

Effect of chitosan-assisted combination of laparoscope and hysteroscope on the levels of IFN- γ and ICAM-1 in treatment of infertility caused by obstruction of fallopian tubes

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ABSTRACT

This experiment was carried out to investigate the effect of combined treatment of tubal obstruction infertility with deacetylated chitosan and two microscopes on the levels of IFN- γ and ICAM-1. In this study, 100 infertile patients with fallopian tube obstruction who were treated in Jiangbei District Hospital of traditional Chinese medicine from January to August 2019 were divided into two groups according to the alternating grouping method, group A (50 cases) received combined surgery, and Group B (50 cases) received combined surgery and chitosan. The curative effect and postoperative pelvic adhesion of the two groups were analyzed, and the levels of IFN- γ , ICAM-1 and IL6(IL-6), laminin (LN), Transforming growth factor beta 1(TGF- β 1) and fibronectin (FN) were observed before and after treatment. Results showed that the total effective rate of Group B was higher than that of Group A (92.00% vs 76.00%). The incidence of pelvic adhesion was lower in Group A (4.00% vs 16.00%) ($P < 0.05$). The levels of IFN- γ , ICAM-1, IL-6, LN, FN and TGF- β 1 in Group B were significantly lower than those in group A ($P < 0.05$). In conclusion, the treatment of tubal obstruction infertility with combined deacetylated chitosan and biendoscopy is effective, which can reduce the levels of IFN- γ and ICAM-1, improve the expression of adhesion-related factors and reduce the occurrence of pelvic adhesion.

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Introduction

Obstruction of fallopian tubes, as a common disease in the clinical gynecological department, refers to the partial or total obstruction of fallopian tubes caused by inflammation, pelvic adhesion and infection and is also the major cause of female infertility (1). In an early stage, obstruction of the fallopian tubes usually presents no evident symptoms, but some patients may demonstrate infectious symptoms in the reproductive tract, including increased vaginal secretion, thickened adnexa area, mass formation and pains in the lower belly (2-3). Published literatures (4-5) have already confirmed that inflammatory cytokines can stimulate the oviductal mucosa to induce a series of pathological changes, like edema, effusion or congestion, and such changes, with the abundant lymphocytes and plasma cells under the mucosa of the oviductal wall, could further induce the fibrotic responses, including the thickening of wall and damage to the functions of wriggling and contraction of epithelium, thereby disturbing the abilities of infundibulum to pick up and transport the egg, eventually triggering infertility. The operation remains the major strategy in clinical treatment for infertility caused by occlusion of fallopian tubes in recent years, and the combination of hysteroscope and laparoscope is widely accepted by clinicians and patients due to the minimal invasion, rapid recovery and painlessness. However, the operation

can only dredge the occluded fallopian tubes to restore the normal anatomical structure, yet remains hopeless in dealing with the changes in the microenvironment, including the pathological inflammation-caused injury and adhesion (6). Chitosan, as the sole cationic biological polysaccharide, is excellent in biological adhesion, antibacterial activity and compatibility and performs well in promoting wound healing and tissue regeneration (7). As such, we aim to elucidate the effect of the chitosan-assisted combination of laparoscope and hysteroscope on the levels of ifn- γ and icam-1 in the treatment of infertility caused by obstruction of fallopian tubes.

Materials and Methods

General data

A total of 100 patients with obstruction of fallopian tubes-caused infertility who visited Chongqing Jiangbei Hospital of Traditional Chinese Medicine between January 2019 and August 2021 were recruited into this study and divided into two groups as per the alternative method.

Criteria for inclusion: 1) Patients conforming to the related criteria for diagnosis and treatment according to the Obstetrics and Gynecology (8), including one of the following criteria: (i) Patient who had normal sexual life with no contraception or pregnancy, and whose husband's reproductive function was normal; (ii) Patient with occlu-

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sion at the proximal end of fallopian tube indicated by uterotubography; (iii) Patient with occlusion at the proximal end of fallopian tube indicated by laparoscopic hydrotubation; 2) Patient who accepted the combination of laparoscope and hysteroscope operation and with no contraindication; 3) Patient who signed the written informed consents after they were informed of the protocols of this study.

