Research Article

Impact of Ethinyl Estradiol to Human Telomerase Reverse Transcriptase Activity on Complete Hydatidiform Mole Culture

Pengaruh Etinil Estradiol terhadap Aktivitas Human Telomerase Reverse Transcriptase (Studi pada Kultur Sel Trofoblas Mola Hidatidosa Komplit)

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Abstract

Objective: To prove the effect of ethinyl estradiol as an activator on human Telomerase Reverse Transcriptase (hTERT).

Method: The experimental study was conducted in vitro by using culture of complete hydatidiform mole trophoblast cell. We exposed the culture to ethinyl estradiol in varied doses and measured the concentration of hTERT through RT-PCR quantitative. There were 40 specimens as control group and 20 specimens exposed to ethinyl estradiol in different doses (10, 20, 40 and 80 mcg) as experimental group. The activity of hTERT was measured by RT-PCR and the concentration of it was assessed by ELISA. We analyzed the variables using ANOVA, Turkey post hoc and Pearson correlation test.

Result: In control group, the concentration of hTERT was not detected. Meanwhile, the concentration among different doses of ethynil estradiol (10, 20, 40, 80 mcg) was 113,117.5; 114,507.6; 102,193.9; 127.546.1 amoles/ml, respectively. Among experimental group, they were significantly different both using F test (ANOVA) (p=0.001) and Turkey post hoc test (p=0.005). The correlation among group was 0.84 which meant higher level of ethinyl estradiol was correlated with higher activity of hTERT.

Conclusion: Ethinyl estradiol impacts to the increase of hTERT activity on complete hydatidiform mole cell culture.

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Keywords: complete hydatidiform mole, ethinyl estradiol, human Telomerase Reverse Transcriptase (hTERT)

Abstrak

***Tujuan**: Untuk membuktikan pengaruh etinil estradiol sebagai aktivator human Telomerase Reverse Transcriptase (hTERT).*

***Metode**: Penelitian eksperimental ini didesain secara in vitro menggunakan kultur sel trofoblas mola hidatidosa komplit. Hasil kultur sel trofoblas mola hidatidosa komplit diberikan etinil estradiol berbagai dosis. Aktivitas hTERT diukur secara kuantitatif dengan RT-PCR. Terdapat 40 spesimen sebagai kelompok kontrol dan 20 spesimen terekspos dengan etinil estradiol berbagai dosis (10, 20, 40, 80 mcg) sebagai kelompok eksperimental. Analisa hasil penelitian menggunakan uji anova, post hoc Turkey dan korelasi Pearson.*

***Hasil**: Konsentrasi hTERT pada kelompok kontrol tidak terdeteksi. Konsentrasi hTERT pada pemberian etinil estradiol (10, 20, 40, 80 mcg) ialah 113.117,5; 114.507,6; 102.193,9; 127.546,1 amoles/ml. Uji beda menggunakan uji F (Anova) diperoleh terdapat perbedaan aktivitas hTERT setelah pemberian Ethinyl Estradiol berbagai dosis (p=0,001). Uji komparasi menggunakan post hoc Turkey diperoleh perbedaan bermakna pada berbagai dosis 0, 10, 20, 40, 80 mcg. Pada uji korelasi menggunakan korelasi Pearson diperoleh hasil (p=0,005) dengan koefisien korelasi 0,84 yang membuktikan tingginya kadar etinil estradiol berhubungan dengan tingginya aktivitas hTERT.*

***Kesimpulan**: Etinil estradiol dapat meningkatkan aktivitas hTERT pada kultur sel mola hidatidosa komplit.*

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***Kata kunci**: etinil estradiol, human Telomerase Reverse Transcriptase (hTERT), mola hidatidosa komplit*

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INTRODUCTION

Hydatidiform mole as trophoblast disease in pregnancy is a differentiation failure from degeneration of placental hydropic. It is the chorion flakes which are usually seen as bubbles.[1,2] Hydatidiform mole is a part of gestational trophoblastic diseases (GTD). WHO classifies GTD into 6 diseases including gestational trophoblastic diseases (complete and partial hydatidiform mole), invasive mola, choriocarcinoma, the placental site trophoblastic tumor, and an unclassified trophoblastic lesions. Complete hydatidiform (CHM) as a common variant has characteristics as followed widened, edematous, and vesicular lesions of placental chorionic villi, also trophoblastic proliferation in various degrees.[3]

The distribution of GTD is varied worldwide, which higher distribution is found in some parts of Asia, Middle East, and Africa. Study conducted by Harmain in 2010 found the incidence of complete hydatidiform mole in Africa was 11.0 per 1,000 births.[4] Meanwhile, epidemiological study in Ban-

dung showed that the incidence of complete hydatidiform mole was 1:500. Patients with complete hydatidiform mole had 15-20% risk to undergo transformation into malignant gestational trophoblastic disease. Unfortunately, the carcinogenesis process in complete hydatidiform mole is still unknown. Some factors which has been known influencing in the carcinogenesis process are DNA ploidy, expression of phospholipids, oncogenes, tumor suppressor genes, nutrition and hormonal status.[5,6]

