
Screening of cellular immunity of T cells in endometrial cancer patients via measurement of interferon gamma, interleukin 22, interleukin 17, interleukin 13, interleukin 10, interleukin 4, interleukin 2, and tumor necrosis factor alpha cytokines

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ABSTRACT

Background and objective: Endometrial cancer (EC) is considered the sixth most prevalent cancer among women worldwide, with over 400,000 new cases annually, and both its incidence and mortality rates are rising. This study attempted to examine the concentrations of interleukins (IL-2, IL-4, IL-10, IL-13, IL-17, IL-22), tumor necrosis factor alpha (TNF- α), and interferon gamma (IFN- γ) in Kurdish women diagnosed with endometrial cancer.

Methods: Seventy patients were included in the study, from which random samples were selected for testing by ELISA multiplex enzyme immunoassay. Serum was separated from the patients' peripheral blood.

Results: The findings revealed a significant ($p < 0.05$) increase in mean concentration of IFN- γ (55 ± 0.2 pg/mL), IL-2 (122 ± 0.33 pg/mL), TNF- α (18.14 ± 9.06 pg/mL), IL-17 (3.06 ± 1.49 pg/mL), IL-22 (10.34 ± 2.34 pg/mL), and IL-4 (9.75 ± 11.35 pg/mL) in patients diagnosed with endometrial cancer compared with their respective healthy individuals, whereas no significant difference was found in the level of IL-10 (3.02 ± 1 pg/mL) in endometrial cancer patients compared with healthy individuals (3.00 ± 0.3 pg/mL). Finally, IL-13 levels showed a significant decrease in EC patients (0.99 ± 0.11 pg/mL) compared with healthy individuals (2.1 ± 0.2 pg/mL).

Conclusion: The study demonstrates a significant elevation of pro-inflammatory cytokines (IFN- γ , IL-2, TNF- α , IL-17, IL-22, and IL-4) and a marked reduction of IL-13 in endometrial cancer patients, suggesting their involvement in tumorigenesis and local immune dysregulation. IL-10 levels remained unchanged, indicating complex immune dynamics.

Keywords: ELISA, Endometrial cancer, Cytokine, T cell

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INTRODUCTION

The second most common gynaecologic malignancy globally is endometrial cancer (EC) (1). The prognosis is optimistic for early-stage disease; however, advanced cancer exhibits a 5-year survival rate of 15% (2). Early identification of high-risk endometrial cancers is essential for enhancing treatment outcomes. Although factors such as pathological type, stage, grade, and molecular type could indicate a high risk, accurate identification of recurrence and metastasis risk in patients still presents significant challenges (3). The main risk factors include unopposed estrogen exposure (e.g., hormone replacement therapy, obesity), late menopause, nulliparity, genetic mutations, and metabolic syndrome (4). Endometrial disease is typically regarded as steroid-sensitive; however, immune system defects, along with genetic and epigenetic predispositions, are also believed to contribute to its development. The immune environment is thought to significantly influence the initiation and progression of endometrial pathology. Immune cells such as B lymphocytes, T lymphocytes, and natural killer (NK) cells play an essential role in influencing implantation outcomes and the proliferation of endometrial cells (5).

The oncogenesis and progression of cancer are closely associated with the immune microenvironment, in which interleukins (ILs) play an integral part. Studies indicate that interleukins significantly influence the oncogenesis and progression of EC through the regulation of cell proliferation, apoptosis, and angiogenesis, among other mechanisms (6). T helper 1 (Th1) cytokines, including IL-2 and IFN- γ , exhibit immune-stimulating functions and have the capacity to inhibit tumor growth (7). IL-2, as a multifunctional cytokine, exerts both suppressive and promoting effects on tumors by regulating the proliferation

