RESEARCH ARTICLE

Evaluation of CA-125 and IL-17 in a Sample of Iraqi Women with Endometriosis

Wassan A. K. Abbas[1], Haneen S. Shaheed[2]*

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq
[2]Department of Pharmacy, Ashur University College, Baghdad, Iraq

ABSTRACT

Endometriosis is a disease with unknown pathogenesis that can lead to infertility; it is benign, an estrogen-dependent gynecological disease characterized by the implantation, growth, and development of endometrial tissue is outside of uterine tissue may lead cause to endometrial cancer because it considered a common, chronic inflammatory disease, and etiology of the disease remains unclear. The main challenge of endometriosis in diagnosis and management is because it considers as a benign gynecological disorder. Many factors play a crucial role in the inflammatory process of endometriosis chronically, such as age, weight, interleukins, hormones, and other factors, which have been implicated in the disease's pathogenesis. The present study was designed to measure the serum cancer antigen 125 (CA-125) and interleukin-17 (IL-17) levels in endometriosis patients to explain the role of these markers in the pathogenesis of disease and their correlation with age, body mass index BMI and correlation of these markers with each other. A total of 30 endometriosis patients and 30 healthy women, matching in average age and body mass index (BMI), were enrolled in this study. Patients with endometriosis and healthy women were attending Kamal Al-Samarrai Fertility and Infertility Hospital. Blood samples were aspirated from both groups to measure the serum CA-125 IL-17 levels by using enzyme-linked immunosorbent assay (ELISA) technique. The obtained results of serum cancer antigen-125 (CA-125) levels showed a highly significant elevation ($p < 0.0001$) in patients when compared with a control group. Also, the results recorded a highly significant elevation ($p < 0.0001$) in serum levels of interleukin-17(IL-17) between patients and the control group. On the other hand, the results declared that there was a highly significant inverse correlation between age and markers cancer antigen-125(CA-125) and interleukin-17(IL-17). The findings of the present study showed no significant correlation between serum cancer antigen (CA-125) levels and interleukin-17 (IL-17) with BMI and, regarding highly direct correlation among the studied markers (CA-125, IL-17), these results explain the role of CA-125 and IL-17in the pathogenesis of endometrioses.

Keywords: Cancer Antigen-125 (CA-125), Endometriosis, Enzyme-linked Immunosorbent Assay (ELISA) technique, Interleukin-17(IL-17).

INTRODUCTION

Endometriosis is a common chronic gynecological estrogen-dependent disorder. The prevalence of endometriosis is between 2–10% in young women and up to 50% in infertile women, and it increases the risk for developing in case of fibromyalgia, autoimmune inflammatory diseases, chronic fatigue syndrome, and atopic diseases more than the general population. High association between endometriosis and stress may be associated with the deregulation of the stress system, riskiness of the disease is that it is one of the causes of female infertility.[1]

Endometrioses characterized by the presence of uterine endometrial tissue external the uterine cavity may present as superficial may be deep in pelvic peritoneal implants, adhesions, and ovarian cysts (endometriomas),[2] endometriosis cause pelvic pain and infertility. Endometriosis is classified into four stages (minimal, mild, moderate, and severe disease) according to the staging system of the American Society for Reproductive Investigation (ASRM). Ultrasound is used to diagnose deep endometriotic nodules, laparoscopic visualization of lesions, and ovarian endometriotic cysts.[3]

Several factors play a crucial role in the chronic inflammatory mechanism of disorder, such as age, weight interleukins, hormones and cancer marker, and others, which have been implicated in the disease's pathogenesis.[4]

This study deals with a correlation of cancer antigene-125(CA-125) and IL-17 with endometriosis and the role of

*Author for Correspondence: ph.haneen2014@gmail.com, haneen.subhee@au.edu.iq

age and body weight in developing a disease in symptomatic women with primary or secondary fertility.

Cancer antigen (CA-125) has been reported to be elevated in endometriosis patients and is the most commonly described, extensively studied endometriosis biomarker to date,[5] it's a high molecular-weight glycoprotein normally expressed on tissues derived from the coelomic and Mullerian epithelia, including the uterus endometrium making it strong pathogenesis factor in the disease.[6]

Interlukine-17 is produced by TCRα/β+CD4−CD8− thymocytes and activated CD4+ and CD4+CD45RO+ memory T cells, play a crucial role in inflammations, polycystic ovarian syndrome (PCOS) and may be involved in the pathogenesis of endometriosis by inducing the production of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α.[7,8]

The current study hypothesized that IL-17 might be involved in the pathogenesis of endometriosis by inducing the production of pro-inflammatory cytokines with the role of other markers evaluated in this study.

