Research Report

Correlation of Anti-Müllerian Hormone Level with Ki-67 Expression in Patients with Ovarian Cancer

Korelasi Kadar Anti-Müllerian Hormone dengan Tingkat Ekspresi Ki-67 pada Penderita Karsinoma Ovarium

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Abstract

Objective: This research was carried out to discover the correlation between the AMH serum in patients with malignant ovarian tumor of epithelial type with the Ki-67 expression as a marker of proliferation, therefore it was expected through the research to know the role of AMH in the inhibition of proliferation and growth of malignant ovarian tumor of epithelial type.

Method: The study design was cross-sectional, with data obtained retrospectively from 24 medical records of patients with malignant ovarian of epithelial type, who had been examined for AMH levels in 2009, along with the sample in the form of preparation of paraffin embedded tissue of malignant ovarian tumor patients, who fulfilled the inclusion criteria. Furthermore examination of Ki-67 expression was performed with the statistical analysis using the rank Spearman analysis.

Result: There was a negative correlation between AMH levels and Ki-67 expression in patients with malignant ovarian tumors of epithelial ($r = -0.652$, $p < 0.05$).

Conclusion: These data indicate a significant negative correlation between serum AMH levels and Ki-67 expression in epithelial ovarian cancer patients.

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Keywords: anti-müllerian hormone, epithelial ovarian cancer, Ki-67, Müllerian inhibiting substance

Abstrak

***Tujuan**: Penelitian ini untuk mencari korelasi antara kadar serum AMH penderita karsinoma ovarium dengan ekspresi Ki-67 sebagai petanda proliferasi, sehingga diharapkan dapat diketahui peran AMH dalam penghambatan proliferasi dan pertumbuhan karsinoma ovarium.*

***Metode**: Rancangan penelitian adalah potong silang, dengan data diperoleh secara retrospektif dari rekam medik 24 penderita karsinoma ovarium, yang sudah diperiksa kadar AMHnya pada tahun 2009, beserta sampel yang berupa sediaan blok parafin penderita tumor ganas ovarium tersebut yang memenuhi kriteria inklusi. Selanjutnya dilakukan pemeriksaan ekspresi Ki-67 untuk dicari korelasinya dengan kadar AMH, menggunakan analisis statistik rank Spearman.*

***Hasil**: Terdapat korelasi negatif antara kadar AMH dan histoskor Ki-67 pada penderita karsinoma ovarium ($r = -0,652$; $p < 0,05$).*

***Kesimpulan**: Data ini menunjukkan korelasi negatif yang signifikan antara kadar serum AMH dan ekspresi Ki-67 pada karsinoma ovarium. Bersamaan dengan literatur terkini, hasil penelitian ini memberikan tambahan data mengenai peranan AMH dalam mengurangi proliferasi sel tumor.*

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***Kata kunci**: anti-müllerian hormone, Ki-67, Müllerian inhibiting substance, karsinoma ovarium*

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INTRODUCTION

Malignant ovarian tumors is an important issue of reproductive health in both Indonesia and another country, because of the high incidence and at early stage is often asymptomatic, so that at the time of diagnosis more than two-thirds of cases of malignant ovarian tumors had reached an advanced stage, with high mortality and often referred to as the silent killer.[1]

The proportion of malignant ovarian tumors in Dr. Hasan Sadikin Hospital in 2008 was 19.77% from 1228 gynecologic cancer,[2] whereas in Dr. Cipto Mangunkusumo in the year 1978 - 1982 was 10.5% from 3874 gynecologic cancer.[3] Based on the Global Cancer Statistics 2002, incidence of malignant ovarian tumors in developing countries is higher than developed countries, ie more than 9/100,000 women, except for Japan (6.4/100,000).[4]

Ovarian cancer comprises a heterogeneous group of tumors of three major types: epithelial, germ cell and sex cord stromal. Epithelial tumors arise from the coelomic mesothelium represent about 82% of all ovarian malignancies. The predominant cell types are: serous, mucinous, endometrioid, clear cell, and Brenner tumor. The malignant germ cell tumors are believed to arise from primitive germ cells in the ovary. These tumors represent about 5% of all ovarian malignancies. They include dysgerminoma, embryonal cancer, endodermal sinus tumor, choriocarcinoma and malignant teratomas. The sex cord stromal neoplasms are derived from mesenchymal stem cells in the ovarian cortex and represent about 10% of all ovarian tumors. They include granulosa-theca cell tumors, granulosa cell tumors (GCTs) and Sertoli-Leydig cell tumors.[5]

