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The effect of hysteroscopic polypectomy on the concentrations of tumour necrosis factor-α (TNF-α) in uterine flushings and serum in infertile women

Ефекат хистероскопске полипектомије на концентрацију фактора туморске некрозе-*α* (*TNF-α*) у испирку материце и серуму код инфертилних жена

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Ефекат хистероскопске полипектомије на концентрацију фактора туморске некрозе-*α* (*TNF-α*) у испирку материце и серуму код инфертилних жена

SUMMARY

Introduction/Objective The aim of this paper is to present changes of tumour necrosis factor- α (TNF-α) levels in uterine flushings and serum of infertile female patients before and after hysteroscopic polypectomy.

Methods A total of 82 infertile female patients were included in the study. The patients were divided into two groups. The first group was the experimental group and comprised 56 infertile women with endometrial polyps, whereas the second group was the control group of 26 infertile women who were not diagnosed with endometrial polyps.

Results The results of this research primarily suggest that TNF-α concentration obtained from uterine flushings and serum of infertile patients diagnosed with endometrial polyps differed before and after a surgical procedure. In the control group of patients, there was no significant difference observed regarding TNF-α concentrations in serum and uterine flushings of women without endometrial polyps. A comparison between these two groups revealed differences in TNF-α concentrations both in venous blood and uterine flushings. These differences were considered statistically significant.

Conclusion Endometrial polyps are one of the causes of higher TNF-α levels both in uterine flushings and serum.

Keywords: cytokines, TNF-α, endometrial polyp, uterine flushings, serum.

САЖЕТАК

Увод/циљ Циљ овог рада је да прикаже промене нивоа фактора туморске некрозе -*α* (*TNF-α*) у испирку утеруса и серуму инфертилних пацијенткиња пре и после хистероскопске полипектомије.

Методе Студија је обухватала 82 инфертилне пацијенткиње. Пацијенткиње су биле подељене у две групе. Прва група је била експериментална група коју је сачињавало 56 инфертилних пацијенткиња са ендометријалним полипом. Друга група је била контролна група коју је сачињавало 26 инфертилних жена без ендометријалног полипа.

Резултати Резултати овог истраживања примарно указују да се концентрације *TNF-α* у серуму и у испирку утеруса инфертилних женаса ендометријалним полипом разликују пре и после хистероскопске полипектомије. У контролној групи, нису уочене значајне разлике у концентрацијама *TNF-α* у испирку утеруса и серуму пацијенткиња без ендометријалног полипа. Поређењем ове две групе уочена је статистички значајна разлика у нивоима *TNF-α* у испирку утеруса и серуму пацијенткиња.

Закључак Ендометријални полипи су један од узрока повећаних нивоа *TNF-α* у испирку утеруса и серуму пацијенткиња.

Кључне речи: цитокини, *TNF-α*, ендометријални полип, испирак утеруса, серум.

INTRODUCTION

Endometrial polyps are benign localized overgrowth of endometrial tissue, composed of glands, stroma, and blood vessels covered by epithelium. They develop once the endometrium becomes hypertrophic, which is a consequence of oestrogen stimulation [1, 2, 3]. They are diagnosed based on hysteroscopic, sonographic or hysterosonographic findings, after a polyp, which can vary in size, has been detected in the uterine cavity [4]. There is a large group of protein molecules that mediate and regulate intercellular communication both

in physiological and pathological conditions and these are called cytokines [5, 6, 7]. Cytokines are produced by different cells that induce chemotaxis, activation, proliferation, and differentiation of other cells. The endometrial tissue also produces cytokines. Cytokines have a significant role in the relationship between the decidua and the embryo during implantation [8, 9]. The most important physiological functions of cytokines include regulating cell growth and humoral immune response, regulation of hematopoiesis, controlling cell proliferation and differentiation, and wound healing [10–13]. Cytokines can act locally or systemically [14]. Cellular response to most cytokines is followed by gene expression, which results in the target cell gaining some new functions and can sometimes lead to cell proliferation [15]. Tumour necrosis factor-α (TNFα) belongs to the family of cytokines that are considered the most versatile. Not only does TNFα play a prominent role in the synthesis of DNA in the early stage of proliferation, but it also contributes to cell differentiation and tissue remodeling. This is essential in terms of embryonic attachment [16].

