Blighted ovum: Roles of human leukocyte antigen-E and natural killer cells

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ABSTRACT

Background: The failure of premature conception product is a specific problem for pregnant women. Blighted Ovum occurred in 37.5% of early pregnancy failure. About 15% of Blighted Ovum is caused by disorders of the immune system. Human Leukocyte Antigen-E and Natural Killer Cell are supposed to be the main factors of this pathomechanism.
Objective: To determine the expression of Human Leukocyte Antigen-E and Natural Killer Cell cells in the Blighted Ovum and normal pregnancy.
Methods: Observational analytic with cross sectional approach. Thirty-two samples were divided into two groups: 16 samples of Blighted Ovum group and 16 samples of normal pregnancy group.

uman Leukocyte Antigen-E and Natural Killer Cell cells in trophoblast of both groups were examined with immunohistochemical methods and statistical analysis that was done by t-test.
Results: The mean of expression of Human Leukocyte Antigen-E in the group of Blighted Ovum are 75.15±6:30, normal pregnancy are 83.13±11:26, p-value=0:02 (P <0.05). The mean of expression of NK cells in the group of a normal pregnancy is 79.72±6.63, while the mean of expression of NK cells in Blighted Ovum is 93.88±14.08, p-value=0.00 (P<0.05).
Conclusion: The expression of HLA-E was lower, and NK cells were higher in Blighted Ovum when compared with normal pregnancy.

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INTRODUCTION

Blighted Ovum also knew as an anembryonic pregnancy where there are pockets of intrauterine pregnancies without their fetus. Blighted Ovum is a form of failure of pregnancy that is common and occurs in the first semester.[1] Failure of the premature conception product is a specific problem for many pregnant women and also constitutes an obstetric complication. Criteria of premature conception product failure are spontaneous abortion, anembryonic pregnancy/Blighted Ovum, and fetal death.[2]

Blighted Ovum often occur at the start or very early in the pregnancy so not all cases can be diagnosed with Blighted Ovum which makes the number of an event difficult to know for certain. Approximately about 20% of all pregnancies end in miscarriage/abortion, and 80% of them occur at the age less than 12 weeks of pregnancy and one-third occur before the age of 8 weeks which is Blighted Ovum. The incidence of the general failure of premature conception product was 2.8%; Blighted Ovum is about 37.5% of premature conception product failure that occurs at 10–13 weeks of pregnancy.[2]

The causes of Blighted Ovum that frequently mentioned are chromosomal abnormalities (duplications or deletions), genetic abnormalities (mutations of genes), lack of good quality of the ovum and sperm, maternal age factor, endocrine, and immunologist factors.[3]

Pregnancy is a biological sample that is semi-allogeneic which may be rejected by the maternal immune system. The fetus is considered a foreign body by the mother that can be destroyed, so it must have the ability to evade the maternal immune system.[4] The fetus that is semi-allogeneic consisting of trophoblast cells at the stage of conception. During the first trimester of pregnancy, the placenta develops into a villous form with trophoblast cell types which has different functions.[5] Trophoblast is a factor that is allegedly essential to this phenomenon because it unfolds in the maternal–fetal interface, where there is a direct contact with the maternal immune system.[6]

The issue that is important in pregnancy is how the fetoplacental relationship avoids the rejection of the maternal immune system, although the fetus was bonded during pregnancy. Sir Peter Medawar, an immunology expert, suggests that a fetus that is semi-allogenic can survive because of their relationships with immune system and emphasis on maternal lymphocytes with the fetus.[7,8] Defects in the bond between the fetus and the mother, in this case, is trophoblast. Associated with various complications of pregnancy such as preeclampsia, intrauterine growth retardation, and failure of conception product (Blighted Ovum, recurrent miscarriage, and IUFD).[9]

Nowadays, Human Leukocyte Antigen (HLA) molecule is suspected to play an important role in maintaining the product of conception.[10]

Human Leukocyte Antigen (HLA)-E is a non-classical HLA class I, which plays an important role to regulate cytokine secretion as controller of trophoblast invasion and regulate immune tolerance locally in the placenta. Human Leukocyte Antigen-E is predominantly expressed in extravillous of trophoblast in the placenta and the formation of the hematopoietic system that is derived from the extraembryonic yolk sac. Human Leukocyte Antigen-E can protect trophoblasts from maternal–fetal immune intolerance and allow trophoblast cells to invade the uterus. Human Leukocyte Antigen-E plays an important role in immune tolerance during pregnancy.[11]

