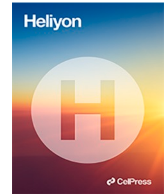
Heliyon 10 (2024) e23294

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



A higher number of exhausted local PD1+, but not TIM3+, NK cells in advanced endometriosis

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ARTICLE INFO

Keywords:
Endometriosis
Exhaustion
NK cell
PD-1
TIM-3

ABSTRACT

Endometriosis (EMT) is a chronic inflammatory disease characterized by the presence and growth of endometrial-like glandular epithelial and stromal cells outside the uterus. Natural Killer (NK) cell dysfunction/exhaustion has been shown in patients with EMT. In this case-control study, we compared the frequency of exhausted PD-1 or TIM-3 positive NK cells in peripheral blood (PB) and peritoneal fluid (PF) of women with advanced endometriosis to control fertile women. PB and PF were collected from women aged 25–40 who underwent the laparoscopic procedure, including 13 stages III/IV endometriosis and 13 control samples. Multicolor flowcytometry was used to compare the frequency of PD-1 or TIM-3 positive NK ($CD3^{-}CD56^{+}$) cells in PB and PF of two groups. We demonstrated a higher percentage of PD-1+ NK cells in the peritoneal fluid of patients with endometriosis rather than controls (P-value = 0.039). This significance was related to stage IV of endometriosis (P-value = 0.047). We can not show any significant difference in the number of PD-1 or TIM-3 positive NK cells in peripheral blood. Our results suggest a local exhausted NK cell response in endometriosis that can be a leading factor in the endometriosis pathogenesis.

1. Introduction

Endometriosis (EMT) is a chronic inflammatory disease that affects 10% of women of reproductive age [1] and is characterized by the presence and growth of endometrial epithelial and stromal cells outside the uterus [2–4]. EMT can manifest as chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility [5]. Microenvironment in endometriosis shares similarities with tumor microenvironment (TME); It has been shown that inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor-α (TNF-α), growth factors (e.g. vascular endothelial growth factor), prostaglandins, reactive oxygen species, and hypoxia have the potential to produce a microenvironment very similar to TME in patients with endometriosis [6–8]. Additionally, several studies indicated that endometriotic lesions increase the risk of ovarian endometrioid, low-grade serous, and clear-cell adenocarcinoma, which then named

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https://doi.org/10.1016/j.heliyon.2023.e23294
Received 9 May 2023; Received in revised form 22 November 2023; Accepted 30 November 2023
Available online 10 December 2023
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endometriosis-associated ovarian cancers [9–12]. Such altered tissue microenvironment can cause suppression of effector immune cells leading to immune cell exhaustion [8,13]. Furthermore, immune dysfunction of natural killer (NK) cells has been proposed to contribute to the persistence of refluxed endometrial cells in the peritoneal cavity. Reciprocal interaction of endometrial stromal cells and macrophages can result in the production of IL-10 and transforming growth factor-β (TGF-β), and subsequent impaired cytotoxicity of NK cells [14].

Natural killer T (NKT) cells are a type of T cells with concurrent invariant T cell receptors and CD56. These cells demonstrate tissue-specific characteristics and function under the influence of cytokines present in the microenvironment [15]. Although there is sparse information regarding the role of NKT cells in endometriosis [16,17]; we proposed that altered NKT cells may somehow contribute to the pathogenesis of endometriosis.

Dysfunctional/exhausted NK cells have been shown in patients with endometriosis. Overexpressed inhibitory receptors characterize immune cell exhaustion in effector cells, decreased effector cytokine production, and cytolytic activity, leading to the failure of immunosurveillance [8,18]. Exhausted NK cells exhibit a phenotype similar to exhausted T cells. Overexpression of inhibitory receptors, including programmed cell death protein 1 (PD-1), T-cell immunoglobulin domain and mucin domain protein 3 (TIM-3), and Natural Killer Cell Inhibitory Receptor (NKG2A), was described on exhausted NK cells [8,19–22].

PD-1 (CD279) is a member of the B7 co-stimulatory factor superfamily, and programmed cell death ligand-1 (PD-L1; CD274) is a ligand of PD-1. PD-1 is usually expressed on the surface of activated T cells, B cells, monocytes, dendritic cells, and NK cells, whereas PD-L1 is expressed on a wide variety of immune and non-immune cells [7,23]. The binding of PD-L1 to PD-1 is involved in the

