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The involvement of multifunctional TGF-β and related cytokines in pathogenesis of endometriosis

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Highlights:

- Deregulated of TGF-β activity has been implicated in pathogenesis of endometriosis
- In endometriosis, TGF-β could affect the differentiation of Th cells towards Th17 or Treg cells,
- Producing more IL-17 and IL-10 to peritoneal fluid might have indirect influence on inflammation.
- tTGF-β may reflect real secretion of this factor in peritoneal fluid of women with endometriosis.

Abstract:

Purpose: Transforming growth factor β (TGF-β) is one of the major immune and inflammation factors responsible for regulating cell proliferation, differentiation, angiogenesis, and immune responses. Deregulated TGF-β activity, especially its influence in peritoneal cytokine cross-talk, has been implicated in pathologies of endometriosis. The aim of this study was to determine whether TGF-β could be involved in the pathogenesis of endometriosis. For this purpose, we evaluated concentrations of TGFβ1, TGF-β2, TGF-β3 and interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-21 and IL-22 in peritoneal fluid (PF) and serum of women with endometriosis.

Methods: A total of 66 women of reproductive age were involved in the study, 51 endometriosis patients, and 15 women from the control group. PF and serum levels of all cytokines were measured with ELISA in women with or without endometriosis.

Results: Higher PF and serum levels of TGF-β1, TGF-β2, TGF-β3, presented also as a total TGF-β in women with endometriosis compared to control were observed. The biggest increase was measured in the case of TGF-β1. The higher levels of IL-1β, IL-6, IL-10, and IL-17 in PF and serum of endometriosis women than control was observed. Higher PF levels of studied parameters in comparison with serum levels were found.

Conclusions: In endometriosis, TGF-β could affect differentiation of T helper (Th) cells, hence produce more IL-17 and IL-10 to PF and might have an indirect influence on inflammation, which is associated with higher IL-1β and IL-6 levels. In consequent, TGF-β in peritoneal fluid may promote an environment favorable to ectopic lesion formation.

Key words: endometriosis, TGF-β, Th cytokines, peritoneal fluid, serum

1. Introduction

Endometriosis is a major reproductive pathology that negatively affects health of 1 in 10 women. It is characterized by the growth of endometrial glands and stroma outside the uterine, primarily into the peritoneal cavity [1]. The physiopathology of endometriosis is not completely understood, however, the disease is known as a complex, chronic inflammation disease and it is closely associated with immune dysregulation [2], particularly with the production of high levels of thymic stromal lymphopoietin (TSLP), depicted as an an important inducer of inflammation [3].

Disorders in the peritoneal fluid, immune cells and their secretory products, create a specific microenvironment facilitating the implantation and proliferation of ectopic endometrial tissue and inducing the development of the disease [4].

Many chronic inflammatory diseases, including endometriosis, are characterized by inappropriate or dysregulated activity of a transforming growth factor β (TGF-β) [5]. It is one of the major immune and proinflammatory factors responsible for regulating of cell proliferation, differentiation, angiogenesis and immune responses [6]. TGF-β is a highly pleiotropic cytokine, that exists in three isoforms (TGF-β1, TGF-β2 and TGF-β3), synthesized by almost all cells and can signal to almost all cells and act in wide-range [7,8]. TGF-β1 is highly homologous to the TGF-β2 and TGF-β3 and it is predominantly expressed by hematopoietic cells [9]. It is secreted in a latent complex including three proteins: TGF-β, an inhibitor latency-associated protein (LAP, which is derived from the TGF-β propeptide) and an ECM-binding protein (one of the latent TGF-β binding proteins, LTBPs) [8]. In this form the associated LAP prevents the mature cytokine from binding to its receptors and inducing TGF-β's known effects [10].

TGF-β is clearly a master regulator of the both immune protection and immune pathology, and it exerts inhibitory effects on cells of all arms of the immune system, especially including CD4+ T

helper (Th) cell response.[11] Depending on cytokine environment, CD4+ T-cells differentiate into Th1, Th2, Th9, Th17, Th22 as well as follicular (Tfh) cells and regulatory (Treg) T-cells, which all are characterized by a different cytokine profile [12,13]. TGF-β, in parallel with IL-1β, IL-6 and IL-21, increased the expression of two important transcription factors, RORγt and RORα, which are involved in Th17 differentiation [14]. Th17 cells produce many cytokines including IL-17A, IL-17F, IL-22 and IL-21 [15].