The aim of this paper is to present changes of tumour necrosis factor-α (TNF-α) levels in serum and uterine flushings of infertile female patients before and after hysteroscopic polypectomy.

METHODS

This was an open cross-sectional study, which included 82 infertile female patients aged 22–42 years. The patients were divided into two groups. Firstly, the experimental group comprised 56 infertile women who were diagnosed with endometrial polyps, whereas the second group was the control group of 26 infertile women who were not diagnosed with endometrial polyps. The study was conducted at the Obstetrics and Gynecology Clinic "Narodni front" in Belgrade and the Centre for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac between May 2012 and November

2013. The inclusion criteria were: a history of infertility (≥ 1 year), age (22–42 years), and regular menstrual cycles, not taking hormonal contraceptives or hormonal medications that could affect the endometrium for the last six months, an endometrial polyp detected by either two-dimensional (2D) ultrasound or saline infusion sonohysterography, no other endometrial pathology observed by a transvaginal ultrasound. The exclusion criteria for both groups of participants were presence of submucous myomas, endometriosis, endometrial cancer, uterine anomalies, history of uterus and fallopian tubes surgery, history of ovulation induction failure, age > 42 years. Furthermore, the research was approved by the Ethical Board of the Obstetrics and Gynecology Clinic "Narodni front" on 24th May, 2012; ref. No.: 04-24/3-1. The diagnosis of polyps was threefold. They were diagnosed by 2D transvaginal ultrasound examinations conducted in the first stage of the cycle, by hysterosonographic examinations, or during hysteroscopy itself. After the intervention, all patients were followed up for one month by taking the samples of peripheral venous blood and determining the levels of the same cytokines. [17] An ultrasound check-up was also performed. Hysteroscopy was performed in a fully equipped operating room using completely adapted instruments. The patients were under general anesthesia during hysteroscopy. Preoperative preparation involved complete diagnostics and routine tests: cervical and vaginal swabs; swabs for chlamydia, ureaplasma, and mycoplasma; Pap smear; 2D ultrasound; blood typing; hematology and biochemical analyses; preoperative assessment by an internist and an anesthetist; giving an informed consent for surgical treatment. The patients underwent surgical procedures prior to the mid-proliferative stage of the cycle, soon after menstrual bleeding had ceased. Hysteroscopy can be performed regardless of the phase of the cycle, providing the patients have been prepared by taking oral hormonal contraceptives. Diagnostic hysteroscopy was performed in the control group of infertile women to detect any pathological changes in the uterine cavity that could cause infertility. Endometrial biopsy was

performed in patients with normal hysteroscopic findings. The endometrial samples were histopathologically examined to detect any abnormalities of the endometrium. During diagnostic hysteroscopy, saline is used for uterine distension, which enables the uterine cavity to be visually examined with 30-degree angle lens. In this way, uterine horns, the fundus, anterior and posterior uterine walls, and the lateral sides of the uterus are visualized. At the level of the intrauterine horns, the hysteroscope allows a panoramic view of the uterine cavity, followed by the visualization of the cervical canal. Due to the muscular structure of uterine walls, the distension pressure of ≥ 40 mm Hg is required. Operative hysteroscopy was performed in the experimental group by using an operative continuous flow hysteroscope with a resectoscope that uses bipolar electrical current. The resectoscope has a cutting loop electrode, which is used for removing endometrial polyps. The procedure for determining the concentrations of TNF-α in uterine flushings was as follows: a Cusco's speculum was inserted once the patient was placed in the lithotomy position, which enabled the visualization of the cervix. After the cervix had been flushed with sterile saline, a pediatric 8F Foley catheter was placed through the cervical canal and into the uterine cavity. Then, 10 ml of sterile saline was injected through the catheter and immediately aspirated without contamination. Uterine flushings were centrifuged at 2,500 x g for 10 minutes and the supernatant was aspirated and stored at -20° C. Afterwards, the ELISA method was used to detect TNF-α and determine its concentration. This was conducted at the Centre for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac. When it comes to measuring serum TNF-α concentrations in the experimental group of patients, ~5 ml of peripheral venous blood was sampled one month before and one month after hysteroscopic surgery. The blood samples were collected into heparinized vacuum tubes and centrifuged at 2,500 x g for 10 minutes. The supernatant was aspirated and stored at -20° C. Again, the ELISA method was used to detect TNF-α and determine its

concentration. Sample size calculation was performed by using a formula for computing a large sample implemented in the PASS software 11.0. Descriptive and analytical statistics were used to process the obtained data. Descriptive methods included absolute and relative numbers, measures of central tendency (mean, median), and measures of dispersion (standard deviation, inverse variance). Analytical statistics methods involved comparison tests (parametric, non-parametric). All results were analyzed using SPSS 22.0 software package. The obtained results were compared to previously reported data. Finally, the conclusions were reached by analyzing and interpreting these results.

