Al-Rafidain J Med Sci. 2024;6(2):178-181.
DOI: https://doi.org/10.54133/ajms.v6i2.939

A J M S
Online ISSN (2789-3219)

Research Article

Relationship Between Human Cytomegalovirus and IL-17A in Iraqi Women with Polycystic Ovary Syndrome

Zahraa Mushreq Hadi Khorsheed[1]*, Ifad Kerim Abd Al-shibly[1], Asmaa Kadhim Gatea[2]

[1]*Department of Microbiology, College of Medicine, Babylon University, Hilla, Iraq;* [2] *Department of Obstetrics and Gynecology, College of Medicine, Babylon University, Hilla, Iraq*

Received: 2 May 2024; Revised: 6 June 2024; Accepted: 15 June 2024

Abstract

Background: Evidence indicates a potential link between PCOS and low-grade infections. IL-17, also known as IL-17A, is an essential immunological regulator, especially in disorders such as polycystic ovarian syndrome (PCOS). The human cytomegalovirus is a β-herpesvirus that causes inflammation and remains dormant in the host for life. The cytomegalovirus has been central to several PCOS-related concepts. The role of IL-17A in CMV infection remains unknown. ***Objective***: To establish the correlation between PCOS and CMV, as well as the connection between PCOS and serum levels of IL17A. ***Methods***: A case-control study included 60 women with PCOS compared to 40 healthy controls. Samples were analyzed regarding CMV via the real-time PCR technique. Furthermore, the ELISA technique measured serum levels of the IL-17A cytokine. Every sample was taken between September 2023 and January 2024. ***Results***: Positive results for CMV were seen in 50 (83.3%) of patients with PCOS compared with 10 (16.7%) who had negative results, while 6 (15.0%) of healthy control subjects had positive results and 34 (85.0%) had negative results; the difference was highly significant. Furthermore, women with polycystic ovary syndrome had a significantly higher IL-17A serum level when compared to healthy controls. ***Conclusions***: In Iraqi women, HCMV Infection in patients with PCOS can be considered as a risk factor. Moreover, the results show that IL-17A is an excellent prognostic marker of polycystic ovary syndrome.

Keywords: Human cytomegalovirus, Interleukin-17, Immunological regulator, Polycystic ovary syndrome.

دراسة العلاقة بين فيروس التضخم الخلوي البشري و الانترلوكين IL-17A في النساء العراقيات المصابات بمتلازمة تكيس المبايض

الخلاصة

الخلفية: تشير الأدلة إلى وجود صلة محتملة بين متلازمة تكيس المبايض والعدوى منخفضة الدرجة. يعد الانترلوكين17-IL منظمًا مناعيًا أساسيًا، خاصة في اضطرابات مثل متلازمة تكيس المبايض. الفيروس المضخم للخلايا البشرية هو فيروس هربس بيتا الذي يسبب الالتهاب ويظل خاملًا في المضيف مدى الحياة. لقد كان الفيروس المضخم للخلايا في قلب العديد من الأفكار المتعلقة بمتلازمة تكيس المبايض. لا يزال دور الانترلوكين-17 Aفي الإصابة بالفيروس المضخم للخلايا غير معروف. **الهدف:** إثبات العلاقة بين متلازمة تكيس المبايض والفيروس المضخم للخلايا، وكذلك العلاقة بين متلازمة تكيس المبايض ومستويات الانترلوكين 17 Aفي مصل الدم . **الطرق:** شملت دراسة المرضى والاصحاء 60 امرأة مصابة بمتلازمة تكيس المبايض مقارنة بـ 40 امرأة سليمة. تم تحليل العينات بخصوص الفايروس المضخم للخلايا عبر تقنية PCR. علاوة على ذلك، قامت تقنية ELISA بقياس مستويات الانترلوكين -17 A في المصل. تم أخذ كل عينة في الفترة ما بين سبتمبر 2023 ويناير 2024. **النتائج:** شوهدت نتائج إيجابية للفايروس المضخم للخلايا في 50 (83.3٪) من المرضى الذين يعانون من متلازمة تكيس المبايض مقارنة بـ 10 (16.7٪) لديهم نتائج سلبية، في حين أن 6 (15.0٪) من الأشخاص الأصحاء لديهم نتائج إيجابية و 34 (85.0٪) لديهم نتائج سلبية. وكان الفرق كبيرا للغاية. علاوة على ذلك، كان لدى النساء المصابات بمتلازمة تكيس المبايض مستوى مصل الانترلوكين -17 Aأعلى بكثير بالمقارنة مع الأصحاء. **الاستنتاج:** في النساء العراقيات، يمكن اعتبار الاصابة بالفايروس المضخم للخلايا في المرضى الذين يعانون من متلازمة تكيس المبايض كعامل خطر. علاوة على ذلك، أظهرت النتائج أن الانترلوكين -A17 يعد علامة تنبؤ ممتازة لمتلازمة تكيس المبايض.