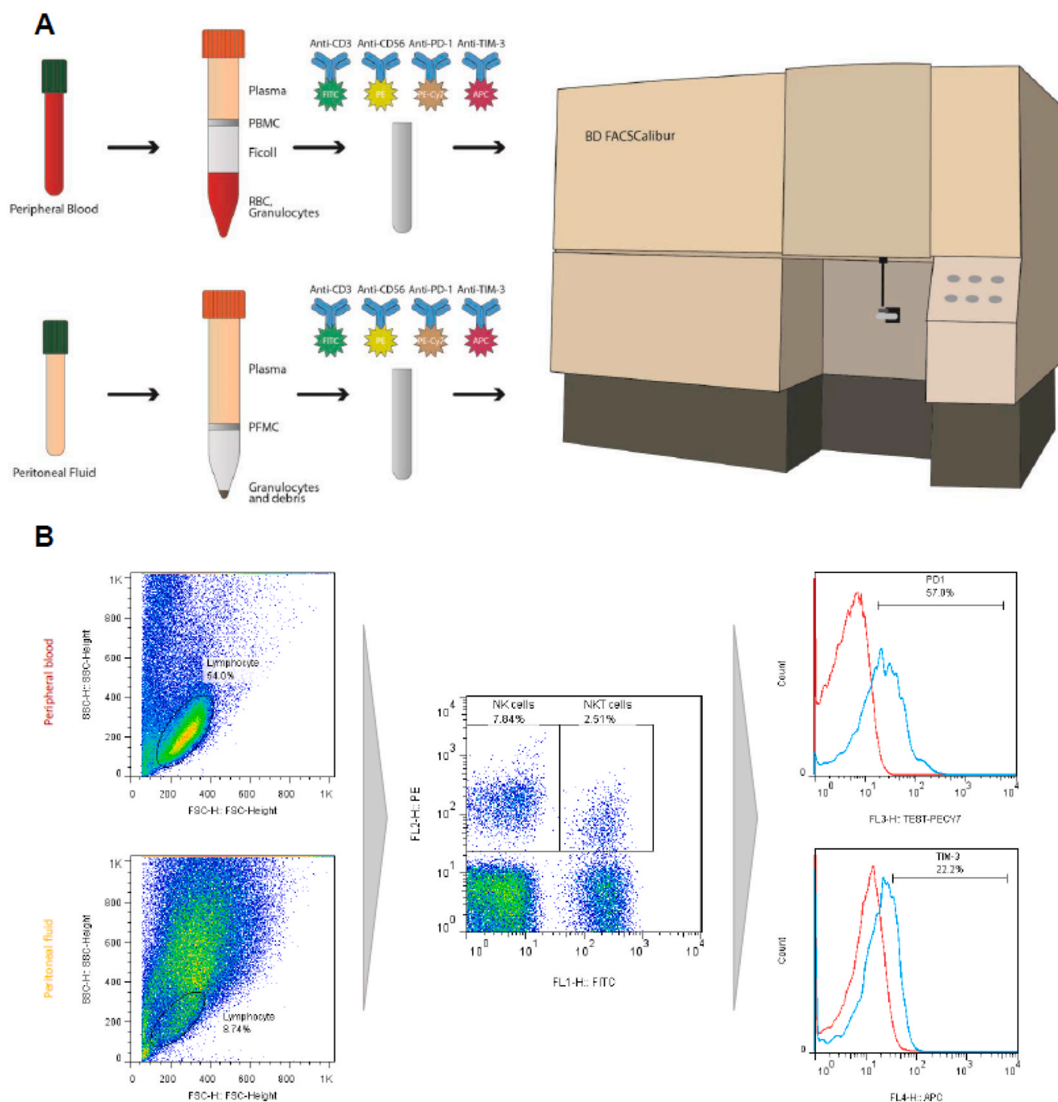

Fig. 1. A) Graphical abstract of the workflow and B) Gating strategy for flow cytometry analysis. The selection method of the investigated peripheral and peritoneal fluid NK ($CD3^{-}CD56^{+}$), NKT ($CD3^{+}CD56^{+}$) cells, and PD-1 or TIM-3 positives were determined.

suppression of effector cells, such as T cells and NK cells, leading to the regulation of the immune system [8,24].

TIM-3 is a member of the TIM-receptor family and galectin-9 (Gal-9) is a ligand of TIM-3. TIM3 is expressed by various immune cells, including T, NK, and antigen-presenting cells, and is implicated in regulating crucial immunological processes such as proliferation, survival, and tissue inflammation [25,26]. The binding of Gal-9 to TIM-3 is involved in the suppression of effector cells which leads to the regulation of the immune system [8,27,28].

Accordingly, immune cell exhaustion is a possible phenomenon in endometriosis patients. Decreased effector functions of T and NK cells in endometriosis were previously described in several studies [9,12,19,21,29]. Here, we assessed the expression of PD-1 and TIM-3 on NK and NKT cells of peripheral blood (PB) and peritoneal fluid (PF) of patients with advanced endometriosis and compared it with fertile women.