Treg cells, as central mediators of immune suppression and immunological tolerance, play crucial roles in many facets of immune systems [16]. The differentiation of induced (adaptive) Treg (iTreg) cells is highly TGF-β dependent. Indeed, TGF-β appears essential for the maintenance of Foxp3+ Treg cells from precursors. Treg maintains peripheral tolerance and suppress antigen specific immune responses by secreting TGF-β, IL-10, and IL-4 [17,18]. Function of TGF-β and differentiation of all CD4+ T cells is presented in Figure 1.

TGF-β, was recently considered as a factor responsible for immune privilege on endometrial cells in endometriosis patients [19,20]. Deregulated TGF-β activity, especially its influence in peritoneal cytokine cross-talk, has been implicated in pathologies of endometriosis. Our aim was to determine whether TGF-β could be involved in pathogenesis of endometriosis. For this purpose, we evaluated concentrations of TGF-β1, TGF- β2, TGF-β3, LAP and IL-1β, IL-10, IL-17AF, IL-21 and IL-22 in peritoneal fluid (PF) and serum of women with endometriosis. Characterization of peritoneal and serum cytokine profiles in patients with and without endometriosis, could illuminate potential differences in immune profiles and so may reflect differences between these two patient populations.

2. Materials and methods

2.1. Patients

66 women aged between 21 and 43 years (mean age ± SD: 32.6 ± 6.4 years), undergoing laparoscopy for unexplained infertility were included in the study. All were patients of the Clinic of Obstetrics and Gynaecology at the Medical University of Silesia in Katowice, Poland in the period from 2011 to 2016, for diagnostic or therapeutic laparoscopy for infertility. All women had normal menstrual cycles (28 to 32 days) and no women had active pelvic inflammatory disease or any autoimmune disease. None had been taking hormonal or anti-inflammatory treatment during the 3-month period preceding the laparoscopy. The surgery was performed during the early proliferative phase of the menstrual cycle, directly after menstruation, to avoid any possible immunological effects of hormones

upon secretion of the studied parameters. Endometriosis was confirmed histologically in 51 women, aged 23 to 43 years (mean age ± SD: 33.7 ± 6.3), whose disease was scored according to revised American Society for Reproductive Medicine (rASRM) classification [21]. Among them there were women with minimal/mild endometriosis (n=30) and moderate/advanced endometriosis (n=21). In 15 women, 21 to 37 years old (mean age: 31.4 ± 5.9 years), used as control, laparoscopic examination demonstrated normal status without evidence of endometriosis, inflammatory diseases or uterine fibroids in the peritoneal cavity. This group included women with idiopathic infertility. Women with other causes of infertility were excluded. The Ethics Committee of the Medical University of Silesia approved this study according to the criteria of the Declaration of Helsinki.

2.2. Cytokine assay

The samples of PF were collected during the laparoscopic procedure from the posterior cul-de-sac, under general anesthesia, immediately after the introduction of the second trocar to minimize blood contamination. Patients with blood contaminated PF were excluded from the study. The PF was centrifuged immediately at 400 × *g* for 10 min and supernatants were stored in aliquots at −80 °C, until assayed. Peripheral blood was collected in a fasting state before laparoscopy and after centrifugation, serum samples were stored at −70°C.

PF and serum levels of all studies cytokines were measured by standard cytokine-specific enzyme-linked immunosorbent assay ELISA in the same time for women with and without endometriosis. All determinations were performed in duplicate, according to the manufacturer's instruction. Dilutions were done wherever necessary to obtain readings in the linear range of the assays. The measurements were repeated in cases of very high or low results. The concentrations of TGF-β1 and TGF-β2 were measured using commercially available kits (Diaclone SAS, Besancon Cedex, France). The sensitivities of these kits were approximately 8.6 pg/ml for TGF-β1 and 6.6 pg/ml for TGF-β2. TGF-β3 was assessed using Cloud-Clone Corp. kit (Uscn Life Science, Wuhan, China) and test sensitivity amounted to 5.7 pg/ml. Levels of LAP, IL-1β, IL-10 and IL-17AF were assessed using Platinum ELISA Kits (eBioscience, Vienna, Austria). The antibodies in this ELISA recognize the LAP/TGF-β complex. The sensitivities of these kits were 0.098 ng/ml for LAP, 1 pg/ml for IL-1β, 1 pg/ml for IL-10 and 8.8 pg/ml for IL-17AF. The concentrations of IL-21 and IL-22 were determined

using BioVendor kits (BioVendor - Laboratorni medicina A.S., Brno, Czech Republic). The sensitivities of these kits were, approximately, 20 pg/ml for IL-21 and 5 pg/ml for IL-22. Precision (intra-assay and inter-assay) was approximately 7% for all the assays.

