HYALURONAN, TNF-α AND ESTRADIOL CONCENTRATIONS IN INFERTILE WOMEN WITH ENDOMETRIOSIS

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ABSTRACT

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic inflammatory reaction. The association between endometriosis and infertility has not yet been clarified but one of the suspected factors is the abnormality of oocyte development. The higher concentration of hyaluronan has been identified as crucial factor for abnormality of oocyte maturation. Estradiol has been correlated with oocyte development. The purpose of this study was to clarify the concentrations of hyaluronan, TNF-α and estradiol in infertile women with endometriosis. Using a cross sectional study, peritoneal and follicular fluid were aspirated laparoscopically from 38 infertile women in mid cycle and divided into 3 groups: non endometriosis, mild and severe endometriosis in tertiary infertility clinic. Elisa laboratory technique was performed to all samples to detect peritoneal fluid TNF-α, estradiol and follicular fluid hyaluronan concentrations. In control, mild and severe endometriosis groups, the concentrations of hyaluronan were as follow: 954.14 ± 294.87; 1499.29 ± 131.49 and 1786.77 ± 44.64 pg/ml, TNF-α: 124.09 ± 99.28; 244.03 ± 106.51 and 409.08 ± 231 pg/ml, estradiol: 298.13 ± 131.37; 567.98 ± 368.82 and 750.50 ± 181 pg/ml, respectively. There were significant difference concentrations of hyaluronan, TNF-α and estradiol between 3 groups (p<0.05). The correlations were found among TNF-α, estradiol and hyaluronan, but the significant correlation was only found between estradiol and hyaluronan (p<0.05). In conclusion, the increase concentration of hyaluronan in endometriosis might be correlated with the abnormality of oocyte maturation. The inflammatory and hormonal milieu play a role in the abnormality of oocyte maturation in infertile women with endometriosis, but more study is needed.

Keywords: hyaluronan, TNF-α, estradiol, endometriosis, infertility

INTRODUCTION

Endometriosis is a disease characterized by the presence of content such as endometrial tissue located outside the uterine cavity and can lead to chronic inflammatory reaction (Kennedy et al., 2005). Pain and infertility are the two most common complaints found in patients with endometriosis. Until this time, the handling of infertile patients with endometriosis is still not satisfactory, it appears from the pregnancy rate is still low at less than 54% compared with other causes of infertility. In the observation of meta-analysis on this IVF fertilization rate also results obtained, the number of oocytes, estradiol levels and implantation rates in endometriosis patients is lower compared with infertile non-endometriosis (Barnhart et al., 2002). Apart from the adhesion of reproductive organs, oocyte maturation due to mental disorders folliculogenesis process also alleged role in the mechanism of occurrence of infertility in endometriosis. But so far the mechanism of oocyte maturation disorders in infertile patients with endometriosis is still unknown.

Folliculogenesis passing growing and maturation process of the granulosa cells, theca and oocyte, so it will produce a mature oocyte ready for fertilization. External and internal environmental conditions determine the success folliculogenesis ovary. One of the important role Hyaluronan, a glycosaminoglycan produced cumulus granulosa cells were functioning in the process of cumulus expansion just before ovulation (Sperroff, 2005; Rajkovic, 2005). The purpose of this research study is to investigate the mechanism of oocyte maturation disorders in infertile patients with endometriosis through observation of the differences and correlation Hyaluronan concentration, TNF-α and Estradiol on non-endometriosis patients, mild and severe endometriosis.

MATERIALS AND METHODS

This study is a cross sectional analytic observational study undertaken in patients with infertility women of
reproductive age (20-35 years) at the Fertility Clinic, Dr Amrita Graha Soetomo the period January to December 2007. The diagnosis of endometriosis using laparoscopy is done just before ovulation. Revised classification of the American Society for Reproductive Medicine is used to determine the diagnosis of endometriosis and subsequently divided into three groups, non-endometriosis, mild endometriosis and severe endometriosis. When laparoscopic fluid follicle aspiration was performed to check levels of Hyaluronan and peritoneal aspiration fluid to check levels of TNF-α and estradiol. All study samples are stored in the closet cooling temperature of minus 80°C and subsequently analyzed using ELISA according to the concentration of the agreed procedures (Abgent San Diego).

Except for endometriosis, the results of the investigation of infertility in the study in normal subjects and all study subjects not using hormonal medications within 6 months prior to laparoscopy. Based on the objectives to be achieved by processing and analyzing the data using statistical computer program SPSS for Windows. Airworthiness research ethics committee was obtained from Dr Soetomo.

**RESULTS**

In this study, 38 study subjects, ie patients infertile women who come to the clinic Fertility Graha Amarta Dr Sutomo Surabaya. Based on the results of 38 laparoscopic patients infertile women were divided into three groups: group 1 (non-endometriosis infertile patients/controls) totaling 16 people, group 2 (patients with mild endometriosis) totaling 13 people and 3 groups (patients with severe endometriosis) totaling 9 people. The research results are described as follows. The measurement of hyaluronan fluid follicles, TNF-α and estradiol fluid peritoneum by Elisa method, with results as listed below.

Table 1. Average concentrations of hyaluronan, TNF-α and estradiol on control group, mild and severe endometriosis.

