Expression of Transforming Growth Factor Beta-1 and Its Correlation with Clinicopathologic Features of Ovarian Epithelial Carcinoma

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***Abstract*- Background:** Ovarian carcinoma is a cancer with high mortality in women and despite comprehensive management with surgery and chemotherapy at an advanced stage, the survival rate is still low. TGFβ-1 contributes to the progression of malignancy and its expression is a prognostic predictor and a potential therapeutic target in several malignancies, but the results are mixed in ovarian carcinoma. **Objective:** To assess the relationship between TGFβ-1 immunohistochemical expression and various clinicopathological parameters of ovarian carcinoma.
Materials and Methods: Formalin-fixed tissue paraffin blocks from 30 patients with ovarian carcinoma were used as samples and TGFβ-1 immunohistochemical staining was assessed. All clinicopathological characteristics were obtained through medical records or pathology files. The relationship between TGFβ-1 expression and clinicopathology was analyzed using statistical tests using the SPSS program.
Results: TGFβ-1 was expressed in 90% of ovarian carcinoma specimens. Positive expression of TGFβ-1 was mostly found in advanced ovarian carcinoma, older age, histopathological types of serous carcinoma and mucinous carcinoma, and low levels of stromal and intratumor TILs.
Conclusion: There is no significant relationship between positive and negative TGFβ-1 expression with the clinicopathological parameters of ovarian carcinoma. The evaluation of the components of TILs and their relation to TGFβ-1 should be investigated further to open up the possibility of other targeted therapies or combinations.

***Index Terms*-** ovarian carcinoma, TGFβ-1, immunohistochemistry, pleiotrophic cytokin

I. Introduction

Ovarian cancer is one of the three most common gynecologic malignancies and has a high mortality rate, with 22,240 new cases and around 140,700 deaths annually worldwide.[1] Based on GLOBOCAN data in 2018, ovarian cancer ranks 8th in diagnosis and death. The most common cancers in women were 295,414 new cases and 184,799 deaths worldwide.[2] In Indonesia, the incidence and mortality of ovarian cancer is in the 10 most common cancers, and is the third most common cancer in women with 13,310 new cases and 7,842 cases of deaths in 2018.[2,3] There is geographic variation in the incidence of ovarian cancer, with an increasing incidence in North America, Central-Eastern Europe, and Southeast Asia.[4] More than 70% of women with ovarian carcinoma were diagnosed at an advanced stage (stage III or IV) based on FIGO stage due to the lack of effective screening strategies at an early stage and early symptoms of non-specific carcinoma. Although comprehensive treatment with debulking surgery with postoperative chemotherapy is performed, the 5-year survival rate is only around 30%. Many studies are aimed to find biomarkers as prognostic and potential targets of therapy with an individual approach based on the genetic profile and molecular characteristics of the patient that can improve patient outcomes.[5]

Transforming growth factor-beta (TGF-β) is a growth factor that regulates cell proliferation, apoptosis, migration, and differentiation in various cell types. TGF-β has 3 known isomers, namely TGF-β1, 2, and 3 which belongs to the same class as 30 other proteins.[5,6] TGF-β is known as a pleiotrophic cytokine with complex functions, where in the early stages of tumorigenesis it acts as a tumor suppressor by inducing cytostasis and apoptosis of normal cells and pre-malignant cells, while at an advanced stage, where cancer cells have oncogenic mutations and/or have lost suppressor gene function, cells are resistant to TGF- induction and change function as tumor promoters by stimulating tumor cells to carry out EMT mechanisms that will cause metastasis, a more aggressive and chemoresistant phenotype. Furthermore, TGF-β plays a role in cancer growth and progression by activating angiogenesis and cancer-associating fibroblasts (CAF) and allowing tumors to evade immune response inhibition.[7] Research on TGF-β immunohistochemical expression in ovarian carcinoma and its relationship with TILs and other clinicopathological parameters and as a prognostic marker in ovarian carcinoma is still limited. This makes researchers interested in assessing the relationship.

II. MATERIAL AND METHODS

This study is an analytical study with a cross-sectional which was conducted in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and General Hospital Haji Adam Malik Medan. This study was done from November 2020 until June 2021, after receiving approval from Health Research Ethics Committee Universitas Sumatera Utara.

Thirty samples in this study were paraffin blocks and slides from postoperative tissue which diagnosed histopathologically as ovarian carcinoma. The clinicopathologic records of samples including age, FIGO stage, pathological type, and tumor infiltrating lymphocytes (TILs) level either intratumoral and stromal were collected and assessed. The exclusion criteria were damaged paraffin blocks that could not be processed for immunohistochemical staining. Each slide was stained with hematoxylin-eosin and immunohistochemical staining of anti-*TGF-β1* rabbit polyclonal antibody (BioEnzy, 1:300).

