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**RESEARCH ARTICLE**

# USED INOSITOL DECREASED Anti-Müllerian Hormone (AMH) IN POLY CYSTIC OVARY SYNDROME WOMEN

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## *Abstract*

Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine-metabolic disorder that implies various severe consequences to female health, including alarming rates of infertility. Although its exact etiology remains elusive, it is known to feature several hormonal disturbances, including hyperandrogenemia, insulin resistance (IR), and hyperinsulinemia, In PCOS, several of the physiological events within the ovarian cycle and folliculogenesis are disrupted. The very beginning of folliculogenesis is compromised due to high levels of Anti-Müllerian Hormone (AMH)]( L. Pellattet al .,2010). In women with PCOS, elevated levels of AMH appear to play an important role in long term disruption of ovarian physiology [A. Karkanaki et al .,2011], with greater AMH concentrations being linked to worse fertility outcomes [A. Pierre et al .,2013].

in women with PCOS advocate for a more complex role of leptin in its pathophysiology. Moreover, reports of elevated leptin in nonobese PCOS patients further question quantitative adiposity as the sole origin of hyperleptinemia in this scenario [R. Yildizhan et al .,2011]. Regardless of its origin, in PCOS hyperleptinemia exerts direct effects on ovarian physiology by arresting follicle development.

**material and method** The study group which complete study period included (95) PCOS women**(group A)** include **(35)** women will treated with **Metoformine** for six months and (**group B)** include **(32)** women will treated with **Choline &Inositol** and **Metoformine** for six months and **(group C)** include **(28)** women will changed lifestyle and followed deit for six months we massacred AMH,Lep and BMI ,before and after treatment by using ELISA .

**RESULT** highly significant decrease ($p<0.01$)in BMI in group B while non significant in group A and C.,significant decrease ($p<0.05$)in Lep level in group( B, C) while non significant in group A.,significant decrease in AMH level in group (A,B)while non significant in group C. **Conclusions** used cholin & inositol plus metformin could be as a first- line treatment in patients with PCOS.

## INTRODUCTION

ovary syndrome (PCOS) is one of the most common and heterogeneous endocrine disorders that occur in 5-10 % of reproductive age women( Kandasamy S.*et al* .,2010). The disorder causes multiple abnormal cysts in enlarged ovaries, so they do not produce the normal number of eggs and do not ovulate ( release eggs ) normally. The disease is present at birth but does not cause symptoms until puberty; clinical features of this disorder may change throughout the lifespan, starting from adolescence to post menopausal age. No effort has been made to define difference in the phenotype and clinical presentation according to age ( Hamzeh R. and Balen, A.,2006).

Not all women with PCOS have polycystic ovaries (PCO), nor do all women with ovarian cysts have PCOS; although a pelvic ultrasound is a major diagnostic tool, it is not the only one. The diagnosis is straightforward using the Rotterdam criteria, even when the syndrome is associated with a wide range of symptoms. (Azziz *et al.*,2004).

The prevalence of PCOS depends on the choice of diagnostic criteria. The World Health Organization estimates that it affects 116 million women worldwide as of 2010 (3.4% of women) .( Vos T, Flaxman AD, *et al* .,2012). One community-based prevalence study using the Rotterdam criteria found that about 18% of women had PCOS, and that 70% of them were previously undiagnosed (H Teede *et al* .,2010).

**Leptin (Lep)**

Leptin (derived from the Greek word LEPTOS, meaning thin) is a protein hormone, discovered in 1974 ( Wilson I., Foster D.,1992), the molecular weight is ~16KDa, 167 amino acid, peptide structural analysis reveals a four alpha helix bundle structural with a three dimensional fold held together by a disulphide link between cys (96) and cys (146) (Zhang Y.,1994 ; Ahima R.,2008)) . It plays a central role as a chemical signal to the brain and other endocrine systems. Leptin inhibit the hypothalamus neuro peptide Y, which inhibitor of GnRH (Maffei M.,1995) .

