Original Article

DOI: 10.7860/JCDR/2024/70153.19672

Differences in Stromal Immunostaining of Tumour Necrosis Factor-alpha and its Receptors in Neoplastic and Non Neoplastic Ovarian Lesions: A Cross-sectional Study

Oncology Section

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ABSTRACT

Introduction: Ovarian cancer has an unknown pathogenesis, and cytokines may play an important role in the aetiology and prognosis. Tumour Necrosis Factor-alpha (TNF-α) and its receptors (TNFR1 and TNFR2) are involved in the biology of ovarian cancer, tumour pathogenesis, and their relationship with prognostic factors. They are involved in biological processes such as immunoregulation, growth modulation, and cell differentiation.

Aim: To evaluate stromal immunostaining of TNF-α and its receptors (TNFR1 and TNFR2) in malignant ovarian neoplasms, comparing it with benign ovarian neoplasms and non neoplastic ovarian lesions.

Materials and Methods: A cross-sectional study was carried out at the Department of Gynaecology and Obstetrics, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil, from January 1997 to December 2020. Patients with ovarian lesions who underwent surgical treatment according to pre-established criteria (n=95) were included in the study. Patients with benign (n=37) and malignant (n=43) ovarian epithelial neoplasms, and non neoplastic ovarian lesions (n=15) were included. Data evaluated included age, parity, hormonal status (menarche or menopause), histological grade, and staging. Immunohistochemical study was performed to evaluate stromal TNF-α, TNFR1, and TNFR2. Data were analysed by GraphPad Prism 6 and IBM Statistical Package for the Social Sciences (SPSS) Statistics 20.0 software. The comparison between non neoplastic tumours, benign and malignant neoplasms was performed by the Fisher's exact test with a significance level below 0.05.

Results: Considering staining intensity 0 and 1 as "weak immunostaining" and 2 and 3 as "strong immunostaining," TNF-α stromal immunostaining was stronger (2/3) in benign ovarian neoplasms compared to non neoplastic tumours (p-value=0.0016) and in malignant neoplasms compared to non neoplastic tumours (p-value<0.0001). TNFR1 immunostaining was stronger (2/3) in the stroma of malignant neoplasms compared to benign neoplasms (p-value<0.0001) and stronger (2/3) when comparing benign neoplasms with non neoplastic ovarian lesions (p-value=0.0002). For TNFR2, stromal immunostaining was stronger (2/3) in malignant neoplasms compared to benign neoplasms (p-value =0.0091) and stronger in malignant neoplasms compared to non neoplastic lesions (p-value=0.0004).

Conclusion: A stronger immunostaining for TNF-α and its receptors was found in ovarian cancer, suggesting that they may be targets for further studies to verify their role in carcinogenesis and the progression of ovarian neoplasms. A better understanding of the role of TNF-α and its receptors in the tumour stroma of ovarian tumours may lead to future studies that may clarify the mechanisms of carcinogenesis and tumour progression.

Keywords: Receptors, Type I receptors, Ovarian neoplasms

INTRODUCTION

Ovarian epithelial cancer has a high lethality among gynaecological malignancies [1]. Cytoreductive surgery followed by chemotherapy is still the main treatment [2]. The American Cancer Society estimates that about 19,880 women will receive a new diagnosis of ovarian cancer, and 12,810 women will die from ovarian cancer in the United States in 2022 [3]. In Brazil, there were 4,037 deaths from ovarian cancer in 2021 [4]. Tumour stroma plays an important role in ovarian cancer [5,6]. In the peritumoural stroma of ovarian cancer, multiple cell types besides cancer cells, coordinating tumour survival, growth, invasion, and progression [6]. The tumour microenvironment has molecules that can be potential targets for new cancer therapies. In ovarian cancer, the stroma contains myofibroblasts, endothelial cells, and leukocytes, which can contribute to disease progression. A network of angiogenic factors, proteases, growth factors, immune response-modulating proteins, antiapoptotic proteins, and signaling molecules promotes tumour cell invasion and metastasis [5].