RESULTS

The study included 56 patients with polyposis (68.3%) and 26 patients without polyposis (31.7%). The age interval was 22 to 42 years. The mean age in the group of patients diagnosed with polyposis was 35.2 ± 5.1, while the mean age in the group of patients without polyposis was 33.2 ± 4.9 years. No significant difference was observed regarding the age ($p = 0.061$). In the group of patients diagnosed with polyposis, there were 32 patients with primary infertility (57.1%); while in the group of patients without polyposis, there were 17 patients with primary infertility (65.4%). The statistical analysis revealed no significant differences between the two groups observed regarding primary infertility ($p = 0.479$). The serum concentrations of TNF-α before hysteroscopic polypectomy were significantly lower in the experimental group of patients than the serum concentrations of TNF-α detected after hysteroscopic polypectomy in the same patients. There was a statistically significant difference in the serum concentrations of TNF-α in infertile patients before and after hysteroscopic polypectomy ($p < 0.005$). This is shown in Table 1 and Figure 1.

The serum concentrations of TNF-α before hysteroscopic polypectomy were higher in the control group of infertile patients than the serum concentrations of TNF-α detected after

hysteroscopic polypectomy in the same patients. However, this difference was not statistically significant ($p > 0.005$). This is shown in Table 2 and Figure 2.

The serum concentrations of TNF-α before hysteroscopic polypectomy were higher in the experimental group of infertile patients than the concentrations of TNF-α detected in uterine flushings of the same patients. The difference was statistically significant ($p<0.005$). This is shown in Table 3 and Figure 3.

The serum concentrations of TNF-α before hysteroscopy were lower in the control group of infertile patients than the concentrations of TNF-α detected in uterine flushings of the same patients. The difference was not statistically significant ($p > 0.005$). This is shown in Table 4 and Figure 4.

The correlation between the TNF-α concentrations detected in all three samples retrieved from the experimental group of patients was examined. The peak values show the sample self-correlation. The correlation values between the samples indicate that there is not a correlation between the detected concentration values of TNF-α. In clinical terms, this means that polyps are associated with endometrial changes even after they have been removed. However, this does not indicate a correlation with serum either before or after polypectomy. This is shown in Table 5 and Figure 5.

The correlation between TNF-α concentrations in all three samples retrieved from the control group of patients was examined. The peak values show the sample self-correlation. The correlation values between the samples indicate that there is a correlation between the detected concentration values of TNF-α, which shows that the detected values are in accordance with similar statistical distributions. In clinical terms, this means that post-hysteroscopy concentration values of TNF-α in the endometrium and serum show a correlating distribution. The obtained results show a correlation between uterine flushings

and serum prior to hysteroscopy. There is also a high correlation between uterine flushings and serum after hysteroscopy. This is shown in Table 6 and Figure 6.

DISCUSSION

Endometrial polyps are among the factors that are linked to certain conditions including infertility and pregnancy loss in the early stages. The frequency of polyps in overall female population is 9–25% [18]. Even though polyps are asymptomatic in many cases, they can result in menstrual cycle disorders (e.g. intermenstrual bleeding). It has been proved that endometrial polyps can lead to infertility and early pregnancy loss. Nevertheless, the pathophysiological processes related to infertility caused by polyps have not been completely elucidated yet. It is believed that polyps may be the cause of abnormal bleeding, thus affecting the endometrial environment or they can have a negative impact on implantation conditions [19, 20]. It is assumed that infertility can be caused by a polyp of 1 cm in diameter. Uterine cavity assessment and endometrial polyp removal are routinely performed in infertile women. There has been evidence suggesting that hysteroscopic polypectomy resulted in increased pregnancy rates in infertile patients who had undergone the surgical procedure [21]. The human endometrium produces cytokines that act as important mediators between the embryo and decidua in the process of implantation. TNF-α belongs to the family of cytokines that are considered to be the most versatile. Not only does TNFα play a prominent role in the synthesis of DNA in the early stage of proliferation, but it also contributes to cell differentiation and tissue remodeling. [22]. Furthermore, TNF-α facilitates apoptotic processes, which initiates menstrual bleeding [23, 24]. In spite of infertility being perceived as a possible consequence of endometrial polyps, the fact is that the influence of endometrial polyps on endometrial implantation factors has not been sufficiently investigated yet. Based on the samples obtained before and after hysteroscopic polypectomy, the present