Expression from human Telomerase Reverse Transcriptase (hTERT) has an important role in the survival of malignant process. This expression can be detected in complete hydatidiform mole and choriocarcinoma, neither in partial hydatidiform mole nor normal pregnancy. Activation of this enzyme is in conjunction with a malignancy and usually occurs after molar pregnancy.[7,8]

Women with complete hydatidiform mole pregnancy should not get pregnant in a year so that they need contraception methods which can return their fertility as soon as possible after discontinuation of the contraception. The choices are both non-hormonal and hormonal contraception. After complete hydatidiform mole, patients in Indonesia like using condom for their husband as non-hormonal method and pills containing ethinyl estradiol as hormonal method. Previous study showed that the ethinyl estradiol effected to carcinogenesis; however, a meta-analysis study released that in women post mola hydatidiform, the use of ethinyl estradiol on hormonal contraception would cause the recurrence of mola.[9] Meanwhile, Esmaili et al. in 2007 said that ethinyl estradiol had carcinogenesis effect in human.[10]

Tamoxifen triphenylethylene as an anti-estrogen is non steroidal component. Tamoxifen binds to estrogen receptor (ER) in order to minimalize the carcinogenic effect of estrogen. Some studies suggested using anti-estrogen to reduce the occurrence of multiple malignancy. However, the occupation of ER is depended on cellular context and the structure of the ligand. The most important of the biological consequence is whether the activated receptor complex can induce estrogenic or anti-estrogenic response. Therefore, we would like to prove the effect of the ethinyl estradiol as an activator on human Telomerase Reverse Transcriptase (hTERT) on complete hydatidiform mole culture.[11]

METHODS

This was an experimental study conducted between May and July 2012. We obtained the samples from complete hydatidiform mole patients with a bubble of mole. We excluded the patients with negative β-hCG test and failure to grow trophoblast cell after culture.

We took the samples (1 cm^3 CHM tissue) by suction curettage after patients signed the informed consent form. The trophoblast tissue with buble of mole was washed by normal saline (NaCl 0.9%), then put in cord solution (medium for trophoblast tissue sampling), which containing Hank's balance salt solution (HBSS (Sigma H 1641)), gentamycine (Gentamerck), sodium hidrogen bicarbonate (SHB (Sigma), phenol red (Sigma P5530), HEPES Solution (Sigma), and deionized water (WFI Otsuka). We performed the process of culture not more than 12 hours after curettage.

The detailed procedures were firstly; the bubbles of mole were washed by NaCl until free from erythrocytes. After that, we washed twice with Dulbecco's phosphate buffered saline (DPBS) added with a final concentration of 100 U/ml penicillin and 100 mcg/ml streptomycine. After the addition of antibiotics, this solution (DPBS) should be kept at 2-8°C and used within one week. Finally, the bubbles were washed with serum free mellin.

To isolate the cells, the bubbles were incubated in a solution of Collagenase (type 1) 7 mg/10 cc for 2 hours and centrifuged 2,000 rpm for 7 minutes. The supernatant was removed and the pellet was resuspended in serum free myelin. The centrifugation was repeated and the resulting pellet was resuspended in 4 ml culture medium, which containing newborn calf serum (NCS, (Sigma N4637)) and 10% FBS, then incubated in 5% CO_2 incubator, in 37°C. Further, the culture was observed daily under a microscope to detect cell growth. Examination of β-hCG was performed to identify the trophoblast cells.

We divided the CHM trophoblast cultures into 4 experimental group in varying doses of ethinyl estradiol (10, 20, 40, 80 mcg/ml) and 1 control group. The hTERT was measured by QTD rt-PCR (quantitative telomerase device real time PCR (Allied Biotech)) and the concentration of it was measured by ELISA at MIPA laboratory Faculty of Medicine Universitas of Islam Negeri (UIN) Malang.

We performed ANOVA test to compare hTERT expression in CHM trophoblast cells and continued by Pearson correlation test to determine the correlation among the groups. Ethical clearance was obtained from Health Research Ethics Committee Saiful Anwar General Hospital no. 132/KILDK-FKUB/05/2012.

RESULTS

Table 1 showed the hTERT activity in CHM cells in various doses of etinhyl estradiol. The data was in homogeneity and normal distribution ($p>0.05$). There was a trend of raising concentration of hTERT as the increase dose of ethinyl estradiol compared to the control group in cell cultures exposed to both tamoxifen and without tamoxifen. Interestingly, there was a tendency of decreasing the hTERT concentration in hydatidiform culture without exposure to tamoxifen in the 40 mg of ethinyl estradiol group (Table 1).

For the group of CHM culture which exposed to tamoxifen, hTERT expression did not occur in the group treated with 10 mcg and 20 mcg of ethinyl estradiol. They expressed same as the control group (Figure 1).

