The values of m_1, m_2, ...……., m_n represent the contribution of each variables towards the outcome. In order to delineate very complex inter-relationships, we have used machine learning based-association rules, which were based on 'IF' and 'THEN' rules[26]. The confidence, support and leverage were used as performance indicators for these association rules. Further, each of these rules was cross-verified based on Fisher exact test for accuracy, sensitivity, specificity apart from determining the odds ratio and 95% confidence interval.

Results

As shown in (Table 1), the demographic details of the study groups were collected. No statistically significant difference was observed between the hysterectomy with or without ovarian function in terms of age, body mass index (BMI), waist circumference (WC), and age at hysterectomy. The percentage of women with premenopausal hysterectomy without ovarian function was high in the age group of 31-35 years and 36-40 years compared to premenopausal hysterectomy with ovarian function (Table 1).

As depicted in (Table 2), AMH levels were used as markers of ovarian function with AMH below detection limit as ovaries without function and categorized as hysterectomy without ovarian function. Serum FSH and LH levels were elevated and estradiol levels were low in hysterectomy group without ovarian function (Table 2). Serum vitamin D3 levels were found to be low in hysterectomy group as compared to control. In addition, we found increased oxidative stress in hysterectomy groups in particularly pre-menopausal hysterectomy group as compared to control.

There was a significant and steady decline in serum AMH and vitamin D levels in patients with increasing years after hysterectomy (Table 3).

As shown in (Fig. 1), the post-hysterectomy duration influence on ovarian function in pre-menopausal women was assessed. When compared to controls, there is a steady decline in ovarian function after every five years post - hysterectomy in early age groups (20-30 years and 31-40 years) followed by loss of ovarian function in the age group of 40-50 years. Hence, pre-menopausal hysterectomy accelerates the early menopause. AMH is positively correlated with

Table 1 — Demographic details of the studied subjects

Parameter	Premenopausal Group			Post-Menopausal Group	
	Control	Hysterectomy with ovarian function	Hysterectomy without ovarian function	Control	Hysterectomy
Demographic Information					
No. of Cases	301	380	131	179	174
Age (years)	31.7 ± 5.5	34.6 ± 4.9	34.5 ± 4.1	50.8 ± 3.5	51.4 ± 3.3
BMI (Kg/m^2)	23.2 ± 4.8	23.1 ± 4.4	22.8 ± 3.8	23.9 ± 5.4	23.5 ± 4.7
WC (cm)	79 ± 8.3	81 ± 10.2	84 ± 9.5	78 ± 9.8	83 ± 10.4
Age at Hysterectomy					
<20 Years.	--	4(1.1%)	0(0%)	--	0(0%)
20 - 25 Years.	--	148(38.9%)	46(35.1%)	--	1(0.6%)
26 - 30 Years.	--	164(43.2%)	53(40.5%)	--	2(1.1%)
31 - 35 Years.	--	47(12.4%)	23(17.6%)	--	7(4.0%)
36 – 40 Years.	--	17(4.5%)	9(6.9%)	--	29(16.7%)
41-45 Years.	--	0(0%)	0(0%)	--	86(49.4%)
>45 Years.	--	0(0%)	0(0%)	--	49(28.2%)
Level of Education					
No Schooling	187(62.3%)	203(53.4%)	87(66.4%)	124(69.3%)	139(79.9%)
Primary Schooling	48(16%)	126(33.2%)	28(21.4%)	37(20.7%)	16(9.2%)
Secondary School	56(18.7%)	43(11.3%)	14(10.7%)	13(7.3%)	14(8.0%)
Higher education	9(3%)	8(2.1%)	2(1.5%)	5(2.8%)	5(2.9%
Place of Surgery					
Govt Hospitals	--	78(20.5%)	21(16.2%)	--	42(24.1%)
Insurance Hospital	--	13(3.4%)	2(1.5%)	--	13(7.5%)
Private Hospital	--	289(76.1%)	107(82.3%)	--	119(68.4%)

Table 2 — Biochemical parameters of premenopausal group were compared with respective age matched control group

Parameter	Hysterectomy (N =380) (a)	Hysterectomy without ovaries function (N =131) (b)	Control (N=301) (c)	*P*-value
Hemoglobin (g/dL)	11.7 ± 1.8	11.9 ± 1.3	11.8 ± 1.2	a - 0.314 b - 0.8113
Glucose (mg/dL)	90 ± 24.5	94 ± 24.3	89 ± 20.0	a - 0.6766 b – 0.0211*
Total Cholesterol (mg/dL)	135 ± 41.9	138 ± 40.5	138 ± 39.8	a - 0.4242 b - 0.9808
HDL Cholesterol (mg/dL)	35.8 ± 7.6	37.9 ± 6.4	37.0 ± 6.4	a - 0.0269* b - 0.1575
Calcium (mg/dL)	8.8 ± 1.6	8.3 ± 0.8	8.9 ± 1.2	a - 0.0638 b - 0.2879
AMH (ng/mL)	1.57 ± 1.90	BLD	2.63 ± 1.93	a - < 0.0001*
FSH (mIU/mL)	9.9±8.5	69.6±20.8	14.4±9.8	a - < 0.0001* b - <0.0001*
LH (mIU/mL)	16.4 ±16.8	46.4 ±18.7	23.9 ±20.5	a - < 0.0001* b - <0.0001*
Estradiol (pg/mL)	45.3 ± 36.4	15.3 ± 17.4	86.4 ± 67.6	a - < 0.0001* b - < 0.0001*
25-OH Vitamin D3 (ng/mL)	23.83±12.67	23.95±12.42	28.21±19.55	a - 0.0004* b - 0.0213*
MDA (nmol/mL)	7.676±4.486	9.057±5.731	4.123±3.313	a - < 0.0001* b - < 0.0001*