study has shown that endometrial polyps found inside the uterine cavity affect TNF-α concentrations in both uterine flushings and serum of infertile women. The TNF-α concentrations obtained from the serum after hysteroscopic polypectomy were significantly higher in comparison to the serum concentrations of TNF-α before hysteroscopic polypectomy. These results are in line with previously reported findings of other authors [8]. The authors were able to prove that TNF-α secretion increased during the menstrual cycle after polypectomy had been performed and that it reached its peak during the mid-luteal menstrual cycle phase. TNF-α has multiple functions and its varying concentrations in different phases of the cycle suggest that it has an impact on the endometrium and has a complex role in the preimplantation of the embryo, which is a prerequisite for a successful implantation [22]. This is why it is important to follow up TNF-α concentrations after polypectomy. Apart from fostering DNA synthesis, TNF-α also improves cell differentiation and endometrial tissue remodeling. This is critical for a successful implantation. Abnormal TNF-α expression may further aggravate infertility linked to polyps and cause pregnancy loss in the early stages. Moreover, the mechanism behind a lower TNF-α synthesis caused by endometrial polyps is still not evident. What differentiates endometrial polyps from the surrounding endometrium is a massive fibrous stroma along with thick-walled dilated blood vessels. Such abnormal endometrial architecture can affect implantation regulators, i.e. it can result in their impaired secretion [25, 26]. It is important to point out that in the stromal component of endometrial polyps there is a lower number of hormone receptors. Therefore, the glands and stroma of polyps do not respond to progesterone stimulation, which may be the cause of abnormal secretion of progesterone in the endometrium [27, 28, 29]. A study that measured TNF-α concentration in uterine flushings of 12 patients before and after hysteroscopic polypectomy showed an increase in TNF-α concentrations after polyp removal with TNF-α values reaching the peak in the mid-luteal menstrual cycle phase [25]. These

results are in line with previously reported findings by other authors [22]. There is a limited number of foreign publications dealing with this subject. No studies examining TNF-α concentration in uterine flushings and serum of infertile women with and without endometrial polyps have been published in Serbia. Similarly, no studies examining the effect of polypectomy in relation to TNF-α concentration in uterine flushings and serum have been published in Serbia. The limitation of the previously reported studies including the present study is the small sample size.

CONCLUSION

TNF-α values in uterine flushings and serum before a surgical procedure were significantly higher in patients diagnosed with endometrial polyps in comparison to the TNF-α values obtained from women who were not diagnosed with endometrial polyps. TNF-α concentration in uterine flushings and serum of women diagnosed with endometrial polyps were significantly lower after surgery. TNF-α concentration in the group of women without endometrial polyps, i.e. the control group, were not significantly different before and after hysteroscopy. Furthermore, no significant difference was observed in the serum TNF-α concentrations in infertile patients who had undergone hysteroscopy in comparison to the infertile women who had no endometrial polyps.

Conflict of interest: None declared.

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Table 1. Serum concentrations of TNF-α in the experimental group of patients before and after hysteroscopic polypectomy

Exp. group of patients	**Mean value**
Serum before hysteroscopy	15.6220 (10.48–28.06) [pg/ml]
Serum after hysteroscopy	22.7302 (13.57–40.79) [pg/ml]
t-test	**Values**
Hypothesis Mean difference	0
Df	110
t Stat	-6.946
P(T <= t)	0.0001
T Critical two-tail	1.980

Figure 1. Serum concentrations of TNF-α in the experimental group of patients before and after hysteroscopic polypectomy