The chronic production of TNF-α in the tumour microenvironment may increase myeloid cell recruitment in Interleukin-17 (IL-17)-dependent manner. This can lead to the tumour-promoting action of this cytokine [7]. Ovarian cancer has immune-suppression capabilities, with regulatory T cells (Tregs) which may contribute to this immune suppression. Patients with ovarian cancer may have high levels of TNF and Tregs expressing TNFR2, which is associated with suppressive capacity [8]. Growing evidence suggests that TNFR2 expression in the cancer microenvironment significantly impacts cancer progression, metastasis, and immune evasion [9]. TNFR2+ Tregs were evaluated in ovarian cancer patients, with TNFR2+ Tregs from tumour-associated ascites being the most potent suppressor T cell fraction, more suppressive than peripheral blood TNFR2+ Tregs [10].

To our knowledge, there are no studies in the literature comparing stromal immunostaining of TNF-α and its receptors in the three groups: non neoplastic lesions, benign neoplasms, and malignant ovarian neoplasms. The objective of the study was to evaluate stromal immunostaining of malignant ovarian neoplasms, comparing

it with benign ovarian neoplasms and non neoplastic ovarian lesions. TNF-α is differentially regulated in ovarian cancer cells compared with untransformed cells, and present study aims to demonstrate the difference in immunostaining not only of TNF-α but also of its receptors in these three different tumour groups.

MATERIALS AND METHODS

A cross-sectional study was carried out at the Department of Gynaecology and Obstetrics, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil, from January 1997 to December 2020. Patients treated at the Pelvic Mass Outpatient Clinic of the Laboratory of Applied Sciences for Women (LaCam)/Department of Gynaecology and Obstetrics, Federal University of Triângulo Mineiro, who underwent surgical treatment according to pre-established criteria, were included [11,12]. After confirming the histopathological diagnosis, patients with benign or malignant ovarian epithelial neoplasms and non neoplastic ovarian lesions were included in the study. Informed consent was obtained from all patients included in the study. The study was approved by the UFTM Research Ethics Committee (protocol number 34770014.4.0000.5154).

Inclusion criteria: Individuals with diagnosis of primary malignant ovarian neoplasm, benign ovarian neoplasm, or non neoplastic ovarian lesion were included in the study. Borderline ovarian tumours were included in the group of malignant neoplasms.

Exclusion criteria: Torsion of the adnexal pedicle, secondary malignant ovarian neoplasm (metastasis), previous antineoplastic treatment, immunosuppressive diseases, and relapse were excluded from the study.

Data from the medical records, including age, parity, hormonal status (menarche or menopause), and for malignant tumours, histological grade and staging (FIGO), were recorded in a specific database for the study. An immunohistochemical study was performed to evaluate stromal immunostaining of TNF-α, TNFR1, and TNFR2.

Anatomopathological Study

The immunohistochemical study was conducted on paraffin sections by the surgical pathology service of UFTM, and the cases were reviewed by an observer from the Surgical Pathology Service to choose the best sections for the study. The anatomopathological evaluation and staging of cases were carried out in accordance with the criteria of the International Federation of Gynaecology and Obstetrics (FIGO) [13]. For histological grading, the recommendations of the World Health Organisation (WHO) were utilised [14].

Immunohistochemistry Study

Specimens obtained by surgical resection were processed in paraffin and reviewed by an experienced pathologist. The selected cases were submitted to new cuts (4 μm) on silanised slides (ATPS-Silane, Sigma® A3648) using the streptavidin-biotin-peroxidase technique, according to the manufacturer's recommendations. Specific primary antibodies against TNF-α, TNFR1, and TNFR2 antibodies were used in the study. Lymphoid tissue from the palatine tonsil was used as a positive control, while smooth and skeletal muscle, vessels, etc., from the palatine tonsil were used as negative controls (internal controls in areas known to be negative). Two observers evaluated the slides, and interobserver agreement was calculated using kappa statistics. Positivity was determined based on the intensity of immunostaining, categorised as follows: 0- absent immunostaining; 1- light immunostaining; 2- moderate immunostaining; and 3- intense immunostaining. Subsequently, immunostaining intensity values of 0 and 1 were considered as "weak immunostaining," while values of 2 and 3 were classified as "strong immunostaining" [15].

In the immunohistochemical study, the agreement between the two observers was performed using the kappa coefficient: $\kappa < 0.4$: weak agreement; $0.4 \leq \kappa < 0.8$: moderate agreement; $0.8 \leq \kappa < 1.0$: strong agreement; $\kappa = 1.0$: perfect agreement [16]. The kappa coefficient for present study was 0.93 (indicating strong agreement). All discordant cases were re-evaluated and the final result was determined by consensus.