Criteria for exclusion: 1) Patient with infertility due to the dysfunction in some organs; 2) Patient with cancer like cervical cancer, endometrium cancer and ovarian cancer; 3) Patient who was allergic to the drugs used in this study. In Group A, there were 50 patients, aged between 25 and 39 years old, with an average age of (31.27 \pm 2.58) years old; the duration of infertility ranged from 1 to 8 years, with an average of (3.38 \pm 1.62) years; there were 15 patients with primary infertility and 35 with secondary infertility. In Group B, there were 50 patients, aged between 24 and 38 years old, with an average age of (30.89 \pm 2.62) years old; the duration of infertility ranged from 1 to 7 years, with an average of (3.40 \pm 1.58) years; there were 17 patients with primary infertility and 33 with secondary infertility. Comparison of the general data between two groups showed no significant difference ($P > 0.05$), suggesting that the data were comparable. This study conformed to the requirement of the *Declaration of Helsinki* of the World Medical Association.

Methods

Patients in two groups underwent the combination of laparoscopic and hysteroscopic operation in the following steps: Operation was chosen within 3 to 7 days after menstruation; patients were required to stay in lithotomy position for general anesthesia; Trocar was placed through the umbilical cord puncture to establish pneumoperitoneum (CO₂: 12mmHg) at the incision in the length of 5 mm at 1.3 cm to the umbilical cord, 1.0 cm and 0.5 cm to the left lower abdomen. After the cervix uteri were dilated, a hysteroscope was inserted; thereafter, the fallopian tube was exposed by separating the pelvic adhesion to examine whether the fallopian tube was blocked by injecting the methylene blue. Under the guidance of a laparoscope, the catheter was delivered to the blocked site through the uterine cavity, where the syringe was pulled and pushed until the resistance disappeared, and then the fallopian tube recanalization was performed. Thereafter, methylene blue was injected and the appearance of blue liquid at the umbrella end indicated that the fallopian tube was unobstructed. After treatment, the pelvic cavity was rinsed with normal saline and antibiotics were given in case of infection. Patients were advised to avoid any sexual intercourse within 1 month after treatment.

Patients in the control group underwent regular treatment: Nutrition supplementation, fasting prior to the ventilation for the gastrointestinal tract and administration of antibiotics according to the condition of the patient. For

patients in the observation group, 3 mL of chitosan gel for medical use was smeared on the peritoneum of the pelvic and abdominal cavity and fallopian tube, followed by the closure of the abdomen.

Indicators for observation

The efficacy of patients in two groups was evaluated according to *Obstetrics and Gynecology* in the following criteria: Excellent for patients with the restoration of patency of oviduct and pregnancy within 6 months after treatment; effective for patients with the significant amelioration of the stenosis in oviduct after treatment; failure for patients with no amelioration of the stenosis in oviduct after treatment. Total effectiveness rate = Rate of excellent + Rate of effective.

Besides, 5 mL fasting venous blood was collected in the morning before treatment and 7 days after treatment, and, from the blood sample, the supernatant was obtained after the sample was centrifuged at 1000 rpm for 10 min. Later, the supernatant was prepared from the determination of inflammatory cytokines [Interferon γ (IFN- γ), intercellular adhesion molecule 1 (ICAM-1), interleukin 6 (IL-6)] and adhesion-related factors [laminin (LN), transforming growth factor β 1 (TGF- β 1) and fibronectin (FN)] via enzyme-linked immunosorbent assay (ELISA). Pelvic floor adhesion was also evaluated for all patients according to the Nair Classification Criteria: Grade I for a patient with only one adhesive band; Grade II for two adhesive bands; Grade III for a patient with bands more than 2 but with no adhesion of organ to the abdominal wall; Grade IV for a patient with bands more than 2 and with the adhesion of organ to the abdominal wall.

Statistical methods

SPSS 23.0 software was used to process and analyze the data. Measurement data were expressed in form of mean \pm standard deviation (SD). Differences between the two groups were validated by the independent sample t -test, while those inside one group were by the pairwise t -test. Enumeration data were expressed in form of a ratio, and the difference was validated by the chi-square test. $P < 0.05$ suggested that the difference had statistical significance.

Results

Patients in Group B had a higher total effective rate as compared to those in Group A (92.00% vs. 76.00%), and the difference had statistical significance ($P < 0.05$; Table 1). Prior to the treatment, levels of IFN- γ , ICAM-1 and IL-6 in patients of two groups had no significant difference ($P > 0.05$); after treatment, levels of indicators above experienced more evident decreases in Group B in comparison with Group A ($P < 0.01$; Table 2). Before treatment, levels of LN, FN and TGF- β 1 of patients in two

Table 1. Comparison of efficacy between two groups [n (%)].

Group	n	Excellent	Effective	Failed	Total effective rate
A	50	17 (34.00)	16 (32.00)	12 (24.00)	38 (76.00)
B	50	20 (40.00)	26 (52.00)	4 (8.00)	46 (92.00)
χ^2					4.762
P					0.029

Table 2. Comparison of the levels of inflammatory factors between two groups ($\bar{x} \pm s$).