Therapy of ovarian malignant tumors, beginning with surgery to remove the tumor burden, up to leave a tumor with a size < 1.5 cm that will be followed by chemotherapy. Giving chemotherapy has severe side effects and still have a fairly high failure due to resistance, therefore another method is required in addition to increasing remission and disease-free period, so that will improve the quality of life.[1] Anti-müllerian hormone/müllerian inhibiting substance (AMH/MIS) that can inhibit growth of Müllerian duct is expected to inhibit the growth of ovarian cancer because of ovarian cancers are also derived from Müllerian duct precursors.

AMH is a member of the transforming growth factor (TGF) superfamily. Anti-Müllerian hormone, has a role in the treatment of epithelial ovarian cancer.[5-6] This glycoprotein has a broad function in epithelial-mesenchymal interactions, cell growth, extracellular matrix production, and tissue remodeling.[6] In physiological conditions AMH inhibited the progression of Müllerian duct male fetuses, whereas in women AMH plays a role in foliculogenesis.[6] Epithelial ovarian cancer is originated from coelomic epithelium which is of Müllerian origin and thus, expresses MIS/AMHRII. Based on the physiological inhibiting role of AMH on the müllerian ducts, researchers have demonstrated that AMH inhibits epithelial ovarian cancer cell both in vitro and in vivo.[7-11] In vitro AMH inhibits the growth of ovarian epithelial cell line (HOSE 6 - 3) and human ovarian cancer cell lines (OVCAR-8) by proliferation of p16 protein, a part of inhibitor of cyclin-dependent kinase (INK4) family.[12] Inhibitors of cyclin-dependent kinase is a family of cyclin-dependent kinase inhibitors (CDKI), which will inhibit cyclin and cyclin-dependent kinase complexes (CDK). Cyclins and CDK complexes have important regulatory roles during cell cycle progression. Based on these findings, there is the possibility that AMH could be used to increase the success of chemotherapy in ovarian cancer.[5,12]

The unpublished study in 2009, on 40 patients with malignant ovarian tumors, found some elevated levels of AMH in 32 patients with ovarian cancer, 6 patients with malignant germ cell tumors, and 2 patients with GCT. Increased levels of AMH may be associated with tumor progression, which among others relate to the level of proliferation. CA125 is commonly used for monitoring ovarian cancer, no increase in 10 - 20% of women with ovarian cancer and does not increase with tumor size. In addition, CA125 can be elevated in benign conditions, such as endometriosis. Therefore, the level of proliferation is better characterized by the proliferation markers, such as Ki-67 expression. Ki-67 protein (antigen) is stable, has a half-life of about 60 - 90 minutes and can be found at all stages of the cell cycle except the G0 phase, so that Ki-67 is a good marker of proliferation,[13] whereas other markers such as proliferating cell proliferation nuclear antigen (PCNA) and JC1 increase also in cells that are not proliferate.[14]

From the prior illustration, AMH including TGF-β family that play a role as a tumor suppressor, preventing proliferation of Müllerian duct during embryogenesis and possibly also growth an ovarian cancer that embriological precursor the same as Müllerian duct, so that alleged that the expression of AMH reduces ovarian cancer cell proliferation that can be characterized by expression of Ki-67.

Based on these reasons, the author wanted to know if there is a correlation between serum AMH levels of ovarian cancer patients who was investigated in 2009 but not yet published, with Ki-67 expression as a marker of proliferation, which is expected to note the role of AMH in the inhibition of proliferation and growth of ovarian cancer.

SUBJECT

The subject is paraffin embeded tissue of ovarian cancer patients who was proven by pathological anatomy and have undergone the examination of serum AMH levels in 2009.

METHOD

The design of this study was a cross-sectional study. The data was obtained retrospectively from medical records of ovarian cancer patients who have examined levels of AMH and paraffin blocks of available stocks in the Anatomical Pathology Department at Dr. Hasan Sadikin Hospital.

RESULTS

Of the 32 patients with ovarian cancer who AMH have examined, a total of 6 patients refused/unable to be contacted to participate in the study and the two stocks paraffin blocks could not be evaluated. The number of samples can be examined in this study had to meet minimum sample size, i.e. 23 subjects.