2. Materials and methods

This case-control study enrolled 13 women with advanced endometriosis (stage III, IV) confirmed by observation of endometriotic lesions during laparoscopy and pathological confirmation of the disease in biopsies that were taken from endometriotic foci as the case group; and a control group comprised of 13 women with no evidence of endometriosis during laparoscopy. All participants were 25–40 years of age. The presence and classification of endometriosis were confirmed by a gynecologist during laparoscopy according to the revised American Society for Reproductive Medicine of endometriosis [30]. Women with autoimmune, inflammatory, malignant disorders, or other gynecological diseases (e.g., polycystic ovary syndrome) and women taking hormone therapy for three months before the study were excluded.

The study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS), Tehran, Iran (Ethics code: IR.TUMS.MEDICINE.REC.1399.1094, IR.TUMS.MEDICINE.REC.1399.1159). The samples were taken from women referred to Arash Hospital, a TUMS-affiliated women's hospital. All women signed an informed consent form for study participation before entering the study.

A total of 5 mL of PB was collected from the antecubital vein of each patient before they underwent general anesthesia. PF was aspirated by the surgeon after the insertion of the second trocar at the beginning of the laparoscopic procedure. The volume of PF varied from 2 to 6 mL in different cases. In each group, the samples were classified as follicular or luteal phase based on the date of the reported patient's last menopausal period at the time of sampling; Also, the menstrual cycle was confirmed by pathologic reports in cases which samples of endometriotic lesions were obtained for pathological investigations. As the majority of stage I and II cases of endometriosis do not routinely undergo laparoscopy for diagnosis, we selected just advanced stages of endometriosis for sample taking.

PB and PF were collected in heparinized tubes and transferred to the laboratory in sterile, cold conditions. The diluted PB or PF with phosphate-buffered saline (PBS) was layered on Ficoll-Hypaque (Inno-train, Germany) and centrifuged (1000 g, 20 min). Collected cells in the interphase layer were entirely washed with PBS, and the precipitated mononuclear cells were suspended in PBS.

For the determination of NK and NKT cells, the mononuclear cells were stained with FITC-labelled anti-CD3 and PE-labelled anti-CD56 (Biolegend, CA, USA) antibodies. Also, two exhaustion markers were stained with PE-CY7-labelled *anti*-PD-1 and APC-labelled anti-TIM-3 (Biolegend, CA, USA). 10^5 stained cells were investigated by the BD FACSCalibur instrument (Becton Dickinson, CA) (Fig. 1A), and the data were analyzed by using FlowJo software (Version 7.6.1).

The lymphocytes were gated based on forward and side scatters in both PB and PF. According to the expression of CD56 and CD3, $CD56^{+}CD3^{-}$and $CD56^{+}CD3^{+}$ cells were considered NK and NKT cells, respectively. The percentage of PD1+ and TIM-3+ cells was determined in each of the NK or NKT cell populations and compared between the case and controls (Fig. 1B).

Because of the non-normal distribution of the samples, we used the Mann-Whitney *U* test to compare the frequency of the NK and NKT cells in PB and PF samples between the two groups. The Wilcoxon test was used to compare the percentage of the NK and NKT cell population in each group between PB and PF. P values < 0.05 were considered to be statistically significant. SPSS version 26 and GraphPad Prism Version 9.1 software were used for data analysis and drawing the plots. The sample size was calculated based on three closely related publications [9,19,22].

3. Results

After considering the inclusion and exclusion criteria, 13 individuals were selected for each group. We collected 11 PB and 11 PF samples from each group, and nine PB and PF samples were paired in each group. Table 1 compares age and body mass index (BMI) between endometriosis and non-endometriosis groups. The clinical characteristics of the patients with endometriosis were

Table 1
Comparing the age and body mass index (BMI) between Endometriosis and Non-endometriosis groups.

	Endometriosis n = 13 Proliferative: 8 Secretory: 5	Non-endometriosis n = 13 Proliferative: 6 Secretory: 7	P-Value
Mean age (years ± SD)	**34.09 ± 5.58**	**31.09 ± 5.59**	**0.082**
Age ranges (years)	**26–40**	**25–39**	
Mean BMI (kg/m^2 ± SD)	**26.95 ± 5.08**	**22.96 ± 2.77**	**0.003**

demonstrated in detail (Table 2).

