

# CORRELATION BETWEEN PLASMA LEAD AND SERUM ANTI MULLERIAN HORMONE LEVELS IN WOMEN WITH PREMATURE OVARIAN FAILURE

By

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## ABSTRACT

**Background:** Lead is a highly toxic metal to humans, with the injurious effects on the hemopoietic, nervous and reproductive system. Lead crosses the placenta during pregnancy and has been associated with intrauterine death, prematurity and low birth weight.

**Objective:** To collate between plasma lead and serum antimullerian hormone levels in women with premature ovarian failure, by estimation of level of Anti Mullerian Hormone (AMH), Follicular Stimulating Hormone (FSH), Estradiol (E2) and plasma Lead level.

**Subjects and Method:** This study was conducted in Al Azhar University Hospital outpatient clinics after approval of the research and ethical committee during the period from December 2015 to December 2018. Study groups were: Group 1 included 50 normal candidate women under 40 years old has regular menstrual cycles (21-35 days), fertile (at least one natural pregnancy carried to term), and serum FSH was less than 10 mIU/ml, Group 2 included 50 patients with premature ovarian failure who aged under 40 years with absence of spontaneous menstrual bleeding for more than 12 months, serum estradiol level was less than 25pg/ml, serum FSH level more than 40 mIU/ml. AMH, plasma lead level in patients with premature ovarian failure diagnosed and correlated with level of serum FSH with the normal control group.

**Results:** There was a statistically significant positive correlation between plasma lead levels with FSH among the study group. On the other hand, there was no statistically significant correlation between plasma lead level and AMH level.

**Conclusion:** The anti mullerian hormone showed a significant difference between the cases of premature ovarian insufficiency and the control group. Moreover, AMH was a good marker for ovarian reserve and ovarian response for gonadotropins simulation. Chronic exposures to heavy metals, especially lead, affected the ovarian function and impairment in folliculogenesis which increased the incidence of Premature Ovarian Failure (POF) especially in rural area due to the low socioeconomic status.

**Key words:** Plasma Lead, Anti mullerian Hormone and Premature Ovarian Failure.

## INTRODUCTION

Women with POF suffer from anovulation and hypoestrogenism and present with primary or secondary amenorrhea, infertility, sex steroid deficiency and elevated gonadotrophins (*Jankowska, 2017*). The condition affects

approximately 1% of women, occurring in 10 –28% of women with primary amenorrhea and 4–18% in those with secondary amenorrhea (*Okeke et al., 2013*). A wide spectrum of pathogenic mechanisms may lead to the development of POF including chromosomal, genetic, autoimmune, environmental, and

iatrogenic causes. In a large proportion of cases, no cause is found, and they are classified as idiopathic or karyotypically normal spontaneous POF (*Ebrahimi and Akbari Asbagh, 2011*). AMH is useful in fertility assessment, as it provides a guide to ovarian reserve. AMH measurement is the best prognostic marker of the ovarian response to controlled ovarian stimulation during IVF cycles, especially when a single marker is determined (*La Marca et al., 2010*). Chronic excessive exposure to lead was associated with increase rates of infertility, miscarriages, still birth and poor infant outcomes. Epidemiological and animal studies have illustrated that the potential to disrupt ovarian function. From high to low doses of lead exposure, there are different responses of lead including reduced fertility, spontaneous abortions, low birth weight, impairment in folliculogenesis, and even damage to the ovaries are also reported (*Sunil, 2011*). Lead is highly toxic to humans, with the injurious effects on the hemopoietic, nervous and reproductive system. Lead crosses the placenta during pregnancy and has been associated with intrauterine death, prematurity and low birth weight (*Flora et al., 2011*).

## PATIENTS AND METHODS

This an observational cross sectional study conducted in Al -Azhar University Hospital outpatient clinics of Al-Hussien and Bab Al-Sharyah during the period from December 2015 to December 2018. All patients were given informed consents and fully informed about the nature and objective of the clinical study, the potential risks, benefits of study participation and their rights as research subjects. The study included 100 women

divided into two equal groups: Group 1 were normal candidate women under 40 years old has regular menstrual cycles (21-35 days), fertile (at least one natural pregnancy carried to term), and serum FSH was less than 10 mIU/ml. Group 2 were premature ovarian oailure who aged under 40 years with absence of spontaneous menstrual bleeding for more than 12 months, serum estradiol level less than 25pg/ml, and serum FSH was level more than 40 mIU/ml. All patients in this study were subjected to detailed history, general and local examination and laboratory study (FSH and E2 serum AMH level and serum lead level).