We analyzed the hTERT concentration data at various dose groups by using Anova test. The ethinyl estradiol in cell culture without exposure of tamoxifen was significantly different to the level of hTERT concentration among groups ($p=0.003$). Apart from that, there was significant correlation between the concentration of ethinyl estradiol and hTERT concentration ($p=0.005$).

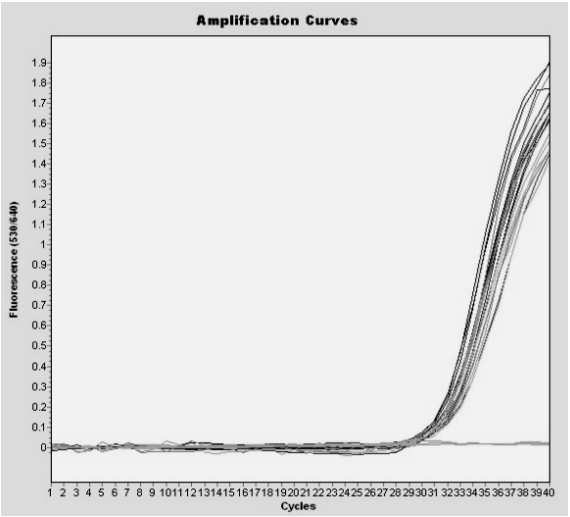

Figure 1. Amplification Curve of Hydatidiform Mole Culture with Ethynil Estradiol.

Table 1. hTERT Activity in CHM Cells in Various Doses of Ethinyl Estradiol Exposure

Estradiol Dose (mcg)	Mean (SD)(amoles/ul)
10 (physiologic)	113117.5 (2.7)
20	114507.6 (4.9)
40	102193.9 (5.5)
80	127546.1 (6.2)
Control (Without EE)	Not detected

DISCUSSION

Complete hydatidiform mole is characterized by edema of the vesicular placenta chorionic villi without the existence of fetus. The pathophysiology of CHM can derive from eggs which not conceived by haploid sperm or one egg is fertilized by two sperms. Histologically, CHM is seen as trophoblast proliferation with various levels of hyperplasia and dysplasia. Apart from that, the chorionic villi were fulfilled by fluid, which made it swell and there were only a few of blood vessels. Some studies suggested that women with gestational CHM was derived from the paternal karyotype 46XX and a little percentage of triploid or tetraploid maternal. Thus, CHM was occurred not only in pathology, but also in genetic abnormality.

Telomerase (hTERT) is an enzyme associated DNA sequence repetitions (TTAGGG) found in eukaryotic chromosomes. Telomerase prevents the loss of an essential component of DNA, so that every time a doubling of the chromosome causes missing of 100-200 nucleotides to prevent the damage in DNA. Most of the normal human somatic cells telomerase are in inactive state and they have a limited replication capacity. Related to several oncogenes, hTERT activity causes malignancy changes. The role of hTERT in this process is to provide unlimited replication capabilities. Inhibition of hTERT in immortal cells will lead to telomere shortening and apoptotic cell death. This shows that hTERT activity is generally required for making immortal and proliferation of cancer cells.

In the genomic pathway, estrogen binding to its receptor activates the estrogen receptor (ER). This activation happens between estrogen and estrogen response element (ERE). Estrogen response element is an area in the region of the hTERT gene promoter. Association of ER with ERE will cause the hTERT gene expression.

In this study, the administration of various doses of ethinyl estradiol was performed. In the control group, we could not detect the hTERT activity. This proved that there was no hTERT activity in trophoblast cell culture. Trophoblast cells which not having hTERT activity was identic to spontaneous regression of mole cell. Giving a dose of 10 mcg, 20 mcg, 40 mcg and 80 mcg EE in trophoblast cell culture resulted the increasing activity of hTERT, whereas the highest activity occurred on 40 and 80 mcg of ethinyl estradiol group.

In the oral contraceptive pills, ethinyl estradiol level is ranged from 20 mcg to 50 mcg, whereas this dose may increase the activity of hTERT. This impacts to a state of post-curettage complete hydatidiform mole, where women should not to become pregnant until one year after the procedure. They are asked to choose hormonal oral contraceptive pill; yet, the pills increase the risk to develop malignant trophoblastic disease (MTD). This event does not happen for all patients taking hormonal oral contraceptive pills because the occurrence of malignancy is also determined by the balance among tumor suppressor gene, oncogene and DNA repair gene.

At the dose of 40 and 80 mcg, ethinyl estradiol increased dramatically the hTERT activity. It could cause the developing of malignant trophoblastic disease complete hydatidiform mole. Therefore, high level of ethinyl estradiol in CHM tissue would increase the activity of hTERT which raised the potency of MTD.

CONCLUSION

Ethinyl estradiol impacts to the increase of hTERT activity on complete hydatidiform mole cell culture.

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