Plasma leptin levels respond slowly to fasting (Wauters M.,1998)and not begin to decrease in humans for 12-14 hour. Leptin gene transcription and plasma leptin are severely reduced by longer starvation (Seow K.,2004) Conversely, the increase in leptin after feeding is delayed. Smaller amounts of leptin are secreted by cells in the epithelium of the stomach and in the placenta ( Panidis D., 2004) . The amount of leptin expressed by adipocytes correlates well with the lipid content of the cells. Although the mechanisms responsible for regulating leptin expression in adipocytes are unknown. It is likely that a number of hormones modulate ob (obese) gene expression, including glucocorticoids and insulin (Kohrt W., Landt M., Birge S.,1996).

Leptin levels in humans are clearly different in men and women, women have much higher leptin levels than men, a difference already observable in children (Hassink S.,1996; Ostlund R . *et al* .,1996; Friedman J., House J.,1998 ;Vrbikova J., HainerV., 2009) .

Women with PCOS are often characterized by visceral obesity, IR, and frequently sub fertility or infertility. Leptin status in these women is not clear ( Haffner S .,1997). Leptin levels in women with PCOS to be higher than expected for their BMI, suggesting abnormalities in leptin signaling to the reproductive system in PCOS ( Montague C.,1996).Other studies, have however, found leptin levels comparable with age and weight matched healthy control women (Sepilian V *et al*., 2006). In the context of PCOS, the role of leptin has been subject to profound controversy, with opposing views regarding its true participation. Because leptin concentrations are consistently found to be strongly correlated with weight, some reports consider the hyperleptinemia seen in PCOS as only a byproduct of this condition [C. S. Mantzoros, A. Dunaif, and J. S. Flier .,1997]. in women with PCOS advocate for a more complex role of leptin in its pathophysiology. Moreover, reports of elevated leptin in nonobese PCOS patients further question quantitative adiposity as the sole origin of hyperleptinemia in this scenario [R. Yildizhan et al .,2011]. Regardless of its origin, in PCOS hyperleptinemia exerts direct effects on ovarian physiology by arresting follicle development

**Anti Mullerian Hormone (AMH):-**

In PCOS, several of the physiological events within the ovarian cycle and folliculogenesis are disrupted. The very beginning of folliculogenesis is compromised due to high levels of Anti-Müllerian Hormone (AMH)]( L. Pellattet al .,2010). In women with PCOS, elevated levels of AMH appear to play an important role in long term disruption of ovarian physiology [A. Karkanaki et al .,2011], with greater AMH concentrations being linked to worse fertility outcomes [A. Pierre et al .,2013].

Anti-Müllerian hormone (AMH), also known as Müller inhibiting factor (MIF) or Müller inhibiting substance (MIS), is ahomodimericglycoprotein linked by disulfide bonds and a molecular weight of 140 KDa, it is

four times larger than LH or FSH. The hormone belongs to the Transforming Growth Factor-β (TGF-β) superfamily ( Panidis ., 2011 ) . AMH plays a role in gender differentiation during embryo development. Under the influence of the AMH formed in the Sertoli cells, the Mullerian ducts degenerate in male fetuses. This leads to the normal development of the male genitals. Female fetuses do not have AMH, and so develop the internal female genital organs. In women, at the onset of puberty AMH is formed by the granulosa cells of the maturing ovarian follicle, but not by the primordial follicles and also not by the antral follicles under direct FSH regulation in the final regulator of folliculogenesis and of primordial follicular rupture. It reduces the rate of follicle conversion from the primordial to the growing stage and regulates follicle growth by inhibiting FSH-induced conversion from the early to the late stage ( Ivana Zec *et al*., 2011) . .In women, AMH is produced by the granulosa cells (GC) of follicles. Specifically, GC produce AMH from the stage of the primary follicle to the initial formation of the antrum. In female neonates, AMH is virtually undetectable but increases gradually until puberty and remains relatively stable thereafter and throughout the reproductive period (Pellatt L, Rice S, Mason H .,2010) . It is widely accepted that the reduction of AMH levels in serum is the first indication of a decline in the follicular reserve of the ovaries. AMH concentration remains stable throughout the menstrual cycle ( SPROUL K. *et al* .,2010) . Recent data, however, have shown that there are fluctuations throughout the cycle (with lower levels during the early secretary phase) or even between consecutive cycles (Cook CL, *et al* .,2000). Nevertheless, these fluctuations are not considered clinically significant to recommend the measurement of AMH concentrations at a specific phase of the menstrual cycle (Streuli I, *et al* .,2009).

