JBS
Research Article

Journal of Biological Studies

eISSN: 2209-2560

V. 9, N. 3: 219-234, 2026

https://doi.org/10.62400/jbs.v9i3.18585

Role of selected interleukins and hormonal biomarkers in screening for human papillomavirus infection and cervical cancer in women

Sura A. Hashim[1], S.K. Al-Qaisi [2], Shahad Saad Alwan [3]

[1]University of Diyala, Diyala, Iraq, 32001.
[2]Department of Biotechnology, college of science, University of Diyala, Diyala, Iraq, 32001.
[3]Department of Biology, College of Science, Diyala University, Diyala, Iraq,32001

*Correspondence author: sura.a@uodiyala.edu.iq

Received 15 June 2026|Accepted 21 August 2026|Published 02 September 2026

Abstract

The roles of immunological and hormonal indicators in screening women with HPV infection and cervical cancer (CC) were investigated. The present study was conducted in Diyala Province during the period from January to March 2026. A total of 90 blood samples were collected from women who visited Al-Batoul Teaching Hospital and women's clinics. Serum levels of IL-1β, IL-2R, FSH, and estrogen, as well as HPV, were detected using ELISA and the Biorex technique. HPV-infected women represented 43.33% of the participants, women with HPV and CC represented 10.00%, and HPV-negative women represented 46.67%. No significant differences ($p > 0.05$) were observed among the study groups regarding marital status, number of children, chronic diseases, and smoking. The highest levels of IL-1β, IL-2R, FSH, and estrogen were observed in women with HPV and CC, followed by HPV-infected women, and then the control group. Exploratory ROC curve analysis showed high within-sample discriminatory performance for IL-1β, IL-2R, FSH, and estrogen in differentiating HPV-infected women from controls and women with HPV-associated cervical cancer from controls. However, the cutoff values, sensitivity, and specificity estimates were derived and evaluated using the same study cohort and should therefore be considered exploratory rather than clinically validated diagnostic measures. Spearman's rank correlation analysis in HPV-infected women showed a significant negative correlation between IL-1β and estrogen ($\rho = -0.430$, $P = 0.006$), and significant positive correlations between IL-2R and estrogen ($\rho = 0.386$, $P = 0.015$) and between FSH and estrogen ($\rho = 0.600$, $P < 0.001$). No statistically significant correlations were observed among the investigated biomarkers in the HPV-associated cervical cancer subgroup. Overall, HPV positivity and HPV-associated cervical cancer were associated with higher serum levels of IL-1β, IL-2R, FSH, and estrogen in the present cohort. Further studies using larger independent cohorts are required to determine the clinical relevance of these findings.

Keywords: HPV, cervical cancer, immunity, hormones, reproductive system, interleukins.

1. Introduction

The most common sexually transmitted virus (STI) in the world is considered to be the human papillomavirus (HPV) (Khan *et al.*, 2025). Despite its substantial public-health importance, knowledge and awareness of HPV and its associated health risks may remain inadequate in some populations (Bakiri *et al.*, 2023). This icosahedral virus has a double-stranded DNA genome and is classified into the alpha, beta, gamma, mu, and nu genera of the *Papillomaviridae* family according to the L1 sequence, which is the gene encoding the viral capsid. More than 200 HPV genotypes have been identified and categorized as either high-risk (HR) or low-risk (LR) HPV based on their carcinogenic potential (Osmani *et al.*, 2025). While high-risk HPV types express oncogenic proteins and have the ability to promote tumor development in the basal layers of infected tissues, thereby primarily causing cervical malignancy, low-risk HPV types are commonly associated with genital warts and normal cervical tissue (Karami *et al.*, 2026).

It is essential to understand how HPV evades the immune system. The majority of HPV infections are eliminated by the immune system, but approximately 10% persist, resulting in lesions that may develop into cancer. HPV employs several strategies to evade immune detection, including suppressing immune responses and altering immune cell recognition (Zhou *et al.*, 2025). Furthermore, HPV uses various mechanisms to evade the host immune response, such as modifying cytokines associated with inflammation and cancer development, as well as altering immune sensors. Cervical cancer progression and potential therapeutic approaches are greatly influenced by this complex interaction between HPV genes and the immune system (Hernández-Silva *et al.*, 2024).

The inflammatory process induced by HPV infection alters the concentrations of several cytokines, including IL-6, IL-1β, and TNF-α. IL-1β activates several inflammatory pathways, damages cervical epithelial DNA, upregulates additional inflammatory mediators and chemokines, suppresses cancer cell death, and accelerates disease progression (Saha et al., 2025). A few studies have suggested mechanisms by which HPV oncoproteins inhibit IL-1β synthesis. According to Niebler *et al.* (2013), HPV16 E6 recruits E6AP to target pro-IL-1β for proteasome-mediated degradation, thereby reducing IL-1β protein levels. In addition, HPV16 E6 suppresses IL-1β through a transcriptional mechanism dependent on IRF6 suppression (Castagnino *et al.*, 2022).

Interleukin-2 is one of the most extensively studied cytokines involved in T-cell survival and proliferation. The IL-2R complex is formed when IL-2 binds to the IL-2 receptor. In addition to monitoring disease activity, severity, and response to therapy, this complex may assist in the diagnosis of certain immunological disorders and malignancies and predict the benefits of specific therapeutic approaches (Lokau *et al.*, 2024). Furthermore, IL-2R has been the focus of numerous studies in cancer immunotherapy and has previously been shown to enhance antitumor immunity in several cancers, particularly cervical cancer, melanoma, and renal cell carcinoma (Buchbinder *et al.*, 2025). Yasin *et al.* (2026) also considered IL-2R to be a useful biomarker for assessing the stage of HPV disease.

Follicle-stimulating hormone (FSH), progesterone (P), estradiol (E2), luteinizing hormone (LH), prolactin (PRL), and testosterone (T) are the six sex hormones most commonly evaluated in clinical testing. Sex hormones are closely associated with HPV replication and transmission, and they also promote uterine growth (Wu *et al.*, 2024). Follicle-stimulating hormone (FSH) and estrogen play important roles in the development of cervical cancer, which is primarily caused by high-risk HPV infection. Elevated FSH levels are associated with increased disease severity and a higher risk of cervical cancer, whereas estrogen promotes the

expression of HPV E6/E7 oncogenes, thereby enhancing tumor development (Bertolazzi *et al.*, 2026).