Appropriate HLA-E expression in the trophoblast is necessary to help trophoblasts invading maternal decidua and vascular system, so there is an increase in uterine perfusion that is needed during pregnancy. However, if the HLA-E was reduced or not being expressed, the trophoblast cells capability will be reduced and prevented from invading the uterus because it was perceived as non-self which has properties as antigens that trigger the formation of antibodies in the mother. These antibodies bind to antigens, and immunological reaction occurs that stimulates activation of proinflammatory cytokines, activated T-cells, and natural killer cells (NK) that will attack trophoblast cell itself, resulting in the failure of the conception product.[10]

Natural Killer cells in pregnant women have the dual function of NK cells through a receptor, which inhibits CD94/ NKG2A and activating via the CD94 receptor/ NKG2C. The imbalance of these receptors can lead to failure in pregnancy, Blighted Ovum as the example. Natural killer cells are activated through CD94 receptor / NKG2C will produce cytokines that attack the trophoblasts and ruin it so that the embryo will experience rejection in uterus.[12]

This study aims to determine the expression of HLA-E and NK cells in trophoblast of Blighted Ovum and normal pregnancy which is expected to be a predictor so that the incidence of the Blighted Ovum can be lowered.

METHODS

This study is an analytic observational with a cross-sectional study; it is carried out at the Obstetrics and Gynecology Hospital Dr. Moewardi Surakarta and hospital networks. Examination of the expression of HLA-E and NK cells with immunohistochemistry method that was performed at the Laboratory of Pathology, Faculty of Medicine, Universitas Sebelas Maret Surakarta.

The sample size was determined based on the formula of Murti (2010) in which each group was 16 samples. Thirteen (?) is the inclusion criteria for Blighted Ovum:

1. Maternal age 20–35 years.
2. The transvaginal ultrasound examination to visualize the intrauterine gestational sac, which is without an embryo or fetal pole structure on gestational age of 8 weeks with a gestational sac size of 2.5 cm, and the normal pregnancy are: aged 37–40 weeks, a single fetus in the intrauterine was found with no complications during pregnancy and childbirth.

All subjects were willing to participate in research. Exclusion criteria are mothers with diabetes mellitus, kidney disease, heart disease, liver disease, chronic hypertension, infectious diseases, smoking, and fetuses with major congenital abnormalities. Both groups were examined for the expression of HLA-E and NK cells in extravillous trophoblast where HLA-E was expressed and decidua NK cells accumulated in the vicinity.

The expressions of HLA-E and NK cells in the immunohistochemical examination is indicated with a bluish tint for weak positive, golden yellow for moderate positive, and brown for strong positive on the trophoblast. Trophoblast cell number was calculated based on the intensity of the expression which is then created with the percentage of overall cell numbers. The obtained percentage was then converted into numbers and calculated according to the formula of the histological score (HS). The ratings of (meaning look of?) HLA-E and NK cells expressed as HS was done based on the formula: $HS = (PK \times IK) + (PS \times IS) + (PL \times IL) + (PN \times IN)$

Formula's explanation:

P=Percentage	K=Strong	N=Negative
I=Intensity	L=Weak	S=Moderate

Histology score of HLA-E and NK cells (percentage of cells: 0–25%=negative; 26–50%=weak positive; 51–75%=moderate positive; 76–100%=strong positive). Histology score of HLA-E and NK cells (qualitative meaning: 0.00–3.75=negative; 3.76–7.50=weak positive; 7.51–11.25=moderate positive; 11.26–15, 00=strong positive).[14]

Reagents used for the expression of HLA-E using HLA-E antibody by Santacruz Biotechnology brand and reagents used for the expression of NK cells is a rabbit polyclonal antibody, an anti-KIR2DL1 bs-2419R BIOSs, Inc that is used to see the expression of NK cell receptors, that is KIR. Observations of the intensity of the color are done

by using a light microscope Olympus CX-21 series, the 400x magnification on a nine field of view. The number of HLA-E and NK cells in trophoblast is calculated based on the intensity of the reddish-brown color and the calculated percentage. The higher the histology score, the stronger the expression will be.

FEASIBILITY OF ETHIC

Feasibility of ethics was obtained from the Ethics Commission of Health Research of Dr. Moewardi Hospital/Faculty of Medicine, Universitas Sebelas Maret Surakarta No. 671 / VIII / HREC / 2016 dated August 4, 2016.