STATISTICAL ANALYSIS

The data were analysed by GraphPad Prism 6 and IBM SPSS Statistics 20.0. The comparison between non neoplastic tumours, benign neoplasms, and malignant neoplasms was performed by the Fisher's exact test with a significance level <0.05.

RESULTS

The study included 95 patients divided into three groups: 37 with benign neoplasms, 43 with malignant neoplasms, and 15 with non neoplastic lesions. Comparison of malignant, benign, and non neoplastic lesion in terms of median age, parity, menarche, and menopause with minimum and maximum values are shown in [Table/Fig-1].

Regarding hormonal status, in the group of malignant ovarian neoplasms, nine (20.9%) patients were in menarche, and 34 (79.1%) patients were in menopause. In the benign neoplasms group, 21 (56.8%) patients were in menarche, and 16 (43.2%) patients were in menopause. In the non neoplastic lesions group, 11 (73.3%) patients were in menarche, and 4 (26.7%) patients were in menopause.

According to FIGO, the stages of malignant neoplasms and histological differentiation grade of malignant tumours, are mentioned in [Table/Fig-2].

Variables	Non neoplastic lesions n=15	Benign neoplasms n=37	Malignant neoplasms n=43
Median age (years); (minimum and maximum)	46 (35-82)	48 (18-69)	56 (18-81)
Median parity (births); (minimum and maximum)	2 (0-5)	2 (0-7)	2 (0-12)
Median age at menarche (years); (minimum and maximum)	13 (11-15)	13 (11-17)	13 (10-16)
Median age at menopause (years); (minimum and maximum)	47 (38-50)	49 (29-55)	49 (33-57)

[Table/Fig-1]: Characteristics of each group in relation to age, parity, age at menarche and age at menopause (median, minimum and maximum).

TNF-α stromal immunostaining was stronger (2/3) in benign ovarian neoplasms compared to non neoplastic tumours (p-value=0.0016) and in malignant neoplasms compared to non neoplastic tumours (p-value<0.0001). However, there was no significant difference when comparing benign and malignant neoplasms (p-value=0.2969) [Table/Fig-3-5a,b].

Regarding TNFR1 immunostaining, it was stronger (2/3) in the stroma of malignant neoplasms compared to benign neoplasms (p-value<0.0001) and stronger (2/3) when comparing benign neoplasms with non neoplastic ovarian lesions (p-value=0.0002). There was no difference when comparing stromal TNFR1 between ovarian cancer and non neoplastic lesions (p-value=0.231) [Table/Fig-3-5c,d].

For TNFR2, stromal immunostaining was stronger (2/3) in malignant neoplasms compared to benign neoplasms (p-value=0.0091) and stronger in malignant neoplasms compared to non neoplastic lesions (p-value=0.0004). However, there was no difference when comparing stromal immunostaining of benign neoplasms and non neoplastic lesions (p-value=0.0933) [Table/Fig-3-5e,f].

DISCUSSION

Studies suggest the role of TNF-α and its receptors (TNFR1 and TNFR2) in the biology of ovarian cancer and tumour pathogenesis [17,18], and relationship with prognostic factors [19]. TNF-α levels were measured in the serum and cytosolic fractions of ovarian

	n (%)
Histological grade	
Grade 1	14 (32.6)
Grade 2	14 (32.6)
Grade 3	15 (34.8)
Staging (FIGO)	
IA	20 (46.5)
IB	1 (2.3)
IC2	3 (7)
IC3	1 (2.3)
IIB	1 (2.3)
IIIA1(i)	2 (4.7)
IIIA2	1 (2.3)
IIIB	3 (7)
IIIC	9 (20.9)
IVB	2 (4.7)

[Table/Fig-2]: Characteristics of the group of patients with ovarian cancer regarding histological grade and staging (n and percentage).

