

# The pentaxin 3 level profile of ovarian follicle fluid in infertile patients with endometriosis

*by* Hendy Hendarto

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## **The Pentraxin 3 Level Profile of Ovarian Follicle Fluid in Infertile Patients with Endometriosis**

**Wisnumurti DP, Primariawan RY, Hendarto H**

Department of Obstetrics and Gynecology  
Faculty of Medicine, Airlangga University,  
Dr Soetomo Hospital, Surabaya

### **ABSTRAK**

*Endometriosis menempati proporsi tertinggi penyebab infertilitas. Salah satu faktor dalam endometriosis yang diduga menjadi penyebab infertilitas adalah penurunan kualitas oosit, yang ditentukan oleh ekspansi kumulus. Ekspansi sel kumulus secara tidak langsung menyebabkan produksi hyaluronic acid (HA), prostaglandin E2 (PGE2), Pentraxin (PTX3), dan tumor necrosis factor alpha-induced protein 6 (TNF- $\alpha$ IP6). Tujuan dari penelitian ini adalah untuk membuktikan perbedaan Pentraxin3 cairan folikel ovarium pada pasien infertil dengan endometriosis stadium berat, endometriosis tahap ringan dan tanpa endometriosis. Penelitian ini dilakukan di Klinik Fertilitas Graha Amerta RSUD dr. Soetomo, Surabaya, dan Laboratorium Biokimia Fakultas Ilmu Universitas Brawijaya, Malang. Penelitian ini merupakan penelitian analitik observasional dengan rancang cross-sectional pada sampel cairan folikel dengan mengamati dan mengukur variabel dari cairan folikel studi subjek wanita infertil yang didiagnosis dengan menggunakan laparoskopi. Pengukuran Pentraxin-3 level pada cairan folikel ovarium menggunakan ELISA. Didapatkan 300 sampel berdasarkan temuan laparoskopi, sampel dikategorikan menjadi tiga kelompok: Non-endometriosis subur/kontrol, endometriosis ringan subur dan endometriosis berat subur. Rerata Tingkat PTX total  $-3.96 \pm 2.71$  ng/ml. Dengan tingkat PTX-3 pada kelompok kontrol  $1.21 \pm 0.23$  ng/ml, endometriosis ringan  $3.47 \pm 0.38$  ng/ml, dan endometriosis berat  $7.21 \pm 1.73$  ng/ml. Analisis korelasi antara Pentraxin-3 tingkat dengan berat endometriosis  $rs = 0.943$  dan  $p = 0.001$ , menunjukkan korelasi yang tinggi. Sebagai kesimpulan, makin berat endometriosis, makin tinggi kadar Pentraxin-3 (MOG 2012;20:8-13)*

**Kata kunci:** PTX-3, endometriosis, infertil, matriks ekstrasel

### **ABSTRACT**

*Endometriosis gives the most proportion as the cause of infertility. One of the factor in endometriosis suspected to be the cause of infertility is the decrease of oocyte quality, which is determined by cumulus expansion. Cumulus cell expansion indirectly causes the production of hyaluronic acid (HA), prostaglandin E2 (PGE2), pentraxin (PTX3), and tumor necrosis factor alpha-induced protein 6 (TNF- $\alpha$ IP6). The objective of this study was to prove the difference in Pentraxin3 level of ovarian follicle fluid in infertile patients with severe stage endometriosis, mild stage endometriosis and without endometriosis. This study was held in Fertility Clinic of Graha Amerta dr. Sutomo Hospital, Surabaya, and Biochemistry Laboratory of Science Faculty Brawijaya University, Malang. This study was an analytic-observational study in human with cross-section study design on follicle fluid samples by observing and measuring the variable from study subject follicle fluid of infertile women that is diagnosed using laparoscopy. The measurement of Pentraxin-3 level on ovarian follicle fluid was done by ELISA. We obtained 300 samples based on laparoscopic findings. The sample were categorized into three groups: Non-endometriosis infertile/control, mild endometriosis infertile and severe endometriosis infertile. The total mean PTX level was  $-3.96 \pm 2.71$  ng/ml. With the level of PTX-3 on control group  $1.21 \pm 0.23$  ng/ml, mild endometriosis  $3.47 \pm 0.38$  ng/ml, and severe endometriosis  $7.21 \pm 1.73$  ng/ml. Correlation analysis between Pentraxin-3 level with endometriosis severity  $rs = 0.943$  and  $p = 0.001$ , showed high correlation. In conclusion, the more severe endometriosis is the level of Pentraxin-3 is also higher. (MOG 2012;20:8-13)*

