SECONDARY MENOPAUSE and its RELATIONSHIP to HORMONAL LEVELS AMONG WOMEN at SALAH AL-DIN HOSPITAL

Mohammed Ahmed Mustafa^{#1,2} & Marwan Q AL-Samarraie³

#1,3Department of pathological Analysis, College of Applied Sciences University of Samarra ²Department of Medical Laboratory Techniques, College of Technology, University of Imam Ja far Al-Sadiq _ Dujail ^{#1,2}Mohammed.alsad3@gmail.com ³marwan.walady@vahoo.com

Abstract:

The research dealt with the study of secondary amenorrhea and secondary amenorrhea and its relationship to the physiological and hormonal status of women, which is one of the gynecological diseases with serious effects on women with this disease, because cardiovascular diseases, osteoporosis, diabetes, infertility and mental state disorder are among the most important complications of this disease. .

The study was conducted on (40) serum samples from Secondary Amenorrhea patients after their condition was diagnosed through clinical and laboratory tests. The ages of the patients ranged between (12-42) years.

The levels of the hormones LH, FSH, E2, and Prog were measured, and the results were the presence of high significant differences (P < 0.05) when measuring the level of hormones LH, FSH, E2, and Prog in the blood serum of the patients with secondary menopause and compared them with their concentrations in the control group.

There were high significant differences (P < 0.05) in serum levels of LH, FSH, and E2 hormones. The presence of high levels of the hormone LH, FSH, in the serum of most patients. The presence of a decrease in the levels of the hormones E2 and Prog in the serum of the majority of patients.

Keywords: Progesterone, Estrogenic, Menopause, LH, FSH.

INTRODUCTION

Gynecological diseases are one of the complex and intertwined topics in medical science, and menstruation problems are among the most common problems that women suffer, as recent studies on menstrual problems indicate that more than half of the world's women suffer from menstrual problems. (Iyengar et al., 2019).

Amenorrhea is one of the most important menstrual problems, as it is one of the endocrine diseases in gynecology, which is a challenge and exhaustion for the clinician. (Gordon et al., 2017).

Amenorrhea simply means menstruation due to many causes, some of which are physiological (before maturity, pregnancy, during the lactation period or at menopause) or it may be caused by a hormonal imbalance, gynecological disease, or a disease in one of the body's systems, and the reasons often overlap and diverge. Leading to menopause as a result of the relationship that binds each to the other (Berga et al, 2019).

In recent years, Arab countries, including Iraq, have witnessed a clear increase in the percentage of women suffering from menopause, especially secondary menstruation. Therefore, this research aimed to study secondary menopause in Iraqi women and the relationship of this interruption to the hormonal status, which included measuring the concentrations of the hormones FSH, LH, Prog, E2, In the blood serum of secondary menopausal patients

MATERIALS AND METHODS

Research period and work location

This study was conducted in the laboratory of Salah al-Din Military Hospital, Tikrit, for the period from December 2019 to February 2020 on 40 female patients suffering from secondary menopause, whose ages ranged between 15-42 years after confirmation of their condition through medical and clinical examinations by specialist doctors In Salah al-Din Military Hospital, Tikrit.

In addition to choosing a random group that included 30 healthy women whose ages ranged between (17-45) years, as the safety of these women from menopause or any hormonal disorders was confirmed by the specialist doctors at Salah al-Din Military Hospital Tikrit in addition to Laboratory tests.

Laboratory materials used

| Name Article | Manufacturer and origin | Name Article | Manufacturer and origin |
|----------------|-------------------------|--------------|-------------------------|
| E ₂ | BioMerieux-France- | FSH ElA Kit | Bio.Cheak-U.S.A- |
| Progesterone | Bio.Merieux-France- | LH ElA Kit | Bio. Cheak–U.S.A– |

Method of sample collection

40 blood samples were collected from women who suffer from secondary menopause, their ages ranged between (15-42) years and 30 samples from healthy females, their ages ranged between (17-45). Blood samples were obtained from one of the veins of the forearm of a size (10). Milliliters per patient. The blood was placed in disposable EDTA-free test tubes.