2.3. Statistical Analysis

All results are presented as mean ± standard deviation or median and interquartile range and were examined for normality of distribution by the Shapiro-Wilk test. Parametric data were analysed using Student's t-test. For nonparametric data, Fisher's exact test (analysis of variance) was applied to indicate statistical significance because it analyses the variance relationship both within and among the groups. Corrections for multiple comparisons were carried out with the step-down Bonferroni method.

Correlations were tested by Spearman's rank correlation test and are presented as correlation coefficient (r). A $p<0.05$ was considered statistically significant.

3. Results

Concentrations of studied cytokines in PF and serum of women with endometriosis and those of the control group are shown in Table 1, Table 2, Table 3 and Figure 2.

3.1. TGF-β

Higher PF and serum levels of all isoforms of TGF-β in women with endometriosis compared to control group were observed. The highest increase was measured in the case of TGF-β1 in both PF and serum of endometriosis patients ($p<0.0001$ for PF and $p<0.001$ for serum). TGF-β1 concentration was nearly 18 times greater in PF of women compared to control, however, in the case of serum only 2.4 times. The lowest level was observed in case of TGF-β3. We decided to show our results as a total TGF-β, which is a sum of all isoforms calculated for each patient. Moreover, higher concentration of TGF-β1 was found in PF and serum of women at the moderate/severe stage of the disease, as compared to women with minimal/mild endometriosis ($p<0.0001$).

There were differences in the concentration of the parameters in the PF and serum of the studied women. Higher concentrations of TGF-β1 and TGF-β2 in PF as compared to serum of women with endometriosis were found.

3.2 LAP

In PF and serum of women with endometriosis the concentration of LAP was significantly higher than control ($p<0.0001$ for PF and $p<0.01$ for serum). LAP level was significantly higher at the moderate/advanced in comparison with the minimal/mild stage of the disease. However, compared to total TGF-β or even TGF-β1 the LAP levels were significantly lower in studied groups, independently on material type (Figure 2).

3.3. Cytokine levels

IL-1β and IL-6 levels in PF and serum were significantly increased in women with endometriosis than in control group. Higher level of the cytokines was observed at the moderate/severe in comparison with the minimal/mild endometriosis. Both IL-1β and IL-6 levels in PF were significantly higher than in serum. Higher levels of IL-10 and IL-17AF in PF and serum of endometriosis women compared to control were observed. Higher levels of the cytokines were observed at the moderate/severe in comparison with the minimal/mild endometriosis. Both IL-10 and IL-17AF levels in PF were significantly higher than in serum. Interestingly, IL-21 and IL-22 were undetectable in PF and serum samples of patients with endometriosis and all samples from control group.

As all studied parameters are involving each other, we evaluated the dependences between the concentrations of those parameters. The positive correlation was only observed between peritoneal levels of TGF-β1 and IL-17 ($r=0.395$ $p<0.05$). Then we calculated the ratio of the concentration TGF-β1 and IL-10, TGF-β1 and IL-17AF, TGF-β1 and IL-6 and also IL-17AF and IL-10 taking into consideration that TGF-β1 is the most important immunological factor involving Th cells development (Table 3). The PF TGF-β1/IL-10 and TGF-β1/IL-17 ratios in endometriosis were significantly higher compared to the control ($p<0.0001$).

4. Discussion

Cytokine-mediated immune response seems to play an important role in endometriosis pathogenesis, but still the aetiology and pathophysiology remain unclear [22]. The dysfunction of TGF-β in women with endometriosis may contribute to the immune escape of ectopic endometrial cells

refluxed to the peritoneal cavity and next the development of the disease. Elevated inflammatory and anti-inflammatory mediators were depicted in serum and PF in endometriosis particularly vascular endothelial growth factor (VEGF) or IL-33 and its receptor [23,24].

Until now, the role of TGF-β, IL-1β, IL-6, IL-10 and IL-17 in the pathogenesis of endometriosis has been studied many times, however the results are still not conclusive [25-53]. For this reason, we tried to determine the concentration of these parameters in the PF and blood serum of women with endometriosis compared to the control group.