* ***Corresponding author***: Zahraa M. H. Khorsheed, Department of Microbiology, College of Medicine, Babylon University, Hilla, Iraq; Email: zhraa.mushrq@gmail.com

Article citation: Khorsheed ZMH, Al-shibly IKA, Gatea AK. Relationship Between Human Cytomegalovirus and IL-17A in Iraqi Women with Polycystic Ovary Syndrome. *Al-Rafidain J Med Sci*. 2024;6(2):178-181. doi: https://doi.org/10.54133/ajms.v6i2.939

INTRODUCTION

Polycystic ovary syndrome (PCOS), commonly known as Stein-Leventhal syndrome, is the most common endocrine reproductive disorder. It is characterized by irregular menstruation, hyperandrogenism, and polycystic ovarian morphology [1]. The prevalence of polycystic ovarian syndrome varies between reports, ranging from 6-20% worldwide [2]. PCOS diagnostic tools include the Rotterdam criteria, the NIH criteria, and the Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) [3]. The 2003 Rotterdam criteria, which define the condition as oligomenorrhea, clinical/biochemical hyperandrogenism, and polycystic ovary (PCOM) on ultrasound [4], are the most commonly used criteria

for PCOS globally. The pathophysiological mechanisms of polycystic ovary syndrome are complex and not entirely comprehended [5]. In the last few years, further research has concentrated on the critical function of chronic, low-grade inflammation in PCOS development [6]. In most Polycystic Ovary Syndrome patients, an increase in the serum level of IL-17 was implicated in the etiology of PCOS by forming an inflammatory response [7,8]. Increased proinflammatory cytokines such as TNF-α, IL-6, and IL-17 cause a low-grade inflammation response [9]. Interleukin-17, also known as IL-17A, is a T-cell-derived inflammatory cytokine that Th17 cells, T cells, and neutrophils primarily produce. Interleukin-17 promotes the production of other proinflammatory cytokines and chemokines that mediate immune responses [10]. It was observed that IL-17A, a proinflammatory cytokine, may be linked to infertility in Polycystic Ovary Syndrome patients [6]. The role of IL-17A in CMV infection remains unknown. Cytomegalovirus (CMV) is the prototype member of Betaherpesvirinae in the subfamily Herpesviridae [11]. With a genome of over 240 kb, it is thought to be the biggest herpes virus that infects people. Both the CDC and the WHO have found that CMV can infect people of any age [12]. Despite the cellular immune response evolved by the host against CMV, the virus avoids the immune attack and stays hidden inside host cells. As a result, CMV is never eliminated in infected individuals, causing persistent asymptomatic infections [13]. In an Iraqi study, Alabassi *et al*. discovered that 16.66% of PCOS patients tested positive for CMV, and they noted that the chronic low-grade inflammation linked to CMV may exacerbate the metabolic and hormonal issues that may lead to the development of PCOS [14]. This study aims to examine whether there is a link between CMV infection and susceptibility to polycystic ovarian syndrome and to measure the levels of IL-17A in women with PCOS.