Then the blood was left at room temperature for (20) minutes, after which the serum was separated using a Centrifuge centrifuge at a speed of (3000) revolutions / minute for a period of (10) minutes, after which the serum was withdrawn using a micropippette. The serum, which was separated, was placed in three diposable tubes (each patient had three test tubes) in order to ensure that the sample was not exposed to thawing and freezing more than once, and then obtaining very accurate results and before the blood was preserved, it was confirmed that none was present. The following factors:

- 1. Hemolysis
- 2. The presence of fats in the blood lipemia
- 3. The presence of Bilirubinemia, which is the disease that results when the formulation of Bilirubin is increased in the blood.

Some of these factors appeared in some samples and accordingly, they were neglected, and new samples were collected in their place.

The blood was then preserved in a deep freezer in order to preserve it at low temperatures until the required tests are carried out.

1. Measuring the concentration of the hormone LH in patients sero Secondary Amenorrhea

The concentration of LH hormone was measured by following the steps provided with its test kit and according to the manufacturer's instructions for the Elisa device (Dawson et al., 1992).

2. Measuring the concentration of the hormone FSH in patients sero Secondary Amenorrhea

The measured concentration of the hormone FSH by following the steps attached with several test their own according to the manufacturer's instructions to your own Elisa. (Dawson et al., 1992)

3. Measuring the concentration of the hormone Progesterone in patients sero Secondary Amenorrhea

The concentration of Prog hormone was measured in the serum of the patients suffering from secondary menopause and the control group using the mini VIDAS device by following the steps included with the test kit for the Prog hormone.

4. Measuring the concentration of the hormone E2 in patients sero Secondary Amenorrhea

The concentration of E2 hormone in the blood serum of the patients suffering from secondary menopause and the control group was measured using the mini VIDAS device, by following the steps attached to the E2 test kit.

Statistical Analysis

The data for the study samples were collected and statistically analyzed using the (SPSS 20) system for Windows (SPSS, Chicago, Illionois and U.S.A.).

RESULTS

The study included (40) serum samples belonging to Secondary Amenorrhea patients, and it was found in the results of the study that there are clear differences for the patients.

1- LH concentration test in Secondary Amenorrhea patients:-

The results of the LH concentration test in the serum of secondary discontinuation patients indicated that there were high significant differences (P <0.01) where its concentration was mUl / ml (2.46 ± 20.25), when compared with the control group where its concentration was mUl / ml (0.33 ± 8.29) and as shown. In Table (1) and Figure (1).

| concentrations in the control group | | | | | | |
|-------------------------------------|------------------------------------|------------------------------|-------------------|--|--|--|
| Hormone | Control (Healthy) (30) | Patients with menopause (40) | Moral differences | | | |
| LH | 2.2 ± 7.2 b | 8.44 ± 19.4 a | P<0.01 | | | |
| FSH | $3.7\pm9.9~b$ | 15.78 ± 18.6 a | P<0.01 | | | |
| Progesterone | $2.07 \pm 3.4 \text{ a}$ | $1.14 \pm 2.26 \text{ b}$ | P<0.05 | | | |
| Estrogen | 24.4 ± 117.6 a | 21.5 ± 73.8 b | P<0.01 | | | |

Table (1) shows the measured hormone concentrations in the patients' serum and its comparison with the measured hormone concentrations in the control group

Different letters mean there is a significant difference. Tabular values represent hormone rate \pm standard error.