3.1. Comparison of the percentage of NK and NKT cells in PF and PB between endometriosis and control groups

The current study indicated that the percentage of PF NK cells is higher in endometriosis patients compared to controls (P-value = 0.017). Specifically, this significance was related to stage III of endometriosis (P-value = 0.044), not Stage IV (P-value = 0.062), relative to controls. (Fig. 2A). There was no significant difference in peripheral blood NK cell percentages between endometriosis and control groups and between different stages of endometriosis compared to controls (Supplementary Figs. 1 and A). Also, there were no significant difference in $CD56^{bright}$ $CD16^{dim}$ NK cell or $CD56^{dim}$ $CD16^{bright}$ NK cell percentage in any of the samples or patient groups when compared to controls.

The percentage of NKT cells was not statistically different between endometriosis and control neither in PF (P-value = 0.438) nor in PB (P-value = 0.270) (Fig. 2B and Supplementary Figs. 1 and B).

The percentage of NK and NKT cells in PB and PF of endometriosis patients in proliferative phase and secretory phase were compared within group and between group; but there were no significant difference in any of the comparisons.

3.2. Comparing the percentage of PD1+NK and PD1+NKT cells in PF and PB between endometriosis and control groups

The number of PD-1-expressing NK cells in the peritoneal fluid of patients with endometriosis was significantly higher than in controls (P-value = 0.039). This significance was related to stage IV of endometriosis (P-value = 0.047) rather than stage III (P-value = 0.159) (Fig. 2C). The percentage of PD1+NK cells was similar in PB between two groups (Supplementary Figs. 1 and C).

The number of PD-1-expressing NKT cells was not statistically different either in PF (P-value = 0.075) or in PB (P-value = 0.797) between women with and without endometriosis (Fig. 2D and Supplementary Figs. 1 and D).

3.3. Paired cell percentage comparisons within endometriosis or control groups

Our results indicated that the percentage of NKT cells in the peritoneal fluid of endometriosis patients was significantly higher than in peripheral blood (P-value = 0.015) (Fig. 3C). In contrast, there was no significant difference in the percentage of NKT cells between control PF and PB samples (P-value = 0.594) (Fig. 3D). Also, there was no significant difference in the percentage of NK cells between PF and PB either in the endometriosis (P-value = 0.250) or control group (P-value = 0.164) (Fig. 3A and B).

3.4. Paired cell percentage correlation analysis between the number of PD1+NK or PD1+NKT cells in PB and PF within endometriosis or control group

It was shown that there is a slightly positive correlation in PD1+NK cells or PD1+NKT cells between PB and PF samples of the control group. In contrast, there is a negative correlation in PD1+NK cells or PD1+NKT cells between PB and PF samples of the endometriosis group (Supplementary Figs. 2 and B).

Table 2
Clinical characteristics of endometriosis patients. In all the categorical variables, the actual case number and percentage of total cases [n (%)] are presented.

	PB (n = 11)	PF (n = 11)
Menstrual Cycle – n (%)		
Proliferative phase	**7 (63.6%)**	**6 (54.5%)**
Secretory phase	**4 (36.4%)**	**5 (45.5%)**
Fertility Data – n (%)		
Previous normal pregnancy	**2 (18.2%)**	**2 (18.2%)**
Abortion	**1 (9.1%)**	**2 (18.2%)**
Infertility	**5 (45.5%)**	**4 (36.4%)**
Intervention Data – n (%)		
Previous laparoscopic intervention	**2 (18.2%)**	**2 (18.2%)**
Clinical Symptoms – n (%)		
Pelvic pain	**5 (45.5%)**	**5 (45.5%)**
Dysmenorrhea	**6 (54.5%)**	**7 (63.6%)**
Dysuria	**0 (0.0%)**	**1 (9.1)**
Dyschesia	**3 (27.3%)**	**4 (36.4%)**
Type of Endometriosis – n (%)		
Peritoneal	**2 (18.2%)**	**3 (27.3%)**
Ovarian endometriosis	**4 (36.4%)**	**3 (27.3%)**
Deep infiltrating endometriosis (DIE)	**1 (9.1%)**	**1 (9.1%)**
Combined (DIE + other)	**4 (36.4%)**	**4 (36.4%)**