Intratumoral TILs are defined as the accumulation of mononuclear immune cells that infiltrate tumor nests. The level of intratumoral TILs was assessed in terms of the percentage of tumor nesting areas infiltrated by mononuclear cells to the total area of tumor cells, and in this study were categorized into: low TILs: <10% TILs, high TILs if found ≥ 10% TILs. Stromal TILs are mononuclear immune cells located in the stromal tissue, between the nests of cancer cells. TILs' level was assessed based on the percentage of stromal area infiltrated by mononuclear cells to the total area of the stroma. The stroma in contact with the fibrovascular cores of the papillary structures was also assessed. The level of stromal TILs in this study was categorized into: low TILs if <50% stromal TILs, high TILs if ≥ 50% stromal TILs found.

Immunohistochemical expression of TGFβ-1 was identified by brown granules in the cytoplasm and tumor cell membrane. Immunoreactivity was assessed semi-quantitatively based on the result of multiplying the area of the tumor that was stained positively with the intensity of staining, which in this study were categorized as follows: The area score is assessed by selecting 5 fields of view with 400x magnification, and taking the average of the percentage of the area of the stained cells, and grouped into:
0: less than 5% staining cells; 1: 5-25% staining cells; 2: 26-50% staining cells; 3: 51-75% of staining cells; 4: > 75% of staining cells. Staining intension was graded as follows: 0 score: no pigment (negative); 1: weak (light yellow); 2: medium (brown/buffy); 3: strong (brown). Both scores were calculated and the interpretation was categorized as follows: negative expression (score 0-4) and positive expression (score 5-12). The evaluation of results was performed by 2 pathologists who were blinded to the specimens' information and labeled antibody.

Data analysis was performed by using SPSS 22 software. Expressions of TGFβ-1 among different clinicopathologic features of ovarian carcinoma samples were compared by chi-square test or Fisher exact test. Results were considered statiscally significant at p<0,05.

III. RESULTS

TGFβ-1 was expressed in the cell membrane and cytoplasm. Immunohistochemical staining of TGFβ-1 revealed that positive expression was found in 27 of 30 ovarian carcinoma samples (90%) whereas 3 samples (10%) showed the negative expression in 3 samples (10%). The positive expression rates of TGFβ-1 were higher in older patients (≥ 50 years old), that is 51,9%. TGFβ-1 was detected in 85% (17 samples) of advanced ovarian carcinoma stage (III-IV FIGO stage). The positive expression rates of TGFβ-1 in serous carcinoma were highest (37,1%) among other pathological types. Both intratumoral and stromal TILs levels were lower in positive TGFβ-1 expression samples. Negative expression rates of TGFβ-1 were all found in advanced stage ovarian carcinoma (100%). Further analysis found that TGFβ-1 expression was not significantly correlated with age, FIGO stage, pathological type, and TILs (stromal and intratumoral) levels (table 1).

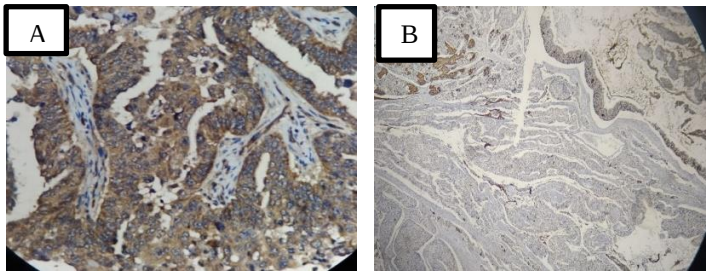

Fig. 1 TGFβ-1 immunohistochemical expression. A. Positve expression. B. Negative expression

Table 1. Association TGFβ-1 expression and clinicopathological features

Features	TGF-β1 Expression				p value
	Positive (n= 27)		Negative (n=3)		
	n	%	n	%	
Age					
< 50 years old	13	48,1	1	33,3	1,00
≥ 50 years old	14	51,9	2	66,7	
FIGO stage					
Stage I-II	10	37,0	0	0	0,532
Stage III-IV	17	85,0	3	100	
Pathological type					
Serous carcinoma	10	37,1	1	33,3	
Mucinous carcinoma	7	25,9	1	33,3	
Endometrioid	6	22,2	1	33,4	0,884
carcinoma	4	14,8	0	0	
Clear cell carcinoma					
TILs intratumoral					
Low	19	70,4	1	33,3	0,251
High	8	29,6	2	66,7	
TILs stroma					
Low	15	55,6	1	33,3	0,586
High	12	44,4	2	66,7	