Figure No. (1): Shows a comparison of the level of hormones in menopausal patients (No. 40) with their level in healthy people (No. 30). P <0.01, compared to the control group (healthy controls.

| Table (2) shows the percentage of hormones in the patients | | | | | | | |
|--|--|-----------|------------|--|--|--|--|
| Hormone | Percentage of female patients with secondary menopause | | | | | | |
| | Hig | h Mo | derate Low | | | | |
| LH | 36.87(74%) | 8.12(16%) | 5(10%) | | | | |
| FSH | 18.12(36%) | 16.8(34%) | 15 (30%) | | | | |
| PROG | 44.37(89%) | 5(10%) | 0.62(1%) | | | | |
| E2 | 35.6(71%) | 7.5(15%) | 6.8 (14%) | | | | |

Where the percentage of patients who had a high level of LH (74%) and the percentage of patients who had a decrease in the level of LH (16%), while the percentage of patients who had a normal level of LH (10%), Table (2) as shown in Figure (2).



Figure No. (2) Percentage of female patients with secondary menopause according to the level of the hormone LH (number of patients = 40)

2- FSH concentration test in Secondary Amenorrhea serum

Table (1) and Fig. (1) indicate high significance (P <0.01) in the level of FSH concentration in the serum of secondary-discontinued patients, as its concentration was mUl / ml (18.6 15.78) when compared with the control group, where its concentration was mUl / ml (9.9 3.7).

Table (2) and Figure (3) indicate that the percentage of patients with an increase in FSH was (36%), the percentage of patients with a decrease in its level was (30%) and the percentage of patients with a normal level of the hormone was (34%).



Figure (3): Percentage of patients with menopause according to the level of the hormone FSH (No. of patients = 40). 3- Concentration level Prog test in patients sero Secondary Amenorrhea.

The results of measuring the level of Prog serum concentration in secondary menopausal patients showed clear significant differences (P <0.05) in its concentration, as it reached ng / ml (2.26 1.14) when compared with its concentration in the control group as it reached ng / ml (3.4 2.07). As shown in Table (1) and Figure (1).

Table (2) and Figure (4) indicate that the percentage of patients who had an increase in the level of Prog was (1%) and the percentage (89%) was for patients who had a decrease in the level of Prog, while the percentage (10%) was for patients who had a normal level of the hormone.



Figure No. (4) Percentage of female patients with secondary menopause according to the level of the hormone progesterone (number of patients = 40)

The results of the study indicated that there were clear significant differences (P < 0.05) when comparing the level of Prog with the time period of patients suffering from secondary menopause.

The level of E2 test in patients sero Secondary Amenorrhea

The results of the research indicate the presence of high significant differences (P <0.01) in the concentration of the hormone E2 in the serum of secondary menopausal patients, as its concentration reached pg / ml (21.5 73.8) when compared with the control group, where its concentration reached pg / ml (24.4 117.6). As shown in Table (1) and Figure (1).

Table (2) and Figure (5) show that the percentage of patients who had an increase in the level of E2 was (14%), and the percentage of patients who had a decrease in the level of the hormone was (71%), and the percentage of patients who had a normal level of the hormone was (%) 14).



Figure No. (5) Percentage of female patients with secondary menopause according to estrogen level (number of patients = 40)

DISCUSSION

The reason for the increase in the LH hormone in the serum of the majority of secondary menopausal patients, whose percentage (73.75%), is due to the infection of a large proportion of patients with polycystic ovaries, where their rate was (68.75%), as the results of patients who have a cyst in the ovaries showed an imbalance in the rate of secretion The hormone LH & FSH, where the ratio was (2: 1, FSH: LH), and this imbalance made the ovaries unable to secrete hormones in the correct way, which leads to disturbance of the menstrual cycle (Al-Hamdany & Gaffar , 2019).

Also, the high level of LH hormone in the serum of the patients came as a result of low levels of E2 and prog, as it is known that LH is inhibited by high levels of E2 and prog through the mechanism of negative feedback. (Warren & Stiehl, 1999)

And since the level of these two hormones was low in the majority of patients, it leads to an increase in the LH hormone, which indicates the occurrence of a state of primary pituitary dysfunction (Kol., 2020; Mustafa et al .,2019; Abdulwahed, et al.,2020).