and function of natural killer (NK) cells and T cells (8). Tumor necrosis factor-alpha (TNF- α) is a multifunctional cytokine primarily associated with inflammation and immune response mechanisms; its activity occurs through binding to tumor necrosis factor receptors 1 and 2 (TNFR1 and TNFR2), leading to activation of various signalling cascades (9). IL-4 is an immunomodulatory cytokine that facilitates the differentiation of T lymphocytes into the Th2 phenotype, thereby improving humoral responses and the production of IgE antibodies (10). IL-10 acts as a vital anti-inflammatory cytokine, generated in response to anti-inflammatory signals by various immune cells, including B cells, T cells, dendritic cells, and macrophages (11). IL-10, mainly secreted by macrophages, is recognized for its role in inhibiting T cell activation (12). Studies have investigated the role of anti-inflammatory cytokines produced by T-helper 2 cells, including IL-4, IL-10, and IL-13, in endometrial lesions (13). IL-17A, the main cytokine generated by Th17 cells, is a pro-inflammatory cytokine linked to the pathogenesis of numerous chronic inflammatory diseases; increases in IL-17A levels in peritoneal fluid have been associated with disease severity and infertility (5).

The aim of the present study is to screen the levels of IL-2, IL-4, IL-10, IL-13, IL-17, TNF- α , and IFN- γ in patients diagnosed with endometrial cancer.

METHODS

Collection and Storage of Blood Serum

The current study received approval from the Ethics Committee of the College of Pharmacy, Hawler Medical University, Erbil, Iraq (Ethical approval no. HMU-EC-Ph-22/9/2024-30).

Blood samples were obtained from women diagnosed with endometrial cancer, classified according to their family history of endometrial and

breast cancer, as well as from women without any family history. To avoid contamination, all samples were collected under sterile conditions. Blood samples were collected and sera were separated via centrifugation for 5 minutes at 6000 rpm, then aliquoted and frozen at -80°C .

Inclusion Criteria

In this case-control study, patients with endometrial cancer were diagnosed based on histopathological examination, had no prior treatment (chemotherapy or radiation), and consented to participate and to the use of their samples.

Exclusion Criteria

Non-Kurdish women, patients with metastatic endometrial cancer at diagnosis, and cases with inadequate sample quality were excluded from the current study.

Multiplex Enzyme Immunoassay

This study used the Human Th1/Th2/Th17/Treg Multiplex ELISA Kit (Arigo Biolaboratories Corporation) to perform semi-quantification of human IFN- γ , IL-2, IL-4, IL-10, IL-13, IL-17A, IL-22, and TNF- α in serum samples. This assay uses the semi-quantitative sandwich enzyme immunoassay method. Standards, controls, and samples were placed into the wells of a CHROMATE microplate reader (Awareness Technology, Inc., Palm City), where any target cytokines present bind to the immobilized specific antibodies.

Assay Procedure

All materials were equilibrated to room temperature ($20\text{--}25^{\circ}\text{C}$) prior to use. The surplus microplate strips were removed from the frame, and 100 μL of standards or samples were added to the wells, which were then covered and incubated for 2 hours at room temperature. Each well was

aspirated and washed, with the procedure repeated for a total of five washes; each well was filled with 300 μL of 1 $\times$ Wash Buffer via a squirt bottle, manifold dispenser, or mechanical washer. Following the final wash, any residual Wash Buffer was removed by aspirating and blotting on absorbent paper towels.

1X Antibody Conjugate Mixture (100 μL) was added to each well, the plate was covered, and incubation proceeded for 1 hour at room temperature. Each well was again aspirated and washed as previously described. 100 μL of 1X HRP-Streptavidin Solution was added to each well; the plate was covered and incubated for 1 hour at ambient temperature. After washing again, each well was filled with 100 μL of TMB Substrate, covered, and incubated for 10 to 20 minutes in a dark location at room temperature. Immediately afterward, 100 μL of Stop Solution was added to each well, changing the color from blue to yellow. Optical density was measured at 450 nm using a microplate reader without delay; absorbance was recorded within 30 minutes of adding the stop solution.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation; significance was set at $p < 0.05$. For parameters exhibiting normally distributed data (e.g., uric acid and TSH), the one-sample t-test was used to determine whether the sample mean deviated substantially from the reference value. For parameters with non-normally distributed data (e.g., glucose, urea, creatinine, GPT, GOT, and bilirubin fractions), the one-sample Wilcoxon signed-rank test was used as a non-parametric alternative, focusing on the sample median. SPSS version 28 was used for the study.