Besides its utilization in in vitro fertilization , determination of AMH may serve as an additional marker in the diagnostics of PCOS, where increased AMH levels reflect the severity of the disease. Positive correlation of serum AMH with the number of antral follicles was found also in patients with PCOS. An increased production of AMH induces a decrease of sensitivity to FSH at receptor level, necessary for the growth of follicles. It leads to an increase of the number of antral follicles on the detriment of their size the number of small antral follicles(2-5) mm in size increases, restraining thus selection of the dominant follicle. Such a situation is clinically characterized by an ovulation cycles, manifesting themselves as oligo- or amenorrhea (Park As *et al* .,2010) . In addition AMH inhibits peripheral aromatase, leading to hyperandrogenemia, another typical feature of PCOS. As mentioned already, secretion of AMH and its serum levels decrease with age. In patients with PCOS this decline was surprisingly less distinct, indicating a slowed-down ovarian ageing, which may be explained by suppression of growth and differentiation of primordial follicles by high levels of AMH ( Panidis ., 2011 ; Ivana Zec *et al* ., 2011). When investigating possible genetically causes of PCOS, the question arose whether described mutations of AMH encoding gene are related to the disease. A genotypic analysis, however, did not reveal association of AMH gene mutations with PCOS, though they may contribute to aggravation of some features of the disease(Park As *et al* . ,2010).

**Treatment of PCOS**

The course of treatment for women with PCOS largely depends on the severity of an individual's symptoms .Well-defined published data indicate a high risk for development of T2DM and CVD in women with PCOS. (Moll E. *et al*.,2007; Ruifrok N. *et al* .,2009) Metformin is now thought to be of therapeutic value directly and/or indirectly in the management of PCOS ( Palomba S. *et al* .,2009) . In addition to the expected improvements in insulin sensitivity and glucose metabolism, metformin therapy also ameliorates hyperandrogenism and menstrual irregularity, the favorable effect of metformin on hyperandrogenism in PCOS.

Treatment with Choline and Inositol "Inositol" is a term used to refer to a group of naturally occurring carbohydrate compounds that exist in nine possible chemical orientations called stereoisomers. The most common being myo-inositol, which is often sold as a dietary supplement labeled simply as inositol. Inositol, particularly myo-inositol and another less common stereoisomer called D-chiro-inositol, plays a critical, but under appreciated, role in insulin signaling. Conditions such as hyperglycemia and diabetes are associated with disrupted inositol signaling, leading many researchers to suggest that this may be a key pathologic feature of insulin resistance (Larner J., 2010).

Natural Treatments for PCOS Over the past few years, research into the naturopathic and nutritional approach to PCOS has revolutionized the condition's treatment. It is important to treat the factors that lead to PCOS. Following The Natural PCOS Diet, making lifestyle changes and taking supplements can influence a healthy outcome.

One of the most important things is to address insulin resistance, if this is an apparent problem. This can be done by incorporating dietary changes and taking suitable nutritional supplements.

What you eat can have a direct influence on how balanced (or unbalanced) your hormones are. This is why it's important to have a healthy diet. .( Jenny Blondel.,2011).

## Material and Methods

In this study , 25 healthy women (without PCOS) and150 women with PCOS were included, they were divided according type of treatment .(55)PCOS women will removed his result because not continue in study period ,and this group included (25)PCOS women which treatment with Choline & Inositol alone and 30 PCOS women of all group which treated but not complete study period so that removed the result.

The study group which complete study period included ninety five(95) PCOS women, (group A) include (35) women will treated with Metoformine for six months and (group B) include (32) women will treated with Choline &Inositol and Metoformine for six months and (group C) include (28) women will changed lifestyle and followed deit for six months.