HDL, high density lipoprotein; AMH, anti-müllerian hormone, FSH, follicle stimulating hormone; LH, Luteinizing hormone; MDA, malondialdehyde; BLD, below limit of detection.
P <0.05 was considered as significant

Table 3 — Serum AMH and Vitamin D levels in patients with different years of hysterectomy

Cases	AGE (years)	No. of cases	AMH (ng/mL)	Vitamin - D (ng/mL)
Controls	31.7 ± 5.5	301	2.63 ± 1.93	28.21 ± 19.55
YSH (1-2 Years.)	32.7 ± 3.6	56	1.94± 1.53*	25.3 ± 16.2
YSH (3-4 Years.)	33 ± 3.5	67	1.76 ± 1.54**	25.0 ± 14.5
YSH (5-6 Years.)	34 ± 3.7	79	1.66 ± 1.7**	24.0 ± 11
YSH (7-8 Years.)	36 ± 2.7	94	1.01± 0.96**	24.0 ± 12.9*
YSH (9-10 Years.)	37 ± 3.5	84	0.82 ± 0.54**	22.3 ± 8.1#

YSH, years of hysterectomy, **P* <0.05 , ***P* <0.001, #*P* <0.005 *vs* control

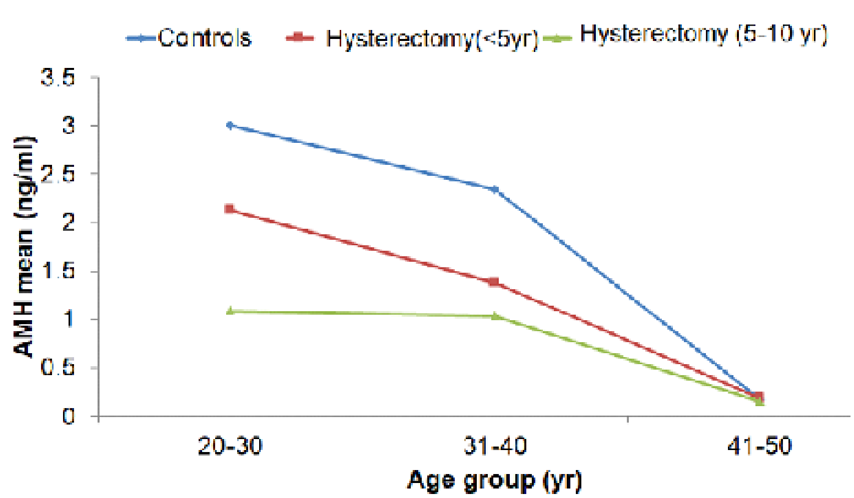
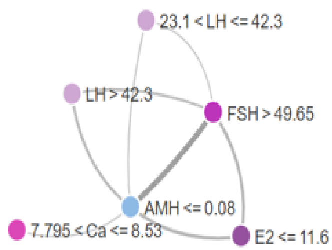


Fig. 1 — Ovarian function depletion in early hysterectomy

Association rules



Performance characteristics

	FSH >49.65	LH >23.1	E2 <11.6	Ca <8.53
OR	1519.18	44.84	5.62	1.59
95% CI	470.76-4902.48	28.02-71.76	3.94-8.05	1.24-2.05
Accuracy	94.9%	80.1%	69.6%	57.7%
Sensitivity	87.3%	94.8%	31.3%	47.4%
Specificity	99.6%	71.3%	92.5%	63.9%

Fig. 2 — Factors influencing the ovarian function

LH and estradiol (E2); and negatively correlated with age, FSH, years since hysterectomy and vitamin D (Fig. 2).

The association statistics followed by Fisher exact test revealed that AMH level <0.08 ng/mL is associated with FSH>49.65 mIU/mL (OR: 1519.18, 95% CI: 470.76 – 4902.48), LH<23.1 mIU/mL (OR: 44.84, 95% CI: 28.02 – 71.76), E2<11.6 pg/mL (OR: 5.62, 95% CI: 3.94 – 8.05) and calcium between 7.795-8.53 mg/dl (OR: 1.59, 95% CI: 1.24 – 2.05). We found serum FSH is the most important predictor with 94.9% accuracy, 87% sensitivity, 99.6% specificity in predicting the AMH-mediated ovarian function. LH has 80.1% accuracy in predicting ovarian function with 94.8% sensitivity and 71.3% specificity. Estradiol has 69.6% accuracy in predicting ovarian function with less sensitivity (31.3%) and high specificity (92.5%). The decreased ovarian function is associated with lower calcium levels, which are likely to influence the bone health.

