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Original Article

The evaluation of fertility biomarkers in women with endometriosis

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Abstract

Endometriosis is a chronic gynecological disease that causes infertility and pain among women of reproductive age, where tissue similar to that of endometrium grows outside the uterus. The current study aims to measure the levels of some fertility-related hormones and their relationship to disease physiology. The study included 90 serum samples divided into 45 women with EMT and 45 healthy women aged between 20 and 41 diagnosed by ultrasound and laparoscopy, and the questionnaire forum obtained all sample information. AMH, inhibin B, DHEA and SHBG were measured by the ELISA method and the results were statistically analyzed SPSS ($P<0.05$). The current results showed a significant decrease in serum AMH and inhibin B, a non-significant decrease in serum DHEA and SHBG in EMT patients compared to healthy women, a significant decrease in serum inhibin B in patients with advanced stage, and a significant decrease in serum AMH in non-fertile patients compared with fertile patients. In conclusion, fertility issues are recorded in many diagnosed cases of EMT, confirming the obvious imbalance in sex hormone levels due to the presence of uterine tissue in other sites of the body, which affects the hormonal response.

Keywords: anti-mullerian hormone, inhibin B, dehydroepiandrosterone, sex hormone binding globulin.

Introduction

Endometriosis (EMT) is a common disease that causes pain and infertility for women as it is characterized by the presence of tissue similar to that of the endometrium outside the uterus, and many factors interfere in the development of the disease, such as hormonal, immunological and neurological factors, where its final diagnosis depends on the surgical procedure, which makes the diagnosis late even after the appearance of symptoms [1]. These tissues have the same ability as the endometrium to hormonal response, where the signs of bleeding, inflammation and fibrosis appear; as a result, it leads to chronic pain and fertility problems. In most cases, EMT includes the pelvic organs and rarely reaches beyond that and usually ovarian cancer develops if the disease spreads to the ovaries [2]. EMT is a chronic gynecological and systemic disease affecting about 5-10% of women of reproductive age worldwide. It is associated with the emergence of pelvic lesions and affects the metabolism of the fatty tissue and the liver, causing inflammation and gene expression in the brain causing pain [3]. Other factors that may explain the pathogenesis of EMT are congenital and allergic factors; also, it is believed that the crossing of menstruation from the fallopian tubes to the pelvis may be one of the etiological factors, but the occurrence of retrograde menstruation has also been observed in healthy women giving way to other factors [4]. There is a long time between the appearance of symptoms, diagnosis and treatment, which makes it a very complex disease and the patient suffers from depression, anatomical changes related to infertility and sexual behavior changes related to pain, where the pain mechanics is physical and sensory pain, as well as EMT, reduces implantation and increase the chances of miscarriage [5]. Recent studies

have shown that stem cells responsible for the endometrium regeneration after menstruation become hyperactive and are stuck outside the uterus in the bloodstream, which leads to the formation of new glands and recruitment of polyclonal stromal cells, which leads to infiltrating EMT that later exposes it to inflammation as an immune response, and the formed glands may harbor some cancerous mutations, like that in ovarian cancer [6]. EMT reduces the ovarian reserve through inflammatory processes, leading to fibrosis and high reactive oxygen species production. Therapeutic surgeries may affect ovarian reserve during the removal of endometriotic tissue, and the pregnancy can be stimulated after surgery by ovarian stimulation or insemination. inside the uterine [7]. Pregnant women with EMT suffer from higher rates of obstetric problems such as cesarean sections, miscarriage and premature deliveries compared to normal pregnant women [8]. It depends heavily on laparoscopy for histological diagnosis of EMT more than the ultrasound because the latter cannot diagnose superficial EMT, but if the result is negative and the symptoms are clear, it is necessary to diagnose by laparoscopy [9]. The diversity of EMT lesions makes the response to treatment unstable, as some lesions are resistant to progesterone or do not need estrogen for their growth. However, if the disease is caused by oxidative stress from the peritoneal microbiome and retrograde menstruation, this treatment may be sufficient in this case to prevent the emergence of new lesions [10]. Anti-mullerian hormone (AMH) is a member of the TGF-β family made in the ovary that acts as an important parameter in measuring ovarian reserve and a woman's fertility; it is related to the development of ovarian follicles during menstruation and plays a vital role in the maturation of internal organs in women [11, 12]. AMH receptors are expressed by bone proteins, estrogens and gonadotropins, where AMH regulates folliculogenesis and has some neuroendocrine functions [13]. Inhibin B is a hormone produced by the small follicles of the ovary, while inhibin A is produced by the corpus luteum and the dominant follicle, where inhibins suppress the production of FSH by blocking the activins signals in the pituitary gland [14]. Dehydroepiandrosterone (DHEA) is a steroidal hormone secreted by the cortex of the thyroid gland, where it is synthesized through the cholesterol-pregnenolone pathway; it plays a role in delaying ovarian aging and has a clear effect on the ovarian reserve when its concentration decreases in the blood [15, 16]. Human sex hormone-binding globulin (SHBG) is a glycoprotein produced in the liver. It binds to blood sex steroids, testosterone and oestradiol to control their levels and has a relationship with some metabolic diseases such as insulin resistance and non-alcoholic fatty liver disease; also, its low concentration leads to elevated androgens leading to impaired ovarian function and anovulation [17, 18]. The current study aims to measure the levels of some fertility parameters that may have an important effect on the fertility of EMT patients and compare it with healthy women in the Basra governorate and link the significant levels of these parameters with some factors related to the disease.