This study was carried out in Kamal AL-Samaraee Hospital- Baghdad during the period from July 2013 to February 2014. It included (95)Iraqi women with (PCOS); their age range was (18-39) years.

***Determination of Serum Anti-Mullerian Hormone**:-*

Serum Anti-Mullerian hormone was measured by ELISA using a kit supplied by CUSABIO -China.

*A. Assay Procedure:-*

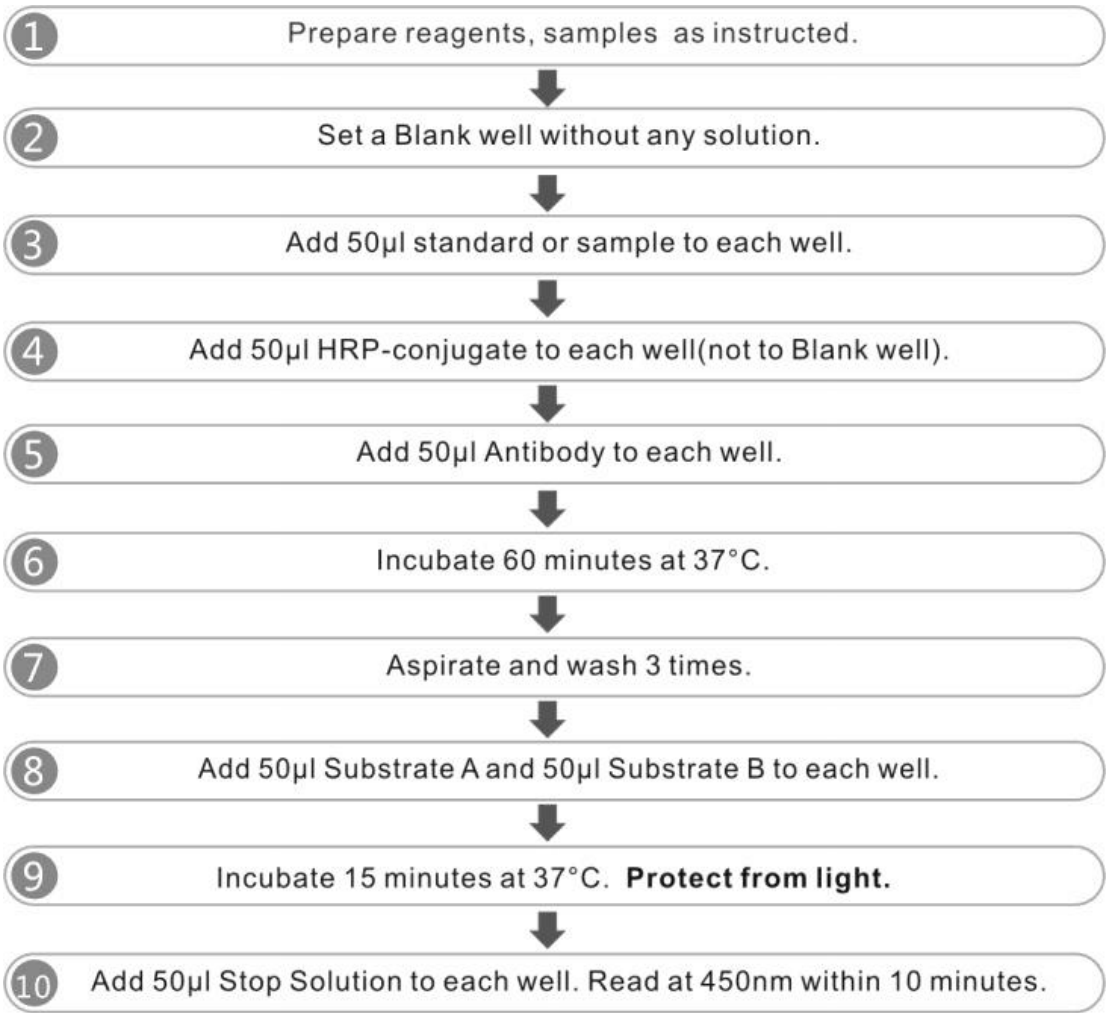
Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25$^{o}$C):