These results were consistent with the results of many researchers (Azziz *et al* .2016; Scholes et al, 1999).

Other studies also indicate that a low FSH level coincides with a low LH level due to an abnormality in the pituitary gland (Beven & Scanlon, 1998; Marchiani et al., 2020).

This is what was observed in the results of the study, where it showed that (30%) of the patients had a decrease in FSH and, accordingly, there were high significant differences.

(P <0.01) When comparing the concentration of the LH level with the FSH level, the relationship was positive as the increase in the two hormones coincided with a decrease in the hormones E2 and Prog in the serum of the patients who had early ovarian deficiency. The decrease in the hormones LH and FSH was observed in the patients who have dysfunction In the pituitary gland and this is what he indicated (De Koning, 1995; Kol, 2020).

These results were consistent with research and studies indicating that a decrease in the level of Prog coincides with an increase in the level of the hormone LH and FSH as a result of a defect in the function of the pituitary gland that causes premature ovarian deficiency (Montagomeg et al, 1998). Therefore, the results showed significant differences (P < 0.05) when comparing the concentration of the hormone Prog with the time period of the disease.

Also, these results were consistent with the findings of (Molitch, 1995), who mentions that the low level of Prog is one of the reasons that lead to an imbalance in the Luteal phase of the ovarian cycle and then the occurrence of menopause in women.

Where the results showed that (71%) of the patients had a decrease in the concentration of the hormone, and this result was consistent with studies and research indicating that the decrease in E2 coincided with an increase in both LH and FSH and a decrease in Prog and that this is evidence of a woman suffering from ovarian impotence. Most secondary menopausal patients suffer from early (Pralong & Crowley, 1997; Sullivan-Pyke *et al.*,2020)

Also, these results were consistent with studies that confirm that women who take hormonal drugs containing Prog suffer from decreased levels of E2 (Glasier, 1999).

CONCLUSIONS

- 1- High significant differences (P < 0.05) in the level of hormones LH, FSH, E2, Prog and serum in patients compared with the control group.
- 2- There was an increase in the levels of the hormones LH, FSH, PRL, Testo in the serum of the majority of patients compared with the control group.
- 3- There was a decrease in the levels of hormones E2 and Prog in the serum of the majority of patients compared with the control group.

RECOMMENDATIONS

- 1- Conducting more studies to find out the extent of women experiencing secondary menopause.
- 2- Conducting more studies to know the effect of drugs, obesity, PCOS, and the presence of a similar case when contracting the disease.
- 3- Conducting more studies to find out the effect of Secondary Amenorrhea on the incidence of heart disease, osteoporosis, infertility and mental state disorder.

4- Conducting extensive Iraqi studies to know the extent of Iraqi women becoming infected with this disease.