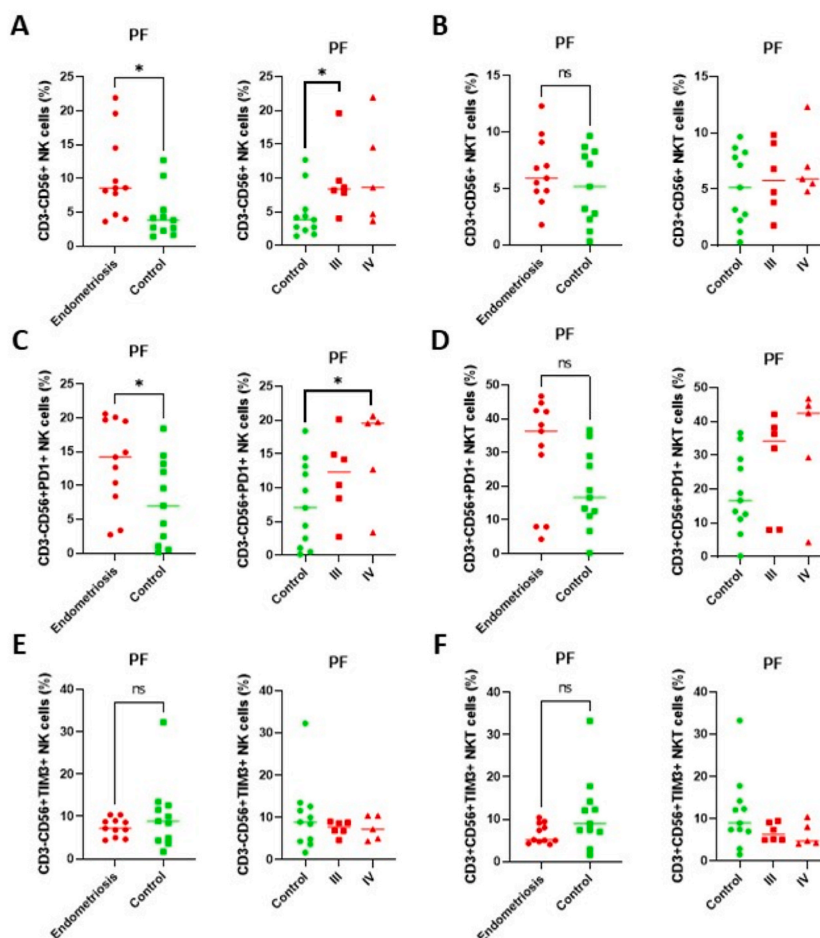

Fig. 2. Comparison of peritoneal fluid cell percentages between endometriosis/subgroups and control group (Endometriosis (n = 11), Stage III (n = 6), Stage IV (n = 5), Control (n = 11)).

3.5. Comparison of the percentage of TIM3+NK and TIM3+NKT cells in PF and PB between endometriosis and control groups

The findings of our study demonstrated no statistically significant difference in the frequency of TIM3+NK and also, of TIM3+NKT cells between endometriosis and controls either in PF or PB (Fig. 2E and F; and Supplementary Figs. 1E and F).

3.6. Comparison of the expression of PD-1 or TIM-3 on the surface of NK and NKT cells between endometriosis and control groups

We detected a higher level of geometric mean fluorescence intensity (GMFI) of PD-1 on NKT cells of peritoneal fluid in patients with endometriosis relative to controls (P-value = 0.045) (Supplementary Figs. 3 and B). Also, there was a higher expression (GMFI) of TIM-3 on NK cells in the peripheral blood of the endometriosis group compared to controls, but it was not statistically significant (P-value = 0.066) (Supplementary Figs. 3 and C).

4. Discussion

Endometriosis is an inflammatory estrogen-dependent disorder with chronic pelvic pain and infertility. Although not much is known about its pathophysiology, it is accepted that dysregulated immune pathways can be involved in the development of this disorder. Previous studies suggest that endometriosis is considered a local disease with systemic subclinical manifestations [31–33]. However, recent studies claim that endometriosis is a systemic disease [34,35]. Accordingly, it is not clear that endometriosis is a local or systemic disease. It is nothworthy that endometriosis is correlated with autoimmune diseases such as systemic lupus erythematosus (SLE), celiac disease (CD), inflammatory bowel disease (IBD), and autoimmune thyroiditis; and shares similarities with autoimmune diseases in lacking self-tolerance, altered immune cell percentage and functions and production of autoantibodies [36,37]. Also, the involvement of immunophenotypic alterations in pathogenesis of endometriosis is still controversial.

In this study, multicolor flow cytometry was used to characterize the abundance and to compare the cell surface expression of