Table 1 shows that the average age of ovarian cancer patients was 44.8 years with a standard deviation of 17.2 years and most are kind serosum (37.5%).

In this study, which compared the characteristics of the study subjects consisted of age and type of histology. According to Berek's literature, the incidence of ovarian carcinoma of age increased from 20 years to 80 years, then subsequently decreased, whereas the peak incidence is age 50 - 60 years.[1] In the research, the results are shown Table 1, the average age of patients with ovarian carcinoma was 44.8 years. This is consistent with the theory that ovarian carcinomas are rare in the young ages.

Table 1. Characteristics of subjects by age and histology results in patients with ovarian cancer (n = 24)

Characteristic	Total
Age (years old)	
• Median: 49	–
• Range: 22 - 72	–
Hystologic type	
• Serous adenocarcinoma	9
• Mucinous adenocarcinoma	8
• Endometrioid adenocarcinoma	6
• Clear cell carcinoma	1

Table 2. The results of Ki-67 expression in ovarian cancer patients

Ki-67	Total	%
Intensity		
• Weak	4	16.6
• Moderate	10	41.7
• Strong	10	41.7
Distribution		
• < 20%	2	8.3
• 20% - 50%	5	20.8
• 50% - 80%	10	41.7
• > 80%	7	29.2

Serous ovarian tumor incidence is 40% of all epithelial ovarian tumors, 50% are malignant, 33% are benign, and 17% are borderline. The incidence of mucinous ovarian tumor is 8 - 10% of all epithelial tumors, as much as 80% are benign, 15% are borderline, and 5% are malignant. The incidence of endometrioid ovarian tumors is 6 - 8% of epithelial malignancies, as much as 80% are malignant and 20% are usually borderline. The incidence of carcinoma of clear cell type as much as 6% of all epithelial malignancies and entirely malignant.[1] In this study the most is the kind serous type (37.5%) and the rarest is the kind of clear cell (4.17%) and this is in accordance with incidence of each type of malignancy.

Table 2 demonstrates the intensity of Ki-67 in patients with ovarian cancer most powerful classified and moderate (41.7%), weakness (16.6%). While most are based on the distribution of between 50% - 80% (41.7%) remaining > 80% (29.2%); 20% - 50% (20.8%) and < 20% (8.3%).

In the study Kupryjanczyk and Isola, Ki-67 as a variable known to increase proliferation in tissues with high mitotic index and may affect the initial spread of malignant tumors ovarium.[15,16]

In this study, as shown in Table 2 obtained intensity of Ki-67 in patients with ovarian carcinoma is a relatively strong majority and moderate (41.7%), whereas the distribution of Ki-67 in patients with ovarian carcinoma the highest is 50% - 80% (41.7%).

Furthermore, based on the value of the intensity and distribution of Ki-67 by making the scoring for the intensity that is:[17]
negative = 0; weak = 1; medium = 2, and strong = 3
and scoring for distribution are:
< 20% = 1; 20% - 50% = 2; 50% - 80% = 3; and > 80% = 4
can be calculated the amount of Ki-67 expression revealed by histoscore:
histoscore = (intensity + 1) x distribution
Next histoscore level be categorized according to the calculation:
Weak: if the score value 1 - 4
Moderate: if the score value 6 - 9
Strong: if the score value of 12 - 16
Histoscore calculation results are presented in the following table.

Table 3. AMH comparison based on histoscore

Ki-67	AMH	
	X	SD
Weak	9.604	± 6.528
Moderate	1.842	± 0.195
Strong	1.229	± 0.409

Table 3 shows the AMH comparison based on histoscore. The more higher AMH level the more lower Ki-67 expression.