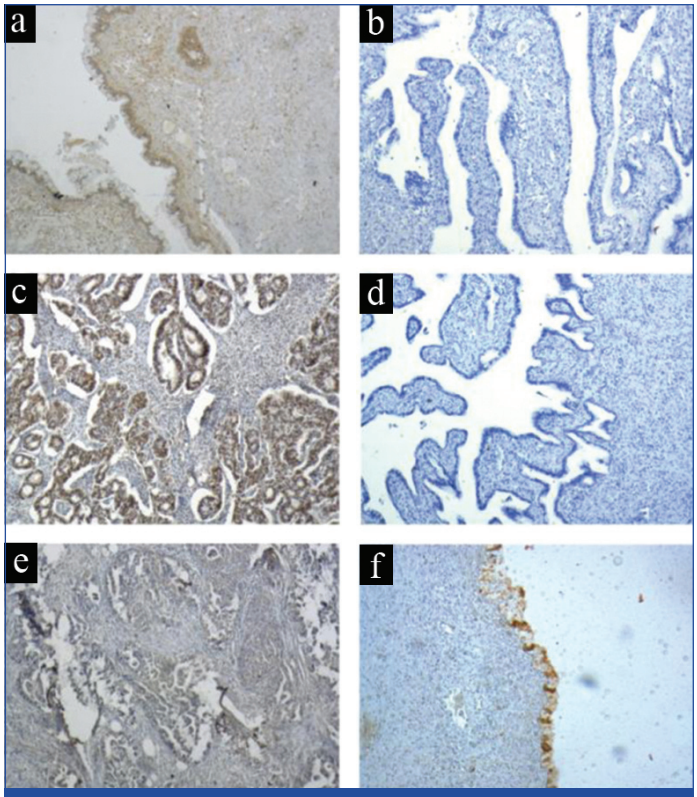
	Neoplasms	Total samples (n)	Weak immunostaining (0/1)	Strong immunostaining (2/3)
TNF-α	Benign	37	11	26
	Malignant	43	8	35
	Non-neoplastic lesions	15	12	3
TNFR1	Benign	37	37	0
	Malignant	43	17	26
	Non-neoplastic lesions	15	9	6
TNFR2	Benign	37	30	7
	Malignant	43	22	21
	Non-neoplastic lesions	15	15	0

[Table/Fig-3]: Distribution of samples in groups based on staining of TNF-α, TNFR1, TNFR2.

	Neoplasms	0/1	2/3	p-value*
TNF-α	Benign	11/37 (29.7%)	26/37 (70.3%)	0.2969
	Malignant	8/43 (18.6%)	35/43 (81.4%)	
TNFR1	Benign	37/37 (100.0%)	0/37 (0%)	<0.0001
	Malignant	17/43 (39.5%)	26/43 (60.5%)	
TNFR2	Benign	30/37 (81.1%)	7/37 (18.9%)	0.0091
	Malignant	22/43 (51.2%)	21/43 (48.8%)	
TNF-α	Benign	11/37 (29.7%)	26/37 (70.3%)	0.0016
	Non neoplastic lesions	12/15 (80.0%)	3/15 (20.0%)	
TNFR1	Benign	37/37 (100.0%)	0/37 (0%)	0.0002
	Non neoplastic lesions	9/15 (60.0%)	6/15 (40.0%)	
TNFR2	Benign	30/37 (81.1%)	7/37 (18.9%)	0.0933
	Non neoplastic lesions	15/15 (100.0%)	0/15 (0%)	
TNF-α	Malignant	8/43 (18.6%)	35/43 (81.4%)	<0.0001
	Non neoplastic lesions	12/15 (80.0%)	3/15 (20%)	
TNFR1	Malignant	17/43 (39.5%)	26/43 (60.5%)	0.231
	Non neoplastic lesions	9/15 (60.0%)	6/15 (40.0%)	
TNFR2	Malignant	22/43 (51.2%)	21/43 (48.8%)	0.0004
	Non neoplastic lesions	15/15 (100.0%)	0/15 (0%)	

[Table/Fig-4]: Differences in stromal immunostaining of TNF-α, TNFR1 and TNFR2 between malignant, benign and non-neoplastic ovarian tumours.
*Fisher's exact test, with a significance level of p<0.05

cancer patients and control patients, demonstrating increased TNF-α levels in the cancer patient group. TNF-α immunostaining



[Table/Fig-5]: Immunohistochemical staining. Histological sections of ovarian lesions. a) Stromal immunostaining (2/3) of TNF-α in mucinous adenocarcinoma (100x). b) Stromal immunostaining (0/1) of TNF-α in non neoplastic tumour (100x). c) Stromal immunostaining (2/3) of TNFR1 in adenocarcinoma (100x). d) Stromal immunostaining (0/1) of TNFR1 in serous cystadenoma (100x). e) Stromal immunostaining (2/3) of TNFR2 in serous adenocarcinoma (100x). f) Stromal immunostaining (0/1) of TNFR2 in mucinous cystadenoma (100x).