METHODS

Study Design and patient's selection

A case-control study involved 60 women with PCOS and 40 healthy controls who attended the Babylon Teaching Hospital for Maternity and Children, as well as the Taiba Centre for Infertility and IVF. The diagnosis was established following the Rotterdam Diagnostic Criteria. The data collection lasted from September 2023 to January 2024.

DNA extraction

DNA was extracted from 2.0 ml of blood samples using Geneaid's gSYAN extraction kit (Frozen Blood), following the instructions provided by the manufacturer (Geneaid, Taiwan).

Quantitative real-time PCR analyses

We used a real-time PCR technique to identify the human cytomegalovirus, utilizing the NCBI-Genbank human cytomegalovirus major capsid protein (MCP) gene sequence (M25411.1) and primer 3 plus, both provided by Macrogen in Korea. We utilized primer and probe sets specifically designed for the human cytomegalovirus, which include the forward primer 5′ TCAAAACCACCGTGACAAGC-3′, the reverse primer 5′-ACAACGTGCTACGAAAGTGC-3′, and the probe (5′/FAMG CTACCTGGTCGACGTGCGTGCTG G-BHQ1/-3′). We carried out the amplification using the RealMODTM probe 2X qPCR mix. The company provided instructions for this master mix, which included a 20 l reaction mixture, 1 l of each primer at 10pmol, 1 l of the probe at 10pmol, 10 l of qPCR master mix, and 2 l of Next, we insert it into a real-time PCR thermocycler (BioRad, USA), where the system adjusts the parameters according to the primer annealing temperature and the qPCR TaqMan kit guidelines. The reaction consisted of a 5-minute cycle at 95 °C, followed by 40 cycles of 20 seconds at 95 °C and 30 seconds at 60 °C.

Measurement of serum IL-17A

We obtained 3.0 ml of blood from each participant, kept it in the gel tube, and centrifuged it at 1500 rpm for 10 minutes. We separated the sera and kept them in Eppendorf tubes, storing them in a deep freezer at -20 °C until we used the Sandwich (ELISA) kit to measure the IL-17A level, following the manufacturer's instructions (Elabscience, USA).

Ethical consideration

The current study followed the ethical principles specified in the Declaration of Helsinki. We asked all enrolled patients for their verbal approval before sampling specimens, and the Committee on Publication Ethics at the College of Medicine, University of Babylon, Iraq, approved this study under reference No. 3-18/Jun 2023.

Statistical analysis

The SPSS version 26 statistical package and Excel 2010 from Microsoft were used for data analysis. Data are presented as mean±standard deviation (SD) after performing the Kolmogorov-Smirnov test for normality. The difference in means between any two groups was studied using an independent sample t-test, provided that the variable was normally distributed. A one-way (ANOVA) analysis of variance was used to evaluate the differences between groups. Two categorical variables were examined for associations using the Chi-square test. The receiver operator curve (ROC) was analyzed, along with the area under the curve (AUC), level of accuracy, sensitivity, specificity, and significance score (P). The odds ratio and confidence interval were used to assess the risk. A *p*-value less than 0.05 was considered statistically significant and a very significant level of 0.01 or less.

RESULTS

Table 1 displays the results of the present study, which enrolled 100 samples from 60 PCOS patients and 40 healthy women as controls to investigate cytomegalovirus (CMV) using a real-time PCR technique. 50 (83.3%) of the PCOS patients had positive results, compared to 10 (16.7%) who had negative results, while 6 (15%) of the healthy control subjects had positive results and 34 (85%) had negative results.

Table 1: Prevalence Cytomegalovirus (CMV) infection according to Real Time PCR technique in studied groups

Technique	PCOS patients (*n*=60)	Healthy women (*n*=40)	*p*
	Real Time PCR technique results		
Positive	50(83.3)	6(15.0)	>0.001
Negative	10(16.7)	34(85.0)	

Values were expressed as frequencies and percentages.

The difference was highly significant (p<0.001). Table 2 demonstrates the results of a comparison of interleukin-17A serum levels between women with PCOS and healthy control patients.