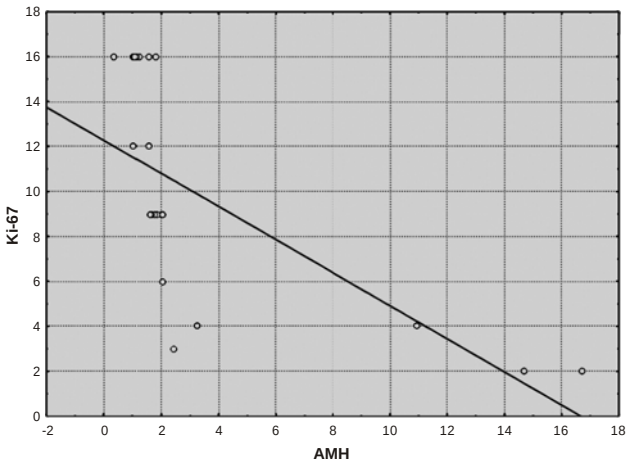

Figure 1. Correlation between elevated levels of AMH and the level of expression of Ki-67

*Note: AMH: Ki-67; y = 12.277 - 0.736*x; r = -0.652; p = 0.001*

Figure 1 shows that, there is strong correlation between AMH levels and the expression level of Ki-67 with r = -0.652. The higher levels of AMH, the lower the level of proliferation that is characterized by Ki-67.

DISCUSSION

In this study, which compared the characteristics of the study subjects consisted of age and type of histology. According to the literature Berek, incidence of age on ovarian cancer increased from 20 years to 80 years, then subsequently decreased, whereas the peak incidence is age 50 - 60.[1] The research results are shown in Table 1, the average age of patients with ovarian cancer was 44.8 years old. This is consistent with the theory that ovarian cancers rarely occur during adolescence/young.

Serous ovarian tumor incidence is 40% of all epithelial ovarian tumors, 50% are malignant, 33% are benign, and 17% are borderline. The incidence of ovarian tumors mucinous is 8 - 10% of all epithelial tumors, 80% are benign, 15% are borderline, and 5% are malignant. The incidence of ovarian endometrioid tumors is 6 - 8% of epithelial malignancies, 80% are malignant and 20% usually are borderline. The incidence of clear cell cancer type as much as 6% of all epithelial malignancies and entirely malignant.[1] In this study the most frequent type is serosum (37.5%) and least common is clear cell type (4.17%) and this agrees with incidence of each type of malignancy.

In research Kupryjanczyk and Isola, Ki-67 as a variable known to increase proliferation in a network with a high mitotic index and can affect the initial spread of malignant tumors ovarium.[15-16]

In this study, as shown in Table 2 obtained intensity of Ki-67 in patients with ovarian cancer is a relatively strong majority and moderate (41.7%), whereas the distribution of Ki-67 in patients with ovarian cancer is the most 50% - 80% (41.7%).[15,16]

Table 3 shows the AMH comparison based on histoscore. The more higher AMH level is the more lower Ki-67 expression. The mean AMH level is 9.604; 1.842; 1.229 which in correlation with a weak, moderate, and strong Ki-67 successful expression.

Anti-müllerian hormone purified in vitro and in vivo inhibits several malignant tumor cell lines derived from human ovarian Müllerian duct,[7,8,10,11] through activation of p16 protein. Protein p16 is a CDKI that slow the cell cycle and decrease the proliferation in a way that phosphorylate proteins CDK inactivation Rb.[12] Anti-müllerian hormone inhibits growth of ovarian epithelial cell line (HOSE 6-3) and malignant tumor cell lines of human ovarian (OVCAR-8) in vivo studies conducted by research conducted Ha et al.[11] Chin et al, rhMIS resulted in inhibition of cell line NIH: OVCAR-3 (ovarian adenocancer).[10]

The level of cell proliferation can be marked for example by expression of Ki-67.[13] Ki-67 has a specific nature that is only found in the cells proliferate and are not affected by DNA damage, making the Ki-67 as a good marker of proliferation.[13,16,18] Research conducted Kohlberger et al, showed proliferative activity with Ki-67 marker, i.e. by 16.7% in benign lesions, 76.5% in cancer ovarium.[19]

Based on the explanation above, the expected levels of AMH is expressed in ovarian cancer patients that the authors examined, can affect the proliferation of these tumors. Figure 1 shows that the statistical test by Spearman rank analysis on the degree of confidence of 80% shows that there is a strong correlation between AMH levels and the expression of Ki-67 are categorized according to the results histoscore in ovarian cancer patients with $r = -0.652$, $p = 0.001$ (p value < 0.05), so the higher the levels of AMH, the lower the level of proliferation that is characterized by Ki-67.

SUGGESTION

These results prove that there is a negative correlation between serum AMH levels with Ki-67 expression in ovarian cancer, so it can support the results of other studies using AMH as an ovarian cancer therapy in vivo and in vitro, and is expected to later be used directly in humans. Futhermore it needs to develop genetically modified to synthesize AMH for therapeutic purposes.