REFERENCES

- [1] Abdulwahed, A.M.H., Alkanaani, M.I.M., Alsamarrai, A.H., ...Ahmed, T., Al-Samarraie, M.Q. (2020) Determination of some visfatin hormone level and lipid profilein some breast cancer patients in Samarra city. Annals of Tropical Medicine and Public Health, 2020, 23(1), S420
- [2] Al-Hamdany, W. A. S., & Gaffar, M. K. (2019). A Comparison Effect of Dysmenorrhea and Secondary Amenorrhea in the Concentration of Some Biochemical and Some Hormones in Women in Tikrit City. Tikrit Journal of Pure Science, 24(2), 37-42.
- [3] Al-Samarrai, M.K.O., Al-Samarraie, M.Q., Alelyan, U.A.A.(2019). Estimation of white blood cells, hemoglobin and copper levels in bakery workers.Biochemical and Cellular Archives, 2019, 19, pp. 2327-2330.
- [4] Azziz, R., Carmina, E., Chen, Z., Dunaif, A., Laven, J. S., Legro, R. S., ... & Yildiz, B. O. (2016). Polycystic ovary syndrome. Nature reviews Disease primers, 2(1), 1-18.]
- [5] Berga, S. L., Genazzani, A. R., Naftolin, F., & Petraglia, F. (Eds.). (2019). Menstrual Cycle Related Disorders: Volume 7: Frontiers in Gynecological Endocrinology. Springer.
- [6] Dawson, G. J., Chau, K. H., Cabal, C. M., Yarbough, P. O., Reyes, G. R., & Mushahwar, I. K. (1992). Solid-phase enzyme-linked immunosorbent assay for hepatitis E virus IgG and IgM antibodies utilizing recombinant antigens and synthetic peptides. Journal of virological methods, 38(1), 175-186.
- [7] De Koning J. 1995. Gonadotrophin surge-inhibiting attenuating factor governs luteinizing hormone secretion during the ovarian cycle: physiology and pathology, Hum Reprod 10:2854.
- [8] Glasier, A. (1999) Contraception. In: Edmonds, D. K., (Ed.) Dewhurst's Textbook of Obstetrics & Gynecology for postgraduates. 6th ed. London: Blackwell Science. 373-386.
- [9] Gordon, C. M., Ackerman, K. E., Berga, S. L., Kaplan, J. R., Mastorakos, G., Misra, M., ... & Warren, M. P. (2017). Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism, 102(5), 1413-1439.
- [10] Iyengar, N. M., Arthur, R., Manson, J. E., Chlebowski, R. T., Kroenke, C. H., Peterson, L., ... & Nassir, R. (2019). Association of body fat and risk of breast cancer in postmenopausal women with normal body mass index: a secondary analysis of a randomized clinical trial and observational study. JAMA oncology, 5(2), 155-163.
- [11] Kol, S. (2020). LH Supplementation in Ovarian Stimulation for IVF: The Individual, LH Deficient, Patient Perspective. Gynecologic and Obstetric Investigation, 85(4), 307-311.]
- [12] Marchiani, S., Tamburrino, L., Benini, F., Pallecchi, M., Bignozzi, C., Conforti, A., ... & Baldi, E. (2020). LH supplementation of ovarian stimulation protocols influences follicular fluid steroid composition contributing to the improvement of ovarian response in poor responder women. Scientific reports, 10(1), 1-12.
- [13] Molitch ME. 1995. Evaluation and Treatment of the patient with a pituitary incidentaioma: J. Clin Endocrinol Metab 80:3,
- [14] Montgomery Rice V; Limback SD; Roby KF; Terranova PF.1998. Differential responses of granulosa cells from small and large follicles to follicle stimulating

hormone (FSH) during the menstrual cycle and acyclicity: effects of tumour necrosis factor- J., Hum Reprod 13:1285.

- [15] Mustafa, H. A., Majid, H. H., Abdulqader, A. T., Mustafa, M. A., & Salih, A. A. Study On Some Physiological, Biochemical And Hormonal Parameters Of Seminal Fluid Of Infertile Men. Biochem. Cell. Arch. Vol. 19, Supplement 1, pp. 1943-1947, 2019
- [16] Pralong FP& Crowley WF Jr. 1997. Gonadotropins: Normal physiology. In Wierman ME (ed): Diseases of the Pituitary: Diagnosis and treatment. Totowa, Humana Press. pp 203-219.
- [17] Scholes, D; Lacronix, A.Z; Ott, S.M; et al. (1999) Bone mineral density in women using depot-medroxyprogesterone acetate for contraception. Obstetrics & Gynecology 93(2), 233-238.
- [18] Sullivan-Pyke, C., Mani, S., Rhon-Calderon, E. A., Ord, T., Coutifaris, C., Bartolomei, M. S., & Mainigi, M. (2020). Timing of exposure to gonadotropins has differential effects on the conceptus: evidence from a mouse model. Biology of Reproduction, 103(4), 854-865.
- [19] Warren, M.P. and Stiehl, A.L. (1999) Exercise and female adolescents: effects on the reproductive and skeletal systems. Journal of the American Medical Women's Association 54 (3), 115-120.