Among the three isoforms, TGF-β1 is the predominant isoform expressed in immune cells [54]. However, indirect evidence suggested that TGF-β2 and TGF-β3 could play an important role in immune regulation, whereby they regulate both pro-inflammatory and anti-inflammatory activities [9-Okamura]. In our work we decided to investigate the changes concentration of all 3 isoforms of TGF-β in PF and serum of studied women. We found higher PF and serum levels of all studied parameters in women with endometriosis compared to control group. The highest level was observed in case of TGF-β1 and the lowest in case of TGF-β3. Most authors confirmed an important role TGF-β1 in the immune response in endometriosis patients [25-26,28-31,55-56]. Interestingly, in our results concentration of TGF-β1 in PF of women with endometriosis was almost 18-times higher than in control group, but in serum this is only 2.4 times. It suggested that TGF-β, especially TGF-β1, may be a potential biomarker for the detection of endometriosis. To support this eventually, we should know the distinction between TGF-β isoforms measured, because in a lot of studies it is not clear if this is TGF-β1 or all TGF-β isoforms or total TGF-β. However, in the case of TGF-β2 and TGF-β3, the results of the studies are divergent. there is no literature directly showing TGF-β2 and TGF-β2 expression, localization and function to endometriosis tissue. Recently, Young *et al*. [28-29] reported TGF-β2 and TGF-β3 to be present within the peritoneal fluid, however, levels of these ligands remained unchanged between women with and without endometriosis. Because of other TGF isoforms also affect the immune system, and their potency can be the resultant of all isoforms [11,57]. Therefore, we decided to present these results as the total TGF-β and confirmed increased of total secretion of the factors in women with endometriosis. All these changes were severe in women with advanced stages of the disease. Moreover, higher levels of the parameters in PF than in serum of studied women were observed.

The multicomplex processing and secretion of TGF-β prompted us to estimate the

concentration of LAP in PF and serum of studied women. Because, LAP can induce epithelial cell migration and promote chemotaxis of monocytes and block inflammation [58], it also seems that this factor can play an important role in the microenvironment of the peritoneal cavity of women with endometriosis. In our knowledge, in the study, for the first time we evaluated the concentration of LAP in PF of women with endometriosis. Moreover, results of Hanada *et al.* [59] demonstrated low LAP expression on the surfaces of macrophages in the PF of patients with endometriosis. Activated TGF-β released from the latent complex on macrophage surfaces may act to decrease LAP expression and increase the concentration of TGF-β in the PF of patients with endometriosis [59]. Potentially, this is a reason of lower LAP level than TGF-β1 or total TGF-β in PF of women with endometriosis.

TGF-β is known to play a pivotal role in the function of all immune cells especially in the regulation of T cell development and in the induction of immunological tolerance [54]. TGF-β inhibits cytotoxic T lymphocyte, Th1 and Th2 cell differentiation. However, TGF-β promotes peripheral Treg, Th17, Th9, and Tfh cell generation [18]. In our study we showed increased concentration of IL-10 in PF and serum of women with endometriosis compared to control group. IL-10, the secretion product of Treg and Th2 cells, is one of the most important anti-inflammatory cytokines. Studies strongly suggested that endometriosis is associated with the elevated levels of IL-10, the marker that has a potential role as a prognostic factor for the disease [50]. In our study we measured concentrations of IL-17AF, IL-21 and IL-22 in PF and serum of affected women. We found higher IL-17AF levels, but no detectable levels of IL-21 and IL-22. It suggested stimulation by TGF-β may be concentrated on activation of Th17 cells, even more we did not-observed secretion of IL-21 and IL-22. To the best of our knowledge there was only one study about the Th9 or Th22 cytokine profile in PF and serum of endometriosis. Guo *et al*. [60] found higher expression of IL-22 and its receptors (IL-22R1 and IL-10R2) in eutopic endometrium and ectopic lesion of women with endometriosis than that from healthy control. Moreover, they found lower concentrations of IL-22 in the serum of patients with ovarian endometriosis than in control women without endometriosis, which was significantly associated with the occurrence of severe deep dyspareunia, however, the association had a moderate correlation coefficient. We do not know what the reason of the differences of divergent results is, however, this confirms that further research about the issue is needed.

Regardless, our research complements the knowledge of the role of TGF-β and IL-17 in

pathogenesis of endometriosis. More, in the PF and serum of women with endometriosis increased secretion of IL-1β and IL-6 were observed. There are a most important cytokines take part in immune and inflammatory response. Moreover, IL-1β and IL-6 can promote the development of Th17. TGF-β may co-operate with these pro-inflammatory cytokines, on the one hand increasing inflammation and, on the other, contributing to the development of IL-17 and Treg. This may be due to elevated factors TGF-β1/IL-10 and TGF-β1/IL-17. Unfortunately, we have not confirmed this trend in the case of TGF-β1, which may be related to other mechanisms of dependence between these factors.