Excluding FSH and LH from the prediction, a multiple linear regression equation was deduced to assess the influence of other variables (independent variables) such as age, BMI, waist circumference (WC), hemoglobin (Hb), glucose, cholesterol (CHOL), HDL-cholesterol, triglycerides (TGL), years of hysterectomy (YSH) and vitamin D on AMH (dependent variable), a marker of ovarian function.

Multiple Linear Regression:
AMH (ng/mL) = 5.8360942276588 –
(0.114063339325046 * Age) + (0.0037324282459407
*BMI) – (0.00158220222787886*WC) +
(0.000221113584605223*Hb) –
(0.00485909394522061*Glucose) +
(0.00354554505347972*CHOL) –
(0.00586280707338296*HDL CHOL) –
(0.00249557097326928*TGL) +
(0.0287730722074681*Calcium) +
(0.00364417158738729*Estradiol) –
(0.93735670504571*Surgery Status) +
(0.0093524909625016*YSH) +
(0.00404664453819367*Vitamin D)

This equation clearly demonstrates that hysterectomy decreases AMH levels by 0.94 ng/mL. Age specific decrease in AMH was evident with 1.14 ng/mL decrease per decade of life. Calcium levels were positively associated with AMH levels. Contribution of other factors towards AMH is not statistically significant. Overall, this equation could explain 32.7% variability in AMH levels.

Discussion

The current study investigated the effect of pre-menopausal hysterectomy on ovarian function following 5 years and 10 years post-hysterectomy period. The results depicted a gradual decline in the ovarian function thus inducing early menopause in the hysterectomy group. Earlier, Abdelzim *et al.*, reported no evidence of ovarian dysfunction till 12 months of hysterectomy in premenopausal women[17]. Similar observation was also reported in a recent study with 3 months follow up data after salpingectomy with no difference in AMH levels following surgery[27]. These studies put together point towards the slow depletion of ovarian function over a period of 5 years or 10 years duration following surgery but not immediately.

The current study demonstrated lower calcium levels in women with loss of ovarian function (AMH <0.08 ng/mL), which corroborates with a recent study that demonstrated an inverse association of annual rate of AMH decline with intake of dairy products, milk, total calcium and dairy calcium[28]. The free estradiol levels were found to decrease in premenopaual women following hysterectomy, which is consistent with a recent study[29]. The association between the oxidative stress and estrogen deficiency has been confirmed in several human studies[30-32]. In a study, increased oxidative stress was observed in postmenopausal women showing elevated levels of serum MDA and oxidized lipoproteins as compared to fertile women[31]. In another study, elevated levels of serum MDA and decreased levels of serum GSH levels were observed in postmenopausal women as compared to the premenopausal women group[32]. The results of the current study corroborated with study by Bellanti *et al.*, in demonstrating reduction of estradiol levels and increased oxidative stress following premenopausal hysterectomy[33]. In addition, we also found that the higher levels of MDA in premenopausal hysterectomy with the decline in the ovarian function as compared to normal ovarian function indicating oxidative stress do contribute to ovarian dysfunction in women under premenopausal hysterectomy.

The current study is the first of its kind to employ machine learning tools to understand premenopausal hysterectomy influence on the ovarian function and bone metabolic markers. Thresholds of the prime determinants *i.e*. FSH, LH, estradiol, calcium were established with reference to AMH threshold that distinguishes women with ovarian function with those without ovarian function. This is further substantiated by Fisher exact test to validate each association in terms of overall accuracy, sensitivity and specificity. This data is consistent with a recent study from China that demonstrated decrease in AMH and increase in FSH after hysterectomy with more severe effect in younger patients[34].

A recent study explored the relation of AMH and antral follicle count with cardiometabolic parameters[35]. Consistent with this study, we observed the high waist circumference in premenopausal hysterectomy women with loss of ovarian function. Consistent with the current study, lower levels of AMH were reported in women with overt hypothyroidism[36].

The major strengths of the current study are: (i) its sample size; and (ii) application of multiple linear regression, machine learning, Fisher exact analysis to establish the inter-relationships of AMH with FSH, LH, E2 and calcium in premenopausal hysterectomy. The limitations are (i) lack of information on the nutritional status of the participants; (ii) other life style risk factors could not be considered in the prediction models. Future studies are warranted to investigate these parameters for a better understanding of ovarian function alterations in the post-hysterectomy periods, specifically by considering efficacy of hormonal therapy in restoring ovarian reserves.

Conclusion

Pre-menopausal hysterectomy show a slow decline in AMH levels probably may have impact on ovarian function after every five years post - hysterectomy in early age groups (20-30 years and 31-40 years) followed by loss of ovarian function in the age group of 40-50 years. By utilizing multiple linear regression and machine learning tools and observed that serum FSH is the most important in predicting the AMH-mediated ovarian function with greater rate of accuracy, sensitivity and specificity. Non-hormonal factors contribute to 32.7% AMH levels.

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