Material and methods

The current study was conducted in Basra governorate/Iraq (June to September 2022) based on serum samples (45 women with EMT and 45 healthy women) during the luteal phase and with ages ranging from 20 to 41 obtained from a private laboratory under the supervision of a specialized gynecologist depending on ultrasound and laparoscopy for diagnosis. It was ensured that all women in the study were free of any other gynecological diseases and chronic diseases; also, it was ensured that these women had not taken any medications in the four months preceding the sampling that may have affected the results of the tests. A questionnaire form was used to obtain the basic information included in the study and the stages of 3 ml of serum were obtained by centrifugation from both patients and control groups. Then, all samples were frozen in the deep freezer at -20°C until the analyses were carried out using ELISA kits: AMH, inhibin B and SHBG (Elabscience/USA), DHEA (SunLong Biotech Co., LTD/China).

Statistical analysis

The statistical analysis is carried out by using SPSS. $P<0.05$ is considered statistically significant, and the difference between patients and control groups was evaluated by (T-test and one-way ANOVA test).

Results

The frequency distribution of the study sample data is indicated in Table 1, where the current study showed a significant decrease in the level of serum AMH and inhibin B in EMT patients when compared with the control group, while there was no significant difference in the level of DHEA and SHBG, Table 2.

Table 1: Demography of the study samples.

Category	Group	Patients (N=45) %	Control (N=45) %
Age (year)	20–30	40.9	62.2
	31–41	59.1	37.8
BMI (kg/m²)	Normal weight	33.3	62.2
	Pre-obesity	43.6	17.8
	Obese	23.1	20.0
Marital	Single	52.9	40
	Married	47.1	60
Fertility	Fertile	39.6	-
	Non-fertile	60.4	-
Pain	Sex pain	77	-
	Period pain	86	-
Menstruation	Normal	20.8	-
	Amenorrhea	3.5	-
	Menorrhagia	75.7	-

The present study showed a significant decrease in the level of serum inhibin B in patients with stage 3 and stage 4 compared with stage 1, while there was no significant difference in the level of AMH, Table 3. Also, the result recorded a significant decrease in the level of AMH in non-fertile patients compared with fertile patients, while there was no significant difference in the level of AMH, Table 4.

Discussion

The current study recorded a decrease in AMH and Inhibin B in women with EMT compared to healthy women, a significant decrease in inhibin B in patients with progressive stages and a significant decrease in the AMH in non-fertile patients compared with fertile patients. Several studies have confirmed that EMT decreases ovarian reserve through low levels of AMH and antral follicle count, also a high follicle-stimulating hormone (FSH) level, especially in the advanced stages of the disease or in the case of ovarian endometrioma [19]. Infertile females with EMT have lower AMH and higher prolactin levels than infertile females without EMT, especially in patients with ovarian endometriomas [20]. Laparoscopic surgery is one of the important factors that reduce the level of AMH in EMT patients, especially after several months of surgery [21]. In the case of ovarian EMT, the ovary reduces the production of AMH by granulosa cells and this, in turn, affects its level in the peritoneal fluid when the disease reaches this site, where the AMHR2 receptors are expressed in this tissue and reflect the role played by this hormone in the development of disease lesions [22]. Another study

Table 2: Serum levels of the study parameters in EMT patients and control.