1. Pipette 50µl of the appropriate calibrators, controls and samples into the assigned wells.
2. Add50µl of standard or sample per well. Standard needed test in duplicate.
3. Add 50µl of Horseradish peroxidase conjugate to each well, then 50µl antibody to each well, and mixed well.
4. Incubated for 60 minutes at 37$^{o}$C.
5. The solution was discarded and washed 3 times.
6. Add 50µL of substrate A and 50µL substrate B to each well and mixed well.
7. Incubated for 15 minutes at 37$^{o}$C, and kept well in the dark.
8. Added 50µL of stop solution to each well, gently tap the plate to ensure through mixed.
9. Read the absorbance in each well at 450nm in a micro plate reader.

*B. Calculation:-*

Average the duplicate reading for each standard and sample and subtract the average optical density of blank . Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit . As an alternative construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph . The data may be linear zed by plotting the log of the AMH concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis . The procedure will produce an adequate but less precise fit of the data .If samples have been diluted ,the concentration read from the standard curve must be multiplied by the dilution factor.

ASSAY PROCEDURE SUMMARY




***Determination of Serum Leptine***

**Diagnostic by (CUSABio - China)**

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody(1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody(1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 100µl of HRP-avid in (1x) to each well. Cover the micro titer plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
11.Determine the optical density of each well within 5 minutes, using a micro plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

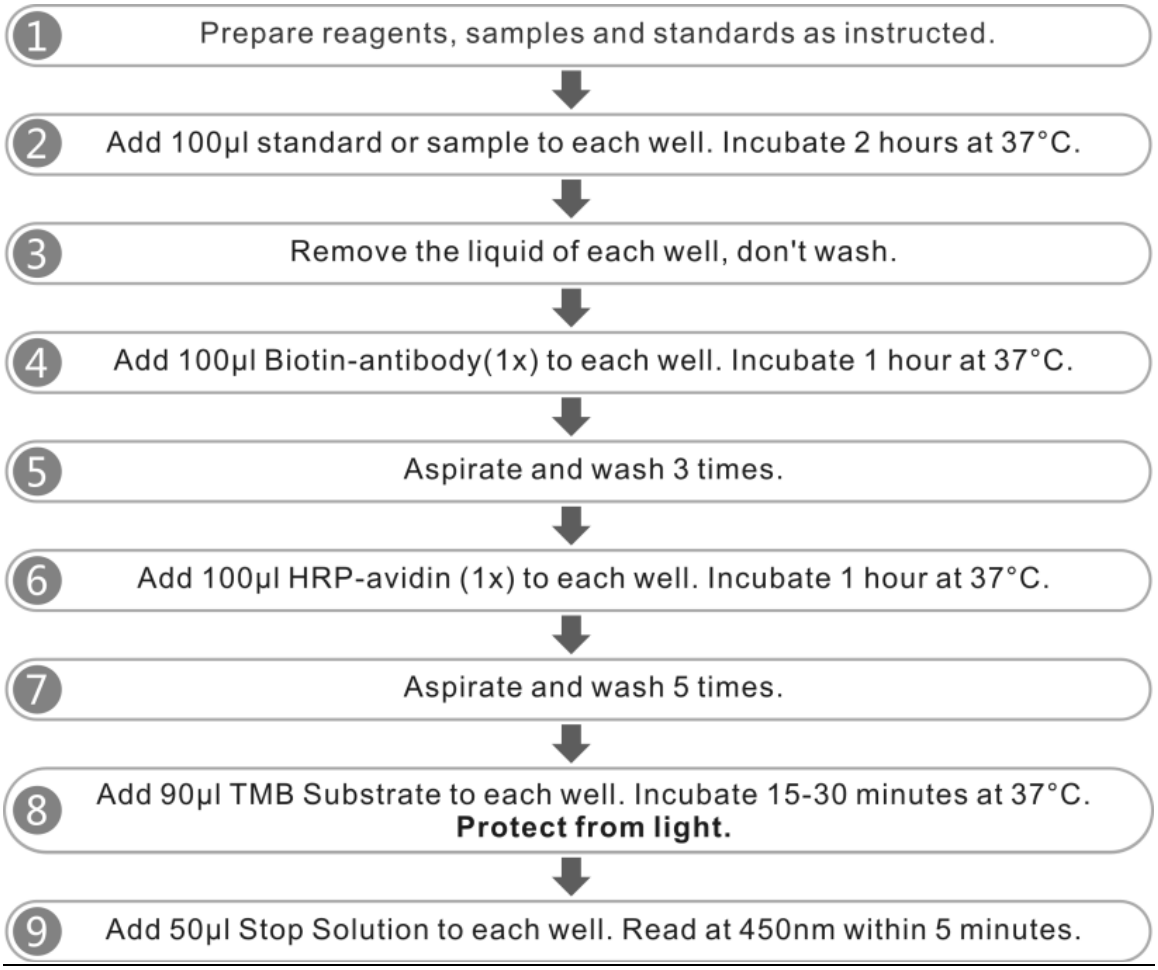
Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linear zed by plotting the log of the LEP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution fact