was positive in malignant lesions and negative for normal ovarian tissue [17]. Present study demonstrated similar results, but compared ovarian neoplasms (benign and malignant) with non neoplastic ovarian lesions rather than with normal tissue. It found that stromal TNF-α immunostaining was stronger in benign and malignant ovarian neoplasms compared to non neoplastic tumours. Studies also show an association of tissue TNF-α with prognostic factors [20] and histological type [21]. One study evaluated the expression of IL-1, IL-6, TGF-β, TNF-α, COX-2, iNOS, and NF-kB in serous and mucinous ovarian cancers using immunohistochemistry and showed that the expression of IL-1, TNF-α, and COX-2 increased with the stage of the disease in serous and mucinous tumours [20]. Expression of TNF-α was assessed in epithelial ovarian carcinomas by ribonucleic acid in situ hybridisation, with expression detected in 46% of tumours. Expression was most common in high-grade serous carcinomas, followed by endometrioid carcinomas [21].

TNF-α plays a role not only in tissue but also in peripheral blood in patients with ovarian cancer [22,23]. Serum levels of IL-6, IL-8, and TNF-α were assessed by ELISA, revealing higher levels of serum IL-8 and TNF-α in patients with ovarian cancer compared to those with benign ovarian cystic lesions. The cut-off levels for IL-8 and TNF-α were 4.09 ng/mL and 2.63 ng/mL, respectively, with sensitivities and specificities of 70% and 96% for IL-8 and 85.7% and 79.3% for TNF-α [22]. Another study evaluated the involvement of T-helper cells and regulatory T cells in epithelial ovarian cancer, examining the percentages of Th22, Th17, Th1, and regulatory T cells in the peripheral blood of patients with epithelial ovarian cancer, benign ovarian epithelial neoplasms, and healthy controls by flow cytometry. The plasma concentrations of IL-22 and TNF-α were significantly elevated in patients with epithelial ovarian cancer compared to the other two groups. Additionally, in ovarian cancer patients, there was an increased trend of Th22, IL-22, and TNF-α in stage III-IV patients compared to stage I-II patients, and a positive correlation between Th22, Th17, and Th1 cells [23].

In recent years, natural killer cell-based immunotherapy for ovarian cancer has shown remarkable potential. Natural killer cells induce antibody-dependent cellular cytotoxicity, cell-piercing release, and granule enzyme release. They also secrete IFN-γ and TNF-α or participate in the Fas/FasL and TRAIL/TRAILR pathways, mediating ovarian cancer cell death [24]. In present study, TNF-α stromal immunostaining was stronger when comparing benign and malignant ovarian neoplasms with non neoplastic tumours. On the other hand, there was no significant difference when comparing benign and malignant neoplasms. The literature also highlights the significance of TNF-α receptors in ovarian tumours [18,25]. Piura B et al., extracted total RNA from normal and malignant ovarian tissues, and mRNA was analysed using semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). An immunohistochemical study was performed for TNFR1 and TNFR2. TNF-α mRNA and TNFR2 mRNA levels were higher in carcinomas compared to normal ovarian tissues, while TNFR1 mRNA levels were similar. TNFR1 and TNFR2 were mainly localised in the epithelial neoplastic cells of the tumour [18].

Regarding TNFR1, a study showed that TNFR1 was overexpressed in ovarian cancer, playing an important role as a prognostic molecule in ovarian malignant tumours [26]. TNFR1 signaling was manipulated in different leukocyte populations, or TNF-α was neutralised by antibody treatment using a mouse model of ovarian cancer. This cytokine maintained TNFR1-dependent IL-17 production by CD4+ cells, leading to the recruitment of myeloid cells into the tumour microenvironment and increasing tumour growth. Furthermore, in patients with advanced cancer treated with infliximab (a specific antibody for TNF-α), there was a reduction in serum levels of IL-17. Thus, the production of TNF-α in the tumour microenvironment increases IL-17-dependent recruitment of myeloid cells [7]. In present study, TNFR1 immunostaining was stronger in the stroma of malignant neoplasms compared to benign neoplasms, and stronger when comparing benign neoplasms with non neoplastic ovarian lesions. There was no difference in stromal TNFR1 immunostaining between ovarian cancer and non neoplastic lesions. Tissue TNFR2 plays crucial roles in ovarian tumours. When studying tissue TNFR2, the proportion of samples positive for TNF-α and TNF-R2, studied by Western blotting and immunohistochemistry, was higher in patients with epithelial ovarian cancer than in benign ovarian lesions [27]. These findings align with those of present study. Present data demonstrated that TNFR2 stromal immunostaining was stronger in malignant neoplasms compared to benign neoplasms, and stronger in malignant neoplasms compared to non neoplastic lesions. There was no difference in stromal immunostaining of benign neoplasms and non neoplastic lesions.

