Research Article

The Serum Level of Vascular Endothelial Growth Factor (VEGF) is Declined after Paclitaxel-Carboplatin Combined Chemotherapy Treatment on Epithelial Ovarian Cancer

Kadar Serum VEGF Menurun setelah Terapi Kemoterapi Kombinasi Paclitaxel-Carboplatin pada Kanker Ovarium Epitelial

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Abstract

Objective: To assess the effect of Paclitaxel-Carboplatin combination on epithelial ovarian cancer by studying the changes in VEGF serum levels after receiving 3 series of chemotherapy.

Methods: This was a cohort study conducted at several teaching hospitals in Obstetrics and Gynecology Department of the Faculty of Medicine, Hasanuddin University from April 2011 to March 2012. The subjects were patients with ovarian cancer who met the inclusion criteria and had undergone surgery. The clinical staging was determined with 2009 FIGO criteria. They went through histopathology examination to determine the histological type and cell differentiation of the lesion. They also went through combined chemotherapy of Paclitaxel and Carboplatin. The data were analyzed with paired t-test.

Results: The study reveals that out of 30 cases of ovarian cancer who received a combination chemotherapy, most were < 45 years of age (53.33%), nulliparous (46.7%), serosum type (53.3%), with moderate differentiation (36.7%), and in advanced stage (73.3%). The VEGF serum level after 3 series of chemotherapy was lower than before (the average value: 294.67 vs 572.77 ng/ml). There was a significant change in VEGF serum level after receiving chemotherapy (p=0.000). The VEGF serum level of advanced-stage and early stage epithelial ovarian cancer after chemotherapy decreases significantly (p=0.000 and p=0.011). The advanced-stage cases showed more responses to chemotherapy than the early-stage did. There was a tendency that adenocarcinoma serosum type was more responsive to the therapy than mucinosum type (p=0.000 vs 0.003).

Conclusion: There is no difference in VEGF serum level based on cell differentiation but there is a tendency that well and moderate differentiated cells have a greater change than the poor differentiated cells (p=0.003, p=0.003 vs p=0.019).

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Keywords: carboplatin, epithelial ovarian cancer, paclitaxel, VEGF

Abstrak

***Tujuan**: Penelitian ini bertujuan menilai respons kemoterapi kombinasi paclitaxel - carboplatin pada kanker ovarium epitelial dengan melihat perubahan kadar VEGF serum sesudah menjalani kemoterapi 3 seri.*

***Metode**: Penelitian ini dilakukan pada periode April 2011 sampai Maret 2012 di beberapa rumah sakit pendidikan Bagian Obstetri dan Ginekologi Fakultas Kedokteran Universitas Hasanuddin. Jenis penelitian ini adalah kohort. Subjek penelitian adalah penderita kanker ovarium yang memenuhi kriteria inklusi dan telah menjalani operasi, penentuan stadium klinis dengan kriteria FIGO 2009, pemeriksaan patologi anatomi yang dilakukan untuk menentukan tipe histologi, diferensiasi sel, dan menjalani kemoterapi kombinasi paclitaxel - carboplatin. Data dianalisis dengan uji t berpasangan (Paired t-test).*

***Hasil**: Hasil penelitian menunjukkan bahwa terdapat 30 kasus kanker ovarium yang menjalani kemoterapi kombinasi paclitaxel-carboplatin dengan kasus terbanyak pada usia kurang dari 45 tahun (53,33%), nulliparitas (46,7%), tipe histologi serosum (53,3%), diferensiasi sedang (36,7%), dan stadium lanjut (73,3%). Kadar VEGF serum sesudah menjalani kemoterapi 3 seri lebih rendah daripada sebelum menjalani kemoterapi (294,67 vs 572,77 ng/ml, mean). Terdapat perbedaan yang signifikan antara kadar VEGF serum sebelum dan sesudah menjalani kemoterapi (p=0,000). Kadar VEGF serum kanker ovarium epitelial stadium lanjut dan stadium awal secara signifikan mengalami penurunan setelah kemoterapi (p=0,000 dan p=0,011), namun stadium lanjut menunjukkan hasil yang lebih responsif terhadap kemoterapi dibandingkan dengan stadium awal. Terdapat kecenderungan pada tipe adenokarsinoma serosum lebih responsif terhadap kemoterapi dibandingkan dengan tipe musinosum (p=0,000 vs 0,003).*

***Kesimpulan**: Tidak ada perbedaan yang signifikan kadar VEGF serum berdasarkan diferensiasi sel, namun ada kecenderungan diferensiasi baik dan sedang mengalami perubahan lebih besar dibandingkan diferensiasi jelek (p=0,003,p=0,003 vs p=0,019).*