RESULTS

In Table 1, it is obtained that the average of maternal age 28.6 years ± 5:07, gestational age 36.71±3.7 weeks, systolic blood pressure of 122.53±33.56 mmHg and diastolic blood pressure of 80.78±18.88 mmHg. Levels of Hemoglobin 12:40±2:04 gr / dl, urea 15:28±4.80 mg/dl, creatinine 0.72±0:20 mg/dl, AST 58.06±109.04 U/I and ALT 29.46±48.84 U/I, random blood sugar of 92.81±10.89 mg/dl, the expression of HLA-E 37.18±30.43% of the cells/field of view and NK cells 49.24±39.40% of the cells/field of view.

Table 1   Characteristics of Research Subject

Variable	N	Min	Max	Mean	SD
Mother age (years)	32	19.00	35.00	28.06	5.07
Pregnancy age (weeks)	32	26.00	40.00	36.71	3.70
Systolic Blood Pressure (mmHg)	32	11.00	190.00	122.53	33.56
Diastolic Blood Pressure (mmHg)	32	60.00	130.00	80.78	18.88
Hemoglobin (gr/dl)	32	6.00	15.70	12.40	2.04
Ureum (mg/dl)	32	7.00	23.00	15.28	4.80
Creatinine (mg/dl)	32	0.40	1.20	0.72	0.20
SGOT (U/I)	32	14.00	587.00	58.06	109.04
SGPT (U/I)	32	6.00	202.00	29.46	48.84
Random Blood Glucose (mg/dl)	32	67.00	110.00	92.81	10.89
HLA-E (% cell/ field of view)	32	0.22	146.46	37.18	30.43
NK Cells (% cell/ field of view)	32	9.98	161.71	49.24	39.40

Table 2   Mean of HLA-E and NK Cells Expression in Trophoblast of Blighted Ovum and Normal Pregnancy

Variable	Blighted Ovum (N=16)	Normal Pregnancy (N=16)	p
HLA-E (% cell/ field of view)	75.15±6.30	83.13±11.26	0.02*
NK Cells (% cell/ field of view)	93.88±14.08	79.72±6.63	0.00*

*Significant p<0.05

From Table 2, it is obtained that the result of the mean of HLA-E expression in the trophoblast of Blighted Ovum group is lower at 75.15±6:30 when compared to the normal pregnant group at 83.13±11:26 with a value of p = 0.02 (p <0.05). The mean of expression of NK cells in trophoblast of Blighted Ovum group is higher at 93.88±14:08 when compared to normal pregnant group 79.72±6.63, with p=0.00 (p <0.05).

Figure 1 shows the expression of HLA-E on trophoblast of Blighted Ovum and normal pregnancy using the immunohistochemical method. The expression of HLA-E is shown with reddish brown color on the core extends into the cytoplasm, to the strong positive, moderate positive: dark brown, weak positive: light brown, and negative: blue with series of Olympus CX21 microscope with a magnification of 400X. The expression of HLA-E on a Blighted Ovum seems lower/ weaker (figure A) when compared to expression in normal pregnancy (figure B).

Figure 2 shows expression of NK cells in trophoblast of Blighted Ovum and normal pregnancy using the immunohistochemical method. Expression of NK cells are shown with reddish brown color on the core extends into the cytoplasm, to the strong positive, moderate positive: dark brown, weak positive: light brown, and negative: blue with series of Olympus CX21 microscope with a magnification of 400X. Expression of NK cells in Blighted Ovum looked stronger/ higher (figure A) when compared to expression in normal pregnancy (figure B).

DISCUSSION

Pregnancy is a unique situation where maternal–fetal tolerance is very important to ensure the development of the placenta and fetus. Inadequate placentation would cause undesirable effects on the mother or fetus, including failure of the premature products of conception such as Blighted Ovum, preeclampsia, slowed fetal growth, and IUFD.[15]

Human Leukocyte Antigen-E has a very important role in the development of pregnancy. Class I antigens are expressed on trophoblast that can help the fetus to avoid the maternal immune system attacks and acts on the scene of the relationship fetoplacental through receptor CD94/ NKG2A NK cells that cause normal process of pregnancy.[10]