SUBJECTS, MATERIALS, AND METHODS

Sixty Iraqi women participated in this study**,** Thirty women were already diagnosed with endometriosis, and the other thirty were healthy apparent. Proceeding to enrollment of participants, history information like age, BMI, concomitant diseases, other treatment were taken after achieving diagnoses by a consultant gynecologist based on abdominal ultrasound.

Measurement of Body Mass Index (BMI)

The BMI was determined by dividing the individual weight (in kilogram) by the squared value of height (in meter) as follow[9]:
$$BMI = Wt\ (kg)\ /\ ht^2\ (m^2).$$

Blood Samples Collection and Measurement of Biochemical and Hormonal Markers

Blood samples were collected from subjects, was placed in gel tubes (contain a clotting activator), and centrifuged within 10 minutes at speed (3000 r.p.m) for 3 min. Then sera of all blood samples were separated and stored at - 20°C until the assay time. The collected sera were used for detection of Cancer antigene-125 (CA-125), Interleukin-17(IL-17) by enzyme-linked immunosorbent assay (ELISA) technique, using readymade kits (Mybioscource, USA for cancer antigen (CA-125), Interleukin-17).

Statistical Analysis

Data were collected and analyzed as the following:
- Statistical Analysis System (SAS) 2012 program was used to show the mean and standard error of the mean (M± SE).
- A dependent t-test was used to compare between mean values.
- The probability considered significantly different when $p < 0.05$, a highly significant difference when $p < 0.01$, and non-significant (NS) when $p > 0.05$.
- Correlation coefficient (*r*-value) was used to test the linear relationship between markers.

The *r*-value or correlation coefficient was expressed as a negative value when there is an inverse correlation, positive value when there is a direct correlation, and zero when there is no correlation.[10]

RESULTS

Demographics and Baseline Characteristics

In the current study, Sixty Iraqi women have participated. Thirty of them were diagnosed with endometriosis; they were considered patients group, other thirty healthy apparent women were considered as a control group. The results recorded matching in mean ages and BMI between two groups. The mean ages of the patient's group were 32.70 ± 0.98 years. The mean age of the control group was 31.46 ± 1.20 years, there BMI mean was29.52 ± 0.54 kg/m^2 of the control group while 28.77 ± 0.34 kg/m^2 of patients, as illustrated in Table 1.

In the current study, the patient group was participated. Nineteen of them were diagnosed with primary infertility, representing 63.33% of the group, while the remaining 36.67%, 11 patients, were diagnosed with secondary infertility, as shown in Table 2.

Serum Level of Cancer Antigen-125(CA-125)

The results demonstrated that the mean level of CA-125 has been elevated significantly in sera of patients group (54.43 ± 2.27 IU/mL) in comparison with a control group (3.92 ± 0.16 IU/mL), ($p < 0.0001$), as clarified in Table 3 and Figure 1.

Serum Level of Interleukin (IL-17)

The results demonstrated that the mean level of IL-17 has been elevated significantly in sera of patients group (197.96 ± 11.77 IU/mL) in comparison with a control group (129.93 ± 5.10 IU/mL), ($p < 0.0001$), as clarified in Table 4 and Figure 2.

Correlation Coefficient between Age, BMI, CA-125, I-17, Inpatient Group.

The results of the current study clarified that age has a non-significant direct correlation ($p > 0.05$) with BMI highly

Table 1: Demographics and Baseline Characteristics.

Group	No	Mean ± SE	
		Age (year)	BMI (kg/m^2)
Control	30	31.46 ± 1.20	29.52 ± 0.54
Patients	30	32.70 ± 0.98	28.77 ± 0.34
T-test	---	3.118 **	2.0017 NS
p-value	---	0.0001	0.2400

** ($p \leq 0.01$).

**= highly significant difference ($p < 0.01$).

Table 2: Caption missing

Infertility	No	Percentage (%)
Primary	19	63.33
secondary	11	36.67
Total	30	100%
P-value	---	0.0002 **

** ($p \leq 0.01$).
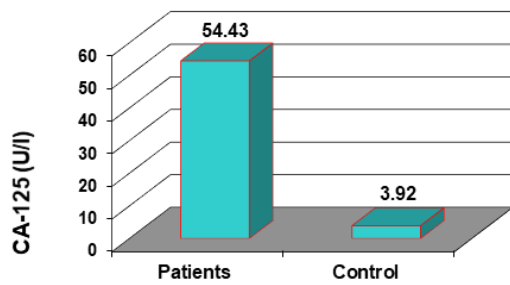
**= highly significant difference ($p < 0.01$).

Figure 1: Mean Serum level of Cancer Antigen-125 in Patients and Control group.
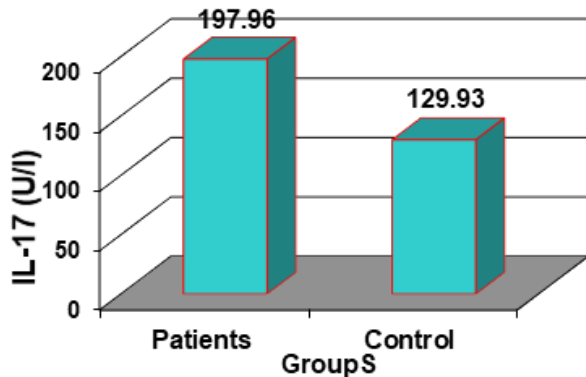CA-125 = Cancer antigen-125, **=highly significant difference (p <0.01).