The present study aimed to detect the roles of immunological biomarkers (IL-1β and IL-2R) and hormonal biomarkers (FSH and estrogen) in screening women with HPV infection alone as well as those with HPV-associated cervical cancer.

2. Materials and Methods

Sample Collection

The present investigation was conducted inDiyala Province from January to March 2026. During this period, 90 blood samples were collected from women attending Al-Batoul Teaching Hospital, the Oncology Center of Baqubah Teaching Hospital, and women's clinics across Diyala Province. The sample included 39 women infected with HPV, 9 women with both HPV infection and cervical cancer, and 42 women who were HPV-negative. Those in the HPV-associated cervical cancer group had been hospitalized at the Oncology Center of Baqubah Teaching Hospital and had previously received a cervical cancer diagnosis from specialist physicians. Their cervical cancer status was confirmed through clinical records, and the participants' demographic and clinical characteristics were documented.

Inclusion criteria

Women aged 21–75 years were included in the study. Based on the diagnostic approach used here, participants were assigned to one of three groups: HPV-18 antigen-positive women, HPV-18 antigen-positive women with cervical cancer, or HPV-negative controls.

Exclusion criteria

Pregnancy, menopause, hormonal therapy, autoimmune diseases, chronic inflammatory diseases, recent infections, immunosuppressive medications, and, HPV-16, 31, 33, 45 genotypes, bacterial infection, parasitic infections, fungal infections, no metastatic cervical cancer, and previous cancer treatment.

Methods

The collected blood samples were centrifuged at 5000 rpm for 5 minutes to obtain serum. HPV-18 positivity in serum was evaluated with a commercially available Human Papillomavirus Type 18 (HPV18) ELISA Kit (MyBioSource, Catalog No. MBS1611969), following the manufacturer's instructions. The assay was used for research purposes to assess HPV-18 positivity in the study samples; HPV DNA testing and molecular genotyping were not performed. Blood samples for hormonal measurements were collected on the second day of the menstrual cycle to minimize physiological variation in FSH and estrogen concentrations across the menstrual cycle. Serum HPV-18 antigen was measured with a commercially available enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions. For this study, HPV status was determined by whether HPV-18 antigen was detected. Because HPV DNA testing and molecular genotyping were not performed, the findings indicate HPV-18 antigen positivity rather than molecular confirmation of the HPV genotype. A Biorex analyzer was used to determine the serum levels of FSH and estrogen in the participants.

Statistical Analysis

Hormonal and immunological indicators were expressed as mean ± standard deviation. Student's *t*-test was performed to calculate the differences between two groups. One-way ANOVA (Duncan's test) was used to determine differences among the mean values of the indicators. Demographic characteristics were expressed as frequencies and percentages. Pear-

son's chi-square test was performed to detect differences among percentages. Receiver operating characteristic (ROC) curve analysis was used to determine the area under the curve (AUC), cutoff values, sensitivity, and specificity of the biomarkers. A *P* value ≤ 0.05 was considered statistically significant. SPSS version 22.0 and GraphPad Prism version 10 were used for the statistical analysis of the present investigation.

Separate age-adjusted binary logistic regression models were fitted for each immunological and hormonal biomarker to account for age as a possible confounder. Age group was entered as an ordinal covariate. Given the small number of women with HPV-associated cervical cancer and the possibility of separation, Firth penalized logistic regression was applied to limit small-sample bias and produce more stable parameter estimates. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were reported. To avoid overfitting, each biomarker was assessed in its own model.

Spearman's rank correlation coefficient assessed the relationships among immunological and hormonal biomarkers. A P value ≤ 0.05 was considered statistically significant.

3. Results

1. Prevalence of HPV Among Participants

The results of the current investigation showed that the distribution of HPV positivity among the women participants (N = 90) was as follows: HPV-infected women (43.33%), women with HPV and cervical cancer (10.00%), and HPV-negative women (46.67%) (Figure 1).

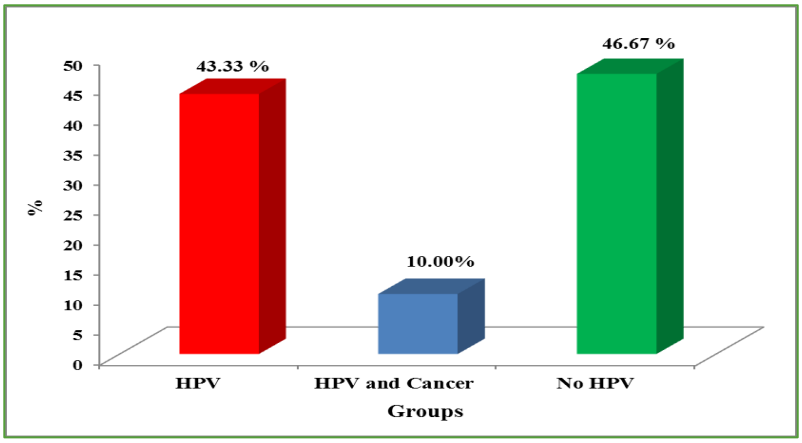

Figure 1. Positivity of HPV among women

2. Baseline Characteristics of the Participants

The findings of the present investigation revealed significant differences ($p < 0.05$) in age distribution among the study groups. The age groups of 21–30, 31–40, and 41–50 years showed the highest percentages among HPV-infected women (23.1%, 25.6%, and 17.9%, respectively), whereas the age groups of 41–50, 51–60, and 61–70 years showed the highest percentages among women with HPV and cervical cancer (22.2%, 66.7%, and 11.1%, respectively). In contrast, the present study did not reveal significant differences ($P > 0.05$) among the study groups with regard to marital status, having children, chronic diseases, and smoking status (Table 1).

Table 1. Demographic and clinical characteristics of the participants.