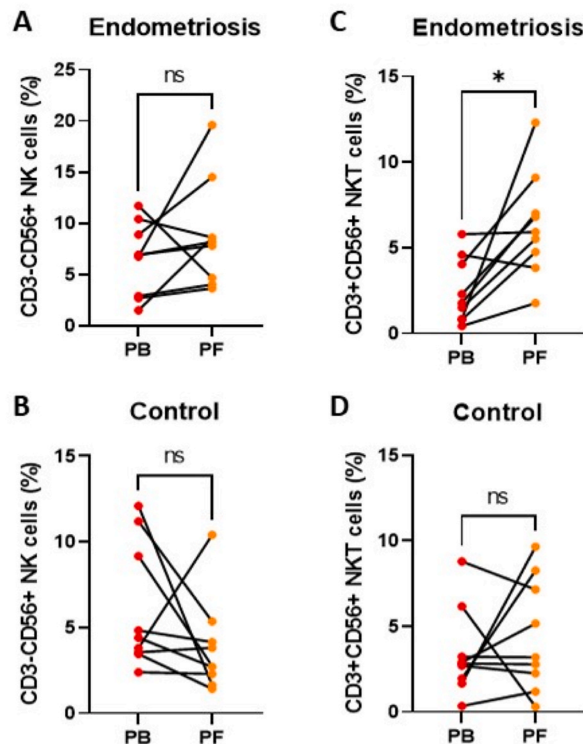

Fig. 3. Comparison of peripheral blood and peritoneal fluid cell percentages within paired samples of endometriosis or control group (Endometriosis (n = 9), Control (n = 9)).

immune checkpoints such as PD-1 and TIM-3 molecules on peripheral blood and peritoneal fluid NK and NKT cell subsets of women with and without endometriosis.

Our findings show no significant difference between the mean ages of endometriosis and non-endometriosis cohorts. However, the mean BMI was higher in endometriosis patients compared to controls. There are controversial results regarding the association between BMI and endometriosis. A meta-analysis performed in 2016 suggested a reverse association between BMI and risk of endometriosis [38]. Contradicting, recent research shows a direct association between BMI and the incidence of endometriosis [39] which is to our results. Considering BMI as a confounding factor, we searched for studies that assessed the impact of BMI on NK cells in the endometrium. In a recent study, Naqvi et al. demonstrated no significant difference in $CD56^+$ NK cell percentage between initial samples and the samples taken after weight loss intervention in the endometrium; furthermore there were no difference in PD-1 expression between endometrium samples before and after intervention [40]. Also, in a study by Palacz et al., there was no correlation between BMI and NK cell percentage in the endometrium [41]. Consequently, it may be appropriate to refrain from categorizing BMI as a confounding variable in relation to NK cell percentage or exhaustion, but rather acknowledge its association with the etiology of endometriosis.

Our results indicated that the percentage of peritoneal fluid NK cells is higher in endometriosis compared to controls while the number of these cells is not different in peripheral blood between the aforementioned groups. This phenomenon could be related to the increased infiltration of NK cells into the peritoneal cavity in endometriosis patients (Fig. 4A). NK cells have an important role in tissue homeostasis and the removal of debris and dead cells from the peritoneum. Accordingly, it could be an indication for the immune system trying to defeat the disease by infiltrating more NK cells into the peritoneum. In a recent study by Wu et al., it was indicated that the percentage of activated NK cells in eutopic endometrium of endometriosis patients is significantly higher relative to controls [42]. Also, we indicated this significance was related to stage III patients, not stage IV, compared to controls. Therefore, we presume that higher infiltration of NK cells happens when the patients are in the lower stage (Fig. 4B).

Here we found that percentage of PD-1+ NK cells in PF samples of patients with endometriosis is significantly higher than in controls (Fig. 4B). On one hand, this can be caused by increased infiltration of PD-1+ NK cells in PF of endometriosis patients. On the other hand, this immunophenotypic change could be due to the exhaustion of NK cells. This exhaustion could be related to epigenetic alterations that happen in the local microenvironment. Both hypotheses require further assessments to be confirmed. Wu L et al. in immunohistochemical and western blot analyses demonstrated that ectopic and eutopic endometrial tissues of endometriosis had significantly higher expression of PD-1 when compared to normal endometrial tissues [10]. Additionally, they confirmed increased PD-1+$CD4^+$ or PD-1+$CD8^+$ T cells in the peripheral blood of endometriosis subjects in comparison to the controls. We did not detect any difference in the frequency of PD1 expressing NK cells in peripheral blood between the groups; this can lead us to the assumption that the accumulation of these PD-1+NK cells is more related locally to the endometriotic microenvironment alterations. Although