Table 2: Interleukin-17A serum level in women with PCOS and healthy controls

Parameters	Cases –control comparison		*p*
	PCOS patients (*n*=60)	Healthy control (*n*=34)	
IL-17A level (ng/ml)			
Mean±SD	324.96±32.81	219.44±19.58	<0.001
Range	220.79-445.94	146.75-272.27	

The mean levels of serum IL-17A in women with polycystic ovary syndrome (PCOS) were 324.96±32.81 ng/ml, while in healthy control participants it was 219.44±19.58 ng/ml. This level was significantly higher in the PCOS group when compared to the healthy control (p<0.001). The ROC curve analysis looked at the IL-17A cutoff value and predicted polycystic ovary syndrome. The results are shown in Figure 1 and Table 3.

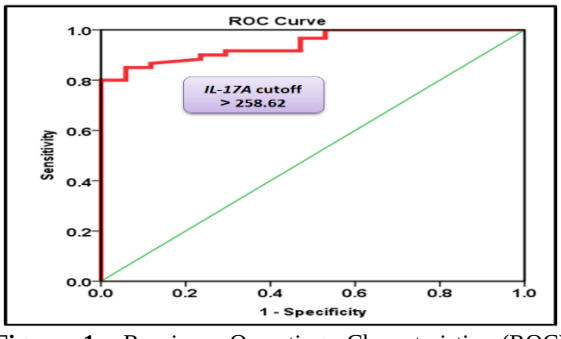

Figure 1: Receiver Operating Characteristic (ROC) Analysis of Serum IL-17A.

Table 3: The Sensitivity and specificity of IL-17A level (< 258.62-fold) in PCOS

IL-17A levels	PCOS patients (*n*=60)	Healthy control (*n*=34)
<258.62 (%)	56	2
>258.62 (%)	4	32
Sensitivity (%)	93.3	
Specificity (%)	94.1	
PPV (%)	96.6	
NPV (%)	88.9	
AUC (95% CI)	0.942 (0.900- 0.985)	

AUC: Area under curve, **CI**: Confidence interval.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve were 93.3%, 94.1%, 96.6%, 88.9%, and 0.942 (0.900–0.985), respectively, with the IL-17A cutoff value being < 258. A comparison of serum IL-17A levels was conducted between patients with positive and negative CMV infections, and Table 4 shows the results. The mean serum IL-17A levels in patients with a positive CMV infection were 335.67± 36.94 ng/ml, while in patients with a negative infection, the level was 271.44±29.62 ng/ml. The level was highly significantly higher compared to patients with positive CMV infection in comparison with patients with negative CMV infection (p<0.001), 62-fold.

Table 4: IL-17A serum level in patients with positive CMV infection and patients with negative CMV infection

Parameter	Positive CMV (*n*=50)	Negative CMV (*n*=10)	*p*
IL-17A level (ng/ml)			
Mean±SD	335.67±36.94	271.44±29.62	<0.001
Range	234.55-445.94	222.79-370.19	

DISCUSSION

The current study elucidates the relationship between PCOS and cytomegalovirus, as well as the relationship between PCOS and interleukin-17A serum levels. Significantly, this study found that 83.3% of patients with PCOS had positive results for CMV, while only 16.7% had negative results, and there was a highly significant difference (p<0.001). Our results were supported by another new study by Alabassi *et al*., who studied the CMV latent infection among PCOS and healthy control subjects and found approximately 16.7% of PCOS patients were found positive for CMV infection. The findings revealed that chronic low-grade inflammation caused by CMV may contribute in some way to hormonal and metabolic abnormalities involving the hypothalamus pituitary adrenal (HPA) axis, our core stress response system, which contributes to the development of PCOS [14]. These findings suggest that CMV infection impacts a subset of Iraqi patients with PCOS. Additionally, this study found that IL-17A levels were elevated in PCOS patients when compared with healthy controls (p<0.001). These findings align with many studies that revealed significantly higher IL-17A levels in PCOS patients [8,15–17]. Despite this, a study by Zangeneh *et al*. revealed that the IL-17 serum level in women with PCO was lower than in the control group (p<0.001) [18]. Elevated IL-17A levels in PCOS women may indicate an inflammatory response within the ovaries. This cytokine is a vital component of the body's immune response, and overproduction of IL-17 may exacerbate inflammatory responses and contribute to tissue damage [19]. Inflammatory responses in PCOS, particularly elevated IL-17A, are associated with complications such as insulin resistance and metabolic disorders [20]. In addition, our findings revealed that the levels of IL-17A in the serum of patients who had a positive CMV infection were significantly higher than those who did not have a positive CMV infection (p<0.001). The pathological