Variables	Categories	Statistic	HPV* (n = 39)	HPV + Cancer (n = 9)	Total	P value
Age groups (years)	**21–30**	**n**	**9**	**0**	**9**	**P < 0.001**
		%	23.1%	0.0%	18.8%	
	31–40	n	10	0	10	
		%	25.6%	0.0%	20.8%	
	41–50	n	7	2	9	
		%	17.9%	22.2%	18.8%	
	51–60	n	5	6	11	
		%	12.8%	66.7%	22.9%	
	61–70	n	4	1	5	
		%	10.3%	11.1%	10.4%	
	>70	n	4	0	4	
		%	10.3%	0.0%	8.3%	
Marital status	Married	n	35	8	43	P > 0.05
		%	89.74%	88.88%	89.58%	
	Single	n	4	1	5	
		%	10.26%	11.12%	10.42%	
Children	No	n	35	7	42	P > 0.05
		%	89.7%	77.8%	87.5%	
	Yes	n	4	2	6	
		%	10.3%	22.2%	12.5%	
Chronic diseases	No	n	26	6	32	P > 0.05
		%	66.7%	66.7%	66.7%	
	Yes	n	13	3	16	
		%	33.3%	33.3%	33.3%	
Smoking status	Non-smoker	n	34	7	41	P > 0.05
		%	87.2%	77.8%	85.4%	
	Smoker	n	5	2	7	
		%	12.8%	22.2%	14.6%	
No significant difference (P > 0.05). P < 0.05 was considered statistically significant HPV*= Human papilloma virus						

3. Levels of Immunological and Hormonal Indicators Among the Study Groups

The outcomes of the current investigation showed the highest levels of IL-1β, IL-2R, FSH, and estrogen in women with HPV and cervical cancer (673.44 ± 64.71, 715.89 ± 133.05, 23.22 ± 3.73, and 557.56 ± 142.49, respectively), followed by HPV-infected women (592.90 ± 145.03, 610.87 ± 204.83, 15.97 ± 4.65, and 452.87 ± 158.38, respectively), compared with the control group (320.36 ± 123.62, 420.83 ± 189.12, 9.81 ± 2.96, and 273.79 ± 121.44, respectively). These differences were statistically significant (P < 0.05) (Table 2 and Figure 2).

Table 2. Comparative Mean Values of IL-1β, IL-2R, FSH, and Estrogen Among the Study Groups

NT=90		N	Mean	Std. Deviation	P value
IL-1Beta	Controls	42	320.36 [c]	123.62	P<0.001
	HPV	39	592.90 [b]	145.03	
	HPV+Cancer	9	673.44 [a]	64.71	
IL-2R	Controls	42	420.83 [c]	189.12	P<0.001
	HPV	39	610.87 [b]	204.83	
	HPV+Cancer	9	715.89 [a]	133.05	
FSH	Controls	42	9.81 [c]	2.96	P<0.001
	HPV	39	15.97 [b]	4.65	
	HPV+Cancer	9	23.22 [a]	3.73	
Estrogen	Controls	42	273.79 [c]	121.44	P<0.001
	HPV	39	452.87 [b]	158.38	
	HPV+Cancer	9	557.56 [a]	142.49	
Different superscript letters (a, b, c) indicate statistically significant differences among groups (P < 0.05)					

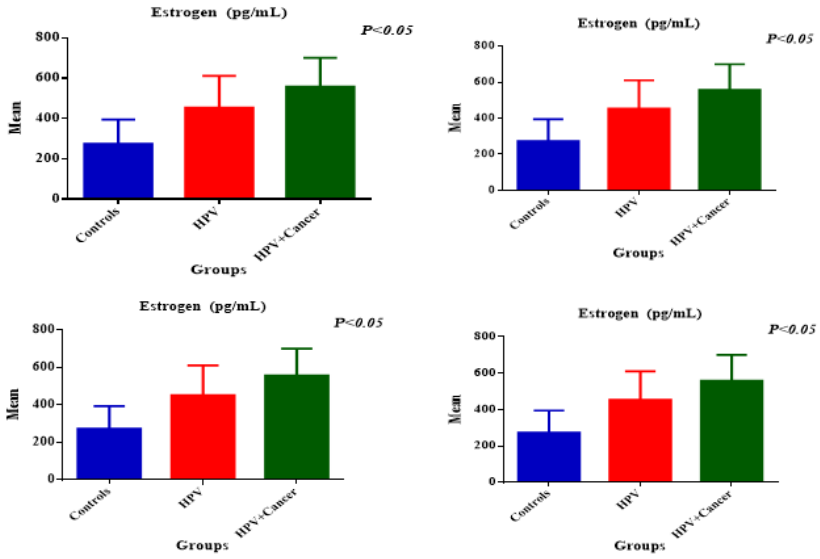

Figure 2. Levels of IL-1Beta, IL-2R, FSH, and Estrogen indicators within study groups

Age-adjusted regression analysis indicated that the observed biomarker associations remained statistically significant after accounting for age group. Among HPV-infected women compared with controls, higher levels of IL-1β, IL-2R, FSH, and estrogen were still significantly associated with HPV positivity (all P < 0.001). The same four biomarkers also remained significantly associated with HPV-associated cervical cancer after age-group adjustment (all P < 0.001). Confidence intervals were much wider, however, in the HPV-associated cervical

cancer subgroup, which included only 9 cases (n = 9). Estimates for this subgroup should therefore be interpreted cautiously, as shown in Table 3.

Table 3. Age-Adjusted Associations of Immunological and Hormonal Biomarkers with HPV Infection and HPV-Associated Cervical Cancer

Comparison	Biomarker	Age-adjusted OR (95% CI)	P
HPV vs Control	IL-1β	4.02 (2.45–7.77)	<0.001
	IL-2R	1.64 (1.29–2.16)	<0.001
	FSH	25.26 (6.56–186.43)	<0.001
	Estrogen	2.12 (1.54–3.10)	<0.001
HPV+CC vs Control	IL-1β	7.79 (2.60–454.97)	<0.001
	IL-2R	3.44 (1.68–14.80)	<0.001
	FSH	25.77 (5.21–4858.27)	<0.001
	Estrogen	5.10 (2.09–52.28)	<0.001

OR = odds ratio; CI = confidence interval. The models were adjusted for age group, with each biomarker analyzed separately. ORs for IL-1β, IL-2R, and estrogen correspond to a 100-unit increase; the OR for FSH corresponds to a 5-unit increase

4. Receiver Operating Characteristic (ROC) Curve Analysis of Immunological and Hormonal Markers

The ROC curve analysis showed high sensitivity and specificity of IL-1β (89% and 86%), IL-2R (92% and 91%), FSH (89% and 100%), and estrogen (97% and 89%) at cutoff values of 426.50, 594.00, 14.50, and 402.00, respectively, in screening women with HPV infection, with statistically significant differences ($P < 0.05$).