TNFR2 plays an important role not only in tissue but also in peripheral blood. Serum TNFR2 levels may be associated with prognostic factors in ovarian cancer [28]. On the other hand, a meta-analysis was performed evaluate the associations between circulating levels of C-reactive protein, IL-6, TNF-α, and soluble TNFR2, and the risk of ovarian cancer. The analysis demonstrated that elevated levels of C-reactive protein, but not circulating IL-6, TNF-α, or soluble TNFR2, are associated with an increased risk of ovarian cancer [29]. TNFR2 is expressed by many immunosuppressive cells during cancer development, leading to immune escape from cancer. Its circulating form may also be associated with the development of cancer. A systematic meta-analysis of cancer studies showed circulating concentrations of soluble TNFR2 (sTNFR2) in cancer patients were recorded and its asso++ciation with cancer risk, showing a consistently significant increase in sTNFR2 levels in several cancer types compared to healthy controls. The malignant tumour types included colorectal cancer, ovarian cancer, breast cancer, non Hodgkin's lymphoma, Hodgkin's lymphoma, lung cancer, hepatocellular carcinoma, and glioblastoma. The study showed an association between circulating TNFR2 levels and the risk of developing cancer at 1.76 (95% CI: 1.53-2.02) [30]. The combination of TNFR2+ Tregs and IL-6 in the pretreatment blood of patients with advanced high-grade serous ovarian cancer masses was effective in differentiating benign or malignant ovarian masses [31].

Tissue TNFR2 is also associated with prognosis and survival. In a study evaluating patients with high-grade serous ovarian cancer who underwent primary surgical cytoreduction followed by platinum-based chemotherapy, the protein expression of TNFR2 and STAT3 was investigated using immunohistochemistry. Strong expression of TNFR2 and STAT3 in ovarian tissue was associated with a significantly longer progression-free survival interval in the platinum-sensitive group in relation to the platinum-resistant group [32]. Ascites is another environment in which TNFR2 plays a role in ovarian cancer. In the evaluation of ascites in patients with ovarian cancer, high levels of immunosuppressive (sTNFR2, IL-10, and TGF-β) and proinflammatory cytokines (IL-6 and TNF) were found in this fluid. TNFR2 expression on all T cell subsets was higher on CD4+CD25hiFoxP3+ Tregs [8].

The literature also demonstrates the potential value of TNFR2 as a possible target for cancer treatment. A novel nanomedicine-based therapeutic strategy may have the ability to target TNFR2 while preventing DNA demethylation, maximising the anticancer potential of nanomedicine-based immunotherapy, and improving treatment outcomes for cancer patients [9]. One study evaluated the effect of genetic ablation of TNFR2 on the in-vitro and in-vivo growth of mouse MC38 and CT26 colon cancer cells. TNFR2 deficiency impairs in-vitro deficiency and colony formation of cancer cells, which is associated with the inhibition of protein kinase B (AKT) phosphorylation and increased cell death caused by autophagy [33].

Present study results demonstrated stronger TNFR2 immunostaining in ovarian cancer compared to both benign neoplasms and non neoplastic lesions. When combined with the evidence described above that the expression of TNFR2 in the tumour microenvironment has important implications for cancer progression, this may be the target of future studies to support the development of TNFR2 antagonist agents in cancer treatment.

Limitation(s)

The main limitation of the study was the heterogeneity of the histological types of ovarian lesions and neoplasms.

CONCLUSION(S)

Stronger immunostaining for TNF-α and its receptors was found in ovarian cancer, suggesting that they may be targets for further studies to verify their role in carcinogenesis and the progression of ovarian neoplasms. A better understanding of the role of TNF-α and its receptors in the tumour stroma of ovarian tumours may lead to future studies that may clarify the mechanisms of carcinogenesis and tumour progression.