IV. DISCUSSION

TGF-β1 is known as a multifunctional growth factor which is known to have a regulatory effect on the signaling development process and shows a dichotomous behavior related to ovarian cancer, namely as a tumor suppressor and tumor promoter. Hurteau, et al. found the presence of TGF-1 which was found in most ovarian tissues, both benign and malignant, and several other studies showed that TGF-1 was not present in all ovarian carcinomas.[7,8] The same thing was found in the study of Yan, et al. where negative expression of TGF-β1 was found in 29.7% of cases of ovarian carcinoma, and also in this study, 10% of samples showed negative TGF-β1 expression. Meanwhile, 90% of ovarian carcinoma samples in this study showed positive

immunohistochemical expression of TGF-β1 on cell membranes and cancer cells. This percentage is higher than the research conducted by Yan, et al. which is 70.3% and Wang et al., which is 78.3%.[9-11]

The TGF-β pathway exhibits pro-tumoral effects by mediating tumor-stromal interactions and remodeling of the tumor microenvironment. Stromal components consisting of extracellular matrix proteins and various cell types (mesenchyme/CAF, pericyte, endothelial, immune cells, bone marrow-derived stem cells) display TGF- receptors, and the TGF-β pathway can influence fibrosis, angiogenesis and immune cell infiltration. Activation of the TGF- pathway contributes to shaping (from a non-tumor environment) and maintaining a tumor microenvironment that is favorable for tumors.[12] Katsuno et al. in his study stated that the high value of positive expression of TGF-β1 indicates that TGF-β1 plays a role in inducing the occurrence of EMT (epithelial mesenchymal transition) which affects gene transcription and causes the accumulation of extracellular matrix proteins through activation of the TGF-β-Smad signaling pathway, which causes the occurrence of TGF--Smad signaling pathway.[13] Mutation in Smad signaling components may cause dysregulation effect of signaling pathway especially in malignancy. TGF-β process can also induce signals outside of Smad, namely by supporting cytoskeletal remodeling, leading to activation of ERK. In interaction with SHC or GRB2, ERK is required to form the SHC-GRB2-ERK complex, which is a key component of TGF-β in inducing tumor invasion and metastasis.[7]

From this study, 51.9% of samples aged 50 years showed positive TGF-β1 expression, and this percentage was higher than the age group < 50 years, which was 48.1%. This is in line with the research of Yan et al. which also showed positive TGF-β1 expression which was more common in older people (60 years) by 57.8%. Based on the results of statistical tests to assess the relationship of TGF-β1 expression with age, there was no significant relationship ($p>0.05$) between age and TGF-β1 expression.

Positive immunohistochemical expression of TGF-β1 was found in the most advanced stages (stages III and IV), which was 63% compared to the early stages (stages I and II), which was 37%, which could indicate that TGF-β1 expression tends to increase in ovarian carcinoma that has metastasized or has expanded invasion. TGF-β1 is one of the cytokines that play a role in the development and progression of ovarian carcinoma, where cytokine dysregulation is thought to cause this to occur. Cytokines act on tumor cells through autocrine and paracrine mechanisms through their interaction with other signaling cascades, such as VEGF, PDGF, Notch, angiopetin which plays a role in angiogenesis and nutrient availability of tumor cells and induces immune suppression.[12]

Specifically, TGF-β1 plays a role as a contributor to ovarian tumor expansion and metastasis. The binding of TGF- and its receptors indirectly affects the cell cycle through the Smad pathway by stopping tumor cells in G1, initiating apoptosis, and inhibiting cell proliferation. Disruption of components of the TGF- β1 signaling pathway will affect signal transduction and dysfunction of pathways associated with tumor cell infiltration and metastasis. Hempel et al. reported that the breakdown of TGF-signal transduction will increase the infiltration and motility of ovarian carcinoma cells.[9] Rodriguez et al. stated that TGF-β increased the infiltration capability of cell lines in most ovarian carcinomas 2-20 times, but had no effect or had an inhibitory effect on the infiltration capability of normal ovarian cells.[14] Hirashima et al. also reported that TGF-β1 produced by ovarian carcinoma cells causes tumor infiltration through an upregulation of plasminogen activator inhibitor-1 (PAI-1) in peritoneal mesothelial cells. Co-occurring will cause tumor growth and progression, and facilitate the growth, relapse, and metastasis of ovarian carcinoma, especially in advanced stages.[9]