RESULTS

Multiplex enzyme immunoassay results revealed a significant increase in mean concentration of IFN- γ (55 ± 0.2 pg/mL), IL-2 (122 ± 0.33 pg/mL), TNF- α (18.14 ± 9.06 pg/mL), IL-17 (3.06 ± 1.49 pg/mL), IL-22 (10.34 ± 2.34 pg/mL), and IL-4 (9.75 ± 11.35 pg/mL) in patients diagnosed with endometrial cancer compared with their respective healthy individuals,

as shown in Figure 1 (A–D) and Figure 2 (A–B). Figure 2/C shows no significant difference in the level of IL-10 (3.02 ± 1 pg/mL) in EC patients compared with healthy individuals (3.00 ± 0.3 pg/mL). Finally, IL-13 levels showed a significant decrease in EC patients (0.99 ± 0.11 pg/mL) compared with healthy individuals (2.1 ± 0.2 pg/mL), as shown in Figure 2/D.

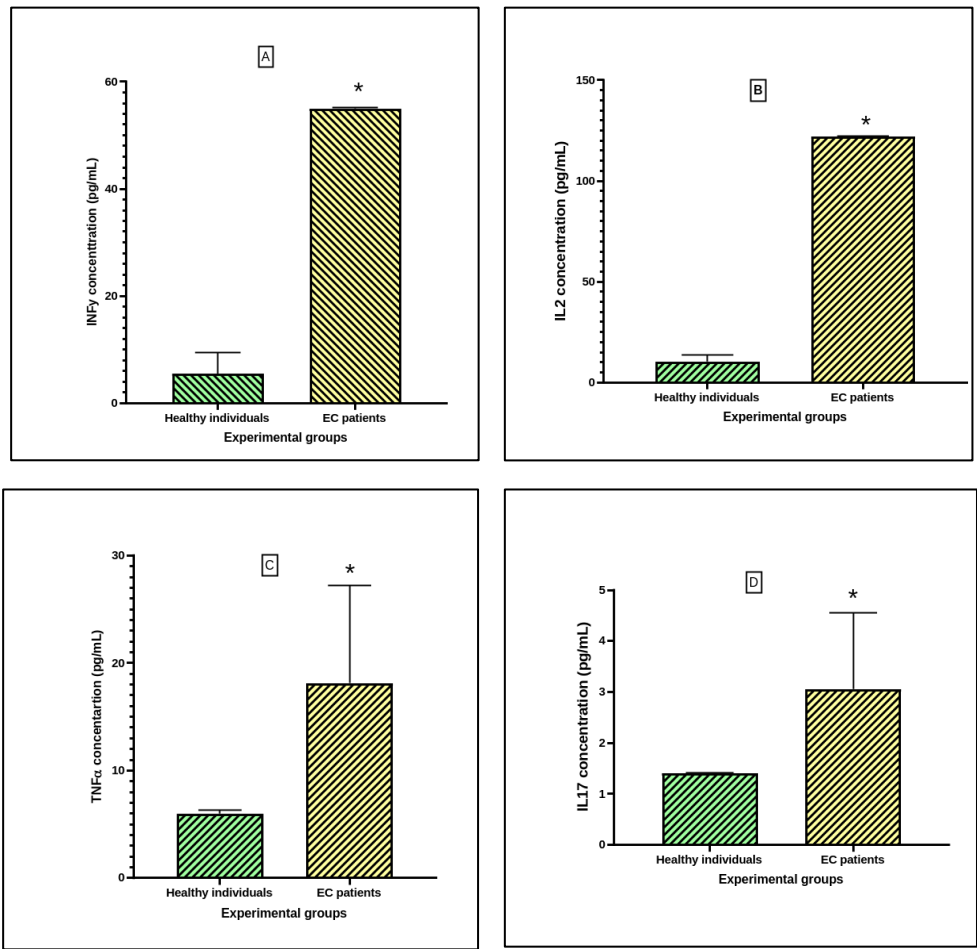


Figure 1. IFN- γ (A), IL-2 (B), TNF- α (C), and IL-17 (D) cytokine expression in healthy individuals and endometrial cancer patients