Additionally, high sensitivity and specificity were found for IL-1β (94% and 87%), IL-2R (94% and 89%), FSH (89% and 90%), and estrogen (95% and 93%) at cutoff values of 426.50, 594.00, 14.50, and 402.00, respectively, in evaluation women with HPV and cervical cancer, with statistically significant differences ($P < 0.05$) (Table 4 and Figure 3).

Table 4. ROC Curve Analysis, Cutoff Values, Sensitivity, and Specificity of Immunological and Hormonal Indicators

Control versus HPV-infected women						
Variables	AUC*	Std. Error	P value	cut off	Sensitivity %	Specificity %
IL-1Beta	.935	.027	<0.001***	426.50	89	86
IL-2R	.916	.037	<0.001***	594.00	92	91
FSH	.914	.041	<0.001***	14.50	89	100
Estrogen	.946	.030	<0.001***	402.00	97	89
Control versus HPV-infected women with cancer						
IL-1Beta	.971	.019	<0.001***	426.50	94	87
IL-2R	.925	.035	<0.001***	594.00	94	89
FSH	.942	.036	<0.001***	14.50	89	90
Estrogen	.962	.026	<0.001***	402.00	95	93
Area under curve (AUC*)			High significant differences (<0.001***)			

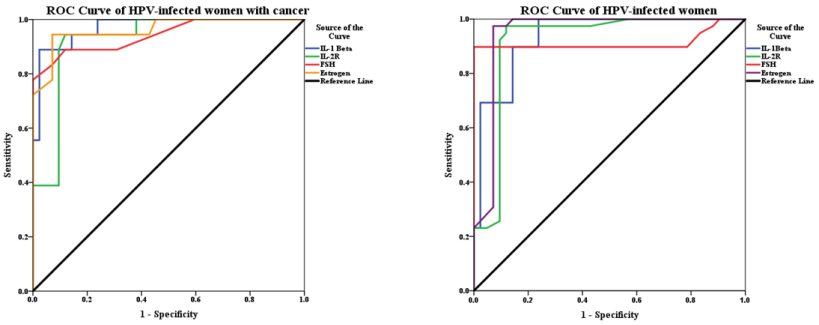

Figure 3. ROC curve immunological and hormonal markers

The ROC-derived cutoff values, along with the sensitivity and specificity estimates, were generated and assessed in the same study cohort. They therefore reflect exploratory discriminatory performance within the sample, not diagnostic estimates validated externally.

5. Correlation Relationship Among Immunological and Hormonal Markers

Spearman's rank correlation analysis among HPV-infected women identified a significant inverse association between IL-1β and estrogen ($\rho = -0.430$, $P = 0.006$). IL-2R was positively correlated with estrogen ($\rho = 0.386$, $P = 0.015$), as was FSH ($\rho = 0.600$, $P < 0.001$). The other correlations did not reach statistical significance ($P > 0.05$), as shown in Table 5.

Table 5. Spearman's Rank Correlations Among Immunological and Hormonal Biomarkers in HPV-Infected Women.

Relationship	ρ	P value
IL-1β – IL-2R	−0.222	0.175
IL-1β – FSH	−0.162	0.324
IL-1β – Estrogen	−0.430	0.006
IL-2R – FSH	0.273	0.093
IL-2R – Estrogen	0.386	0.015
FSH – Estrogen	0.600	<0.001

ρ = Spearman's rank correlation coefficient; P = probability value

Spearman's rank correlation analysis found no statistically significant correlations among IL-1β, IL-2R, FSH, and estrogen in women with HPV-associated cervical cancer ($P > 0.05$). Still, the results warrant cautious interpretation given the small number of women in this subgroup ($n = 9$), as shown in Table 6.

Table 6. Spearman's Rank Correlations Among Immunological and Hormonal Biomarkers in Women with HPV-Associated Cervical Cancer.

Relationship	ρ	P value
IL-1β – IL-2R	0.333	0.381
IL-1β – FSH	0.607	0.083
IL-1β – Estrogen	−0.302	0.429
IL-2R – FSH	0.508	0.162

Relationship	ρ	P value
IL-2R – Estrogen	0.119	0.760
FSH – Estrogen	−0.110	0.778

ρ = Spearman's rank correlation coefficient; P = probability value

4. Discussion

The study assessed immunological and hormonal biomarkers among women with HPV infection and HPV-associated cervical cancer. HPV was detected in 53.33% of participants: 43.33% had HPV infection, while 10.00% had HPV-associated cervical cancer. This proportion exceeded rates reported in several earlier studies from Iran and Iraq. HPV prevalence and genotype distribution may vary considerably among populations and geographic regions, as also demonstrated among unvaccinated women in Albania (Bakiri *et al.*, 2025). Variations in the study populations, sample sizes, geographic settings, and HPV detection methods may account for the differences between studies. Age varied significantly between the study groups. HPV infection occurred across a wide age range, but women with HPV-associated cervical cancer were generally older, with the largest concentration in the 51–60-year age group. That pattern agrees with earlier reports linking advancing age to a higher likelihood of persistent HPV infection and cervical cancer. Since age may confound the results, it was included in the adjusted statistical analysis.

Age varied substantially across the study groups, making it a potential confounder, especially when interpreting FSH and estrogen levels. Even after adjustment for age group, IL-1β, IL-2R, FSH, and estrogen remained significantly associated with HPV positivity and HPV-associated cervical cancer. The cervical cancer subgroup, however, included only nine women, resulting in wide confidence intervals around its estimates. These adjusted associations are therefore preliminary and need to be confirmed in larger cohorts.

Importantly, the present study revealed no significant differences ($P > 0.05$) among the study groups with respect to marital status, smoking status, chronic diseases, and having children (Table 1 and Figure 1).