ASSAYPROCEDURESUMMARY



**Anthropometric Measurements**

These include: weight (kg), height (m), From these measurements the following variables are calculated:
Body mass index (BMI): weight (kg)/ height ($m^2$).

**Statistical Analysis**

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study. Estimate of correlation coefficient between difference variables.

## Result

The result of the body mass index, leptin hormone level and Anti – mullerian hormone (AMH) table(a) showed, Group (A) the women with PCOS it is the first time massacred AMH level after treatment which treated with meftormin (850 mg tow time /day) we found the anti – mullerian hormone we found before treatment (13.30±0.66 a) ng / ml become (10.35±0.44b) ng/ml after treatment the statically analysis showed significant decreased in anti-mullerian hormone level ($P \geq 0.5$)during (0and 3)month and during (3and 6)month. Body mass index (BMI)before treatment (32.81 ± 0.83 a)became (31.15 ± 0.73 a) after treatment and leptin hormone level before treatment (6.20 ± 0.26 a) became( 5.41 ± 0.23 a ) after treatment the result was decrease in (BMI) and leptin hormone level but the statistical analysis not significant of BMI decrease and leptin decrease during (0,3 and 6)month .

Group (B) in the same table (a) the women with PCOS treated with cholin & inesitol (500/500 mg tow time /day and metformine ( 850 mg tow time /day) we found significant decrease in BMI ($P \leq 0.01$), leptine level ($P \leq 0.05$) and AMH level ($P \leq 0.05$).

Group (C) in the same table( a) showed no significant decreased in BMI, significant decrease in leptin hormone level and non significant decrease in AMH level.

Table (a) shows mean and standard errors of BMI ,LEP and AMH hormone for women patient of PCOS which treated with different treatment during (0,3 and 6)month.

| Group | Duration of treatment | Mean ± SE | | |
|---|---|---|---|---|
| | | BMI (Kg/m2) | Lep. (ng/ml) | AHM ( ng/ml) |
| A | 0 | 32.81 ± 0.83 a | 6.20 ± 0.26 a | 13.30 ± 0.66 a |
| | 3 | 32.59 ± 0.81 a | 6.05 ± 0.26 a | 11.87 ± 0.58 ab |
| | 6 | 31.15 ± 0.73 a | 5.41 ± 0.23 a | 10.35 ± 0.44 b |
| LSD value | | 4.622 NS | 0.952 NS | 2.095 * |
| B | 0 | 40.56 ± 8.92 a | 6.78 ± 0.32 a | 12.51 ± 0.74 a |
| | 3 | 33.24 ± 1.01 b | 5.79 ± 0.26 b | 10.99 ± 0.63 ab |
| | 6 | 29.71 ± 0.85 b | 4.87 ± 0.26 c | 9.97 ± 0.52 b |
| LSD value | | 7.063 ** | 0.8931 * | 1.745 * |
| C | 0 | 31.44 ± 0.93 a | 7.07 ± 0.31 a | 13.74 ± 0.70 a |
| | 3 | 31.01 ± 1.01 a | 6.48 ± 0.35 a | 14.20 ± 0.85 a |
| | 6 | 29.86 ± 0.28 a | 5.69 ± 0.28 b | 13.36 ± 0.83 a |
| LSD value | | 6.541 NS | 0.793 * | 1.773 NS |