Lead was measured in blood after collection by Graphite Furnace Atomic Absorption (GFAAS) based on a method described by *Amiri and Amini (2012)*, and AMH was measured in serum by ELIZA based on a method described by *Hampl et al., (2011)*.

Statistical analysis: Data was collected, coded, and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 under windows 7. Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test: For quantitative parametric data: Independent student's t-test used to compare measures of two independent groups of quantitative data For qualitative data, Fisher's exact test to compare two of more than two qualitative groups, and Bivariate correlation test to test association between quantitative variables. The level  $P < 0.05$  was

considered the cut-off value for significance.

## RESULTS

All POF candidates were married, 30 patients (60%) were from rural areas, while 20 patients (40%) were from urban

areas. Mean standard deviation and range of the age were 26.5 yrs, 5.8 and 18-39 yrs respectively (**Table 1**).

**Table (1): Demographic characters of the POF women.**

Variables	Characters	N (%)
<b>Marital status</b>	Single	0(0%)
	Married	50(100%)
<b>Residence</b>		
	Urban	20 (40%)
	Rural	30 (60%)
<b>Age</b>	26.4yrs(mean), +/-5.8(SD), 18-39yrs(range)	

According to the parity, there was a statistically significant difference between case and controls with p-value <0.001. The multipara women were 50 (100%), and 22 (44%) in the control group and the

case group respectively. The nullipara women were 0 and 28 (56%) in the control group and the case group respectively (**Table 2**).

**Table (2): The parity among cases and controls.**

Parity \ Groups	Control (n=50)		case (n=50)		p-value
	No.	%	No.	%	
Nullipara	0	0	28	56%	<0.001
Multipara	50	100%	22	44%	

As regards to age, there was no statistically significant difference between cases and controls with p-value 0.5. On the other hand, there was a statistically significant difference between cases and controls with p-value <0.001. As FSH level and plasma lead levels were with high mean among cases, and AMH with high mean among controls. The mean

FSH was 4mIU/ml and 52.1mIU/ml in controls and cases respectively. The mean AMH was 1.1 and 0.52 ng/ml (normal range: 0.5-25ng/ml) in controls and cases respectively. The mean plasma lead was 10 and 4.1ug/dl (normally up to 20ug/dl) in controls and cases respectively (**Table 3**).

**Table (3): Comparison of age and basal hormonal profile FSH, AMH and Lead among cases and controls.**

Parameters \ Groups	Controls (n=50)		Case (n=50)		p-value
	Mean	SD	Mean	SD	
Age	27.5	5.7	26.4	5.5	0.5
FSH	4	1.2	52.1	25	<0.001
AMH	1.1	1	0.52	0.2	<0.001
Plasma Lead	4.1	1.7	10	3.6	<0.001

Among different residences, there was statistically significant difference between them with p-value <0.001, plasma lead level among cases with high mean among

rural residence. The mean plasma lead level was 7.6 and 11.4ug/dl among urban and rural cases respectively (Table 4).

**Table (4): Comparison of plasma lead level in different residence among cases.**

Parameters \ Residence	Urban (n=20)		Rural (n=30)		p-value
	Mean	SD	Mean	SD	
Plasma lead level	7.6	1.4	11.4	3.8	<0.001

According to correlation between AMH level with age, FSH and lead among controls, there were statistically significant negative correlations between AMH level and each of age and FSH (r-0.8.p<0.001, r-0.8; p<0.001) respectively, and there was no statistically significant correlation between AMH level and plasma lead with p-value 0.06. On the

other hand, among cases there was high statistically significant negative correlation between AMH level with FSH with p-value 0.001 (r-0.5; p=0.001), and there was no statistically significant correlation between AMH level with age and plasma lead with p-value 0.1 and 0.4 respectively (Table 5).