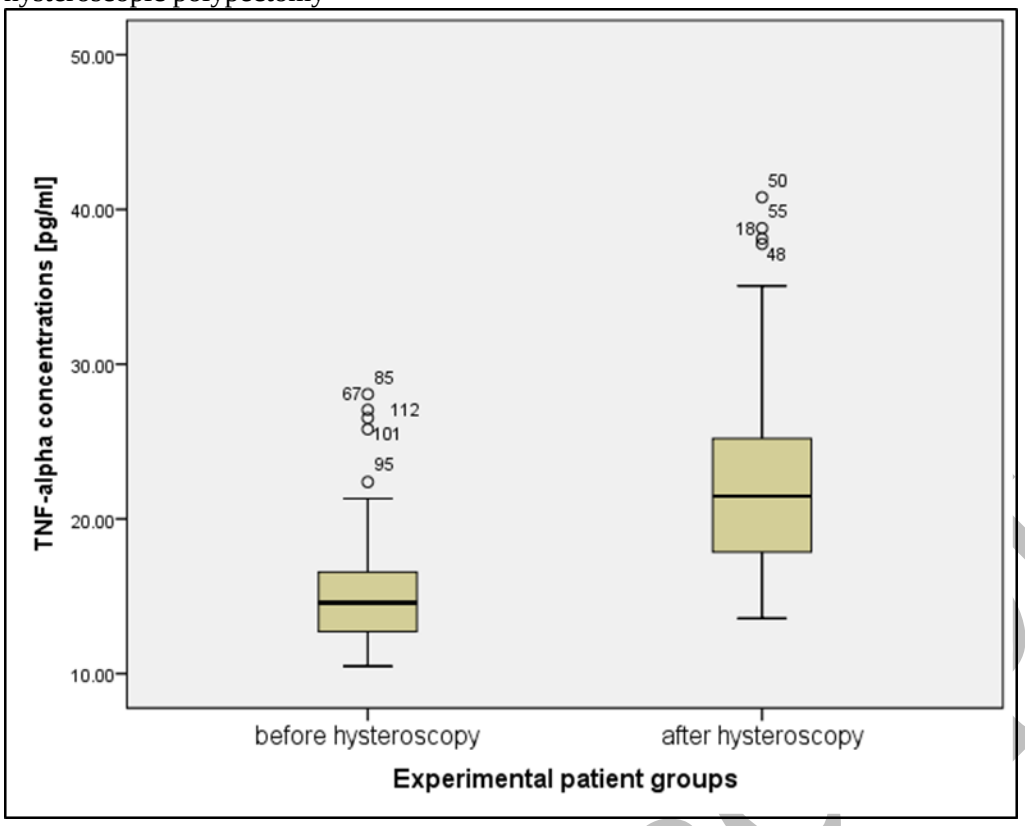

Table 2. Serum concentrations of TNF-α in the control group of infertile patients before and after hysteroscopy

Cont. group of patients	Mean value
Serum before hysteroscopy	13.5908 (6.70-29.26) [pg/ml]
Serum after hysteroscopy	14.5127 (4.12-42.56) [pg-ml]
t-TEST	Values
Hypothesis Mean difference	0
Df	110
t Stat	-0.917
P(T <=t)	0.361
T Critical two-tail	2.871

Figure 2. Serum concentrations of TNF-α in the control group of infertile patients before and after hysteroscopy