Parameter	Endo. patients (N=45) Mean±SD	Control (N=45) Mean±SD	P-value
AMH(ng/ml)	1.01±0.87	2.61±2.02	0.003*
Inhibin B(pg/ml)	26.26±18.99	82.90±37.23	0.025*
DHEA (pg/ml)	1590.84±283.49	1402.41±178.91	0.214*
SHBG (pmol/ml)	65.77±24.19	39.46±30.06	0.258

Note: * – Significant at P≤0.05.

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Table 3: Serum levels of the study parameters in EMT patients and control according to the disease stages.

Parameter	Stage 1 N=12 Mean±SD	Stage2 N=10 Mean±SD	Stage3 N=12 Mean±SD	Stage4 N=11 Mean±SD	P-value
AMH (ng/ml)	1.09±.921	1.10±1.010	0.99±0.95	0.87±0.67	0.92
Inhibin B (pg/ml)	42.49±23.43	27.95±16.09	19.14±14.46	14.93±6.69	0.002* stage 3 and stage 4 *vs*. stage1

Note: * – Significant at P≤0.05.

Table 4: Serum levels of the study parameters in EMT patients and control according to the disease stages.

Parameter	Fertile patients N=19	Non-fertile patients N=26	P-value
AMH (ng/ml)	1.17±1.01	0.8635±0.71	0.026*
Inhibin B (pg/ml)	27.94±22.27	24.49±15.14	0.118

Note: * – Significant at P≤0.05.

showed that AMH levels in women with EMT over the age of 40 had low AMH, while EMT women with large ovarian endometriomas had elevated AMH [23]. A decrease in inhibin B level was observed in women with EMT through ovarian stimulation by giving HCG, which leads to a weakening of the function of the granulosa cell in the ovaries, where inhibin B can be used as a marker to measure the growth of the ovarian follicles or to identify the retrieved oocytes numbers [24]. A decreased level of serum inhibins was also observed in EMT patients women who experienced ovarian stimulation for the purpose of external fertilization during egg collection and embryo transfer [25]. In another study, lower serum Inhibin A was recorded compared with cystic and peritoneal fluids, and the higher level was in cystic fluid, and α and βA subunits were clearly expressed in the stromal and epithelial elements in the affected ovary [26].

The present study showed a non-significant increase in DHEA and SHBG between patients with EMT and control. The levels of serum testosterone, androstenedione, DHEA and DHEA-S showed a non-significant increase in the female patients with EMT compared to the healthy women [27]. In another study, there was a significant association between cancer antigen-19-9 and cancer antigen-125 with DHEA-S; this indicates that elevated androgens are an important marker of endometrial pathology [28]. Elevated testosterone and DHEA were significantly observed in endometriosis lesions in the ovary compared to its concentration in serum, especially in women treated with contraceptive medication, and these high quantities are produced from these lesions and the tissues adjacent to it in the ovary [29]. An increase in the SHBG gene expression was observed in the endometriotic tissues, which led to providing a suitable environment for the activity of estrogen, and the estrogen-related to SHBG is protected from liver metabolism and existing in the endometrial cells, which leads to the emergence of endometriosis in the pelvic region [30]. The increased SHBG was recorded in EMT women aged between 21 to 37 years, which may be associated with some of the pathological mechanisms of infertility issues in EMT patients [31].

Conclusion

The current study shows the clear effect of the spread of uterine tissues outside the uterine itself, such as the ovaries, where its effect on ovarian functions and the normal response to the hormonal mechanics associated with fertility quality, and the general level of these hormone concentrations that related to the general health of the EMT patients. On this basis, it is preferable to conduct additional studies to cover all the physiological aspects of other factors associated with study hormones that affect the fertility in these patients.

Conflict of interest

The author declares no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the College of Health and Medical Techniques, Southern Technical University (approval ID: 204).

Consent to participate

All informed consent was obtained from all cases included in this study.