Group	n	IFN- γ (ng/mL)		ICAM-1 (μ g/mL)		IL-6 (pg/mL)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
A	50	44.78 \pm 9.74	37.41 \pm 6.83*	283.56 \pm 30.47	237.63 \pm 30.38*	12.23 \pm 1.25	9.64 \pm 1.31*
B	50	45.23 \pm 8.86	32.15 \pm 6.42*	281.49 \pm 29.71	174.76 \pm 31.62*	12.08 \pm 1.14	7.68 \pm 1.22*
<i>t</i>		0.242	3.968	0.344	0.138	0.627	7.742
<i>P</i>		0.809	<0.01	0.732	<0.01	0.532	<0.01

Note: * $P < 0.05$ vs. the level before treatment in the same group.

Table 3. Comparison of the levels of adhesion-related factors between two groups ($\bar{x} \pm s$).

Group	n	LN (μ g/L)		FN (ng/mL)		TGF- β 1 (μ g/L)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
A	50	110.32 \pm 8.85	94.72 \pm 6.38*	15.48 \pm 4.68	11.42 \pm 3.63*	60.35 \pm 12.40	51.42 \pm 8.73*
B	50	111.83 \pm 7.98	85.68 \pm 7.31*	15.76 \pm 4.93	8.76 \pm 3.44*	61.08 \pm 11.74	42.34 \pm 7.61*
<i>t</i>		0.896	6.588	0.291	3.761	0.302	5.544
<i>P</i>		0.372	<0.01	0.772	<0.01	0.763	<0.01

Note: * $P < 0.05$ vs. the level before treatment in the same group.

Table 4. Comparison of the incidence of pelvic adhesion between two groups [n (%)].

Group	n	Grade I	Grade II	Grade III	Grade IV	Total incidence rate
A	50	5 (10.00)	1 (2.00)	1 (2.00)	1 (2.00)	8 (16.00)
B	50	1 (2.00)	1 (2.00)	0	0	2 (4.00)
χ^2						4.000
<i>P</i>						0.046

groups had no significant difference ($P > 0.05$); after treatment, patients in Group B had lower levels of LN, FN and TGF- β 1 as compared to those in Group A ($P < 0.01$; Table 3). For pelvic adhesion, the incidence rate of patients in Group B was much lower than that in Group A (4.00% vs. 16.00%; $P < 0.05$; Table 4).

Discussion

Fallopian tube obstruction, as one of the major factors contributing to female infertility, can induce the hydrosalpinx and stenosis of the cavity to trigger the dysfunction in sperm transport, eventually leading to infertility. As the technique of endoscopes continues to improve, hysteroscope and laparoscope have been widely applied in the treatment of gynecological diseases by visualizing the site of the lesion and condition of the patient as well as the advantages, like slight trauma and good prognosis; besides, a combination of hysteroscope and laparoscope can further increase the effectiveness of surgery and ameliorate the clinical symptoms (9-10). However, surgery can only dredge the obstructed fallopian tube and restore the normal anatomic structure but fail to deal with the inflammation-induced pathological injury and maintain the long-term unblocked status of the fallopian tube, so medication in combination with surgery is quite meaningful for improving the efficacy and reducing the level of inflammatory factors. Results of this study demonstrated that as compared to Group A, patients in Group B had a higher total effective rate but a lower incidence rate of pelvic adhesion, suggesting that chitosan in combination with combined laparoscope and hysteroscope did increase the effective rate

of treatment, increase the openness of fallopian tube and prevent the pelvic adhesion, which, according to our analysis, may relate to the good biological compatibility and degradability of chitosan that is conducive to the postoperative prophylaxis of adhesion.

Existing studies (11-12) have shown that surgical treatment can induce the injury, bleeding or stimuli of serosa, thereby inducing the hyperinflammatory responses in the serum of patients with fallopian tube obstruction. Local adhesion, as the inflammatory responses secondary to the fibroplasia, is somehow related to the level of inflammatory factors. Cell adhesion molecules can mediate allergic reactions, inflammation and thrombosis, where ICAM-1, as a key molecule, can recognize the specific receptor on the surface of cells to induce the adhesion, thereby being involved in the immune regulation and inflammation (13-14). IFN- γ , secreted by the nature of killing cells and T lymphocytes, can assist immune regulation and resist viral infection, showing a correlation with the severity of inflammation and injury to fallopian tubes (15). Zhao M Z *et al.* (16) have found that ICAM-1 and IL-6 are involved in the pathological and inflammatory responses which can further give rise to pathological injury to the lung in the mouse. Li X *et al.* (17) predicted the pregnancy of patients with fallopian tube obstruction after the combined laparoscopic and hysteroscopic treatment by the levels of IFN- γ and ICAM-1 in serum and found that increased levels of IFN- γ and ICAM-1 are major risk factors inducing the postoperative infertility of patients. IL-6, as a kind of bioactive glycoprotein, plays a role in local or general anti-infection, and a high level of IL-6 can induce fibroplasia, thereby aggravating the injury to the fallopian tube