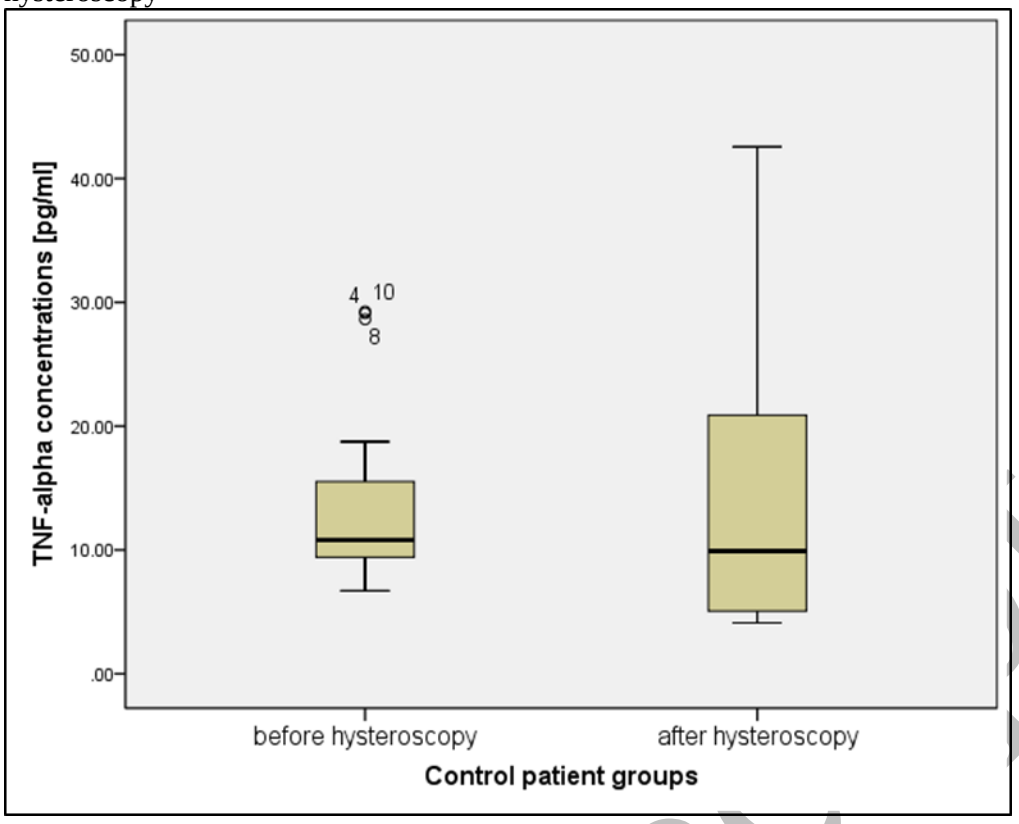

Table 3. Concentrations of TNF-α in serum and uterine flushings in the experimental group of infertile patients before hysteroscopic polypectomy

Exp. group of patients	Mean value
Serum before hysteroscopy	15.6220 (10.48-28.06) [pg/ml]
Flushings	12.6046 (6.43.43-23.81) [pg/ml]
t-TEST	Values
Hypothesis Mean difference	0
Df	110
t Stat	4.450
P(T <=t)	0.0001
T Critical two-tail	1.980

Figure 3. Concentrations of TNF-α in serum and uterine flushings in the experimental group of infertile patients before hysteroscopic polypectomy