**Table (5): Correlation between AMH level with age, FSH and lead among controls and cases respectively.**

Parameters \ AMH (n=100)	R	p-value
<b>Controls</b>		
Age	-0.8	<0.001
FSH	-0.8	<0.001
Lead	-0.2	0.06
<b>Cases</b>		
Age	0.2	0.1
FSH	-0.5	0.001
Plasma lead level	-0.12	0.4

According to correlation between plasma lead level with age, FSH, and AMH, among controls, there was no statistically significant correlation between plasma lead level with age, FSH, and AMH level with p-value (0.92, 0.83

and 0.61) respectively. On the other hand, among cases, there was no statistically significant correlation between plasma lead level and age, FSH, and AMH level with p-value (0.72, 0.11 and 0.41) respectively (Table 6).

**Table (6): Correlation between plasma lead level with age, FSH, and AMH among controls and cases respectively.**

Parameters \ Plasma lead level (n=50)	R	p-value
<b>Controls</b>		
Age	0.03	0.92
FSH	0.05	0.83
AMH	-0.08	0.61
<b>Cases</b>		
Age	-0.13	0.72
FSH	0.23	0.11
AMH	-0.13	0.41

## DISCUSSION

Premature ovarian insufficiency (POI), also known as premature ovarian failure (POF), is the condition when women experience early menopause. It is defined as ovarian failure before age 40 years (which is two standard deviations below the age of normal menopause) (*Mann et al., 2012*). In infancy, the FSH levels are greater than the levels reached during the normal adult menstrual cycles, decreasing to low levels usually by one year, but sometimes later, LH levels are in the range of lower adult levels, this early activity is accompanied by inhibin levels comparable to the low range observed during the follicular phase of the menstrual cycle (*Andersen et al., 2010*). AMH is initiated as soon as primordial follicles are recruited to grow into small pre-antral follicles and its highest expression is observed in pre-antral and small antral follicles (*Dunlop and Anderson, 2014*).

AMH, produced by the granulosa cells of small growing follicles, inhibits initial follicle recruitment and FSH-dependent growth and selection of pre antral and small antral follicles (*van Houten et al.,*

*2010*). Lead is stored in soft tissue; autopsy studies show the liver to be the largest repository of soft tissue lead (33%), followed by kidney cortex and medulla, pancreas, ovary, spleen, prostate, adrenal gland, brain, fat, testis, heart and skeletal muscle. Levels of lead in soft tissue appear to be relatively constant during life, despite a fairly high turnover rate (*S Sharma et al., 2011*). The present study shows that there is high statistically significant difference between cases and controls as regards to AMH with p-value<0.001 with high mean among controls.

*La Marca et al. (2010)* supports the hypothesis that in women with hypergonadotrophic amenorrhea (POF) serum AMH levels are very low or undetectable, also hypothesized that the determination of serum AMH levels in POF patients could help in evaluating the persistence of follicles and possibly of the fertility potential and in some patients could also help in clarifying the mechanisms of ovarian dysfunction. *Hagen et al. (2010)* in cross sectional study of young girls with Turner Syndrome were investigated which factors

had the highest predictive value for finding follicles, the most powerful were the presence of 46XX/XO chromosomal mosaicism, serum FSH levels below 11 mIU/ml and serum AMH levels  $> 0.28$  ng/ml. AMH may therefore have a role in the diagnostic work-up and fertility counseling of patients with Turner Syndrome. In the present study there is statistically significant negative correlation between AMH and FSH with p-value  $<0.001$  among cases and controls.

*Freeman et al. (2012)* reported that the reproductive ageing related to changes in the hypothalamic-pituitary-ovarian axis associated with physiological and endocrine changes. One of the earliest signs of reproductive ageing is the FSH rise observed throughout the menstrual cycle, but most prominently noted in the early follicular phase of older ovulatory women. The physiological mechanisms behind the FSH rise are not completely understood, but probably involve decreased negative feedback (lower inhibin B secretion) from the diminishing number of pre-antral and early antral follicles in older reproductive aged women associated with decreased AMH level which secreted by the granulosa cell of the pre-antral follicles. Our study shows that there is statistically significant negative correlation between AMH and age with p-value  $<0.001$  among controls but not among cases.