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***Kata kunci**: carboplatin, kanker ovarium epitelial, paclitaxel, VEGF*

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INTRODUCTION

Ovarian cancer is one of the five most common cancers in women and it was the fifth most common cause of death in women with cancer.[1] In USA, 2009, there were 21,550 new cases of ovarian malignancy, with 14,600 deaths per year. Ovarian malignancy most commonly found in women aged 65 - 74 years old (25.3%).[2,3]

This high mortality rate is mainly caused by the lack of complain in the main stages of this disease, leading to a difficulty in making an early diagnosis. It is estimated that about 75% of ovarium cancer has already spread outside the ovarium or metastized to distant site when the diagnosis is made, causing an unsatisfying result and low 5-year survival rate (± 40%).[4,5]

The management of ovarium cancer is deciced based on the stages, differentiation, preservation of fertility and the general condition of the patient. The management of late stages ovarium cancer patient consists of the combination of cytoreductive operation and chemotherapy. The objective of chemotherapy is mainly to prevent the multiplication of cancerous cells, their invasions and metastasis. The combination of taxane and platinum/paclitaxel with carboplatin or cisplatin) is one of the chemotherapy regiment used for the management of ovarium cancer.[6]

A study of GOG 158 compared the effectivity of carboplatin and paclitaxel combination wth cisplatin and paclitaxel combination. The result is a similiar survival rate, but more severe cases of gastrointestinal problem and neurotoxicity in the group receiving cisplatin. Based on these studies, the recommended chemotherapy combination for late stage ovarium cancer is the combination of paclitaxel and carboplatin.[6-8]

For the last few years, the understanding on the molecular biology, especially angiogenesis, in ovarium cancer has improved. It brought a new finding on the target of therapy and a new molecular based prognostic factor. This caused a development of more radical treatment on ovarium cancer. Factors influencing angiogenesis are playing a key role in understanding the mechanism and management of ovarium cancer. One of the aforementioned factors is the vascular endothelial growth factor (VEGF).

VEGF is the main signal, which utilized by the oxygen-deprived cells to induce the development of new blood vessels. VEGF is the main regulator of angiogenesis, performing its action by stimulating mitogenesis from endothelial cells and increasing the vascular permeability. VEGF also plays a role in lymphangiogenesis, which is the development of new lymphatic vessels. The lymphatic drainage system is important in maintaining the fluid balance in the tissues, mediating afferent immune response and also in the metastation of malignant tumor to the regional lymph node.[9]

Several studies has already insinuated that VEGF-induced angiogenesis is a major component in the development of ovarium cancer. The immunostaining of VEGF has shown in the epithelial cells of benign ovarium neoplasma. The microvascular density and expression of VEGF and its receptor in the ovarium tumor are associated with poor prognosis, and this suggest that angiogenesis, possibly through VEGF, influences the progresivity of the disease.[10]

Mesiano et al had found the role of VEGF in the occurrence of ascites fluid in epithelial cell type ovarium cancer. In the end, they used the human ovarium cancer cell, SKOV-3, to develop an in vivo model of ovarium cancer in immunodeficient mices. The cancer developed into intraperitoneal cancer and there is also ascites fluid build up commonly seen in ovarium cancer patients.[11]

Some researches examined the level of VEGF in the tissue of the tumor. The findings from several studies showed that the level of circulating VEGF was associated with the progressivity of the disease, response to chemotherapy and the life expectancy rate. This showed a possible use of examining the level of VEGF in the blood serum or plasma.[11]

The combination of chemotherapy in the therapy of ovarium cancer influences the angiogenic factor, including VEGF. The general characteristic of cancer cells is their fast multiplication caused by angiogenesis mechanism. Platinum-based drugs will influence the cell cycle in mitotic phase by preventing the multiplication of cells and inducing apoptosis and the Taxane-based drugs will interfere with DNA synthesis. These mechanisms will cause the expression of VEGF declined.[12]

The role of VEGF in the growth and development of the ovarium cancer is very important. By understanding its role on the growth, invasion and the spread of cancer cells, we could understand more about the development of ovarium cancer and the potential of VEGF as a target for therapy. Studies need to be performed to further evaluate whether the currently standard regiment of chemotherapy resulting in decreasing level of VEGF, thus preventing the growth, invasion and spread of cancer cells.