Funding: The authors wish to acknowledge the funding received from CNPq (National Council for Scientific and Technological Development- Brazil, process No.: 01115/2018-4 and research grant for Rosekeila Simões Nomelini process No.: 303086/2022-0), FAPEMIG (Minas Gerais Research Funding Foundation, process No.: APQ-00888-21 and RED-00011-14), FUNEPU (Uberaba's Teaching and Research Foundation, process No.: 02 2011), and CAPES (Brazilian Federal Agency fot the Support and Evaluation of Graduate Education, process No.: 32012012001P5).

REFERENCES

[1] Henley SJ, Thomas CC, Lewis DR, Ward EM, Islami F, Wu M, et al. Annual report to the nation on the status of cancer, part II: Progress toward Healthy People 2020 objectives for 4 common cancers. Cancer. 2020;126(10):2250-66.

[2] Randall TC, Rubin SC. Cytoreductive surgery for ovarian cancer. Surg Clin North Am. 2001;81(4):871-83.
[3] American Cancer Society. Cancer facts & figures 2022. [Internet]. [cited 2022 Jul 22] Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2022.html.
[4] INCA. Câncer de ovário. 2022. [Internet]. [cited 2024 Feb 13] Available from: https://www.gov.br/inca/pt-br/assuntos/cancer/tipos/ovario.
[5] Davidson B, Trope CG, Reich R. The role of the tumor stroma in ovarian cancer. Front Oncol. 2014;4:104.
[6] Silva AC, Jammal MP, Crispim PCA, Murta EFC, Nomelini RS. The role of stroma in ovarian cancer. Immunol Invest. 2020;49(4):406-24.
[7] Charles KA, Kulbe H, Soper R, Escorcio-Correia M, Lawrence T, Schultheis A, et al. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest. 2009;119(10):3011-23.
[8] Kampan NC, Madondo MT, McNally OM, Stephens AN, Quinn MA, Plebanski M. Interleukin 6 present in inflammatory ascites from advanced epithelial ovarian cancer patients promotes tumor necrosis factor receptor 2-expressing regulatory T cells. Front Immunol. 2017;8:1482.
[9] Al-Hatamleh MAI, E A R ENS, Boer JC, Ferji K, Six JL, Chen X, et al. Synergistic effects of nanomedicine targeting TNFR2 and DNA demethylation inhibitor-an opportunity for cancer treatment. Cells. 2019;9(1):33.
[10] Govindaraj C, Scalzo-Inguanti K, Madondo M, Hallo J, Flanagan K, Quinn M, et al. Impaired Th1 immunity in ovarian cancer patients is mediated by TNFR2+ Tregs within the tumor microenvironment. Clin Immunol. 2013;149(1):97-110.
[11] Murta EFC, da Silva CS, Gomes RA, Tavares-Murta BM, Melo AL. Ultrasonographic criteria and tumor marker assay are good procedures for the diagnosis of ovarian neoplasia in preselected outpatients. Eur J Gynaecol Oncol. 2004;25(6):707-12.
[12] Murta EFC, Nomelini RS. Early diagnosis and predictors of malignancy of adnexal masses. Curr Opin Obstet Gynaecol. 2006;18(1):14-19.
[13] Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynaecol Obstet. 2014;290(5):839-42.
[14] WHO Classification of Tumours. Female Genital Tumours. 5th Edition, Volume 4, 2020. Available from: https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Female-Genital-Tumours-2020.
[15] Georgiannos SN, Renaut A, Goode AW, Sheaff M. The immunophenotype and activation status of the lymphocytic infiltrate in human breast cancers, the role of the major histocompatibility complex in cell-mediated immune mechanisms, and their association with prognostic indicators. Surgery. 2003;134(5):827-34.
[16] Arango, HG. Bioestatística teórica e computacional. (Biostatistics-Theoretical and Computational) Rio de Janeiro: Editora Guanabara Koogan, 2001; p. 93-113. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/sus-23066?lang=en.
[17] Hassan MI, Kassim SK, Saeda L, Laban M, Khalifa A. Ovarian cancer-induced immunosuppression: relationship to tumor necrosis factor-alpha (TNF-alpha) release from ovarian tissue. Anticancer Res. 1999;19(6C):5657-62.