Figure 2: mean serum level of interleukin-17 in Patients and Control Group.
IL-17 = Interlukin, **=highly significant difference (p <0.01).

Table 3: Mean Serum level of Cancer Antigen-125 in patients and control group.

Group	Mean ± SE of CA-125 (IU/mL)
Control	3.92 ± 0.16
Patients	54.43 ± 2.27
T-test	4.567 **
p-value	0.0001

CA-125 = Cancer antigen, **=highly significant difference (p <0.01).

Table 4: Mean serum level of interleukin-17 in patients and control group.

Group	Mean ± SE of IL-17 (IU/mL)
Control	129.93 ± 5.10
Patients	197.96 ± 11.77
T-test	25.686 **
P-value	0.0001

IL-17 = Interleukin, **=highly significant difference (p <0.01).

significant inverse correlation ($P < 0.01$) with CA-125 and IL-17.

Regarding BMI, there was a non-significant inverse correlation coefficient (p >0.01) with CA-125 and inverse correlation coefficient at a non-significant level (p >0.01) with IL-17a, while CA-125 recorded a highly significant correlation (p <0.01) with IL-17a, as shown in Table 5.

Table 5: correlation coefficient between difference variables in patients.

Variables	Correlation coefficient-r	Sig.
Age & BMI	0.18	NS
Age & CA-125	-0.77	**
Age & IL-17a	-0.54	**
BMI & CA-125	-0.11	NS
BMI & IL-17a	-0.16	NS
CA-125 & IL-17a	0.48	**

** (p ≤0.01), NS: Non-Significant.

DISCUSSION

Endometriosis and infertility are associated clinically; the current study was to explain the relation between endometriosis and CA-125, IL-17and show the role of these biomarkers in the development of disease complications.

An elevated level of CA-125 in serum may also be caused by the inflammatory reactions that alter the endothelial permeability leading to the liberation of the marker into circulation.[11] Depending on the results of this study that shows a highly significant difference between patients and control group in serum level of CA-125, as shown in Table 3 and Figure 1, this result agrees with the study explain the role of CA-125 levels to identify endometriosis-associated ovarian cancer, and agree with the result of Budak E *et al*. (2019) study showed significant elevation of serum level of CA-125 levels in endometrial carcinomas.[12]

As a result, showed in this study, CA-125 showed a high inverse correlation coefficient with ages of patients; this result agrees with the result of Deutsch K *et al*. (2019) study shows the correlation between CA-125 and Grade 1 endometrial cancer.[13] And disagree with the study of Karimi-Zarchi M *et al*. (2016) explain the Correlation of CA-125 serum level of patients with endometriosis.[14]

Interleukin-17 (IL-17) plays a very important role to increase immunity in general, by induction the immune response against the foreign body, helper T cell 17 (Th17) cell also play a pivotal role in the pathogenesis of endometriosis, miscarriage, preterm labor, and preeclampsia, and complete pregnancy process.[15] The current study shows the relation between IL-17 and endometriosis in Table 4 and Figure 2. The results demonstrated that the mean level of IL-17 has been elevated significantly in sera of patients group in comparison with the control group; these results agree with many studies Tarokh M *et al*. (2019),[16] Miller JE *et al*.(2020)[17] and Kitazawa J *et al*.(2020).[18]

On the other hand, the results of the current study have shown inverse correlation of serum IL-17 levels with the age of patients at a highly significant level because the decrease of immunity with increasing age and bad life style,[19] otherwise the result show a direct correlation coefficient at highly significant level between CA-125 and IL-17 that ensure the role of CA-125 in stimulation immune system to protect the body and useful prognostic power to predict severity of the disease.[20]

CONCLUSIONS

Serum cancer antigen 125 (CA-125) and interleukine-17 (IL-17) levels are elevated in the serum of endometriosis patients and play an important role in the pathogenesis of disease by different mechanisms like stimulation immune system and produced cancer antigens and interleukins.

ACKNOWLEDGMENTS

REFERENCES

1. Kajiyama H, Suzuki S, Yoshihara M, Tamauchi S, Yoshikawa N, Niimi K, Shibata K, Kikkawa F. Endometriosis and cancer. Free Radical Biology and Medicine. 2019 Mar 1;133:186-92.

2. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. Annual Review of Pathology: Mechanisms of Disease. 2020 Jan 24;15:71-95.