METHOD

This was a cohort study aimed to evaluate the response to Paclitaxel-Carboplatin chemotherapy combination in epithelial cell ovarium cancer by

examining the change in the serum level of VEGF. This study was performed in several hospital acquintanced with Department of Obstetrics and Gynecologic in Faculty of Medicine Hasanuddin University, Makassar: Dr. Wahidin Sudirohusodo Hospital, Pelamonia Hospital, Faisal Hospital, Labuang Baji General Hospital, and Bhayangkara General Hospital. This study was conducted from 1st April 2011 to 31st March 2012. The subject of this study is ovarium cancer patient who had been operated on and given 3 series of Paclitaxel-Carboplatin chemotherapy combination. The patients must never had chemotherapy before, had no other malignancies such as colon cancer, liver cancer, lung cancer and breast cancer. The level of serum VEGF was examined with sandwich enzyme immunoassay technique (Immunology assay with quantitatively layered enzyme). This study used human VEGF immunoassay, catalog number DVEoo, R&D systems product. A VEGF-spesific polyclonal antibody was alyered on a microplate. The sample was taken with a pipette, put into a tube, and the VEGF would be bound by immobilized antibody. After being cleaned from unbound compounds, a polyclonal antibody bound to an enzyme and specific for VEGF would also be put into the test tube. After washing to eliminate unbinding antibody-enzyme reagent, a substrate would be added into the crook, making an appearance of color consistent with the level of VEGF (pro and/or active), which was bound, in the early steps. The color changing process than stopped and the color intensity was measured in ng/ml.

RESULT

This study was performed on 30 patients, 43.86 years old on average (the age range was 19-55 years old). Sixteen patients were under 45 years old and 14 cases were over 45 years old. Most patients were nulliparous. The most common histological type was adenocarcinoma serosum, found in 16 cases (46.7%). There were 9 well-differentiated cases (30%), 11 moderately differentiated cases (36.7%), and 10 poorly differentiated cases (33.3%). Most cases (73%) were in the advanced stages.

Table 1 showed that the mean level of serum VEGF before chemotherapy was 572.77 (SD±346.21) and after chemotherapy was 294.67 (SD±144.11). Statistically, there was a significant difference of VEGF serum level before and after chemotherapy, with p value of 0.000 (p<0.05).

Table 1. The comparison of serum VEGF level before and after Paclitaxel-Carboplatin chemotherapy regiment in epithelial cell type ovarium cancer

n=30	Min	Max	Mean	p*
VEGF(ng/ml)				
Before	176.40	1521.70	572.77	0.000
After	85.40	590.30	294.67	

**Paired t-test*

Table 2 showed that the VEGF serum level in epithelial cell type ovarium cancer declined significantly after chemotherapy, both in the early stage

Table 2. The comparison of serum VEGF level before and after Paclitaxel-Carboplatin chemotherapy regiment in epithelial cell type ovarium cancer based on the stages

Stages	VEGF (ng/ml)						p*
	Before chemotherapy		After chemotherapy		Change		
	Mean	SD	Mean	SD	Mean	SD	
Early	484.66	272.59	313.95	147.32	170.71	140.95	0.011
Advanced	604.81	369.75	287.65	145.78	317.15	309.10	0.000

**Paired t-test*

Table 3. The Comparison of serum VEGF level in epithelial cell type ovarium cancer before and after Paclitaxel-Carboplatin chemotherapy regiment based on the histologic type

Histologic type	VEGF (ng/ml)						p*
	Before chemotherapy		After chemotherapy		Change		
	Mean	SD	Mean	SD	Mean	SD	
Adenoca mucinosum	447.25	189.21	332.65	141.52	114.59	106.69	0.003
Adenoca serosum	699.98	407.59	271.68	152.73	428.30	301.89	0.000

*Paired t-test

cases (p-0.011) and the advanced stage cases (p=0.000). It also showed that the change in VEGF level was greater in the advanced stage cases.

Table 3 showed that the VEGF serum level in epithelial cell type ovarium cancer declined significantly after chemotherapy, both in the adenocarcinoma mucinosum (p=0.003) and serosum (p=0.000). We could see that the change in VEGF level was greater in adenocarcinoma serosum, which showed that the adenocarcinoma serosum type gave a better response to Paclitaxel-Carboplatin chemotherapy combination.