**Keywords :** PTX-3, endometriosis, infertile, extracellular matrix

**Correspondence:** Wisnumurti DP, Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Dr Soetomo Hospital, Surabaya, wisnumurti\_dr@yahoo.com

### **INTRODUCTION**

Endometriosis gives the most proportion as the cause of infertility. Based on study held in 1988-2000 the percentage of endometriosis on infertile patients is 33% and mild endometriosis gives 68% from total endometriosis cases. As reported by Gupta on 2008, the

incidence of endometriosis on infertility increases 60,7%.<sup>1</sup> As reported by Samsul Hadi on 2002, there is an increase in endometriosis incidence on infertility cases after laparoscopy at infertility clinic Faculty of Medicine Airlangga University/dr. Soetomo hospital, Surabaya.<sup>2</sup>

It has been known that there are several factors related to infertility on endometriosis patients, one of which is the mechanical disturbance such as reproductive organs adhesion that makes the alteration of oocyte uptake by fimbria during ovulation and resistance of sperma-oocyte union at fallopian tube.<sup>3</sup> This mechanical disturbance can explain the infertility on severe endometriosis, while on mild endometriosis without reproductive organs adhesion the mechanism is still difficult to be explained.<sup>4</sup> One of the factor in endometriosis suspected to be the cause of infertility is the decrease of oocyte quality.<sup>1</sup>

From the previous study, there is chronic inflammation on endometriosis that increases the inflammation cytokines and TNF- $\alpha$  level has the highest level than the other cytokines. TNF- $\alpha$  cytokines on granulosa cells on women with endometriosis can alter the synchronization between oocyte maturation, ovulation, oocyte uptake, the decrease on granulosa cells and the increase of apoptotic cells that result in the decrease in fertilization rate.<sup>5</sup> Before ovulation there will be a cumulus expansion that will determine the oocyte quality.<sup>6</sup> Moreover, the succesful follicle rupture and fertilization are influenced by disturbance of cumulus matrix composition and functional capacity.<sup>7,8</sup> Cumulus cell expansion is a process like inflammation reaction that causes cells musification that surrounds oocyte. This process causes the cumulus cells to be attached to oocyte inside extracellular matrix sheath so it can protect oocyte during follicle extrusion and accommodate the binding of sperm and fertilization process.<sup>9</sup> Cumulus cell expansion indirectly causes the production of hyaluronic acid (HA), prostaglandin E2 (PGE2), pentraxin (PTX3), tumor necrosis factor alpha induced proitein 6 (TNFAIP6), that is regulated by GDF9 in vitro.<sup>10</sup>

On in vitro study it is stated that in rat cumulus Ptx3-/- can't bind hyaluronic acid inside matrix structure, but, although it has been proven that the binding of HA-heavy chain and binding of TNFAIP6-heavy chain have a role in cumulus matrix stabilization by binding the HA molecules that is separated one another, there's an instability of cumulus matrix on rat with Ptx3 deficiency where the both complexes are inside. So, it can be said that PTX3 has a role on cumulus matrix stabilization via independent mechanism.<sup>10,11</sup> PTX3 construct a binding with TNFAIP6 on the different surface by binding HA and this binding creates a stable matrix from peripheral cumulus for pellucida zone.<sup>12</sup> How is the role of PTX3 on endometriosis? Until now, the role of PTX3 in the decrease of oocyte quality on endometriosis is still unknown.