Women with HPV-associated cervical cancer had significantly higher FSH and estrogen levels than both HPV-infected women and controls. This agrees with earlier observations that changes in sex hormones are linked to HPV-associated cervical carcinogenesis (Wu *et al.*, 2024). Estrogen may aid HPV-related tumor development by increasing viral oncogene expression and encouraging cellular proliferation. Elevated FSH, by contrast, may indicate hormonal changes related to age and disease status (Hu *et al.*, 2024; Pitambra et al., 2026). Estrogen receptor-mediated signaling has also been associated with cervical cancer progression (Hong *et al.*, 2025). Blood samples were collected on the second day of the menstrual cycle, reducing variation in FSH and estrogen measurements caused by differences in menstrual-cycle phase. Researchers have reported that reproductive tract inflammation is an additional risk factor for persistent high-risk HPV infection in women with cervical intraepithelial neoplasia (CIN). Inflammation of the reproductive tract can impair local immune responses by disrupting the normal physiological environment of the cervix (Huang *et al.*, 2024). This compromised immune response reduces the body's ability to eliminate high-risk HPV, allowing the virus to persist and replicate. Furthermore, the inflammatory response may alter cervical mucus by increasing its permeability and disrupting epithelial barrier function, thereby facilitating HPV attachment, invasion, and persistence. In addition, inflammatory mediators and cytokines produced during inflammation may influence the interaction between HPV and host cells, further promoting the persistence of high-risk HPV infection (Wang *et al.*, 2025).

Approximately 90% of cervical cancer cases are attributed to HPV infection, making it one of the most important global health concerns, particularly among women (Jensen *et al.*, 2024). In addition, HPV is the primary cause of genital warts and is the most common sexually

transmitted disease. Understanding the underlying immunological imbalance in HPV-infected individuals is therefore essential. IL-2R was investigated in the present study because of its important immunoregulatory role. Yasin *et al* (2026) reported significantly higher IL-2R concentrations in women with HPV infection and HPV-associated cervical cancer than in healthy women, and these findings are consistent with those of the current investigation. Elevated serum IL-2R levels indicate sustained T-cell activation. During chronic HPV infection, continuous exposure to viral antigens prolongs the activation of $CD4^+$ and $CD8^+$ T cells. Activated T cells express high levels of membrane-bound IL-2Rα (CD25), which can be cleaved and released into the circulation as soluble IL-2R (Oportus et al., 2025). Elevated IL-2R not only reflects persistent immune activation but also suggests immune dysfunction because prolonged stimulation may exhaust T-cell responses, alter cytokine signaling pathways, and reduce the efficiency of viral clearance (He *et al.*, 2024). This immune exhaustion and dysregulation may contribute to viral persistence and promote the progression of HPV-associated lesions toward cervical intraepithelial neoplasia and cervical cancer. Zhu *et al.* (2023) demonstrated that women with HPV infection and cervical cancer had significantly higher serum IL-2R levels than healthy women, which is consistent with the findings of the present study. Elevated circulating IL-2R (CD25) may function as a decoy receptor by binding free IL-2, thereby limiting its availability to effector T cells. Consequently, effector T cells may undergo apoptosis because of insufficient IL-2-mediated survival signals (Zhang *et al.*, 2026). This mechanism may contribute to the immune dysregulation observed in these patients. According to Thappa and Chiramel (2016), these findings may also explain the beneficial effects of immunotherapeutic approaches, such as the *Mycobacterium* vaccine, in treating recurrent warts by enhancing T-helper 1 cytokine responses, including IL-2 production. These observations also support the development of novel immunotherapeutic strategies, including the systemic administration of low-dose IL-2. Moreover, IL-2 has been investigated in immunotherapy models for cervical cancer, where it enhanced the efficacy of genetically modified T cells (Yasin *et al.*, 2026). Trujillo-Cirilo *et al.* (2023) suggested that tumor cells may exploit the relatively low concentrations of IL-2 produced by immune cells through IL-2R-mediated mechanisms to support tumor growth while simultaneously suppressing effective antitumor immune responses. Zhu et al. (2023) also reported that IL-2 concentrations in vaginal fluid were independently associated with the risk of cervical intraepithelial neoplasia (CIN). Future large-scale prospective studies should focus on clarifying the relationship between IL-2 expression, persistent high-risk HPV infection, and the development of CIN.

IL-1β is an important pro-inflammatory cytokine involved in the immune response to viral infections (Khalaf, 2024). Excessive cytokine production, including IL-1β, IL-6, and TNF-α, may amplify systemic inflammation and contribute to tissue injury during severe viral infections (Muhsin *et al.*, 2025). In general, HPV-infected cells express numerous genes associated with pro-inflammatory mediators, including IL-1β. Furthermore, in response to viral infection, inflammasomes, which are multiprotein complexes, produce pro-inflammatory cytokines such as IL-1β and IL-18. These complexes play a critical role in antitumor immunity by promoting antigen presentation through antigen-presenting cells (APCs), particularly dendritic cells, and by stimulating the secretion of pro-inflammatory cytokines (Aghbash et al., 2025). The present study revealed higher IL-1β levels in HPV-infected women than in the control group, and these findings are consistent with those reported by Olukomogbon et al. (2024). Following viral infection, infiltration of macrophages and dendritic cells into infected tissues stimulates the release of several inflammatory mediators, including IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-15, MCP-1, and MIP-1β, all of which play important roles in the host immune response against HPV (Olukomogbon *et al.*, 2024).

According to a previous study, patients with cervical cancer had significantly higher IL-1β levels than healthy individuals (Saha *et al.*, 2025). These findings are consistent with the results of the present study. Long *et al.* (2021) also reported markedly elevated IL-1β levels during

different stages of cervical precancer. Higher numbers of IL-1β-expressing keratinocytes and high-risk HPV stimulate the release of IL-1β from keratinocytes in cervical intraepithelial neoplasia (CIN). This process promotes the proliferation of cervical epithelial cells, induces DNA damage, and ultimately facilitates HPV DNA integration and the expression of the E6 and E7 oncoproteins, thereby promoting cervical carcinogenesis (Saha et al., 2025). Castagnino *et al.* (2022) investigated IL-1 cytokine signaling in several HPV biological and disease models and demonstrated that the HPV16 E6 and E7 oncoproteins strongly suppress cellular responses to IL-1β during both the early and late stages of cancer development.