References

1. Saunders P.T., Horne A.W. Endometriosis: Etiology, pathobiology, and therapeutic prospects. Cell. 2021;184(11):2807-2824. doi: 10.1016/j.cell.2021.04.041. PMID: 34048704.
2. Arafah M., Rashid S., Akhtar M. Endometriosis: a comprehensive review. Advances in Anatomic Pathology. 2021;28(1):30-43. doi: 10.1097/PAP.0000000000000288. PMID: 33044230.
3. Taylor H.S., Kotlyar A.M., Flores V.A. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. The Lancet. 2021;397(10276):839-852. doi: 10.1016/S0140-6736(21)00389-5. PMID: 33640070.
4. Smolarz B., Szyłło K., Romanowicz H. Endometriosis: epidemiology, classification, pathogenesis, treatment and genetics (review of literature). International Journal of Molecular Sciences. 2021;22(19):10554. doi: 10.3390/ijms221910554. PMID: 34638893; PMCID: PMC8508982.
5. Gruber T.M., Mechsner S. Pathogenesis of Endometriosis: the origin of pain and subfertility. Cells. 2021;10(6):1381. doi: 10.3390/cells10061381. PMID: 34205040; PMCID: PMC8226491.
6. Wang Y., Nicholes K., Shih I.M. The origin and pathogenesis of endometriosis. Annual review of pathology. 2020;15:71. doi: 10.1146/annurev-pathmechdis-012419-032654.
7. Lee D., Kim S.K., Lee J.R., Jee B.C. Management of Endometriosis -related infertility: Considerations and treatment options. Clinical and experimental reproductive medicine. 2020;47(1): 1.
8. Porpora M.G., Tomao F., Ticino A., Piacenti I., Scaramuzzino S., Simonetti S., *et al.* Endometriosis and pregnancy: a single institution experience. International Journal of Environmental Research and Public Health. 2020;17(2):401. doi: 10.3390/ijerph17020401. PMID: 31936225; PMCID: PMC7014217.
9. Chen-Dixon K., Uzuner C., Mak J., Condous G. Effectiveness of ultrasound for Endometriosis diagnosis. Current Opinion in Obstetrics and Gynecology. 2022;34(5):324-331. doi: 10.1097/GCO.0000000000000812. PMID: 36036477.
10. Koninckx P.R., Fernandes R., Ussia A., Schindler L., Wattiez A., Al-Suwaidi S., *et al.* Pathogenesis Based Diagnosis and Treatment of Endometriosis. Frontiers in Endocrinology. 2021;12:745548. https://www.frontiersin.org/articles/10.3389 / fendo.2021.745548/full
11. Josso N., Picard J.Y. Genetics of anti-Müllerian hormone and its signaling pathway. Best Practice & Research Clinical Endocrinology & Metabolism. 2022;101634. doi: 10.1016/j.beem.2022.101634.
12. Cedars M.I. Evaluation of Female Fertility—AMH and Ovarian Reserve Testing. The Journal of Clinical Endocrinology & Metabolism. 2022;107(6):1510-1519. doi: 10.1210/clinem/dgac039. PMID: 35100616.
13. Di Clemente N., Racine C., Pierre A., Taieb J. Anti-Müllerian hormone in female reproduction. Endocrine reviews. 2021;42(6):753-782. doi: 10.1210/endrev/bnab012.
14. Goney M.P., Wilce M.C., Wilce J.A., Stocker W.A., Goodchild G.M., Chan K.L., *et al.* Engineering the ovarian hormones inhibin A and inhibin B to enhance synthesis and activity. Endocrinology. 2020;161(8): bqaa099. doi: 10.1210/endocr/bqaa099.
15. Jankowska K., Maksym R., Zgliczynski, W. DHEA in infertility–can you reverse the flow of time?. In Endocrine Abstracts (Vol. 70). Bioscientifica. Endocrine Abstracts. 2020; 70 EP404 | DOI: 10.1530/endoabs.70.EP404.
16. Sahu P., Gidwani B., Dhongade H.J. Pharmacological activities of dehydroepiandrosterone: A review. Steroids. 2020;153:108507. doi: 10.1016/j.steroids.2019.108507.
17. Qu X., Donnelly R. Sex hormone-binding globulin (SHBG) as an early biomarker and therapeutic target in polycystic ovary syndrome. International journal of molecular sciences. 2020;21(21):8191. doi: 10.3390/ijms21218191.
18. Bourebaba N., Ngo T., Śmieszek A., Bourebaba L., Marycz, K. Sex hormone binding globulin as a potential drug candidate for liver-related metabolic disorders treatment. Biomedicine & Pharmacotherapy. 2022;153:113261. doi: 10.1016/j.biopha.2022.113261.
19. Tian Z., Zhang Y., Zhang C., Wang Y., Zhu, H.L. Antral follicle count is reduced in the presence of endometriosis: a systematic review and meta-analysis. Reproductive biomedicine online. 2021;42(1):237-247. doi: 10.1016/j.rbmo.2020.09.014.
20. Pedachenko N., Anagnostis P., Shemelko T., Tukhtarian R., Alabbas L. Serum anti-Mullerian hormone, prolactin and estradiol concentrations in infertile women with endometriosis. Gynecological Endocrinology. 2021;37(2):162-165. doi: 10.1080/09513590.2020.1855634.
21. Nankali A., Kazeminia M., Jamshidi P. K., Shohaimi S., Salari N., Mohammadi M., Hosseinian-Far A. The effect of unilateral and bilateral laparoscopic surgery for Endometriosis on Anti-Mullerian Hormone (AMH) level after 3 and 6 months: a systematic review and meta-analysis. Health and quality of life outcomes. 2020;18(1):1-9. doi.org/10.1186/s12955-020-01561-3.
22. Kitajima M., Matsumoto K., Murakami N., Kajimura I., Harada A., Kitajima Y., *et al.* AMH Concentrations in Peritoneal Fluids of Women With and Without Endometriosis. Frontiers in Surgery. 2020;7:600202. doi: 10.3389/fsurg.2020.600202. eCollection 2020.
23. Roman H., Chanavaz-Lacheray I., Mircea O., Berby B., Dehan L., Braund S., *et al.* Large ovarian endometriomas are associated with high pre-operative anti-Müllerian hormone concentrations. Reproductive BioMedicine Online. 2021;42(1):158-164. doi: 10.1016/j.rbmo.2020.09.008. Epub 2020 Sep 11. PMID: 33060013.
24. Dokras A., Habana A., Giraldo J., Jones E. Secretion of inhibin B during ovarian stimulation is decreased in infertile women with endometriosis. Fertility and sterility. 2000;74(1):35-40. doi: 10.1016/s0015-0282(00)00568-9.
25. Babčová K., Ulčová-Gallová Z., Rumpík D., Mičanová Z., Bibková K. Inhibin A and B levels in serum and follicular fluids of women with various reproductive failures undergoing in vitro fertilization. Ginekologia Polska. 2015;86(10): 726-730. doi: 10.17772/gp/57844.