## MATERIALS AND METHODS

This study is an analitic-observational study in human with cross-section study design using consecutive sampling technique on follicle fluid samples by observing and measuring the variable from study subject follicular fluid sample from all patients with infertility on Fertility Clinic Grha Amerta dr. Soetomo hospital, Surabaya on April until June 2010 and has been diagnosed as sever and mild and non-endometriosis by laparoscopic surgery. The study was held in Fertility Clinic of Graha Amerta dr. Sutomo Hospital, Surabaya and Biochemistry Laboratory of Science Faculty Brawijaya University, Malang. The time and place of study was around March. Inclusion Criteria: 20-40 years old, regular menstrual cycle 28 days  $\pm$  3 days, laboratory parameter is within normal limit, LH (+) and from transvaginal sonography the minimum diameter of follicle is 17 mm, willing to be included in study by signing the informed consent. Exclusion Criteria: internal genitalia organs adhesion which is not endometriosis, pelvic inflammatory disease, intraabdominal infection (eg: appendicitis), PCOS, obesity, ovarian tumor except endometrioma, compliation during diagnostic laparoscopy, already received hormonal therapy within last 6 months.

The sample was taken from follicular fluid of infertile patients had been diagnosed by laparoscopy in the middle of menstruation cycle near ovulation. The ovulation time was detrmind using serial urine LH test to observe the LH surge. If LH test (+) then we do the transvaginal USG, if the minimun follicular diameter is 17 mm we continue to laparoscopy. During laparoscopy, we determine the presence of endometriosis or not. If there is an endometriosis, then we classify by the severity using rAFS (Stadium 1 until 4) In this study, stadium 1 and 2 is included in mild endometriosis and stadium 3 and 4 is included in severe endometriosis. The sample in this study is divided into 3 which is the non-endometriosis patients, mild endometriosis patients and severe endometriosis patients. We do the follicle fluid aspiration as much as 1 cc using laparoscopic needle. The follicle fluid is centrifused to separate the granulosa cell with follicle fluid. Supernatan is stored in -80°C temperature. The sample is stored in cold storage -80C. Sample has been isolated in the same amount, 200 microlyte, then we check using ELISA method.

The hypothesis of this study is based on the fact that on endometriosis there is apoptosis on granulosa cells of ovarian follicle caused by inflammation reaction that causes the increase of TNFa peritonealfuid and oxidativve stress. The decrease of granulosa cells number causes the alteration in nutritional supple



needed to produce some growth factors which has role in oocyte maturation, one of which is GDF-9. The decrease of GDF-9 can cause disturbance of granulosa cell proliferation so the granulosa cells won't develop. The apoptosis process increases with the decrease of granulosa cell proliferation will also decrease the Pentraxin3 level produced by granulosa cells so the Pentraxin3 level on follicle fluid is also decreased. The decrease of Pentraxin-3 level will make the extracellular matrix needed for ovulation having early degradation during ovulation and causes infertility.

The data of this study is recorded in data sampling form specially designed for this study. Based on the main purpose of the study the data processing and analysis is done using descriptive and inferential statistic by SPSS for Windows program and we do comparison test using Anova with the variable of Pentraxin3 on each group of severe endometriosis, mild endometriosis and control group.

## RESULTS AND DISCUSSION

This study was designed to learn the role of Pentraxin3 on infertile patients with endometriosis, so we must observe the sample characteristic that can influence the fertility like age and the marital year.

We got the total mean age of study object  $32.93 \pm 4.73$ . The youngest subject is 23 years old and the oldest is 40

years old. From the normality test using Kolmogorov Smirnov test on the age of study subject we got normal distribution data on control group ( $p = 0.440$ ), mild endometriosis ( $p = 0.814$ ) and severe endometriosis ( $p = 0.899$ ).

The average rate of marital year of study subject is  $6.03 \pm 3.81$ . The marital year variable is used to determine the infertility duration although there are several subjects which is included in secondary infertility category. From the result of normality test using Kolmogorov Smirnov test on the marital year characteristic on each subject group we got normal distribution data on control group ( $p = 0.830$ ), mild endometriosis ( $p = 0.936$ ) and severe endometriosis ( $p = 0.937$ ). To determine whether there is a difference of characteristic between the age and marital year on each group we use ANOVA comparison test. There is no significant difference statistically ( $p = 0.744$ ) on age and marital year characteristic ( $p = 0.248$ ).