DISCUSSION

In this study, most cases were over 45 years old (53.3%). Theoretically, ovarium cancer is rarely found in women below 40 years old. The incidence will rise along with age, from 15 per 100,000 in women aged 40-44 years old, to 57 per 100,000 in women aged 70-74 years old. The average age of the patients by the time of diagnosis was 63 years old and 48% patients were over 65 years old. The study performed by Okayasa et al in 2007 found that women over 45 years old had 2.8 times more risk to suffer ovarium cancer.[6,13]

There were 16 patients younger than 45 years old in this study. This could happen since there is a lot of predispositing factor of ovarium cancer, such as hereditary factor, early menarche, the high rise of infertility treatment, endometriosis, hormonal therapy, and pelvic inflammatory disease, which occurring more often in women. Those factors could cause a spontaneous mutation, which in turn could end up as the development of ovarian cancer.[14]

Most patients were nulliparous, which supported the incessant ovulation hypothesis by Fathalia (1972) that cellular trauma in the surface epithelial ovarium during each ovulation, happening regularly without a break, would cause the epithelial cell to undergo a neoplastic change.[14]

Epidemiologically, more than half of ovarium cancer cases are adenocarcinoma m=serosum, and only 5-10% are adenocarcinoma mucinosum.[15] In this study, most cases are adenocarcinoma serosum (53,3%) and the rest were adenocarcinoma mucinosum.

The level of serum VEGF after chemotherapy regiment declined significantly (from 572.77 ng/ml to 294.67 ng/ml; p=0.000). This indicated that there was a response towards the chemotherapy based on the VEGF level. The target of chemotherapy is to prevent the multiplication, invasion and spread of the cancer cells. VEGF, an angiogenesis factor responsible in the process of cancer cells invasion and spread, turned out to have a good response towards Paclitaxel-Carboplatin chemotherapy regiment, except in adenocarcinoma mucinosum, which will be explained later. Until recently, there was no study that examined the correlation of the change in VEGF level with the prognosis of ovarium cancer.[9,15]

In this study, the change in VEGF level was greater in the advanced stage cases compared to the early stage cases (604.81 ng/ml vs 484.66 ng/ml), eventhough there was still a significant change before and after chemotherapy in both stages. This pointed to the fact that angiogenesis palys a great role in the growth and spread of ovarium cancer, considering that in the advanced stage, a massive angiogenesis and lymphangiogenesis had already happened because of the high level of VEGF. This shows that there is a significant relation between the activation of VEGF and invasion and metastasis of ovarium cancer.

VEGF caused angiogenesis, tumor growth and tissue metastasis due to hypoxia in cancer cells. The rising level of VEGF found in tumor cells is related to malignant transformation. Several studies proved that the expression of VEGF was an independently significant predictor for relapse rate and the overall prognosis.[10]

The higher responsiveness of cancer in the advanced stage could be explained by the pattern of growth known as Gompertz growth. When a cancer is small in size and the mass is still unpalpable, it will grow exponentially. In this phase, the cells will be more sensitive towards chemotherapy because most cells are in the active phase of the cell cycle. Thus, the metastazing cells are more sensitive towards the chemotherapy compared with the primary tumor itself. In other words, late stages ovarium cancer, which has already metastasized, will be more sensitive towards chemotherapy.[16]

In this study, the VEGF serum level in epithelial cell type ovarium cancer declined significantly after chemotherapy, both in the adenocarcinoma mucinosum (p=0.003) and serosum (p=0.000). We could see that the change in VEGF level was greater in adenocarcinoma serosum (428.30 ng/ml) compared to adenocarcinoma mucinosum (114.59 ng/ml).

A genetic and molecular study shows a difference between adenocarcinoma mucinosum and serosum, which support the concept that these two types of tumor are separated in the development. Several studies showed that adenocarcinoma mucinosum had a differen pattern of molecular expression with other type of cancer such as serosum and endometrioid. Several other studies also showed that a mutation on RAS (especially KRAS on codon 12) is oftern found in the adenocarcinoma mucinosum, but not in other types. On contrary, a mutation on P53 gene, which associated with the sensitivity towards Paclitaxel, is rarely found in adenocarcinoma mucinosum. Besides, the experession of COX-2 is also rarely found in adenocarcinoma mucinosum, but more often in adenocarcinoma serosum.[17]

The histological difference of the type of ovarian cancer is still under research, considering the relatively poor response of adenocarcinoma mucinosum towards both single or combination chemotherapy. Adenocarcinoma mucinosum also has a poorer life expectancy rate, making it a priority to determine a chemotherapy regiment specifically designed for it.[17,18]

Clinically, a grading system is important on the management of cancer, because it could influence the prognosis and recurrency rate. Higher grade mostly means that the mitotic process on the cancer is alos greater, thus making it more responsive towards chemotherapy. In this study all degree of differentiation showed a significant response towards the chemotherapy regiment. But it could be observed that the well or moderate differentiated cancer gave better response compared to the poor differentiated. But this also influenced by the histological type and stage of the cancer when the chemotherapy was performed.[17,18]

CONCLUSION

There was a significant difference of VEGF serum level before and after Paclitaxel-Carboplatin chemotherapy regiment, in all stages, histologic type and level of differentiation of ovarium cancer.

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