** ($P \leq 0.01$).

group( A) which treated with metformine(850mg tow time /day).
group (B) which treated with Choline&Inositol (500\500tow time /day) and Metoformin (850 mg tow time /day).
group (C) change life style and followed diet.

## Discussion

this result was in agreement with anther studies, Piltonen T. *et al*(2005) showed serum AMH levels decreased significantly during metformin treatment.

In our study, serum AMH levels were significantly higher in patients with PCOS when compared to healthy women, patients observed with metformin treatment showed lower AMH level when compared to untreated patient group. AMH was similar between control and metformin groups. (Falbo A ., *et al*, 2010).

serum AMH levels significant decrease after metformin treatment , probably due to decrease in hyperinsulinemia .(Kamala KZ. *et al* ., 2011).

The present study the result was disagree with many studies which reported AMH was not significantly decreased under metformin treatment (Aykut M.D., *et al*, 2007 ; Panidis D., *et al*., 2011;Nascimento AD, *et al.*, 2013).

La Marca A *et al* (2009) reported our data seem to suggest that AMH might play a key role in the intra -ovarian mechanisms regulating the ovarian function .In fact , significant changes in serum AMH levels in PCOS patients ovulating under metformin treatment .the reason for the reduction in AMH concentration after metformin remains still controversial

. In the present study, leptin concentrations were correlated with BMI, in PCOS patients, This is in accordance with reports in the literature that show that leptin is strongly correlated with BMI (Zheng Z., *et al*., 2002; Sepilian V. *et al*., 2006).

The present study result was : agreement with

study which reported that no significant differences in serum leptin in women with PCOS which treated with metformin (Abdulla. Th ., *et al.*, 2012).

On the other hand, anther studies suggested that there was no statistically significant change in weight or BMI after treatment with metformin (Ashraf M. *et al*., 2013; Kurzthaler , *et al*., 2014).

Another authors have suggested that the decrease in leptin could not, however, be explained by the changes in body weight, becomes the BMI of their patients remained constant during therapy. (Morin P., *et al*., 1998).

Leptin concentration is closely related to body fat mass , yet reduction in leptin level con not be fully explained by the reduction in weight and BMI because metformin is found to reduce leptin concentration even in normal weight patients.( Fruehwald- Schultes *et al* .,2002).

Several researches have been done to analyses the molecular mechanism behind the effect of metformin on leptin levels ,An in -vitro study reports that metformin inhibits leptin secretion by inhibit in MAPK signaling path way in adipocytes .(Klein *et al* ., 2004).

The present study disagree with another study which reported the weight and BMI reduced significantly after metformin treatment ($P < 0.01$). the hormonal assays showed significant reduction in the level of insulin, and leptin. (Prerna U. *et al*., 2011).

Aleyasin *et al* (2011) also conducted a study on patient suffering from PCOS with different BMI they observed that metformin was effective only on patients with BMI greater than 35 to 40 kg/m2 ,there fore this study explain the result in the present study about decrease in BMI.

The present study group (B) result was in agreement with anther studies which reported in an Italian study of 92 PCOS patients, almost 50% showed significant weight loss and reduced leptin levels after receiving myo-insotol. (Gerli S *et al* 2007; Genazzani AD, *et al*., 2012).

Another studies reported, there was an inverse relationship between body mass and treatment efficacy. In fact, a significant weight loss and leptin reduction was recorded in the myo-inositol group.

Another studies showed there was serum AMH level and indexes of insulin resistance significantly decreased during the treatment with DCI. (Gerli S, *et al*., 2007; La. Marca A, *et al.*, 2014).