The results of statistical tests showed that there was no significant relationship between TGF-β1 expression and the FIGO stage in this study. This is in line with Liu et al. which stated that there was no difference in clinicopathological factors of age, clinical stage, histological grade between weak TGF-β expression and strong expression which implied that TGF-β could be an independent prognostic factor in ovarian carcinoma.[15] However, this is not in line with the research of Yan et al. al. which shows that overexpression of TGF-1 is associated with higher clinical stages, where TGF-β1 plays an important role in the progression of ovarian carcinoma and is a prognostic factor in ovarian carcinoma.[10] In advanced ovarian carcinoma, the inhibitory effect of TGF -β1 decreases and will increase the malignant behavior of tumor cells. TGF-β1 can inhibit proliferation and abolish the destructive activity of various cell types involved in cellular immunity, including cytotoxic T lymphocytes, natural killer, and lymphokine-activated killer cells.[9]

In various histopathological types of ovarian carcinoma, positive immunohistochemical expression of TGF-β1 in this study was found to be the most in serous carcinoma type, which was 37.1% compared to other types, such as mucinous carcinoma (25.9%), endometrioid carcinoma (22.2%) and clear cell carcinoma where all samples showed positive TGF-β1 expression. This is not in line with Wang et al. which shows that the mucinous type has more positive TGF-β1 expression, which is 53.2% compared to the serous type, which is 46.8%. There were 3 samples of negative TGF-β1 expression found in high grade serous carcinoma, endometrioid carcinoma and mucinous carcinoma with the same percentage of 33.3% respectively. There was no significant relationship ($p>0.05$) between the immunohistochemical expression of TGF-β1 and histopathological type. This is also in line with Wang et al. who also reported that TGF-β1 intensity expression in ovarian carcinoma was not associated with histologic type.[9]

TILs reflect a local immune response (antitumor immune response) that plays a role in regulating mechanisms of tumor growth and progression but can also help to create an immunosuppressive environment in the area where tumors develop.[16] Assessment of the immune response to tumors plays an important role in the implications of prognosis and immunotherapy in various types of tumors. Several studies regarding the prognosis of ovarian carcinoma associated with TILs are still controversial. Research by Zhang et al. showed that intratumoral TILs were an independent prognostic factor in ovarian carcinoma. Stumpf et al., and several other studies reported that high levels of intratumoral TILs were associated with good survival. James et al. in his study reported that there was an association between intratumoral TILs and stromal TILs in primary and secondary tumors, while in another study it was

reported that there was no relationship between TILs and ovarian carcinoma prognosis.[16-18]

Positive TGF-β1 expression was found to be more common at low levels of stromal and intratumoral TILs, 55.6% and 70.4%, respectively, while negative TGF-β1 expression was mostly found at high levels of TILs stroma and intratumoral (66.7%). This shows the degree of TILs tends to be inversely related to TGF-β1 expression, although after statistical tests were carried out to assess the relationship between the degree of stromal TILs and intratumoral TILs with TGF-β1 expression, $p>0.05$ was obtained, which means no significant relationship was found. Researchers cannot compare the results of this study with other studies, because to the author's knowledge no one has investigated this in ovarian carcinoma. In this study, high levels of stromal and intratumoral TILs were found in stage IIIC HGSC and stage IIIB mucinous carcinoma samples. This may indicate a tendency for negative TGF-β1 expression accompanied by high levels of TILs to be found in advanced tumors that have undergone invasion and/or metastasis, with a poor prognosis. However, further research related to this needs to be done.

In malignancies, including ovarian cancer, TGF-β is a strong immunosuppressor in the tumor microenvironment, influencing natural killer cells, dendritic cell activity, cytokine production, and T cell function. regulatory T cells (Tregs). Increased secretion of TGF-β in the tumor microenvironment recruits Tregs via FoxP3 display, which leads to a decrease in cytotoxic T cells. Tregs are elevated in patients with carcinoma and are associated with a poor prognosis. TGF-β1 in the tumor microenvironment can lead to the formation of Tregs from CD4+, CD25+ cells. The ability of tumors to increase TGF- levels and enhance TGF-β signaling facilitates tumor growth and dissemination by weakening cytotoxic immune defense. [10,15,19] TGF-β1 is strongly expressed in tumor tissue and functions as an immunosuppressive factor, forming a barrier around tumor tissue. which protects tumor cells from host immunosurveillance and this effect causes tumor cell infiltration and metastasis.[9]

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