In this context, higher levels of TGF-β may occur in the regeneration process, inducing adhesion formation and the appearance of fibrotic tissue and stimulating Treg cells to regulate the exacerbated immune response. Because TGF-β influence on Th17 and Treg development, it seems likely that with so much production of this factor, these two cell populations and their secretion products will be strongly involved in the pathogenesis of endometriosis. Treg cells play a key role in T cell-mediated immune response and development of immune disorders, their significance in endometriosis remains to be elucidated [61,62]. This strongly suggests that endometriosis can be associated with the disturbance of circulating Treg cells. We hypothesize about the role Treg and Th17 cells might play in the survival of endometriosis foci in ectopic localization and in the evasion of such lesions from host immune surveillance but also to promote chronic inflammation (Figure 3). Recently IL-37 an anti-inflammatory cytokine was reported to suppress IL-6 and IL-17 levels [63].

In conclusion, TGF-β is a pleiotropic factor that oversees many processes, such as immune and inflammatory statements. For the first time, we presented our results of studied parameters as total TGFβ and tTGFβ in comparison to LAP in PF of women with endometriosis. TGF-β could affect not only the differentiation of Th cells towards Th17 or Treg cells, hence produce more IL-17 and IL-10 to PF and might have indirect influence on inflammation. In consequent, TGF-β in peritoneal fluid of women with endometriosis may promote an environment favourable to lesion formation. However, this may require further research.

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Conflict of interest

None of the Authors has any proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The design of the study was approved by Ethics Committee of the Medical University of Silesia in Katowice, Poland (KNW/0022/KB/123/14).

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Legend of Figures:

Figure 1. Role of TGF-β.

TGF-β affects multiple components of the immune system and inflammation, especially, TGF-β inhibits the function of NK and cytotoxic T lymphocytes. Additionally, TGF-β inhibits dendritic cell function, block type 1 macrophage and neutrophil development, but promotes type 2 macrophages and neutrophils, in consequent TGF-β leads to the immune suppression state. TGF-β induces also Treg and Th17 cell differentiation. All T helper lymphocytes are differentiated from T helper progenitor (Th0). Depending upon what cytokines the Th0 cells are exposed to, they may differentiate into one of several different T helper phenotypes: Th1, Th2, TH9, Th17, Th22, Treg and Tfh.

Figure 2. Concentrations of total TGF-β and LAP in PF and serum of women with endometriosis and control

Results are presented as median and interquartile range

Figure 3. Potential role of TGF-β in endometriosis development.

In endometriosis, TGF-β could influenced on Th cells differentiation towards Th17 cells, hence produce more IL-17 and IL-10 to peritoneal fluid, but also might have indirect influence on inflammation. This suggests significant inadequacy in the specific TGF-β activity at the peritoneal cavity level. The imbalance between all studied cytokines in endometriosis may escalate peritoneal inflammation and, in consequence, develop endometriosis. In consequent, TGF-β in peritoneal fluid of women with endometriosis may promote an environment favourable to lesion formation.