The present study group (C) was in agree with another study which investigation in obese patients demonstrated inconsistency in term of weight reduction after 12 – months on low – carbohydrate diets. (Dansiger ML., *et al*., 2005; McAuley KA., *et al*., 2005).

The effects of type of diet on PCOS have been evaluated in two experiments.

Both of these studies reported no significant differences in weight loss. (Moran LJ, *et al*., 2003; Stamets K, *et al*., 2004).

Carmina E. *et al* (2003) showed that diet alone does not explain differences in body mass.

In other hand, studies showed decreased in leptine level and small decrease in body weight during diet (Eveline w.*et al*., 2002).

The present study result well disagree with Vosnakic C., *et al*(2013) which reported that the resulted in significant weight loss, and increased serum AMH level.

Group (A) which include women with PCOS treated with metformne (850 mg tow time /day ) for sixth months. The result in this group will good and that metformin therapy restores normal levels of insulin and testosterone, AMH and anther hormone in study this result was similar to results pawekczyk *et al* (2004) report that metformin therapy not only restores normal levels of insulin and testosterone but also decreases the lipid profile . the role of metformin in improving the frequency Ovulation and balance the anther hormone will observed by other authors (Ashraf M. *et al*., 2005 ).

The present study showed group A results lower effective than group B, may need long term period of treatment .

Table (a) hormonal profile and biochemical parameter in healthy women (without PCOS)

| Parameter | Mean ± SE |
|---|---|
| BMI | 28.35±3.17 |
| AMH | 8.54±0.17 |
| Lep | 4.38±0.18 |

The present study showed group B which treatment with Cholin & inositole 500 / 500mg(tow time /day ) and metformine (850mg tow time /day ) this type of treatment used first time in Iraq and now used as a first line of treatment PCOS patent in Kamal AL-Samaraee Hospital -Baghdad

Showed the best result because all parameter which was massacred in the study retrain to the normal level or near to it when compared with healthy women in table(a)women without PCOS ) ,the women with PCOS can become pregnant in shorter time and the rate of pregnancy higher than group A which treatment with metformin alone. Cholin & incsitol and metformin will give the best result D-chiro inositol (DCI) administration a meliorates insulin resistance, hyperandrogenmia and improves ovulation in this disorder, while metformin does not increase the amount of DCI available, it may act to release the DCI – IPG mediator that to the cell to use and / or store glucose, Deficiency of DCI in women with PCOS may be why metformin is effective in some but not all PCOS patients (Raffone E. *et al*., 2010), so that we give two drug together .

The present study is the first to treated patient with PCOS by giving two insulin agents(inositol and metformin together)because not found data poplished similar to the present study .

The group( C) which change life style and followed diet did not give the best result because used diet alone without any chemical treatment did not have role to management the PCOS because big number of patient in this group will flowed diet by meal replacement and this type of lifestyle modification (diet alone)an affective in weight loss and hormonal balance in PCOS patient , this result was similar to the result Moran LJ *et al* (2009)which reported that the use of twice - daily meal replacements was an affective for achieving weight loss and gaining associated hormonal and clinical benfits in women with PCOS and that long -term weight loss may not be needed for fertility improvement in all women with PCOS .

The present study showed the dietary approaches may be useful additional strategies for women with PCOS . Lifestyle modifications in the treatment of PCOS do not escape criticism and controversy despite being widely accepted recommendations. Physical activity (PA) has been reported to ameliorate anovulation, IR, blood pressure, and lipid profiles in women with PCOS, sometimes independently of weight loss( C. L. Harrison *et al* ., 2011); yet PA alone does not seem to be able to equal these parameters to non-PCOS subjects (C. L. Harrison *et al*.,2012). Therefore, it should be accompanied by a complementary diet plan in order to fully potentiate the effects of a lifestyle-modification therapeutic program.

In this study combine between choline & inositiol and metformin offered a significant advantage over another type of management in addition patients on this management reported no side effects during the course of treatment .cholin & inositol and metformine could be as a first – line treatment in patients with PCOS.

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