 https://doi.org/10.46389/rjd-2023-1331

26. Reis F.M., Di Blasio A.M., Florio P., Ambrosini G., Di Loreto C., Petraglia F. Evidence for local production of inhibin A and activin A in patients with ovarian endometriosis. Fertility and sterility. 2001;75(2):367-373. doi: 10.1016/s0015-0282(00)01720-9. PMID: 11172841.
27. Evsen M.S., Sak M.E., Soydinc H.E., Guven S., Basaranoglu S., Hatipoglu N.K., *et al*. Serum levels of androgens and prostate-specific antigen in EMT. Clinical and Experimental Obstetrics & Gynecology. 2014;41(4):432-435. PMID: 25134292.
28. Fiala L., Bob P., Raboch, J. Oncological markers CA-125, CA 19-9 and endometriosis. Medicine. 2018;97(51): e13759. doi: 10.1097/MD.0000000000013759. PMID: 30572523; PMCID: PMC6320090.
29. Huhtinen K., Saloniemi-Heinonen T., Keski-Rahkonen P., Desai R., Laajala D., Ståhle M., *et al*. Intra-tissue steroid profiling indicates differential progesterone and testosterone metabolism in the endometrium and endometriosis lesions. The Journal of Clinical Endocrinology & Metabolism. 2014;99(11):E2188-E2197. doi: 10.1210/jc.2014-1913.
30. Huang R., Ma Y., Holm R., Trope C.G., Nesland J.M., Suo, Z. Sex hormone-binding globulin (SHBG) expression in ovarian carcinomas and its clinicopathological associations. PloS one. 2013;8(12): e83238. https://doi.org/10.1371/journal.pone.0083238
31. Dinsdale N.L., Crespi B.J. Endometriosis and polycystic ovary syndrome are diametric disorders. Evolutionary applications. 2021;14(7):1693–1715. https://doi.org/10.1111/eva.13244.