In this study, the mean of expression of HLA-E on trophoblast was lower, and expression of NK cells was higher in Blighted Ovum when compared with normal pregnancy. The results of the present study according to research conducted by Fotoohi (2016) showed that HLA-E was lower in spontaneous
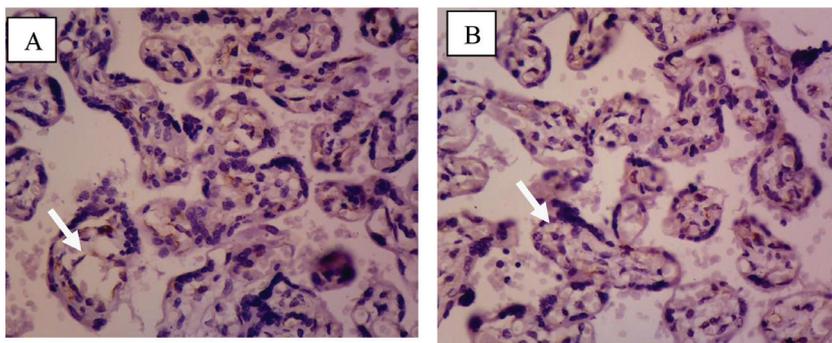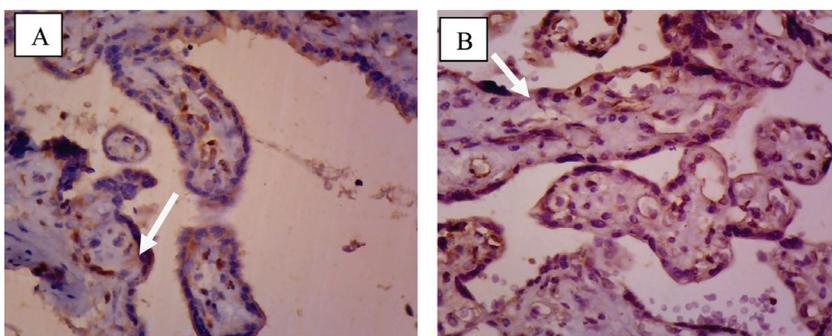
A. HLA-E Expression in Trophoblast of Blighted Ovum

B. HLA-E Expression in Trophoblast of Normal Pregnancy

Figure 1 HLA-E Expression in Trophoblast of Blighted Ovum and Normal Pregnancy

A. NK Cells Expression in Trophoblast of Blighted Ovum

B. NK Cells Expression in Trophoblast of Normal Pregnancy

Figure 2 NK Cells Expression in Trophoblast of Blighted Ovum and Normal Pregnancy

abortion repeatedly. This includes Blighted Ovum than normal pregnancy because of HLA-E is an antigen that interacts with NKG2A which is a receptor inhibitor of NK cells. That help the fetus during maternal immune response so that when there is a deficiency of HLA-E, the fetus will be more vulnerable to be attacked by NK cell activity which would lead to Blighted Ovum.[11] Human Leukocyte Antigen-E increased the success of pregnancy since HLA-E is believed to protect the fetus from maternal immune attacks through the interaction of CD94/ NKG2A and NK cells.[10] The study according to research that was conducted by Gelmini et al. (2016), which says that the Human Leukocyte Antigen-E has an important role in modulating the immune response in conjunction with the success and viability of a conception of pregnancy.[16]

Morandi and Pistoia in 2014 revealed that during pregnancy, Human Leukocyte Antigen-E is expressed in trophoblast cells and plays an inhibitory role of NK cells function. Human Leukocyte Antigen-E will interact with less CD94 to turn on the receptors of NK cells. The Association of HLA-E with NKG2A receptors is

six times more powerful than NKG2A receptor that bound with NK cells. Mechanisms in maternal fetal surface is important in regulating the immune response to prevent the destruction of fetal tissues by NK cells.[17]

Fotoohi et al. 2016 said that the expression of HLA-E on cytotrophoblast suggested an important role in creating good conditions at the surface of the fetus and the mother so that the fetus can be protected from the maternal immune rejection response. Human Leukocyte Antigen-E interacts with CD94/ NKG2A, a lectin which acts in NK cell inhibitory receptors, which play an important role in the inhibition of NK cells activity.[11]

Matter in contrast to this study in 2013 which found that HLA-E on recurrent spontaneous abortion including a Blighted Ovum is not significantly different when compared with normal pregnancy, it is possible the number of samples is much less.[6]

As in this study, Haumonte (2014) also obtained (?) high levels of NK cells in cases of a Blighted Ovum.[15] Failure of maternal immune adaptation will generate increased Th1 which will increase the activation of NK cells and secrete cytokines that are detrimental to the trophoblast, which are TNF-α, IFN -ɣ, TGF-β, and IL-2. This situation will lead to Blighted Ovum.[18] The CD56 bright NK cells greatly increased in recurrent spontaneous abortion and Blighted Ovum. This is because of the NK cells to produce Th1 cells which would cause pregnancy outcomes were not as good as Blighted Ovum.[19]

CONCLUSION

The expression of HLA-E was lower, and NK cells were higher in Blighted Ovum when compared to normal pregnancy.

CONFLICT OF INTEREST STATEMENT

Authors declare that there are no conflicts of interest related to this research, the writer or publication of this article.

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