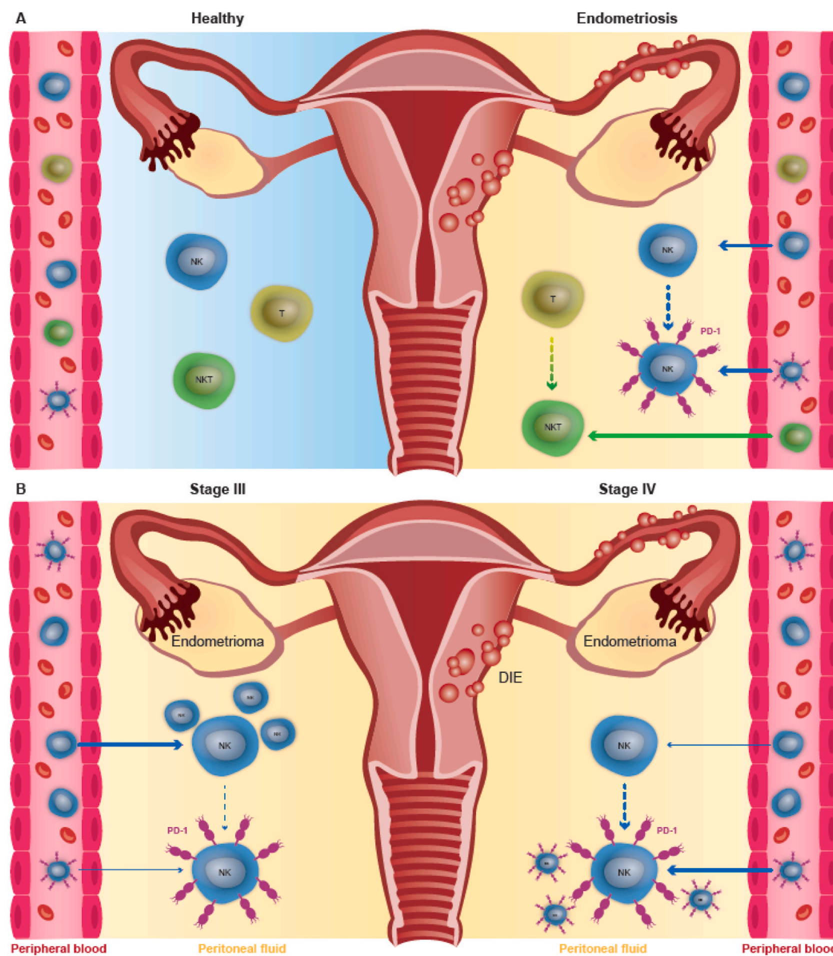

Fig. 4. A) NK cell, PD-1+ NK cell, and NKT cell infiltration and/or T cell transition to NKT cell phenotype in endometriosis patients (Dashed lines are representative of immunophenotypic change/transition). B) NK cell and PD-1+ NK cell infiltration in Stages III and IV of patients with endometriosis. (Dashed lines are representative of immunophenotypic change).

several studies demonstrated higher expression of PD-1 and PDL1 expressing circulatory immune cells of endometriosis patients including $CD8^+$ and $CD4^+$ T cells [9,10], $CD19^+$ B cells [9] and neutrophils [12], this dominance was not observed in our experiment about peripheral blood PD1+NK cells in endometriosis. In addition, a recent study demonstrated that the PB and PF levels of soluble PDL1 in endometriosis patients were significantly higher compared to controls [13]. Besides we have compared the number of PD1+NK cells in stage III and IV patients with controls and indicated that this significance was related to stage IV patients rather than Stage III.

Concerning NKT cell numbers, our study did not show any significant difference between different stages of endometriosis and control either in PF or PB. However, we demonstrated that the percentage of NKT cells in peritoneal fluid is significantly higher than peripheral blood in endometriosis but not in control group (Fig. 3C and D). In concordance with our study, in a study by Matyas Meggyes et al., it was shown that percentage of NKT cells in peritoneal fluid is significantly higher than in peripheral blood in endometriosis patients [19]. This phenomenon could be due to NKT cell infiltration into PF and/or local T cell transition to NKT cell phenotype in endometriosis patients. In a study by Good et al., it was indicated that chimeric antigen receptor (CAR) T cell dysregulation/exhaustion is associated with a CD8+T-to-NKT cell transition. Also, in recurrent cancers after CAR T cell therapy percentage of CAR NKT cells becomes significantly higher than in controls [43]. Accordingly, a novel hypothesis is formed that T cells might be transitioned into NKT cells in the peritoneal fluid microenvironment in patients with endometriosis (Fig. 4A). However, it requires further assessments to be proved.

Our findings support that there is a negative correlation in either PD1+NK or PD1+NKT cells' frequency between PB and PF of the endometriosis group; however, the correlation in the control group was positive. This finding can be interpreted in the context of endometriosis as a local disease. Furthermore, we detected a higher expression level of PD-1 (GMFI) on NKT cells of peritoneal fluid in endometriosis relative to controls (Supplementary Figs. 3 and B). In a study conducted by Young et al., it was indicated that the effect of PD-1 on NKT cell emigration was dependent upon the direct PD-1/PD-L1 interaction and that PD-1 and PD-L1 would play a role in chemotaxis and chemokine receptor expression [44].