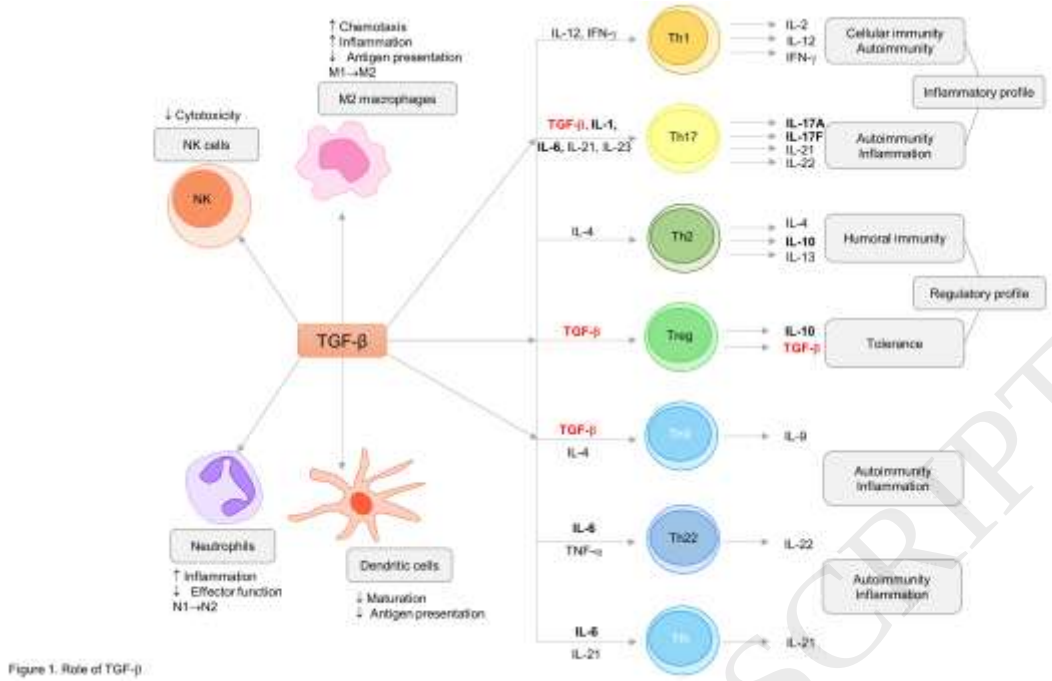

Figure 1. Role of TGF-β

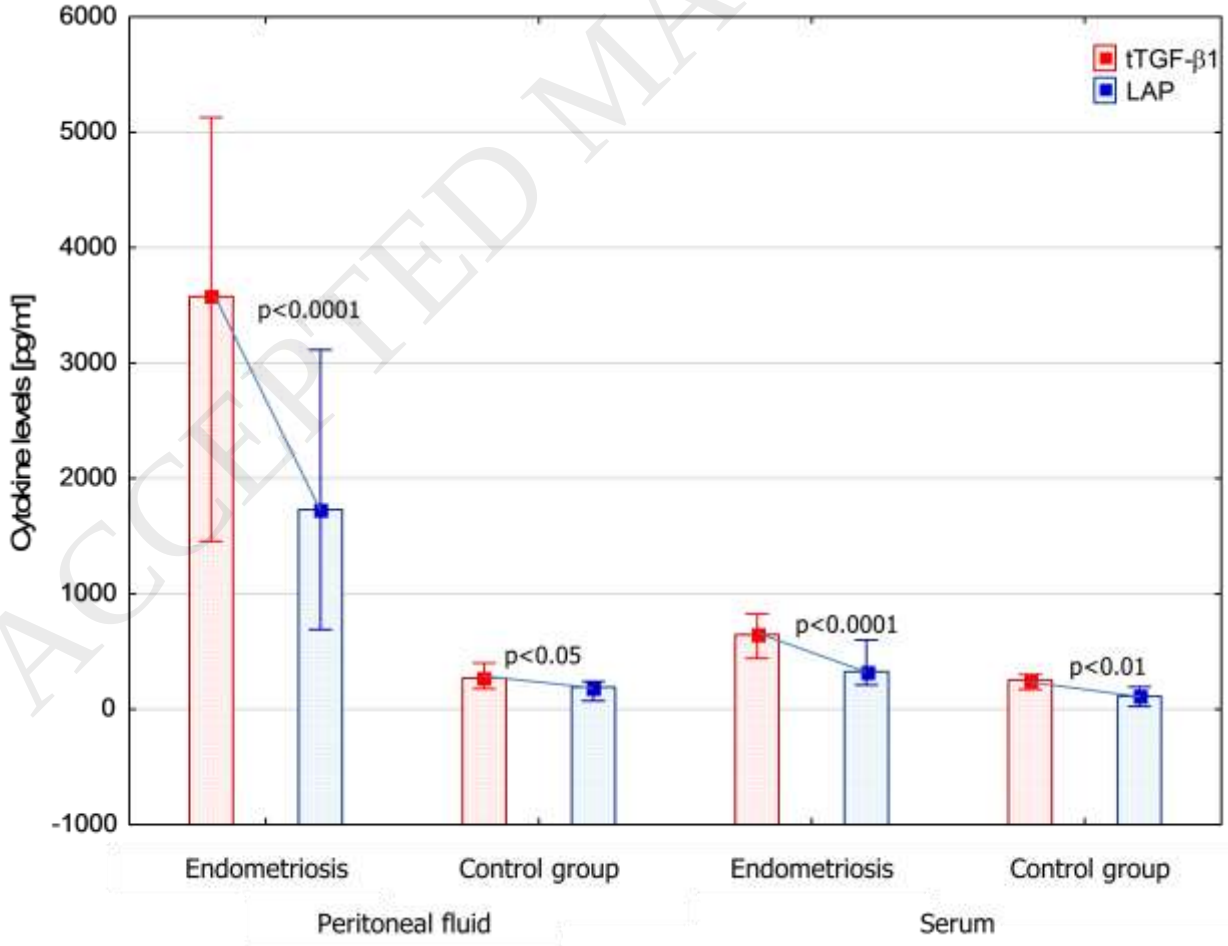

Figure 2. Concentrations of total TGF-β and LAP in PF and serum of women with endometriosis and control