Previous studies have suggested that the cervical carcinoma microenvironment stimulates neighboring monocytes to produce IL-1β and activate the inflammasome, which may contribute to poor clinical outcomes in cervical cancer (Fernandes *et al.*, 2023). More recently, Mantoani et al. (2025) reported that patients with larger colposcopic cervical lesions had higher IL-1 levels. This finding may explain the pro-inflammatory role of IL-1 in the progression of CIN 2/3 lesions, particularly in lesions larger than 1 cm^2.

Cytokine profiling has demonstrated significant alterations in the vaginal immune microenvironment of women co-infected with high-risk human papillomavirus (hrHPV) and asymptomatic sexually transmitted infections (STIs). Previous studies have suggested that concomitant STIs modify the inflammatory response during hrHPV infection, thereby influencing disease progression. Elevated IL-1β concentrations in vaginal samples indicate a proliferative and inflammatory microenvironment that may contribute to the initiation and progression of cervical dysplasia (Kebede *et al.*, 2025) (Table 2 and Figure 2).

The present results showed that the AUC, sensitivity, and specificity of FSH for screening women with HPV-associated cervical cancer were 0.942, 94%, and 89%, respectively. These values were higher than those reported by Wu *et al.* (2024), who found an AUC of 0.712, sensitivity of 64%, and specificity of 70%. Previous studies have suggested that FSH is a potential risk factor for cervical cancer, and elevated FSH concentrations are associated with an increased risk of disease. Therefore, FSH may serve as a predictive biomarker for cervical cancer (Ilia *et al.*, 2026). These findings are consistent with the results of the present study.

Kim *et al.* (2018) reported that the AUC, sensitivity, and specificity of estrogen for detecting cervical cancer were 0.831, 85%, and 75%, respectively, indicating diagnostic performance that was better than or comparable to conventional HPV testing. These values were lower than those obtained in the present study, in which the AUC, sensitivity, and specificity of estrogen were 0.962, 95%, and 93%, respectively.

Recent studies have suggested that alterations in hormone receptor expression, particularly decreased ERα expression and increased progesterone levels, together with abnormalities in circulating sex hormones, may contribute to the development of cervical cancer. These hormonal and biochemical profiles, together with possible genetic alterations, highlight the importance of hormone profiling in the clinical assessment of cervical cancer (Hong *et al.*, 2025).

Yasin et al. (2026) reported high sensitivity and specificity of IL-2R (93.33% and 100%, respectively) for screening HPV-infected women. These findings are comparable to those of the present study (92% and 91%, respectively). In addition, Yasin *et al.* (2026) reported a sensitivity and specificity of IL-2R of 81.32% and 69.54%, respectively, for screening women with HPV-associated cervical cancer. These values were lower than those observed in the present study (94% and 89%). The similarities and differences among studies indicate that further investigations are needed to evaluate the diagnostic performance of IL-2R.

Kebede *et al.* (2025) reported that the AUC, sensitivity, and specificity of IL-1β for diagnosing women with HPV-associated cervical cancer were 0.821, 100%, and 11%, respectively, which were lower than those reported in the present study (0.971, 94%, and 87%, respectively). Similarly, Mantoani *et al.* (2025) reported an AUC, sensitivity, and specificity of IL-1 for differentiating CIN stage II from stage III of 0.670, 66.7%, and 65.2%, respectively.

Importantly, the present investigation demonstrated high sensitivity and specificity of IL-1β, IL-2R, FSH, and estrogen for screening women with HPV infection and HPV-associated cervical cancer. Importantly, the present investigation demonstrated high within-sample discriminatory performance of IL-1β, IL-2R, FSH, and estrogen in distinguishing women with HPV infection and HPV-associated cervical cancer from controls. However, these findings are exploratory and require validation in larger independent cohorts before any clinical application can be considered (Table 4 and Figure 3).

Although the ROC analyses produced high AUC, sensitivity, and specificity values, the cutoff values were both derived and tested in the same study population. No independent validation cohort was available, so these estimates should be considered exploratory. Larger independent populations must be used for validation before the biomarkers or cutoff values are considered for clinical screening or diagnostic use.

High estrogen concentrations, particularly in the presence of high-risk HPV infection, may act as a co-carcinogenic factor by promoting a pro-inflammatory microenvironment through elevated IL-1β expression and facilitating immune evasion, as indicated by increased soluble IL-2R levels. Estrogen promotes HPV-induced tumorigenesis, whereas the HPV E6/E7 oncoproteins increase IL-1β production and suppress cellular immune responses (Li *et al.*, 2024). IL-1β, a cytokine produced by activated inflammasomes, is one of the major pro-inflammatory cytokines involved in the immune response to viral infections. It enhances both innate and adaptive immunity through the activation of immune cells and cytokine signaling pathways. Inflammation-induced IL-1β production may promote tumor development and HPV DNA integration, whereas elevated FSH may act as a potential risk factor. Increased concentrations of these biomarkers, which are frequently associated with menopause or persistent HPV infection, may indicate a higher risk of cervical cancer (Hernández-Silva *et al.*, 2024).

Elevated estrogen and follicle-stimulating hormone (FSH) levels are associated with an increased risk of HPV-positive cervical cancer. Estrogen, particularly through estrogen receptor alpha (ERα), promotes HPV-associated malignant transformation, whereas FSH may serve as a predictor of disease progression. Estrogen enhances the expression of HPV oncogenes, while elevated FSH is strongly associated with tumor progression (Wu *et al.*, 2024).

Spearman's rank correlation analysis identified significant relationships among several immunological and hormonal biomarkers in HPV-infected women. IL-1β was negatively correlated with estrogen, whereas IL-2R and FSH each showed positive correlations with estrogen. Together, these results may indicate interactions between hormonal regulation and immune responses during HPV infection. No statistically significant correlations were found among the biomarkers examined in the HPV-associated cervical cancer subgroup. That result warrants caution, given the small sample size (n = 9), as shown in Table 5 and Table 6.

Limitation

The small number of women in the HPV-associated cervical cancer group (n = 9) represents an important limitation of the present study. This limited sample size may reduce the precision and stability of the ROC estimates, including AUC, sensitivity, and specificity, and may also affect the reliability of correlation analyses within this subgroup. Therefore, findings related to the HPV-associated cervical cancer group should be interpreted cautiously and considered exploratory. Larger studies are required to confirm these observations.