*La Marca et al. (2010)* demonstrated that AMH may constitute an important measure of ovarian reserve as serum AMH level fall throughout the reproductive life and the level become undetectable after spontaneous menopause. *Van Helden and Weiskirchen*

*(2017)* was suggested that AMH levels were measured and used to determine an estimate of mean AMH as a function of age. It was found that there was good conformity between the observed distribution of age at menopause and that predicted from declining AMH levels. Our study shows that there is statistically significant difference between different parity with p-value 0.01 as regards to AMH level with high mean among multipara group.

*Freeman et al. (2012)*, in a prospective cohort study, showed that women who have given birth to more children are likely to have significantly higher AMH levels. These mean that women that having a high AMH is associated with a higher age at menopause.

Also, our study showed that there was a statistically significant difference between case and controls. As regards to plasma lead level with high mean among the cases, this study was in agreement with *Qureshi et al. (2010)*.

*Sharma and Bhattacharya (2014)* concluded that Animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis. A low Lead concentration in the mice ovary caused dysfunction of folliculogenesis with fewer primordial follicles and an increase in atretic antral follicles.

*Qureshi et al. (2010)* noted that ovary of lead treated animals showed damaging pattern in its structure and distribution of various components when compared to control. There was an evident damage in germinal epithelium, cortex and inner medullary region. Lead treated ovary showed damage in different types of developing follicles and various ovary

components, i.e. germinal epithelium, cortex and inner medullary region. The lead affects the follicle and the extent of damage increase with the concentration of lead. Theca cells may also be a major target for heavy metal injury. Lead treated animals showed reduced number of primordial follicles, the granulosa cells gathered in the centre of the follicle and oocyte was not apparent and marked increase in atretic follicles. The study shows that there is statistically significant positive correlation between plasma lead level with FSH with p-value <0.001 among the study group.

*Sharma et al. (2013)* suggested that exposure of the ovary to heavy metals can directly cause ovarian failure by extensive follicular destruction. This targeting can result in the loss of ovarian steroid hormones and ultimate disruption of neuroendocrine feedback causing increased levels of FSH and LH. Our study also shows that there is statistically significant difference as regards to parity with p-value <0.001 between POF cases and controls with low parity among cases.

The association between parity and preterm ovarian failure was limited to sporadic cases of preterm ovarian failure (i.e. women without a family history of preterm ovarian failure), and disappeared when patients with familial preterm ovarian failure were considered separately, these women having a similar number of pregnancies. A possible explanation is that patients with a history of familial preterm ovarian failure may pay more attention to their reproductive patterns: they are aware of the earlier age of their relatives at menopause and as a

consequence, tend to conceive earlier (*Torrealday et al., 2017*).

Finally, there was a statistically significant difference between different residence with p-value <0.001 as regards to Plasma lead with high mean among rural. This difference can be attributed to the life style, the socioeconomic status or possible different surrounding environments.

A larger sample of population is needed to determine the AMH and Plasma lead in cases with premature ovarian insufficiency.

## CONCLUSION

The majority of cases are presented after marriage may be due to lack of awareness before marriage about reproductive health. High prevalence of Premature Ovarian Failure in the poor rural areas arouses attention to importance of improvement of the reproductive health care. In the present study measurement of basal day 3 FSH has a highly significant difference between the two studied groups with high mean among the case group. The Anti Mullerian Hormone showed highly significant difference between the cases of premature ovarian insufficiency and the control group. Moreover AMH is a good marker for ovarian reserve and ovarian response for gonadotropins simulation. Chronic exposures to heavy metals specially lead affecting the ovarian function and impairment in folliculogenesis which increasing the incidence of POF especially in rural area due to the low socioeconomic status.

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## العلاقة بين مستوى الرصاص و مثبط هرمون مولر بالدم في النساء المصابات بفشل المبيض المبكر

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**خلفية البحث:** يعد معدن الرصاص من المعادن السامة التي لها تأثيرا سلبيا على أجهزة الجسم المختلفة كالجهاز العصبي والجهاز التناسلي ويزيد من تأثيره السلبي أنه يعبر الحاجز المشيمي أثناء الحمل مما قد يؤدي إلى وفاة الجنين أو الولادة المبكرة أو قلة وزن الجنين.