The measurement of Pentraxin-3 level on ovarian follicle fluid is by ELISA method. In this study, we got the minimal PTX3 level 0.755 ng/ml on control group and the highest level at 10.544 ng at severe endometriosis group. We got average total PTX3  $3.96 \pm 2.71$  ng/ml with the PTX-3 level on control  $1.21 \pm 0.23$  ng/ml, mild endometriosis  $3.47 \pm 0.38$  ng/ml, and severe endometriosis  $7.21 \pm 1.73$  ng/ml.

Table 1. Age normality test on control group, severe endometriosis group, mild endometriosis group

Groups	Age(year)						Normality
	n	X	SD	Minimum	Maximum	p	
Control	10	32.50	4.65	27	38	0.440	Normal
Mild Endometriosis	10	32.40	5.44	23	38	0.814	Normal
Severe Endometriosis	10	33.90	4.41	27	40	0.899	Normal
Total	30	32.93	4.73	23	40	0.341	Normal

Table 2 Marital age normality test control group, severe endometriosis group, mild endometriosis group

Groups	Age(year)						Normality
	n	X	SD	Minimum	Maximum	p	
Control	10	7.10	4.46	3	17	0.830	Normal
Mild Endometriosis	10	4.40	2.17	1	8	0.936	Normal
Severe Endometriosis	10	6.60	4.20	1	15	0.937	Normal
Total	30	6.03	3.81	1	17	0.350	Normal

Table 3. Anova of age and marital age between control group, severe endometriosis group, mild endometriosis group

Variabel	Test	
	P	Note
Age	0.74	Unsignificant
Marital	0.248	Unsignificant

Table 4. Pentraxin3 level on infertile women without endometriosis, with mild and sever endometriosis

Grade	Pentraxin-3 Concentration				Brown Forsythe
	X	SD	Min	Max	
Control	1.21 <sup>a</sup>	0.23	0.755	1.500	F=86.519 p = 0.001*
Mild	3.47 <sup>b</sup>	0.38	2.877	3.902	
Severe	7.21 <sup>c</sup>	1.73	5.132	10.544	

Note: \* significant at  $\alpha=0.05$

a,b,c superscript shows the difference between group (based on multiple comparison metode Games-Howell)

The correlation analysis Pentraxin-3 level with the severity of endometriosis  $r_s = 0.943$  dan  $p = 0.000$ , shows significant correlation. The more sever the endometriosis the more Pentraxin-3 level exists.

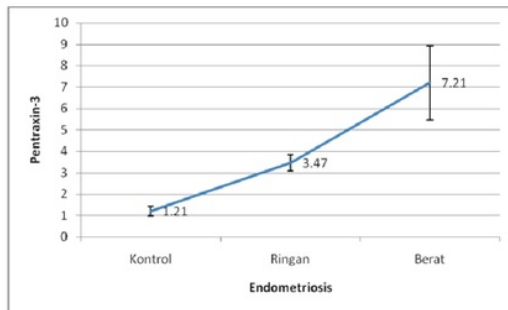


Figure 1. Concentration of PTX-3 on control group, mild and sever endometriosis

From total 30 study subjects, we got average age of all subject is 32.93 years old. Average age of subject with mild endometriosis is 32.40 years old, while severe endometriosis group is 33.90 years old. This suits the study held by Verselini (2007) that the most endometriosis cases is at the age range of 31-35 years old (29.7%).<sup>13</sup> The duration of infertility from this study is at the average marital year of 6.03 years with the least is 1 year and the longest is 17 years. If compared between each group and marital year we can conclude that there's no significant difference between each group. This suits the Khadem's study (2003), there's no

correlation between the duration of infertility with the severity of endometriosis (One-way ANOVA,  $F=1.6$ ,  $df=3.13$ ,  $P = 0.237$ ).<sup>14</sup>