<table>
<thead>
<tr>
<th></th>
<th>Hyaluronan</th>
<th>TNF-α</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
<td>X</td>
<td>SD</td>
<td>X</td>
</tr>
<tr>
<td>Control</td>
<td>11.23</td>
<td>204.87</td>
<td>99.28</td>
</tr>
<tr>
<td>Mild Endometriosis</td>
<td>1499.29</td>
<td>131.49</td>
<td>244.03</td>
</tr>
<tr>
<td>Severe Endometriosis</td>
<td>1700.77</td>
<td>44.64</td>
<td>409.08</td>
</tr>
</tbody>
</table>

Kolmogorov Smirnov one sample at levels of hyaluronan, TNF-α and oestradiol in the control group, mild endometriosis and severe endometriosis all showed normal distribution (P > 0.05). Using ANOVA showed that the levels of hyaluronan, TNF-α and oestradiol in the control group, mild endometriosis and severe endometriosis all significantly different (P < 0.05). Post Hoc LSD test showed that hyaluronan levels were significantly different on all the different test groups, control, endometriosis light and heavy endometriosis (P <0.05). The correlation between these three variables showed that only estradiol levels and hyaluronan Representatives have significant correlation, whereas TNF-α and hyaluronan; TNF-α and estradiol were not significant. (Figure 1, 2 and 3)
Hyaluronan, TNF-α and Estradiol Concentrations in Infertile Women with Endometriosis (Hendarto H et al.)

DISCUSSION

Folliculogenesis is growing and maturation process of female germ cells within the ovarian somatic cells to become mature oocyte ready for fertilization (Rajkovic, 2006). Ovarian follicle is the functional unit of female reproduction, the cell consists of oocyte, granulosa cells and thecal cells. Folliculogenesis involves close cooperation between the three cells above and involves many processes including: proliferation of granulosa cells, oocyte meiosis, steroidogenesis theca cells, cumulus expansion and others (Knight, 2006). Shortly after Lutenizing Hormone surge in the menstrual cycle can cause cumulus expansion is a viscoelastic extracellular matrix synthesis by cumulus granulosa cells for oocyte complexes wrap. Hyaluronan is a dominant component in the matrix ekstraseluseler useful to prepare and protect the oocyte against proteolytic enzymes and mechanical stress during ovulation. Hyaluronan or hyaluronic acid is also called glycosaminoglycan with high molecular weight consisting of acid and N-acetylglucosamine glucoronat shaped viscoelastic solution. Although an inert molecule with a structure, known as a ligand Hyaluronan is involved in the control of dynamic multiple biological functions such as cell migration, proliferation, wound healing and inflammation (Stock et al., 2002). In this study, the results of Hyaluronan levels in fluid follicles in the control group, mild endometriosis and severe endometriosis are: 954.14 ± 294.87, 1499.29 ± 131.49 and 1786.77 ± 44.64 pg/ml. In Kruskal Wallis test showed significant differences between follicular fluid levels of Hyaluronan the three groups were (p = 0.000). These results indicate that the more severe stages of endometriosis the higher levels of Hyaluronan. Elevated levels of Hyaluronan is expected to cause disruption of extracellular matrix maturation of oocytes and become thick so hard penetrated by sperm during fertilization.

In a study by Saito who examined levels of Hyaluronan on fluid follicles taken from patients following in vitro fertilization programs get the results that the content of Hyaluronan in the group that failed fertilized oocyte higher than the group that with successful oocyte sterilization. Saito concludes that high levels of lead Hyaluronan become overmature oocytes thus losing the ability to normally fertilized (Saito et al, 2000). Saito research results could be the basis that the high levels of Hyaluronan fluid follicles in patients with endometriosis will interfere with the final result of the process folliculogenesis overmature oocytes resulting in infertility.

In this study also showed that the levels of TNF-α fluid peritoneum in the control group, mild endometriosis and severe endometriosis all significantly different (P <0.05). These results indicate that the more severe stages of endometriosis the higher levels of TNF-α. Fluid peritoneum is a dynamic environment that connects with the reproductive organs of the immune system. The flow of blood through menstruation through the fallopian tubes into the pelvic cavity in endometriosis peritoneal fluid changing atmosphere (Diaa et al., 2003). The number and activity of peritoneal macrophages in fluid increases. The inflammatory response, and tissue repair neovascularization highly dependent on macrophages and cytokines production, namely Interleukin (IL) -1, IL-2, IL-6, IL-8, IL-10, TNF-α (Carlberg et al. , 2000). Bedaiwy mention of the examined cytokines, namely IL-10, IL-6, IL-8, IL-12, IL-13, and TNF-α. TNF-α was only having a more meaningful predictive value as a diagnostic tool endometriosis (Bedaiwy et al., 2002).
Our research findings strengthen the existing theory that is an increase in immunological components fluid peritoneal endometriosis patients, especially TNF-α has an essential role in the pathogenesis, and progression of endometriosis.

Estradiol levels fluid peritoneum in the control group, severe endometriosis and mild endometriosis showed significantly different (P <0.05). It appears that the more severe stages of endometriosis the higher levels of estradiol. This is in accordance with the opinion that the development of endometriosis is dependent to estrogen. Apart from the granulosa cells, elevated levels of estradiol in endometriosis peritoneal fluid derived from local estradiol production in peritoneal implants of endometriosis (Bulun et al., 2000). High estradiol levels is expected to increase through the activation of enzyme secretion Hyaluronan Hyaluronic acid synthetase, which is in line with research conducted by Uzuka of Hyaluronic acid synthetase enzyme induction by estrogen in mouse skin. (Uzuka et al., 1981). In our study proved that in patients with endometriosis there was significant correlation between levels of estradiol and hyaluronan (P <0.05), while the correlation between levels of TNF-α and Hyaluronan not prove meaningful.

CONCLUSION

The conclusion of this research is on infertile patients with endometriosis have increased levels of peritoneal fluid Hyaluronan possible disturbances associated with oocyte maturation. Environmental hormone levels are higher in endometriosis estadiol which are associated with increased levels of Hyaluronan. To the fore further research is still needed to complete the data associated with impaired oocyte maturation in infertile patients with endometriosis.

REFERENCES


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