Prognostic value of p53 gene in ovarian cancer

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Tujuan: Mengetahui peranan gen p53 dan mengidentifikasi nilai prognostik ekspresi protein p53 mutan terhadap kanker ovarium.

Rancangan/rumusan data: Penelitian survei analitik dengan desain *cross sectional* pada rumah sakit pendidikan di Makassar.

Bahan dan cara kerja: Pasien dinyatakan kanker ovarium berdasarkan hasil pemeriksaan histopatologi, dianalisis ekspresi protein p53-nya dengan teknik imunohistokimia.

Hasil: Derajat ekspresi protein p53 ditemukan lebih tinggi pada kanker ovarium stadium lanjut. *Follow-up* penderita antara 6 bulan sampai 2 tahun setelah operasi menunjukkan bahwa penderita yang mempunyai ekspresi protein p53 mutan yang tinggi mempunyai angka kematian yang tinggi.

Kesimpulan: Analisis p53 dapat dipakai sebagai indikator prognostik terhadap kanker ovarium.

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Kata kunci: p53, kanker ovarium, prognosis.

Objective: *To investigate the role of p53 gene and to identify prognostic value of p53 mutant expression in ovarian cancer*

Design/data identification*: Analytical survey with cross sectional approach in institutional hospitals in Makassar.*

Material and methods: *The immunohistochemical of p53 expression analysis of ovarian cancer were performed. All samples have been diagnosed by histopathological examination.*

Results: *The p53 overexpression was found in the advance stage of ovarian cancer. Follow-up of patients for 6 months - 2 years after the operation showed that the higher p53 overexpression, the higher mortality.*

Conclusion: *p53 analysis can be used as a prognostic indicator of ovarian cancer.*

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Keywords: *p53, ovarian cancer, prognosis.*

INTRODUCTION

Ovarian cancer is the most cause of death among gynecological cancer in the western countries[1], and is the second most cancer prevalence after cervical cancer. In the last 10 years the incidence of this disease increase about 30 %, lead to mortality increased of 18 %[2]. The incidence of ovarian cancer is 15 cases per 100,000 women every year and 141,000 new cases found with 106,000 death cases every year around the world[3]. In United States, 25,400 new cases of ovarian cancer in 2003, which 14,300 among those was death[2].

The incidence of ovarian cancer is increase at every age increasing of a woman, from 15.7 in 100,000 women at 40 years old, to 54 in 100,000 women at 79 years old[2]. Overall, it could be state that 1 of 70 women will have ovarian cancer in her life.

The hypothesis about ovarian cancer pathogenesis is still unclear and need further investigation. Until now, the hypothesis about ovarian cancer pathogenesis include inclusion bodies formation lead to malignant transformation in ovary's stroma[4], incessant ovulation[5], androgen and progesterone hormones influence[6,7,8]. Some risk factors of ovarian cancer have been identified, including parity, oral contraception, and ovulation induction drugs. However, these factors still need further study. The obstacle in ovarian cancer research is that of this disease is diagnosed at advance stage because no signs and symptoms at early stage, also the location is more hiding compare to other reproduction organs[5].

The morbidity and mortality rates of ovarian cancer are still high in the last 3 decades, because the etiology and pathogenesis of this disease is still unclear. One of the reason is limitation of molecular biology underlie this disease and limitation of biomarker for detection.

p53 gene is the most study gene in the prognosis and chemotherapy response prediction of ovarian

cancer patients. It has been demonstrated that mutation and accumulation of p53 can be used as chemosensitivity prediction of ovarian cancer, which mutation and expression of p53 proteins were increased after chemotherapy[9]. This results demonstrated that apoptosis by p53 gene was related to ovarian cancer chemosensitivity.

In this study, we perform the analysis of p53 overexpression in ovarian cancer tissues. The p53 gene is known to play a role in cell survival and that makes it interesting to study the role of this gene in ovarian cancer pathogenesis since the incidence, morbidity, and mortality rates of ovarian cancer is high in Indonesia. The knowledge of ovarian cancer pathogenesis lead to prompt treatment to the patients includes chemotherapy.

This study also analyzed the role of p53 gene in the prognosis of ovarian cancer. This issue is important in antiresistant drugs development, since the resistant of ovarian cancer chemotherapy is still high. Furthermore, molecular study of ovarian cancer in Indonesia is still limited and this study could gain the development of basic research for clinical applications.

MATERIALS AND METHODS

This study was an analitical survey using cross sectional study. The research location was at Teaching Hospital Dr Wahidin Sudirohusodo Faculty of Medicine Hasanuddin University Makassar between 2003 - 2005. Samples were patient suspected ovarian cancer patients by clinical examinations, ultrasound, Doppler trans-abdominal/vaginal, and CA-125 tumor marker examinations. Disease stages were determined using FIGO 2000 criteria. Ovarian cancer diagnosis was established by histopathological examinations.