Results are presented as median and interquartile range

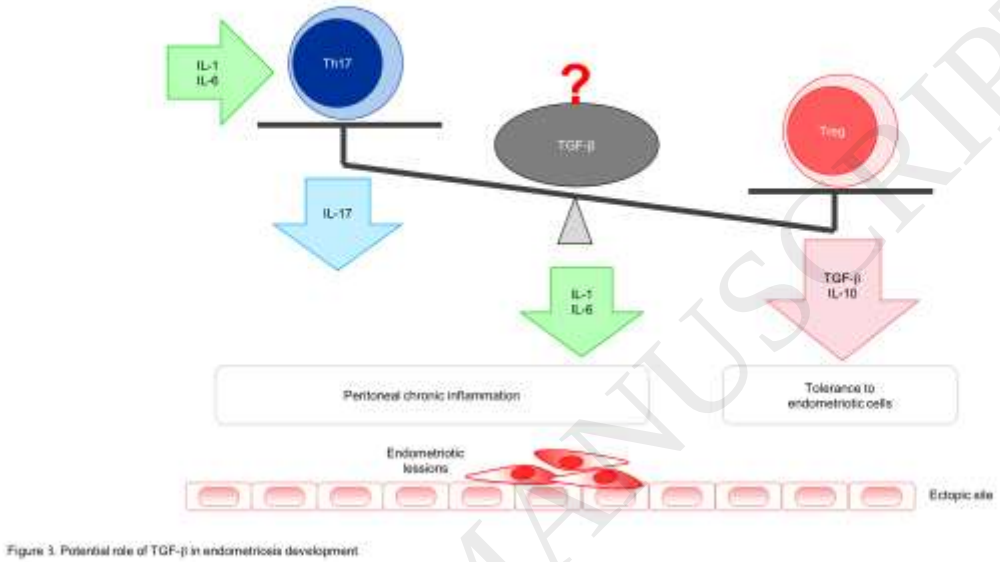

Figure 3. Potential role of TGF-β in endometriosis development

Legend of Tables:

Table 1. The cytokine levels in the peritoneal fluid and serum of women with endometriosis and control group

Table 2. The cytokine levels in the peritoneal fluid and serum of women with minimal/mild and moderate/advanced endometriosis

Table 3. Peritoneal and serum cytokine ratios in women with endometriosis and control group

Table 1. The cytokine levels in the peritoneal fluid and serum of women with endometriosis and control group

Cytokines	Endometriosis	Control	*p* value*
TGF-β1 (pg/ml) PF	3160.50 (1870.53 – 4259.20)	173.50 (145.50 – 218.09)	<0.0001

TGF-β1 (pg/ml) S	451.13 (355.05 – 541.53)	191.50 (150.12 – 220.25)	<0.001
	<0.0001^	NS^	
TGF-β2 (pg/ml) PF	286.83 (208.37 – 349.21)	38.00 (29.69 – 46.12)	<0.0001
TGF-β2 (pg/ml) S	140.41 (107.96 – 166.47)	19.89 (9.54 – 16.73)	<0.0001
	<0.001^	<0.01^	
TGF-β3 (pg/ml) PF	64.40 (55.32 – 73.45)	53.00 (37.39 – 62.52)	<0.05
TGF-β3 (pg/ml) S	54.38 (46.21 – 60.32)	33.50 (20.38 – 39.46)	<0.01
	NS^	<0.001^	
LAP (ng/ml) PF	1.73 (0.88 – 2.37)	0.19 (0.10 – 0.24)	<0.0001
LAP (ng/ml) S	0.33 (0.27 – 0.39)	0.11 (0.06 – 0.16)	<0.01
	<0.0001^	<0.05^	
IL-1 (pg/ml) PF	26.04 (22.82 – 31.65)	7.00 (5.74 – 7.83)	<0.0001
IL-1 (pg/ml) S	14.66 (12.85 – 16.39)	5.61 (4.65 – 6.64)	<0.0001
	<0.001^	NS^	
IL-6 (pg/ml) PF	52.55 (43.55 – 55.72)	4.13 (2.71 – 5.42)	<0.0001
IL-6 (pg/ml) S	15.63 (13.75 – 17.35)	6.10 (4.80 – 6.73)	<0.001
	<0.0001^	NS^	
IL-10 (pg/ml) PF	18.16 (16.12 – 20.57)	4.90 (4.13 – 6.44)	<0.0001
IL-10 (pg/ml) S	12.73 (10.04 – 14.01)	4.10 (2.73 – 4.62)	<0.001
	<0.01^	NS^	
IL-17AF (pg/ml) PF	69.82 (64.58 – 80.92)	22.75 (17.40 – 25.93)	<0.0001
IL-17AF (pg/ml) S	47.36 (37.65 – 55.70)	20.80 (15.8 – 24.62)	<0.0001
	<0.01^	NS^	

Note:Data are presented as median concentrations and interquartile range
PF- peritoneal fluid
S- serum
**endometriosis compared to control
^ PF compared to S
All p-values were corrected for multiple comparisons using the Stepdown Bonferroni method.