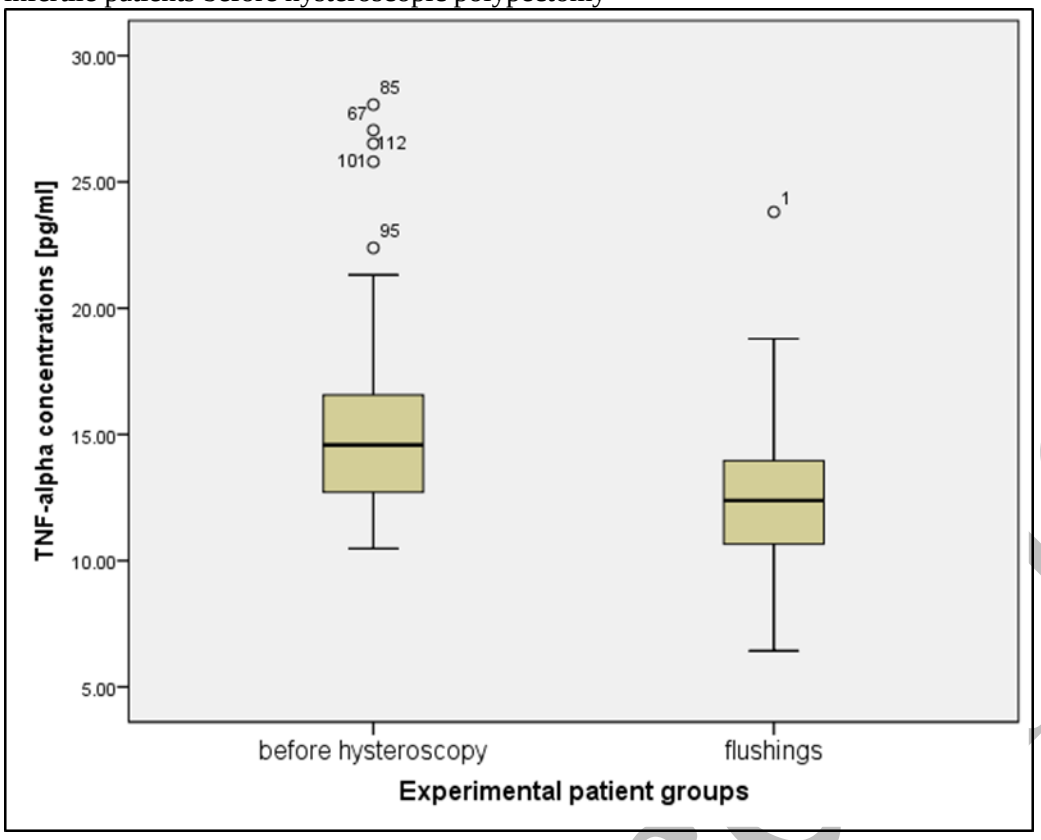

Table 4. Concentrations of TNF-α in serum and uterine flushings in the control group of infertile patients before hysteroscopy

Cont. group of patients	Mean value
Serum before hysteroscopy	13.5908 (6.70-29.26) [pg/ml]
Flushings	15.6673 (8.31-27.61) [pg/ml]
t-TEST	Values
Hypothesis Mean difference	0
Df	47
t Stat	-1.140
P(T <=t)	0.260
T Critical two-tail	2.011

Figure 4. Concentrations of TNF-α in serum and uterine flushings in the control group of infertile patients before hysteroscopy

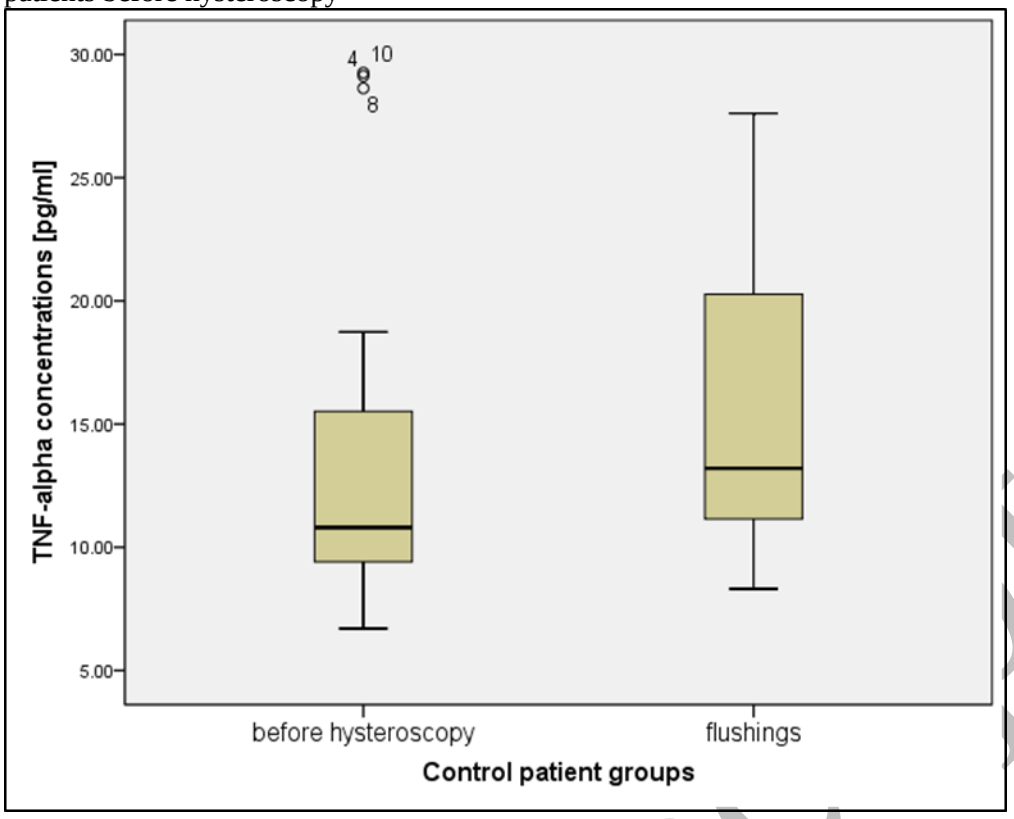

Table 5. Correlation of TNF-α concentrations in uterine flushings and serum in the experimental group of infertile patients before and after hysteroscopic polypectomy