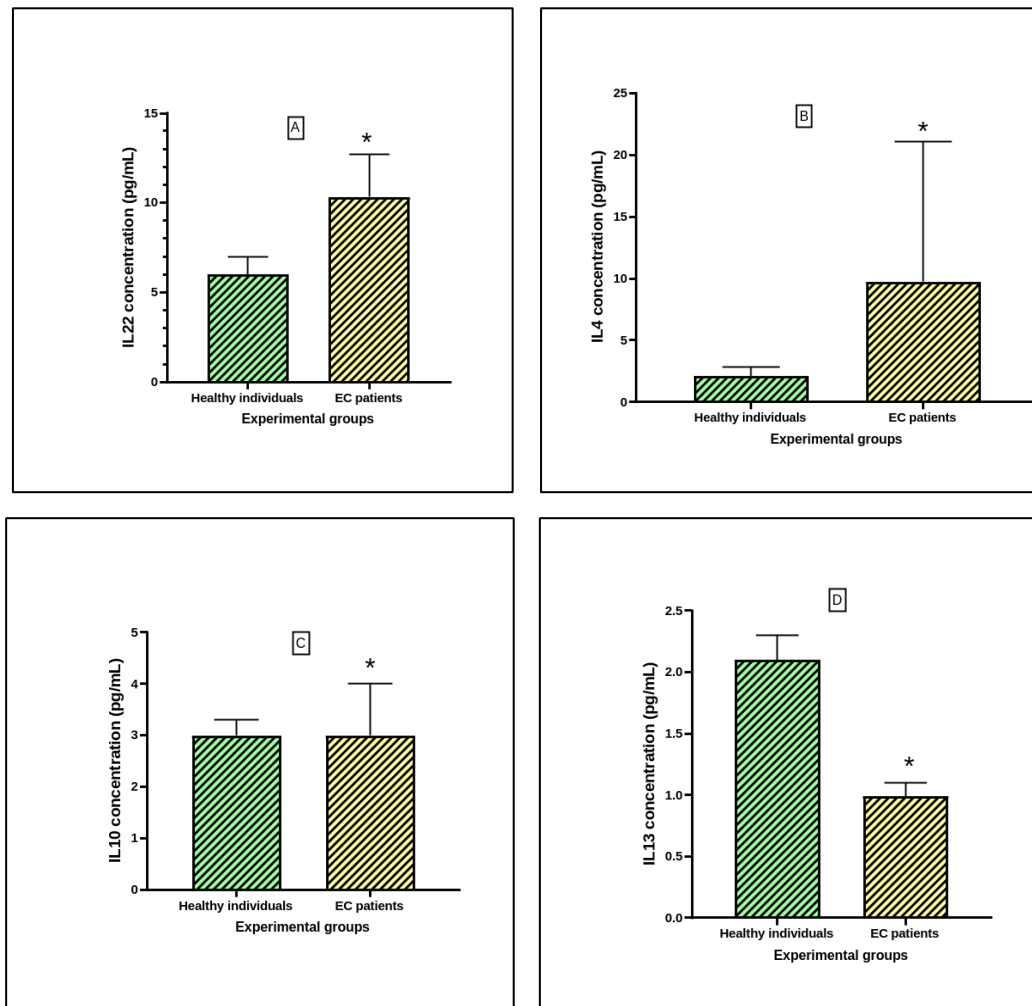


Figure 2. IL-22 (A), IL-4 (B), IL-10 (C), and IL-13 (D) cytokine expression in healthy individuals and endometrial cancer patients