On table 3 we got PTX-3 level in follicle fluid on endometriosis group is higher than control group and severe endometriosis group has higher PTX-3 level than control group and mild endometriosis group. The correlation analysis between Pentraxin-3 level with the severity of endometriosis  $r_s = 0.943$  and  $p = 0.000$ , shows very significant correlation. The more severe the endometriosis the higher the Pentraxin-3 level is. From this study we got different result from the hypothesis. The difference of the result of this study shows that there might be another pathway that is more dominant in the process of Pentraxin3 increase rather than granulosa cells apoptosis pathway. From the exploration deduction and extrapolation result from many studied shows that Pentraxin3 which is one of the main component of extracellular matrix is connected with inflammation process. As been stated by Han on 2005, the production of PTX-3 is induced by lipopolysaccharide (LPS), interleukin-1 (IL-1) and TNF- $\alpha$ .<sup>15</sup>

Peritoneal fluid is a specific environment that is dynamical, connects the reproductive organs with immune system. The presence of menstrual backflow with endometriotic cells flows through fallopian tube into pelvic cavity will alter the peritoneal fluid environment.<sup>16</sup> Bedaiwy (2002) reported that the increase of macrophage numbers and activity in peritoneal fluid with the production of cytokines : IL-1, IL-2, IL-6, IL-8, IL10, TNF- $\alpha$  induces inflammatory response. From the studied cytokines IL-1 $\beta$ , IL-6, IL-8, IL-12, IL-13 and TNF- $\alpha$  that increase on peritoneal fluid because of inflammation response, only TNF- $\alpha$  has significant predicting value as diagnostic tool for endometriosis.<sup>17,18</sup>

It has been proven that in ovarium TNF- $\alpha$  is produced by granulosa cells and macrophage.<sup>5</sup> Wu reported, there is 5-15% macrophages and monocytes inside follicular fluid. In most organs, macrophage is involved in tissue homeostasis via various functional abilities. One of which is by producing cytokines. On flow cytometric test in infertile patients with endometriosis there is higher macrophage level than infertile patients without

endometriosis. In the end, there will be an increase of cytokine level especially TNF- $\alpha$ .<sup>19</sup> There are various mechanism that can explain the increase of PTX-3 on ovarium follicle fluid endometriosis patients. On endothelial cells, IL-1 and TNF- $\alpha$  induces PTX-3 expression in minimum level. While PTX-3 expression on dendritic cell and macrophage is induced by LPS, IL-1 and TNF- $\alpha$ .<sup>20</sup> It has been reported by Han, TNF- $\alpha$  can induce PTX-3 expression on epithelial cells on pulmonary epithelial cells via JNK pathway by activating JNK.<sup>15</sup> Most likely the PTX-3 level on follicle fluid is really induced by macrophage and the increase of TNF- $\alpha$  level on follicle fluid in endometriosis patients.<sup>5</sup> The increase of Pentraxin3 level is an adaptation response to inflammation so the oocyte can be matured to maintain the life cycle in a stable extracellular matrix. So the increase of Pentraxin level is favorable process. On the other side, the increase of Pentraxin3 level and extracellular matrix will cause sperm becomes difficult to penetrate and fertilize. As seen from the successful fertilization, the increase of Pentraxin3 is an unfavorable event to sperm. The presence of inflammation reaction marked with the high rate of TNF- $\alpha$  on peritoneum fluid and follicle fluid in endometriosis patients might influence the production of Pentraxin3. As we know before, there is an increase of Pentraxin3 level locally on the inflamed tissue. As a adaptation mechanism, inside the ovarium, macrophages will be activated to secrete Pentraxin3. On the other way, on follicle fluid, beside binds to the death receptor on granulosa cells, TNF- $\alpha$  will also activate JNK so the granulosa which doesn't have apoptosis process will secrete more Pentraxin3.

## CONCLUSION

1 The Pentraxin-3 level profile of ovarian follicle fluid in infertile patients with endometriosis is high. The level of Pentraxin-3 is along with the severity of endometriosis. The more severe endometriosis, the higher the level of Pentraxin-3.

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