CONCLUSION

There is a strong correlation between elevated levels of AMH and AMH decrease in proliferation.

REFERENCES

1. Berek JS. Ovarian cancer. In Berek J, editors. Novak's gynecology. 12th ed. Philadelphia: Lippincott Williams and Wilkins; 2002. 1245: 53-8
2. Laporan Tahunan 2008. Bagian/SMF Obstetri dan Ginekologi Fakultas Kedokteran Universitas Padjadjaran Rumah Sakit dr. Hasan Sadikin Bandung.
3. Yatim F. Penyakit kandungan. Myoma, kanker rahim/leher rahim dan indung telur, kista, serta gangguan lainnya. Kanker indung telur. Edisi ke-2. Jakarta: Pustaka Populer Obor; 2008: 28
4. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. Cancer J Clin. 2005; 55: 74-108
5. La Marca A, Volpe A. The-antimüllerian hormone and ovarian cancer. Hum Reprod. 2007; 13: 265-73
6. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Müllerian hormone. Reprod. 2002; 124: 601-9
7. Stephen AE, Pearsall LA, Christian BP, Donahoe PK, Vacanti JP, MacLaughlin DT. Highly Purified Müllerian Inhibiting Substance Inhibits Human Ovarian Cancer in Vivo. Clin Cancer Res. 2002; 8: 2640
8. Masiakos PT, MacLaughlin DT, Maheswaran S, Teixira J, Fuller AF Jr, Shah PC. Human ovarian cancer, cell lines, and primary ascites cells express the human Müllerian inhibiting substance (MIS) type II receptor, bind, and are responsive to MIS. Clin Cancer Res. 1999; 5: 3488-99
9. Behringer R. The in vivo roles of Müllerian-inhibiting substance. Curr Top Dev Bio. 1994; 29: 171-87
10. Chin TW, Parry RL, Donahoe PK. Human müllerian inhibiting substance inhibits tumor growth in vitro and in vivo. Cancer Res. 1991; 51: 2101-6
11. Ha TU, Segev DL, Barbie D, Masiakos PT, Tran TT, Dombkowski D. Müllerian inhibiting substance inhibits ovarian cell growth through an Rb-independent mechanism. J Biol Chem. 2000; 275: 37101-9
12. Song JY, Chen KY, Kim SY, Kim MR, Ryu KS, Cha JH. The expression of müllerian inhibiting substance/anti-mullerian hormone type II receptor protein and mRNA in benign, borderline and malignant ovarian neoplasia. Int J Oncol. 2009; 34: 1583-91
13. Ross W, Hall PA. Ki67: from antibody to molecule to understanding. J Clin Pathol: Mol Pathol. 1995; 48: 113-7
14. Rose DSC, Maddox PH, Brown DC. Which proliferation markers for routine immunohistology? A comparison of five antibodies. J Clin Pathol. 1994; 47: 1010-4
15. Kupryjanczyk J, Bell DA, Yandell DW, Scully RE, Thor AD. P53 expression in ovarian borderline tumors and stage I carcinomas. Am J Clin Pathol. 1994; 102: 671-6
16. Isola J, Kallioniemi OP, Korte JM. Steroid receptors and Ki-67 reactivity in ovarian cancer and in normal ovary: Correlation with DNA flow cytometry, biochemical receptor assay and patient survival. J Pathol. 1990; 162: 295-301
17. Helin HJ, Isola JJ, Helin MJ, Helle MJ, Krohn KJE. Imprint cytology in immunocytochemical analysis of oestrogen and progesterone receptors of breast carcinoma. J Clin Pathol. 1989; 42: 1043-5
18. Van Dierendonck JH, KeijzerR, Van de Velde CJ, Cornelisse CJ. Nuclear distribution of the Ki-67 antigen during the cell cycle: comparison with growth fraction in human breast cancer cells. Cancer Res. 1989; 49: 2999-3006
19. Kohlberger PD, Kieback DG, Mian C, Wiener H, Kainz C, Gitsch G. Numerical chromosomal aberrations in borderline, benign, and malignant epithelial tumors of the ovary: Correlation with p53 protein overexpression and Ki-67. J Soc Gynecol Invest. 1997; 4: 262-4