Inconsistent with a prior study by Meggyes et al. that indicated higher circulatory TIM3+NK in non-endometriosis compared to endometriosis [19], We could not detect a significant difference in the frequency of TIM3+ NK or TIM3+NKT cells neither in PB nor in PF between endometriosis and control. This inconsistency could be due to the dynamic nature of the endometriosis disease. Although we detected higher expression of TIM-3 on NK cells of PB in the endometriosis relative to controls, it was near a statistically significant level (P-value = 0.066) (Supplementary Figs. 3 and C).

Another finding by Yeo et al., which was based on a real-time PCR, demonstrated that the expression of TIM-3 mRNA was significantly higher in PF of endometriosis patients compared to PF of gynecologic cancer patients; but the difference was not significant between endometriosis patients and healthy controls [20]. In addition, a study by Brubel et al. demonstrated that the PB levels of soluble galectin-9, as a ligand of TIM3, in endometriosis patients were significantly higher compared to controls [45].

The immune checkpoint molecules are located on the surface of exhausted T and NK cells (PD-1 and TIM-3) [8,28,29,46], and their ligands (PD-L1 and Gal-9) are elevated in endometriosis tissue [9,10,13,21,45,47]. Under normal circumstances, these checkpoint molecules prevent autoimmune disease and protect healthy tissues against damage after activation of the immune system [48]; Within the tumor microenvironment, checkpoint molecules prevent T cells from attacking the tumor, weakening the ability of the immune system to recognize and destroy tumor cells [49]. By blocking these molecules using monoclonal antibodies (mAbs), the immune response of T cells can be largely activated, thereby reestablishing antitumor immune effects [50]. To date, three mAbs targeting PD-1 (Pembrolizumab, Nivolumab, and Cemiplimab) and three mAbs targeting PD-L1 (Atezolizumab, Durvalumab, and Avelumab) have been approved by the US Food and Drug Administration (FDA). Currently, several clinical trials are being pursued to evaluate the therapeutic potential of immune checkpoint inhibitors (ICIs) in gynecologic cancers. Considering the similarities of the microenvironment in endometriosis and tumor, immune cell exhaustion is an inevitable phenomenon in endometriosis patients which implies that ICIs may have a therapeutic value for treatment of endometriosis. In conclusion, further studies are required to determine if PD-1 and/or TIM-3 expression on T and/or NK cells of patients with endometriosis has diagnostic/therapeutic value in patients with endometriosis.

This research was subjected to some limitations that are better to be considered and could be addressed in future research. The first limitation is related to the restricted sample size that was used for analysis. The second limitation is the lack of cytotoxicity marker assessment. Despite these limitations, the strength of our present work is the assessment of inhibitory markers (PD-1 and TIM-3) on NK and NKT cells in peripheral blood and peritoneal fluid simultaneously, in endometriosis and control groups.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ramin Hosseinzadeh: Writing - review & editing, Writing - original draft, Methodology, Investigation, Conceptualization. **Ashraf Moini:** Resources. **Reyhaneh Hosseini:** Resources. **Mina Fatehnejad:** Resources. **Mir Saeed Yekaninejad:** Software, Formal analysis. **Moslem Javidan:** Software, Investigation. **Mostafa Changaei:** Software, Investigation. **Fahimeh Feizisani:** Investigation. **Samira Rajaei:** Writing - review & editing, Writing - original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was part of an M.Sc. thesis [9811127004-50402] supported by the Tehran University of Medical Sciences [Grant No: 99-3-101-50840] and a research grant [Grant No: 99-3-101-50419] from the Tehran University of Medical Sciences, Iran.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23294.

Abbreviations

EMT	Endometriosis
PB	Peripheral blood
PF	Peritoneal fluid
PBMC	Peripheral blood mononuclear cell
PFMC	Peritoneal fluid mononuclear cell
rASRM	revised American society for reproductive medicine
LMP	Last menstrual period

DIE	Deep infiltrative endometriosis
NK	Natural Killer cell
NKT	Natural Killer T cell
PD-1	Programmed cell death Protein 1
PD-L1	Programmed death-ligand 1
TIM-3	T cell immunoglobulin and mucin domain-containing protein 3
Th	T helper cell
IL	Interleukin
CD	Cluster of differentiation
ns	Non-significant
MFI	Mean Fluorescent Intensity
GMFI	Geometric Mean Fluorescent Intensity
FBS	Fetal bovine serum
D-PBS	Dulbecco's phosphate buffer saline
FITC	Fluorescein isothiocyanate
PE	Phycoerythrin
PE/Cy7	Phycoerythrin Cyanine 7
APC	Allophycocyanin

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