3. Vanhie A, O D, Peterse D, Beckers A, Cuéllar A, Fassbender A, Meuleman C, Mestdagh P, D'Hooghe T. Plasma miRNAs as biomarkers for endometriosis. Human Reproduction. 2019 Sep 29;34(9):1650-60.

4. Choi YS, Kim S, Oh YS, Cho S, Hoon Kim S. Elevated serum interleukin-32 levels in patients with endometriosis: A cross-sectional study. American Journal of Reproductive Immunology. 2019 Aug;82(2):e13149.

5. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, *et al*. Blood biomarkers for the non-invasive diagnosis of endometriosis. The Cochrane database of systematic reviews. 2016(5):CD012179. pmid:27132058

6. Sasamoto N, DePari M, Vitonis AF, Laufer MR, Missmer SA, Shafrir AL, Terry KL. Evaluation of CA125 in relation to pain symptoms among adolescents and young adult women with and without surgically-confirmed endometriosis. PLOS One. 2020 Aug 24;15(8):e0238043.

7. Shaheed HS, Jasim SY, Abbass WA. Studying Some Novel Biochemical and Immunological Markers in a Sample of Iraqi Women with Polycystic Ovarian Syndrome. Research Journal of Pharmacy and Technology. 2020 Jul 1;13(7):3171-8.

8. Wang WJ, Zhang H, Chen ZQ, Zhang W, Liu XM, Fang JY, Liu FJ, Kwak-Kim J. Endometrial TGF-β, IL-10, IL-17 and autophagy are dysregulated in women with recurrent implantation failure with chronic endometritis. Reproductive Biology and Endocrinology. 2019 Dec;17(1):1-9.

9. Nickerson BS, Esco MR, Bishop PA, Fedewa MV, Snarr RL, Kliszczewicz BM, *et al.* Validity of BMI-based body fat equations in men and women: A 4-compartment model comparison. The Journal of Strength & Conditioning Research. 2018 Jan 1;32(1):121-9.

10. Statistical Analysis System, User's Guide. Statistical. SAS. 2012. Version 9.1th ed. SAS. Inst. Inc. Cary. NC. USA.

11. Li M, Tan J, Zhang Y, Ai C, Wang H, Zhang H, Jin Y, Chen Y. Assessing CT imaging features combined with CEA and CA125 levels to identify endometriosis-associated ovarian cancer. Abdominal Radiology. 2020 May 18.

12. Budak E, Kahraman DS, Budak A, Yanarateş A, Inan AH, Kanmaz AG, Beyan E. The prognostic significance of serum CA125 levels with ER, PR, P53 and Ki-67 expression in endometrial carcinomas. Ginekologia Polska. 2019;90(12):675-83.

13. Deutsch K, Cekova E, Manahan KJ, Geisler JP. CA 125 and Grade 1 endometrial cancer: analyzing the risk of metastases. *European* Journal of Gynaecological Oncology. 2019 Apr 10;40(2): 246-8.

14. Karimi-Zarchi M, Dehshiri-Zadeh N, Sekhavat L, Nosouhi F. Correlation of CA-125 serum level and clinicopathological characteristic of patients with endometriosis. International Journal of Reproductive BioMedicine. 2016 Nov;14(11):713.)

15. Crosby DA, Glover LE, Brennan EP, Kelly P, Cormican P, Moran B, Giangrazi F, Downey P, Mooney EE, Loftus BJ, McAuliffe FM. Dysregulation of the interleukin-17A pathway in endometrial tissue from women with unexplained infertility affects pregnancy outcome following assisted reproductive treatment. Human Reproduction. 2020 Aug 1;35(8):1875-88.

16. Tarokh M, Ghaffari Novin M, Poordast T, Tavana Z, Nazarian H, Norouzian M, Gharesi-Fard B. Serum and peritoneal fluid cytokine profiles in infertile women with endometriosis. Iranian Journal of Immunology. 2019 Jun 1;16(2):151-62.

17. Miller JE, Ahn SH, Marks RM, Monsanto SP, Fazleabas AT, Koti M, Tayade C. IL-17A modulates peritoneal macrophage recruitment and M2 polarization in endometriosis. Frontiers in Immunology. 2020;11.

18. Kitazawa J, Kimura F, Nakamura A, Morimune A, Hanada T, Amano T, Tsuji S, Kasahara K, Satooka H, Hirata T, Kushima R. Alteration in endometrial helper T cell subgroups in chronic endometritis. American Journal of Reproductive Immunology. 2020 Nov 6:e13372.

19. Prinz I, Sandrock I. Dangerous γδ T cells in aged mice. EMBO reports. 2019 Aug;20(8):e48678.

20. Gupta PN. Molecular basis and biomarkers of disease activity. Endometriosis: An Enigma. 2020 Mar 2:9.