Pearson correlations	Flushings (Exp.)	Serum before (Exp.)	Serum after(Exp.)
Flushings (Exp.)	1	-0.18	0.108
Serum before (Exp.)	-0.18	1	-0.132
Serum after(Exp.)	0.108	-0.132	1

Figure 5. Correlation of TNF-α concentrations in uterine flushings and serum in the experimental group of infertile patients before and after hysteroscopic polypectomy

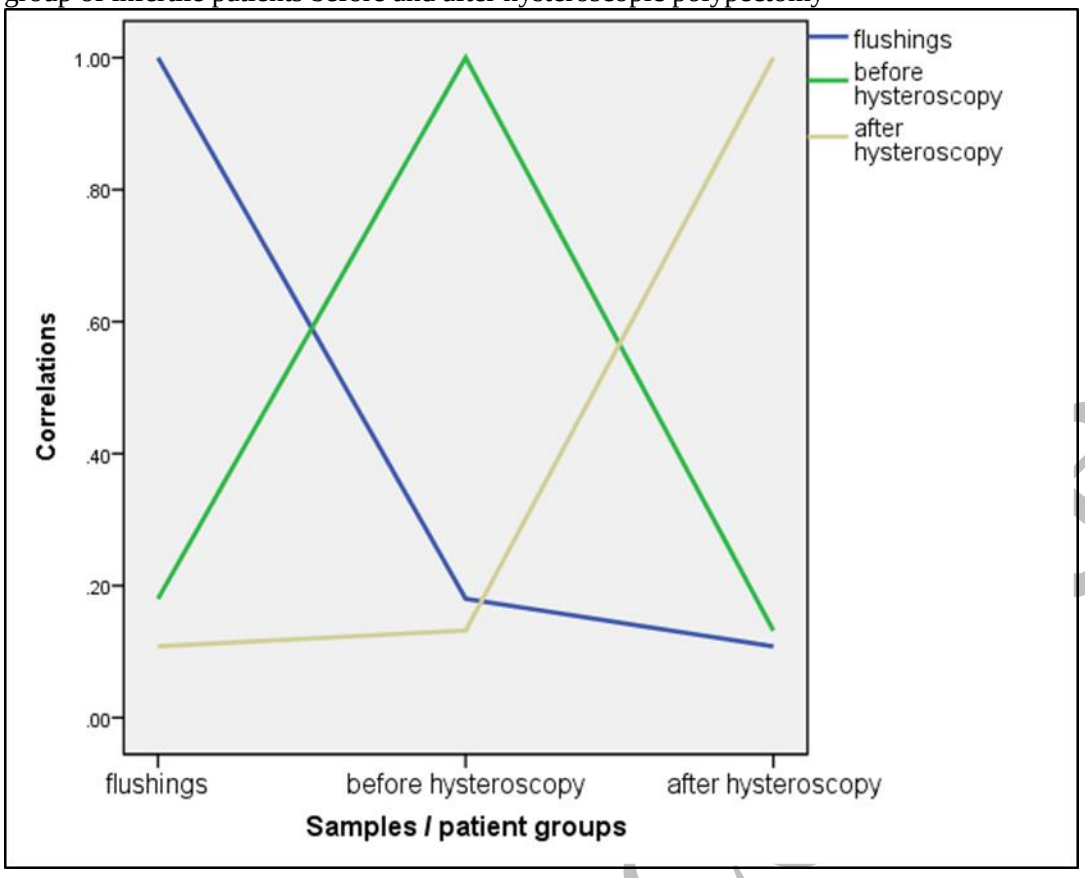

Table 6. Correlation of TNF-α concentrations in uterine flushings and serum in the control group of infertile patients before and after hysteroscopy

Pearson correlations	Flushings (Control)	Serum before (Cont.)	Serum after (Cont.)
Flushings (Cont.)	1	0.482	0.696
Serum before (Cont.)	0.482	1	0.398
Serum after (Cont.)	0.696	0.398	1

Figure 6. Correlation of TNF-α concentrations in uterine flushings and serum in the control group of infertile patients before and after hysteroscopy