Samples were obtained from 41 malignant ovarian tumor. None of patients received therapy before surgery, and all patients had been followed up to have the survival data. The observation were performed from 6 months to 2 years after surgery.

Immunohistochemistry was performed using Novocastra® *mouse monoclonal antibody antihuman* p53, and Novocastra® *Biotinylated Universal secondary antibody* p53. The positive p53 overexpression was determined by brown granule in the tumor's cell nucleus. Scoring was performed by counting the positive cells as follow: score 1 = ≤ 25 %, score 2 = 26 - 50 %, score 3 = 51 - 75 % and score 4 = 76 - 100 %. Tumor types, tumor differentiation, and expression grade of p53 were determined by a pathologist. Data were analyzed using *Statistical Program for Social Sciences (SPSS) for Windows version 11.5*.

RESULTS

Samples characterized showed that most of samples were below 55 years of age (82.9 %), and there were 8 years and 12 years old among them. Most histological type was epithelial cancer (80.5 %), while the rest 19.5 % were non-epithelial types such as germ cell origin (malignant teratoma, dysgerminoma), and struma ovarii. The well differentiated cancer was the most frequent (68.3 %), while 31.7 % were poorly differentiated. Patients were mostly in stage III of disease (48.8 %), followed by stage I (26.8 %), stage IV (14.6 %), and stage II (9.8 %).

We found that 58.5 % of samples were overexpress p53, and most of them was grade 4 (58.3 %), while grade 1 was 20.8 %, grade 2 was 12.6 %, and grade 3 was 8.3 %. The epithelial type of ovarian carcinoma demonstrated higher overexpression of p53 (63.6 %), while the non-epithelial types showed less or no expression of p53 (62.5 %). Most of poorly differentiated tumor (92.3 %) showed p53 overexpression, while well differentiated tumor tent not to show the p53 overexpression (57.1 %). The p53 overexpression was mostly found in the stage IV, folowed by stage III, II, and I.

Yate's correction X^2 statistical analysis was applied to find the relation between p53 overexpression and histological types and differentiation of ovarian cancer. We found that the overexpression of p53 has significant relation with histological type of ovarian cancer ($p = 0.0241$). Samples that have positive p53 overexpression were majority epithelial type. We also found that there was significant relation between overexpression of p53 and differentiation of ovarian cancer cells ($p = 0.0080$). The poorly differentiated samples have more p53 overexpression.

X^2 statistical analysis was applied to find the correlation between p53 overexpresion and ovarian cancer stage. We found that there was significant correlation between p53 overexpression with ovarian cancer stage ($p = 0.0020$). Samples that have p53 overexpression mostly in advance stages disease (stage III and IV), while samples that were not expressed p53 more in early stages (stage I and II).

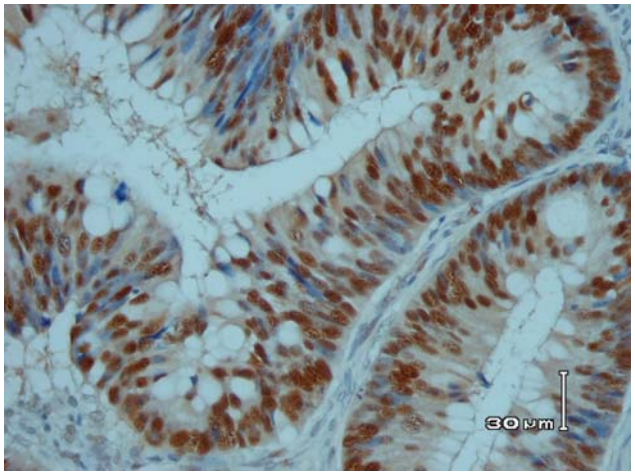

Figure 1. Overexpression of p53 in ovarian cancer tissue (400 x magnifications)

The expression of p53 in poorly differentiated ovarian cancer is shown in figure 1. The p53 protein was accumulated in the nucleus of tumors and the expression degrees were increased as histopathological grade increased. In this study, we did not find significant relation between p53 overexpression with ages of patients ($p > 0.05$).

Prognostic value analysis found that there was significant relation ($p = 0.0050$) between p53 overexpression with the outcome of the patients. Those who have higher p53 overexpression were mostly died (83.3 %) in the follow-up time compared with those who have no overexpression of p53 (35.3 %). The logistic regression analysis found that there was positive significant relation ($p = 0.0130$) between p53 overexpression with the patients outcome (OR = 10.5), means that the mortality rate patients who have p53 overexpression were 10.5 times higher than the patients who have no p53 overexpression.

We also found that the capability of life of ovarian cancer was 12 - 14 months after first diagnosis established. In the first 5 months after diagnosis, about 50.0 % patients who have p53 overexpression were died, while patients without p53 overexpression 80.0 % still alived. After 8 months, both p53 overexpression patients and no p53 overexpression have similar *cumulative survival* (around 20.0 %). Survival time of p53 overexpression patients were 5.72 months (range 4.09 - 7.35 months), while in no p53 overexpression patients were 6.74 months (range 5.16 - 8.31 months).