Detailed information regarding histopathological confirmation, histological subtype, and clinical stage was not available for all patients in the HPV-associated cervical cancer group. Therefore, this limitation should be considered when interpreting the findings related to this subgroup.

5. Conclusion

In this study, women with HPV positivity and HPV-associated cervical cancer had higher serum levels of IL-1β, IL-2R, FSH, and estrogen than the controls. The biomarkers also showed promising discriminatory performance within the sample, although the ROC-derived cutoff values, sensitivity, and specificity were calculated from the same study population. They should therefore be regarded as exploratory, not clinically validated. Among HPV-infected women, selected immunological and hormonal biomarkers were significantly correlated. No statistically significant correlations were found in the HPV-associated cervical cancer subgroup. That group contained only nine women, so its findings require cautious interpretation. Larger independent studies are needed to confirm these associations and assess the possible clinical relevance of the biomarkers investigated.

Acknowledgements

We thank all staffs and the Director of Al-Batoul Teaching Hospital in Diyala as well as to women's clinics for their support and coordination. We thank all laboratory staffs who helped us during the data collection period and for their cooperation during the study.

Author contributions

Sura A. Hashim: study design, data collection, lab work, writing. S.K. Al-Qaisi: supervision, conceptualization, revision. Shahad S. Alwan: data analysis, interpretation, editing.

Sources of funding

Authors did not receive any grants from funding agencies.

Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis, or presentation of the research.

References

Aghbash, P. S., Rasizadeh, R., Nahand, J. S., & Baghi, H. B. (2025). The role of immune cells and inflammasomes in modulating cytokine responses in HPV-related cervical cancer. International Immunopharmacology, 145, 113625.

Bakiri, F., Çullhaj, B., Kaziaj, S., & Lika, M. (2025). Human papillomavirus genotypes in unvaccinated women in Albania: A public health perspective. Journal of Biological Studies, 8(1), 111–121. https://doi.org/10.62400/jbs.v8i1.13857

Bakiri, F., Abazi, E., & Çekani, M. L. (2023). Albanian male students perception and knowledge of human papillomavirus (HPV). Journal of Biological Studies, 6(4), 273–281. https://doi.org/10.62400/jbs.v6i4.8727

Bertolazzi, M., Genta, M. L., Lopes, A., Fernandes, R., Maciel, G., Lopez, R., ... & Carvalho, J. (2026). Randomized trial of autologous ovarian graft to preserve ovarian function in young women undergoing pelvic radiotherapy for cervical cancer. European Journal of Surgical Oncology, 111496.

Buchbinder, E., Lotze, M. T., Margolin, K. A., Amaria, R., Sarnaik, A., Seery, V., ... & Silk, A. W. (2025). Role of high-dose interleukin-2 for melanoma in the age of cellular therapy. Journal for ImmunoTherapy of Cancer, 13(5), e011119.

Castagnino, P., Kim, H. W., Lam, L. K. M., Basu, D., & White, E. A. (2022). Systematic analysis of IL-1 cytokine signaling suppression by HPV16 oncoproteins. Journal of Virology, 96(22), e01326-22.

Fernandes, F. P., Cambui, R. A. G., Soares, J. L. D. S., Reis, E. C. D., Leal, V. N. C., & Pontillo, A. (2023). Cervical carcinoma induces NLRP3 inflammasome activation and IL-

1β release in human peripheral blood monocytes affecting patients' overall survival. Clinical and Translational Oncology, 25(11), 3277–3286.
He, N., Yi, C., Zeng, Q., Jing, W., & He, W. (2024). Advancements in the vaginal microenvironment and regression of high-risk human papillomavirus. Indian Journal of Microbiology, 1–10.
Hernández-Silva, C. D., Ramírez de Arellano, A., Pereira-Suárez, A. L., & Ramírez-López, I. G. (2024). HPV and cervical cancer: Molecular and immunological aspects, epidemiology, and effect of vaccination in Latin American women. Viruses, 16(3), 327.
Hong, M. K., Chiang, C. H., Cheng, C. H., & Chu, T. Y. (2025). Anti-estrogen therapy achieves complete remission and stability in recurrent cervical cancer: A case study. The American Journal of Case Reports, 26, e946296.
Hong, M. K., Wang, J. H., Li, M. H., Su, C. C., Cheng, C. H., & Chu, T. Y. (2025). Topological distribution of the sex hormone receptor expressions highlights the importance of stromal ERα and epithelial PR in malignant transformation of the uterine cervix. International Journal of Molecular Sciences, 26(9), 4418.
Hu, H., Wang, Z., Xue, L., Zhang, T., Jing, Y., & Li, Y. (2024). Effects of neoadjuvant chemotherapy combined with radical laparoscopic surgery with quality nursing care on clinical efficacy and sex hormones in patients with cervical cancer. American Journal of Translational Research, 16(2), 477.
Huang, R., Liu, Z., Sun, T., & Zhu, L. (2024). Cervicovaginal microbiome, high-risk HPV infection, and cervical cancer: Mechanisms and therapeutic potential. Microbiological Research, 287, 127857.
Ilia, G., Thomopoulos, A., & Chronas, D. (2026). Preserved menstruation after chemoradiotherapy in stage IIIC1 cervical cancer: A unique case. Journal of Clinical Medicine, 15(4), 1550.
Jensen, J. E., Becker, G. L., Jackson, J. B., & Rysavy, M. B. (2024). Human papillomavirus and associated cancers: A review. Viruses, 16(5), 680.
Karami, A., Rastin, A., Shahani, T., Robati, A. K., Ahmadi, F., & Jozi, M. (2026). Determination of human papillomavirus prevalence in the female population from Zanjan, Iran. Preventive Medicine Reports, 103406.
Kebede, H. B., Tsehay, D., Mekuria, S., Forslund, O., Borgfeldt, C., Jerkeman, M., ... & Mihret, A. (2025). Cytokine profiling in vaginal specimens from asymptomatic women with human papillomavirus and/or sexually transmitted infections. DOI:10.20944/preprints202502.1372.v1.
Khalaf, S. A. (2024). Serum level of some immunological markers in COVID-19 patients in Diyala Province, Iraq. Infectious Epidemiology and Microbiology, 10(4), 329–333.
Khan, A., Pillay, M., Bipath, R., Msimang, M., Harry, J., Sibiya, A. L., & Msomi, N. (2025). Evolution of testing for the diagnosis of human papillomavirus (HPV) status in head and neck squamous cell carcinoma: Where from and where to?. Oral Oncology, 162, 107208.
Kim, G., Yu, K., Kim, J., Kim, S., Park, S., Ahn, S., ... & Lee, D. (2018). Diagnostic availability of estrogen receptor alpha mRNA in cervical cancer tissue. Korean Journal of Clinical Laboratory Science, 50(4), 449–456.
Kushwah, A. S., Gupta, M. K., Srivastava, K., & Banerjee, M. (2025). Cytokine gene variants as risk predictors and prognostic biomarkers for cervix cancer: A revisit. Current Cancer Therapy Reviews, *21*, 1–11.
Li, Q., Kaidong, L., Tian, Z., Diao, W., Sun, Y., Bai, Y., ... & Zhao, W. (2024). Association of inflammatory factors with cervical cancer: A bidirectional Mendelian randomization study. Journal of Inflammation Research, 17, 10119–10130.
Lokau, J., Petasch, L. M., & Garbers, C. (2024). The soluble IL-2 receptor α/CD25 as a modulator of IL-2 function. Immunology, 171(3), 377–387.