[18] Piura B, Medina L, Rabinovich A, Dyomin V, Levy RS, Huleihel M. Distinct expression and localization of TNF system in ovarian carcinoma tissues: possible involvement of TNF-α in morphological changes of ovarian cancerous cells. Anticancer Res. 2014;34(2):745-52.
[19] Martins Filho A, Jammal MP, Côbo EC, Silveira TP, Adad SJ, Murta EF, et al. Correlation of cytokines and inducible nitric oxide synthase expression with prognostic factors in ovarian cancer. Immunol Lett. 2014;158(1-2):195-99.
[20] Plewka D, Kowalczyk AE, Jakubiec-Bartnik B, Morek M, Bogunia E, Kmiec A, et al. Immunohistochemical visualization of pro-inflammatory cytokines and enzymes in ovarian tumors. Folia Histochem Cytobiol. 2014;52(2):124-37.
[21] Gupta M, Babic A, Beck AH, Terry K. TNF-α expression, risk factors, and inflammatory exposures in ovarian cancer: Evidence for an inflammatory pathway of ovarian carcinogenesis? Hum Pathol. 2016;54:82-91.
[22] Pawlik W, Pawlik J, Kozłowski M, Łuczkowska K, Kwiatkowski S, Kwiatkowska E, et al. The clinical importance of IL-6, IL-8, and TNF-α in patients with ovarian carcinoma and benign cystic lesions. Diagnostics (Basel). 2021;11(9):1625.
[23] Wang T, Zhang Z, Xing H, Wang L, Zhang G, Yu N, et al. Elevated Th22 cells and related cytokines in patients with epithelial ovarian cancer. Medicine (Baltimore). 2017;96(43):e8359.
[24] Fan Z, Han D, Fan X, Zhao L. Ovarian cancer treatment and natural killer cell-based immunotherapy. Front Immunol. 2023;14:1308143.
[25] Miura R, Yokoyama Y, Shigeto T, Futagami M, Mizunuma H. Inhibitory effect of carbonyl reductase 1 on ovarian cancer growth via tumor necrosis factor receptor signaling. Int J Oncol. 2015;47(6):2173-80.
[26] Peng C, Su J, Zeng W, Zhang X, Chen X. TNFR1 regulates ovarian cancer cell tumorigenicity through PIK3CB-p110beta. Curr Mol Med. 2015;15(5):487-96.
[27] Dobrzycka B, Terlikowski SJ, Garbowicz M, Niklińska W, Bernaczyk PS, Nikliński J, et al. Tumor necrosis factor-alpha and its receptors in epithelial ovarian cancer. Folia Histochem Cytobiol. 2009;47(4):609-13.
[28] Nomelini RS, Borges Júnior LE, de Lima CA, Chiovato AFC, Micheli DC, Tavares-Murta BM, et al. TNF-R2 in tumor microenvironment as prognostic factor in epithelial ovarian cancer. Clin Exp Med. 2018;18(4):547-54.
[29] Zeng F, Wei H, Yeoh E, Zhang Z, Ren ZF, Colditz GA, et al. Inflammatory markers of CRP, IL6, TNFα, and soluble TNFR2 and the risk of ovarian cancer: A meta-analysis of prospective studies. Cancer Epidemiol Biomarkers Prev. 2016;25(8):1231-39.
[30] Kartikasari AER, Cassar E, Razqan MAM, Szydzik C, Huertas CS, Mitchell A, et al. Elevation of circulating TNF receptor 2 in cancer: A systematic meta-analysis for its potential as a diagnostic cancer biomarker. Front Immunol. 2022;13:918254.
[31] Kampan NC, Kartikasari AER, Deceneux C, Madondo MT, McNally OM, Flanagan KL, et al. Combining TNFR2-expressing tregs and IL-6 as superior diagnostic biomarkers for high-grade serous ovarian cancer masses. Cancers (Basel). 2023;15(3):667.
[32] Silva Raju J, Abd Aziz NH, Atallah GA, Teik CK, Shafiee MN, Mohd Saleh MF, et al. Prognostic value of TNFR2 and STAT3 among high-grade serous ovarian cancer survivors according to platinum sensitivity. Diagnostics (Basel). 2021;11(3):526.
[33] Li P, Yang Y, Yang X, Wang Y, Chou CK, Jiang M, et al. TNFR2 deficiency impairs the growth of mouse colon cancer. Int J Biol Sci. 2023;19(4):1024-35.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 15, 2024
- Manual Googling: Mar 13, 2024
- iThenticate Software: May 03, 2024 (20%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: As declared above.
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Feb 14, 2024**
Date of Peer Review: **Mar 15, 2024**
Date of Acceptance: **May 04, 2024**
Date of Publishing: **Jul 01, 2024**