DISCUSSION

P53 tumor suppressor gene is the most mutated gene in human cancers. It has been found that 50 % or all human cancer contain p53 mutations[10]. However, the role of p53 in ovarian carcinogenesis is still unclear, although some studies found that p53 mutant expression could be a prognostic indicator of ovarian cancer patients.

In this study, most samples were above 55 years old (82.9 %), and there were samples who 8 and 12 years old among them. This data support previous studies that ovarian cancer incidence is increase with age, and reach the peak at the fifth decade of life[2,3].

The very young age samples showed that the tend of ovarian cancer express in young age, and probable of more malignant types and hereditary factors based on mutation in germ line cells[4]. This assumption was supported by our finding, that the 8 years and 12 years old patients have malignant teratoma, which origin from germ lince cells. Although hereditary ovarian cancer only 5 - 10 % occur in all ovarian cancer statistically, study of hereditary ovarian cancer is very important to be done in the future especially for genetic councelling purpose.

The most histological type of ovarian cancer in this study was epithelial type (80.5 %). This finding support some previous studies that also find similar results[10,11], and this support the hypothesis that majority of ovarian cancer was originated from the epithel surface of ovarian cancer. The majority of our samples was at stage III (48.8 %), and this indicated that patients came to the hospital already in advance stages. Another reasons are most ovarian cancer has no signs and symptoms and has hide location, lead to the late diagnosis. However, we found more well differentiated ovarian cancer grade (68.3 %), which indicate that clinical stage has less or no relation to the histopathological differentiation grade.

Immunohistochemistry analysis showed that p53 protein expression is located in cell nucleus. The accumulated p53 protein was mutant proteins that have longer half life and more stable than the wild-type p53. Although normal cell also express p53, this p53 is not stable and will immediately degraded and did not or very faint appear using immunohistochemistry technique[12].

We found that 58.5 % of samples overexpressed p53, and there was significant relation between p53 overexpression and clinical stages, histological types, differentiation grades. P53 overexpression was

found more in stage III and IV, more in epithelial types, and more in poorly differentiated grade. These results support the statement that p53 gene play a role in pathogenesis and ovarian cancer progression. Furthermore, these results also support the previous studies that p53 overexpression increased as stage increase and did not found in normal ovarii and benign ovarii tumor[13,14].

It has been known that the relationships among genotype, phenotype, and clinical manifestation are very complex. The gene expression has many steps from the DNA to protein. Some authors found that neoplastic cell that have p53 *missense* mutation, could be observed by immunohistochemistry technique because this mutation produced stable p53 proteins and longer half-life. They also found that the *frame-shift* or *nonsense* (*chain termination/protein truncated*) p53 mutation produced unstable p53 protein and easily degraded[14], and could not be detected by immunohistochemistry. This might be the reason why some of our samples did not express p53. This result needs further study to identify the type of p53 mutation of our ovarian cancer samples.

The prognosis determination is very important to increase the quality of life and of patients, such as determination of the suitable antiresistant chemotherapy. Until now, the efficacy of chemotherapy is limited by resistant capability of tumor to the chemotherapy. Most ovarian cancer has high respond to level one chemotherapy (*initial chemotherapy*), but the patients frequently has recurrence and resistance to the chemotherapy. These make the prognosis of ovarian cancer is still poor until now, and the survival rate is still low[13,14].

The p53 tumor suppressor gene is the most study gene for prognosis and prediction of chemotherapy respond of ovarian cancer patient, but the prognostic value of p53 in ovarian cancer is still controversial. In this study, we found that the most patient who has high p53 overexpression, died (83.3 %) compared to patients who has not p53 overexpression. Statistical analysis demonstrated that the life capability or ovarian cancer patients was 12 - 14 months after first diagnosis established, and in the first 5 months around 50 % patients who have high p53 overexpression were dead. This results support that p53 gene is an independent marker to poorly prognosis and is a prognosis indicator of patients survival.

These results can be used in various clinical application of ovarian cancer. If p53 expression of ovarian cancer tissue patients is high, we can predict the poorly prognosis because it resistant to chemotherapy, and the clinician could choose a suitable chemoterapy drugs. Furhter study of p53 mutation analysis of ovarian cancer before cisplatin, doxorubicin, and cyclophosphamide treatment is also needed, because this factor is related to ovarian cancer chemosensitivity [15,16,17].

This study is still limited in elucidate of pathogenesis and prognosis of ovarian cancer because sample number limitation and only analysed one gene that play the role in carcinogenesis apoptosis. However, our results could be use as basic data for further ovarian cancer study.

CONCLUSIONS

1. The p53 overexpression is related to the advance stages, epithelial type, and poorly differentiated grade of ovarian cancer.
2. The p53 overexpression has a prognostic value to the survival rate of ovarian cancer patients.

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