Long, D. L., Song, H. L., & Qu, P. P. (2021). Cytokine profiles in cervical mucosa in patients with cervical high-risk human papillomavirus infection. The Journal of Infection in Developing Countries, 15(5), 719–725.

Mantoani, P. T. S., Micheli, D. C., Jammal, M. P., Vieira, J. H., Michelin, M. A., Ferreira, C. G. X., ... & Nomelini, R. S. (2025). High-grade cervical intraepithelial neoplasia: Impact of colposcopic lesion area on systemic immune responses. International Journal of Women's Health, 17, 345–353.

Muhsin EA, Alkhayat SA, Nsaif RH. Hemorrhagic fever viruses: Epidemiology, pathogenesis and emerging control strategies. Novel Research in Microbiology Journal, 9(4):322–331. doi:10.17582/journal.NRMJ/2025/9.4.322.331.

Niebler, M., Qian, X., Höfler, D., Kogosov, V., Kaewprag, J., Kaufmann, A. M., ... & Rincon-Orozco, B. (2013). Post-translational control of IL-1β via the human papillomavirus type 16 E6 oncoprotein: A novel mechanism of innate immune escape mediated by the E3 ubiquitin ligase E6-AP and p53. PLoS Pathogens, 9(8), e1003536.

Olukomogbon, T., Akpobome, B., Omole, A., ACCME Research Group, Adebamowo, C. A., & Adebamowo, S. N. (2024). Association between cervical inflammatory mediators and prevalent cervical human papillomavirus infection. JCO Global Oncology, 10, e2300380.

Oportus, J., Hojman, L., Gonzalez, V., & Karsulovic, C. (2025). Soluble IL-2R as a marker of T-cell activation in immune-mediated diseases: Review and case-based interpretation. Lymphatics, 3(1), 1.

Osmani, V., Rossiter, M., Hörner, L., Nkurunziza, T., Rank, S., Tanaka, L. F., & Klug, S. J. (2025). Worldwide burden of cervical human papillomavirus (HPV) in women over 50 years with abnormal cytology: A systematic review and meta-analysis. BMJ Global Health, 10(4).

Pitambra, A., Nigam, J. S., Pradeep, I., Rath, A., Palo, S., & Kumar, N. (2026). Immunohistochemical expression of Ki-67, estrogen receptor, and human epidermal growth factor receptor 2 in p16-positive premalignant and malignant cervical squamous lesions: Associations with clinicopathological parameters. Ewha Medical Journal.

Saha, S., Sultana, S., Rahmat, R., Akther, T., Nessa, A., & Jahan, M. (2025). Patterns of different cervical cytokine expression in high-risk human papillomavirus-infected patients with cervical cancer and its precancerous lesions. Clinical Medicine Insights: Oncology, 19, 11795549251316767.

Thappa, D. M., & Chiramel, M. J. (2016). Evolving role of immunotherapy in the treatment of refractory warts. Indian Dermatology Online Journal, 7(5), 364–370.

Trujillo-Cirilo, L., Weiss-Steider, B., Vargas-Angeles, C. A., Corona-Ortega, M. T., & Rangel-Corona, R. (2023). Immune microenvironment of cervical cancer and the role of IL-2 in tumor promotion. Cytokine, 170, 156334.

Wang, J., Tian, Z., & Wang, J. (2025). Risk factors for persistent infection of high-risk HPV in patients with cervical intraepithelial neoplasia. American Journal of Translational Research, 17(4), 2992.

Wu, X., You, Y., Guan, Z., Ban, Y., Wang, Y., Du, D., ... & Mo, L. (2024). A correlation study between cervical cancer and sex hormones. Frontiers in Oncology, 14, 1475052.

Yasin, M. M., Hassan, R. A., El Sayed, A. A., Ezzelarab, S. S., Helal, M. M., & Abdelhady, A. S. (2026). Serum level of soluble interleukin-2 receptor among human papillomavirus-infected female patients. Infectious Agents and Cancer, 21(1), 7.

Zhang, J., Wang, Y., & Li, H. (2026). Research progress of adoptive tumor-infiltrating lymphocyte therapy for cervical *cancer. International Journal of Gynecology & Obstetrics, 172*(1), 104–114.

Zhou, X., An, R., & Li, X. (2025). Cervical cancer immune microenvironment: Mechanisms of HPV-mediated immune evasion and advances in immunotherapy. Oncology Letters, 31(1), 22.

Zhu, R., Wang, W., Yang, A., Zhao, W., Wang, W., Wang, Z., ... & Hao, M. (2023). Interactions between vaginal local cytokine IL-2 and high-risk human papillomavirus infection with cervical intraepithelial neoplasia in a Chinese population-based study. Frontiers in Cellular and Infection Microbiology, 13, 1109741.