Note: the values above for IFN- γ , IL-2, TNF- α , and IL-22 have been re-matched to their correct cytokines after cross-checking against the source data in Figures 1 and 2. The original manuscript's Abstract and Results text listed these four values in an order that did not match the corresponding figure panels (e.g., the text paired "122 $\pm$ 0.33 pg/mL" with IFN- γ , but Figure 1 Panel B shows this value belongs to IL-2, with IFN- γ at 55 $\pm$ 0.2 pg/mL in Panel A). IL-17 and IL-4 were already correctly matched in the original text. The Discussion section's panel-by-panel interpretation was already consistent with the figures throughout and required no changes. We recommend verifying this correction against the original SPSS/GraphPad output before final submission. Separately, the source chart for IL-10 (Figure 2, Panel C) displays a significance asterisk even though the reported means (3.02 vs. 3.00 pg/mL) and the manuscript's own text describe this as a non-significant difference — this apparent plotting artifact is reproduced as supplied and should also be checked against the underlying statistical output.

Statistical evaluation of biochemical markers indicated that all parameters were within normal physiological limits. Tests included glucose, urea, creatinine, uric acid, liver enzymes (GPT, GOT), bilirubin fractions (direct, indirect, and total), and thyroid-stimulating hormone (TSH). Depending on data distribution, the Wilcoxon signed-rank test or one-sample t-test was applied to compare sample means with their respective theoretical/reference values. All p-values were high (ranging from 0.875 to 1.000), and none of the differences were statistically significant, confirming alignment with standard clinical norms. Measured means for

glucose (128.45 mg/dL), urea (30.18 mg/dL), and creatinine (0.77 mg/dL) reflect normal metabolic and renal function. Liver enzymes (GPT: 23.72 U/L, GOT: 29.56 U/L) and bilirubin levels (total: 0.46 mg/dL, direct: 0.19 mg/dL, indirect: 0.26 mg/dL) also fell within acceptable ranges, suggesting no hepatic abnormalities. Similarly, uric acid (4.5 mg/dL) and TSH (2.09 μ IU/mL) values support normal renal and thyroid function. Overall, this analysis confirms a healthy biochemical profile in the study sample, with no evidence of pathological deviations, as shown in Table 1.

Table 1. Biochemical markers in endometrial cancer patients

Test	Mean	SD	p
Glucose (mg/dL)	128.45	45.48	0.999
Urea (mg/dL)	30.18	9.78	1.000
Creatinine (mg/dL)	0.77	0.20	1.000
Uric acid	4.50	1.26	0.995
GPT (U/L)	23.72	24.32	1.000
GOT (U/L)	29.56	43.16	1.000
Indirect bilirubin (mg/dL)	0.26	0.19	1.000
Direct bilirubin (mg/dL)	0.19	0.14	0.875
Total bilirubin	0.46	0.27	1.000
TSH (μ IU/mL)	2.09	1.05	1.000

Blood test results showed that all measured hematological parameters, including red blood cell indices (HGB, HCT, RBC, MCH, MCHC, MCV, RDW-SD, RDW-CV), white blood cell counts and differentials (WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils), and platelet indices (PLT, PCT, MPV, PDW, P-LCR), were within the normal range compared with their respective

upper normal limits. All p-values were greater than 0.05, most notably at 1.000, indicating no statistically significant difference between the observed means and the upper reference values. This suggests a stable and healthy hematological profile with no signs of anemia, infection, inflammatory conditions, or platelet abnormalities in the current dataset, as shown in Table 2.

Table 2. Blood parameters in endometrial cancer patients

Test	Mean	SD	p
RBC	4.742	0.646	1.000
HGB	12.633	1.447	1.000
HCT	37.769	4.593	1.000
MCV	80.101	8.082	1.000
MCH	26.879	2.929	1.000
MCHC	33.547	1.567	1.000
RDW-CV	13.559	1.946	1.000
RDW-SD	47.737	8.742	1.000
WBC	8.718	3.518	1.000
Neutrophil %	67.526	7.801	0.999
Lymphocyte %	28.222	8.364	1.000
Monocyte %	6.848	1.821	0.409
Eosinophil %	1.557	0.840	0.279
Basophil %	0.383	0.244	0.191
PLT	288.010	84.870	1.000
PCT	0.334	0.408	0.875
MPV	9.361	1.315	1.000
PDW	28.374	14.749	1.000
P-LCR	23.926	8.730	1.000