**الهدف من البحث:** دراسة قيمة مقياس الهرمون المضاد للمولرين ومستوى الرصاص في البلازما في الدم في حالات فشل المبيض المبكر بالمقارنة مع النساء العاديات كأداة تنبؤية لتقييم مخاطر فشل المبيض المبكر.

**الاشخاص وطرق البحث:** أجريت هذه الدراسة في العيادات الخارجية بمستشفيات جامعة الأزهر بعد موافقة لجنة البحث والأخلاقيات في الفترة من ديسمبر ٢٠١٥ إلى ديسمبر ٢٠١٨ عن طريق قياس نسبة هرمون منشط محفز اعلى و المصل الهرمون المضاد للمولرين و مستوى الرصاص في البلازما لكل من مجموعتي البحث :

**المجموعة الاولى:** شملت ٥٠ امرأة مصابة بمرض فشل المبيض المبكر.

**المجموعة الثانية:** شملت ٥٠ امرأة أخرى طبيعية غير مصابة بالمرض.

**نتائج البحث:** هناك فروق ذات دلالة إحصائية في القيم المتوسطة لمصل الهرمون المحفز للحويصلة و الهرمون المضاد للموليريان والرصاص بالبلازما في مجموعة فشل المبيض المبكر مقارنة مع مجموعة التحكم.

**الاستنتاج:** يمكن استخدام الهرمون المضاد للمولرين للتنبوء باحتياطي المبيض أكثر من التنبوء بحالات فشل المبيض المبكر ويمكن من مستوى الهرمون المحفز

للحويصلة تشخيص حالات قصور المبيض المبكر، كما أن مستوى الرصاص بالدم يؤثر تأثيراً واضحاً على المبيض مما قد يساعد على زيادة نسبة حدوث الفشل المبكر للمبيض، ولكن هذا المجال لا يزال يحتاج إلى مزيد من البحث.

**الهدف من البحث:** معرفة تأثير التحميل الدوري على قوة إصلاح وتر الإنقباض باستخدام خياطة تانج ذات الست خيوط بشكل تجريبي.

**مواد وطرق البحث:** احتوت هذه الدراسة التجريبية على خمسين عينة من الأوتار تم تشريحها من أرجل الماعز تم قطعهم ثم اصلاحهم بطريقة تانج باستخدام خيط البولي بروبيلين ٠/٤ و خيط ٠/٥ بولي بروبيلين للغرزة الطرفية. مقسمه إلى مجموعتين احتوت كل واحدة على خمسة وعشرين عينة من الأوتار. وتمت اختبار المجموعتين باستخدام جهاز قياس قوة الشد (Zwick/Z010) المجموعة (أ) تم استخدام بروتوكول تحميل دوري الدرج. تم تحميل جميع الأوتار مبدئياً بحمولة أقصاها ٣١,٧ نيوتن لمدة ٥٠٠ دورة. تم زيادة التحميل بعد ذلك بمقدار ٥ نيوتن لمدة ٥٠٠ دورة إضافية للإصلاحات المتبقية. تم تكرار هذا الإجراء حتى حدث الفشل (فشل أكثر من ٢ مم). المجموعة (ب) و تم اختبارها بواسطة التحميل المستمر

**نتائج البحث:** المجموعة (أ) أظهرت تقنية تانج بقاء ٨٠٪ من العينات في اختبار ٣١,٧ نيوتن و ٧٢٪ في اختبار ٣٦,٧ نيوتن و ٤٤٪ في اختبار ٤١,٧ نيوتن دون دليل على وجود فجوة كبيرة أو تمزق. المجموعة (ب) كان متوسط قوة قطع الوتر ٥٥,٩٥ نيوتن طريقة تانج طريقة قوية وأمنة مما تسمح بحركة نشطة ومبكرة مابعد الاصلاح مع مود حركى افضل وقدر افضل على تنى الاصابع ضد مقاومة.

**الخلاصة:** توفر طريقة خياطة تانج مقاومة كافية للفجوة وقوة الشد قادرة على تحمل التعبئة النشطة المبكرة بعد إصلاح وتر العضلة المرنة. ولكن مع بعض العيوب المتعلقة باستهلاك الوقت أثناء الجراحة وصعوبة استخدامها في طب الأطفال والأوتار الصغيرة.