or protective roles of interleukin-17 during viral infections remain contentious [21]. Recent studies indicate that IL-17 is involved in both the antiviral immune response and the promotion of virus-mediated illnesses [22]. A study demonstrated that mice infected with murine CMV had higher IL-17 levels compared to controls [23]. Additionally, the results align with studies that demonstrate elevated Th17 cell activity during acute rejection following kidney transplant recipients' CMV infection, resulting in increased blood levels of IL-17A production [24]. Also, a study revealed a link between IL-17 and CMV in chronic heart failure patients [25].

Study limitations

The study does not mention specific limitations; however, potential limitations could include the study's limited duration.

Conclusion

This study identified higher IL-17A levels in individuals with PCOS compared to healthy control subjects and is thus considered an excellent prognostic marker of PCOS. Additionally, the study found that a subset of PCOS patients in Iraqi women have CMV infection.

Conflict of interests

No conflict of interests was declared by the authors.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Kiran GU, Vali DY, Shankar DY, Lokesh G, Chaitanya PK, Sujitha S. Overview of polycystic ovary syndrome (PCOS). *World J Adv Engineer Technol Sci*. 2023;8(2):011–22. doi: 10.30574/wjaets.2023.8.2.0052.
2. Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: A brief systematic review. *J Hum Reprod Sci*. 2020;13(4):261-271. doi: 10.4103/jhrs.JHRS_95_18.
3. Yan D, Yan-Fang W, Shi-Yang Z, Rui-Lin M, Xue-Song D, Xiao M, et al. Is polycystic ovary syndrome appropriately diagnosed by obstetricians and gynaecologists across China: a nationwide survey. *J Ovarian Res*. 2021;14(1):25. doi: 10.1186/s13048-021-00780-6.
4. Rasquin LI, Anastasopoulou C, Mayrin JV, (Eds.), Polycystic Ovarian Disease. StatPearls Publishing, Treasure Island (FL); 2023. PMID: 29083730.
5. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. *Mol Metab*. 2020;35:100937. doi: 10.1016/j.molmet.2020.01.001.
6. Rostamtabar M, Esmaeilzadeh S, Tourani M, Rahmani A, Baee M, Shirafkan F, et al. Pathophysiological roles of chronic low-grade inflammation mediators in polycystic ovary syndrome. *J Cell Physiol*. 2021;236(2):824-838. doi: 10.1002/jcp.29912.
7. Özçaka Ö, Buduneli N, Ceyhan BO, Akcali A, Hannah V, Nile C, et al. Is interleukin-17 involved in the interaction between polycystic ovary syndrome and gingival inflammation? *J Periodontol*. 2013;84(12):1827–1837. doi: 10.1902/jop.2013.120483.
8. Foroozanfard F, Soleimani A, Arbab E, Samimi M, Tamadon MR. Relationship between IL-17 serum level and ambulatory blood pressure in women with polycystic ovary syndrome. *J Nephropathol*. 2017;6(1):15–24. doi: 10.15171/jnp.2017.04.
9. Kalyan S, Patel MS, Kingwell E, Côté HCF, Liu D, Prior JC. Competing factors link to bone health in polycystic ovary syndrome: Chronic low-grade inflammation takes a toll. *Sci Rep*. 2017;7(1):3432. doi: 10.1038/s41598-017-03685-x.
10. Mills KHG. IL-17 and IL-17-producing cells in protection versus pathology. *Nat Rev Immunol*. 2023;23(1):38-54. doi: 10.1038/s41577-022-00746-9.