DISCUSSION

Figure 1 shows a significant rise in the expression of pro-inflammatory cytokines — IFN- γ , IL-2, TNF- α , and IL-17 — in patients with endometrial cancer relative to healthy individuals. This finding illustrates a distinct immune activation profile linked to the tumor microenvironment in EC. Panel A shows a significant increase in IFN- γ levels among patients with EC. IFN- γ , a Th1 cytokine, serves a vital function in macrophage activation and antigen presentation; however, its overproduction is linked to chronic inflammation and tumor progression in various malignancies, including gynecological cancers (14). Similarly, IL-2 (Panel B), another cytokine associated with Th1, was significantly elevated, suggesting enhanced T cell proliferation and potential immunological dysregulation (15). IL-2 is essential for anti-tumor responses; however, its dysregulation may facilitate immune evasion mechanisms in tumor cells. Panel C indicates elevated TNF- α levels in patients diagnosed with EC. TNF- α serves as a key mediator of inflammation and is linked to the promotion of angiogenesis, tumor proliferation, and metastasis in endometrial cancer (16). IL-17 levels (Panel D) are significantly increased in EC patients, suggesting enhanced Th17 cell activity. IL-17 promotes tumor-associated inflammation and is associated with negative prognosis in endometrial cancer (17). The results indicate a pro-inflammatory immunological profile in EC patients, characterized by elevated levels of Th1 and Th17 cytokines. This suggests a chronic inflammatory condition that contributes to tumor development and progression, which might provide targets for immunotherapy.

Figure 2 presents a cytokine profile revealing notable changes in the expression levels of IL-22, IL-4, IL-10, and IL-13 in patients with EC compared with healthy individuals. This indicates a complex

interaction between pro-inflammatory and anti-inflammatory responses within the tumor microenvironment. Panel A demonstrates a significant elevation of IL-22 levels in patients with EC. IL-22, a cytokine generated by Th22 and Th17 cells, serves a dual function in tissue protection and tumorigenesis; increased levels of IL-22 have been linked to enhanced cancer cell proliferation, survival, and invasion via activation of STAT3 signaling (18). Elevated levels of IL-22 in EC patients may consequently facilitate tumor progression and immune evasion. Panel B indicates a notable increase in IL-4, a characteristic cytokine of the Th2 response. IL-4 enhances tumor growth by fostering a suppressive immune microenvironment and directing macrophages toward a tumor-supportive M2 phenotype (19). This matches the tumor's approach to inhibiting effective anti-tumor immunity. Panels C and D show a reduction in IL-10 and IL-13 levels among EC patients. IL-10 is typically classified as an anti-inflammatory cytokine with immunosuppressive properties; however, recent data indicate a context-dependent function in which its downregulation may result in unchecked inflammation and tumor advancement (20). IL-13, another Th2 cytokine, is similarly decreased, potentially indicating a compromised regulatory response and a decreased anti-inflammatory balance in EC. The results point to a shift toward an imbalanced immune environment in EC that promotes tumor growth and diminishes regulatory cytokine activity.

This study has some limitations that should be considered when interpreting the results. First, the sample size would ideally have been larger, as the data were collected from a single hospital. Second, although ELISA is quite sensitive and widely used, using additional methods such as flow cytometry or gene expression profiling might have enhanced the accuracy and range of cytokine detection.

CONCLUSION

This study highlights the significant elevation of pro-inflammatory cytokines IFN- γ , IL-2, TNF- α , IL-17, IL-22, and IL-4 in patients with endometrial cancer, supporting their role in tumorigenesis and immune dysregulation. The notable reduction of IL-13, alongside stable IL-10 levels, indicates complex immune interactions in the tumor microenvironment. Cytokine profiles may serve as potential indicators for diagnosis and prognosis. The absence of significant alterations in biochemical and hematological indicators suggests that immunological changes in EC are localized rather than systemic. Further study is needed to confirm these findings and to investigate their therapeutic implications.

COMPETING INTEREST

The authors declare that they have no competing interests.

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