Table 2. The cytokine levels in the peritoneal fluid and serum of women with minimal/mild and moderate/advanced endometriosis

Cytokines	**Endometriosis**	***p* value[#]**

	minimal/mild	moderate/advanced	
TGF-β1 (pg/ml) PF	2256.86 (1246.00 – 3120.50)	4262.44 (3492.32 – 4412.27)	<0.0001
TGF-β1 (pg/ml) S	362.63 (350.15 – 440.48)	538.67 (496.31 – 576.48)	<0.001
	<0.0001^	<0.0001^	
TGF-β2 (pg/ml) PF	272.54 (199.43 – 357.51)	290.35 (246.64 –341.43)	NS
TGF-β2 (pg/ml) S	140.41 (101.89 – 185.37)	140.91 (119.10 – 164.34)	NS
	<0.001^	<0.001^	
TGF-β3 (pg/ml) PF	61.59 (52.32 – 72.35)	67.83 (58.47 – 74.68)	NS
TGF-β3 (pg/ml) S	55.18 (48.55 – 59.23)	52.50 (46.36 – 61.92)	NS
	NS^	NS^	
LAP (ng/ml) PF	1.08 (0.88 – 1.96)	2.32 (1.52 – 2.81)	<0.001
LAP (ng/ml) S	0.27 (0.24 – 0.32)	0.41 (0.37 – 0.51	<0.05
	<0.001^	<0.0001^	
IL-1 (pg/ml) PF	22.88 (20.99 – 24.33)	31.90 (29.49 – 34.15)	<0.0001
IL-1 (pg/ml) S	13.20 (12.55 – 15.28)	16.29 (14.52 – 18.51)	<0.01
	<0.001^	<0.001^	
IL-6 (pg/ml) PF	52.80 (43.70 – 61.90)	69.25 (52.30 – 74.60)	<0.05
IL-6 (pg/ml) S	13.95 (13.50 – 15.10)	16.73 (14.64 – 21.91)	NS
	<0.0001^	<0.0001^	
IL-10 (pg/ml) PF	16.33 (15.56 – 17.94)	23.54 (18.52 – 26.21)	<0.01
IL-10 (pg/ml) S	10.90 (9.58 – 13.58)	12.91 (11.17 – 14.58)	NS
	<0.05^	<0.01^	
IL-17AF (pg/ml) PF	65.85 (59.30 – 72.80)	82.65 (70.40 – 86.90)	<0.001
IL-17AF (pg/ml) S	40.55 (33.51 – 48.24)	59.74 (47.58 – 69.70)	<0.001
	<0.001^	<0.001^	

Note:Data are presented as median concentrations and interquartile range

PF- peritoneal fluid

S- serum

#* minimal/mild compared to moderate/advanced endometriosis

^ PF compared to S

All p-values were corrected for multiple comparisons using the Stepdown Bonferroni method.

Table 3. Peritoneal and serum cytokine ratios in women with endometriosis and control group

Ratio	Peritoneal fluid		p value	Serum		p value
	Endometriosis	Control		Endometriosis	Control	
TGF-β1/IL-10	166.72* (109.22 – 208.57)	36.54[#] (25.10 – 43.14)	<0.0001	38.92 (29.10 – 47 .89)	42.63 (29.15 – 55.73)	NS
TGF-β1/IL-17	41.98* (24.17 – 58.98)	7.68[#] (5.20 – 9.16)	<0.0001	9.86 (6.21 – 11.72)	9.93 (5.31 - 14.79)	NS
TGF-β1/IL-6	59.13* (33.32 – 80.44)	45.97[#] (30.77 – 65.56)	NS	28.00 (22.82 – 36.82)	32.37 (23.22 – 40.40)	NS
IL-17/IL-10	3.97^ (3.11 – 4.89)	4.30[#] (2.91 - 5.47)	NS	3.53 (1.99 – 6.02)	5.54 (1.92 – 8.24)	<0.001

Note: Data are presented as median concentrations and interquartile range

*p<0.0001compared to serum level in endometriosis patients

^NS compared to serum level in endometriosis patients

[#]NS compared to serum level in control

All p-values were corrected for multiple comparisons using the Stepdown Bonferroni method.

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