11. Payne S, (Ed.) Viruses: from understanding to investigation, (2nd Ed.), Elsevier; 2022.
12. Al Mana H, Yassine HM, Younes NN, Al-Mohannadi A, Al-Sadeq DW, Alhababi D, et al. The current status of cytomegalovirus (CMV) prevalence in the MENA region: A systematic review. *Pathogens*. 2019;8(4). doi: 10.3390/pathogens8040213.
13. Fulkerson HL, Nogalski MT, Collins-McMillen D, Yurochko AD. Overview of Human Cytomegalovirus Pathogenesis. In: Yurochko AD, (ed.), Human Cytomegaloviruses: Methods and Protocols. New York: Springer US; 2021:1–18. doi: 10.1007/978-1-0716-1111-1_1.
14. Alabassi HM, Kadri ZHM, Mahmood MM, AL-Kubisi MI. The possible etiological role of CMV and EBV latent infections in polycystic ovary syndrome Iraqi patients. *Syst Rev Pharmacy*. 2020;11(7):51–53. doi:10.31838/srp.2020.7.08.
15. Zhou H, Xu J, Hong L, Jia Y, Burk LV, Chi F, et al. The alterations of circulating mucosal-associated invariant T cells in polycystic ovary syndrome. *Front Endocrinol (Lausanne)*. 2022;13:1038184. doi: 10.3389/fendo.2022.1038184.
16. Vasyukova E, Zaikova E, Kalinina O, Gorelova I, Pyanova I, Bogatyreva E, et al. Inflammatory and anti-inflammatory parameters in PCOS patients depending on body mass index: A case-control study. *Biomedicines*. 2023;11(10). doi: 10.3390/biomedicines11102791.
17. Kuang H, Duan Y, Li D, Xu Y, Ai W, Li W, et al. The role of serum inflammatory cytokines and berberine in the insulin signaling pathway among women with polycystic ovary syndrome. *PLoS One*. 2020;15(8):e0235404. doi: 10.1371/journal.pone.0235404.
18. Zafari Zangeneh F, Naghizadeh MM, Masoumi M. Polycystic ovary syndrome and circulating inflammatory markers. *Int J Reprod Biomed*. 2017;15(6):375-382. PMID: 29177240.
19. Abraham Gnanadass S, Divakar Prabhu Y, Valsala Gopalakrishnan A. Association of metabolic and inflammatory markers with polycystic ovarian syndrome (PCOS): an update. Archives of Gynecology and Obstetrics. 2021;303(3):631–43.
20. Zhai Y, Pang Y. Systemic and ovarian inflammation in women with polycystic ovary syndrome. *J Reprod Immunol*. 2022;151:103628. doi: 10.1016/j.jri.2022.103628.
21. Ma WT, Yao XT, Peng Q, Chen DK. The protective and pathogenic roles of IL-17 in viral infections: friend or foe? *Open Biol*. 2019 ;9(7):190109. doi: 10.1098/rsob.190109.
22. Sahu U, Biswas D, Prajapati VK, Singh AK, Samant M, Khare P. Interleukin-17-A multifaceted cytokine in viral infections. *J Cell Physiol*. 2021;236(12):8000-8019. doi: 10.1002/jcp.30471.
23. Liu LL, Li XF, Qin WQ, Liu XL, Li G, Shu SN, et al. Is IL-17 an accomplice contributing to salivary gland damage during CMV infection? *Future Virol*. 2013;8(9):861–869. doi: 10.2217/fvl.13.78.
24. Dhital R, Anand S, Zeng Q, Velazquez VM, Boddeda SR, Fitch JR, et al. Th1/17 cells infiltrate murine cytomegalovirus-infected renal allografts via virus-induced CCL20 and promote Th1 cells through IL-17A. *bioRxiv*. 2021;2021.08.05.455061. doi: 10.1101/2021.08.05.455061.
25. García-Torre A, Bueno-García E, López-Martínez R, Rioseras B, Díaz-Molina B, Lambert JL, et al. CMV infection is directly related to the inflammatory status in chronic heart failure patients. *Front Immunol*. 2021; 12:687582. doi: 10.3389/fimmu.2021.687582.