and local pelvic adhesion; moreover, IL-6 can promote the formation of inflammatory scar, which could also contribute to the local adhesion and obstruction of the fallopian tube, eventually leading to infertility. Results of this study showed that after treatment, patients in Group B had lower levels of IFN- γ , ICAM-1, IL-6, LN, FN and TGF- β 1 when compared to Group A, revealing that for fallopian tube obstruction, chitosan in combination with combined laparoscopic and hysteroscopic treatment can reduce the levels of IFN- γ and ICAM-1 and improve the levels of adhesion-related factors. Jiang *et al.* (18) evaluated the efficacy and safety of chitosan in preventing postoperative intestinal adhesion in 40 cases, and the results showed that chitosan can inhibit the levels of IL-1, TNF- α and TGF- β , thereby reducing the incidence of intestinal adhesion, which coincided with our findings. This is possible because the gel of chitosan can act as the lubricating factor and biological barrier that can prevent adhesion, and chitosan can promote the growth of epithelial cells but suppress the fibrotic cells selectively, thereby advancing the repairing of serosal cells.

In conclusion, chitosan in combination with combined laparoscopic and hysteroscopic treatment performs well in the treatment of fallopian tube obstruction by reducing the levels of inflammatory factors (IFN- γ and ICAM-1), improving the expression of adhesion-related factors and minimizing the incidence of pelvic adhesion.

References

- Arustamyan K, Totoyan E, Karapetyan A, Gasparyan A. The state of fallopian tubes in women with urogenital chlamydia and infertility. *Georgian Med News*. 2017 Jul-Aug;(268-269):80-885. Russian. PMID: 28820419.
- Lee SK, Kim CJ, Kim DJ, Kang JH. Immune cells in the female reproductive tract. *Immune Netw*. 2015 Feb;15(1):16-26. doi: 10.4110/in.2015.15.1.16. Epub 2015 Feb 17. PMID: 25713505; PMCID: PMC4338264.
- Bai W, Xu X, Xie H, Sun C, Che K, Liu M, Liu J, Shi Y, Ma H. Adnexal torsion in the third trimester of pregnancy: a case report and diagnostic value of MR imaging. *BMC Med Imaging*. 2020 Feb 17;20(1):19. doi: 10.1186/s12880-020-00422-1. PMID: 32066402; PMCID: PMC7027050.
- Pyra K, Szmygin M, Dymara-Konopka W, Zych A, Sojka M, Jargiełło T, Leszczyńska-Gorzela B. The pregnancy rate of infertile patients with proximal tubal obstruction 12 months following selective salpingography and tubal catheterization. *Eur J Obstet Gynecol Reprod Biol*. 2020 Nov;254:164-169. doi: 10.1016/j.ejogrb.2020.08.008. Epub 2020 Sep 16. PMID: 32979628.
- Li X, Luan T, Zhao C, Zhang M, Dong L, Su Y, Ling X. Effect of sildenafil citrate on treatment of infertility in women with a thin endometrium: a systematic review and meta-analysis. *J Int Med Res*. 2020 Nov;48(11):300060520969584. doi: 10.1177/0300060520969584. PMID: 33176524; PMCID: PMC7673063.
- Stewart JK, Hipolito Canario DA, Daso G, Thapa D, Montgomery S, Kohi M. Use of n-Butyl-2-Cyanoacrylate for Fallopian Tube Embolization via Selective Catheterization in a Rabbit Model: Feasibility Study for Potential Nonsurgical Sterilization. *J Vasc Interv Radiol*. 2023 Feb;34(2):225-233. doi: 10.1016/j.jvir.2022.10.026. Epub 2022 Oct 25. PMID: 36306987.
- XIE Xing, GOU Wen-li. *Obstetrics and Gynecology* (8th edition) [M] Beijing: People's Medical Publishing House, 2013; 369-372.
- Doulaveris G, Vani K, Saccone G, Chauhan SP, Berghella V. Number and quality of randomized controlled trials in obstetrics published in the top general medical and obstetrics and gynecology journals. *Am J Obstet Gynecol MFM*. 2022 Jan;4(1):100509. doi: 10.1016/j.ajogmf.2021.100509. Epub 2021 Oct 14. PMID: 34656731.
- Kamo N, Takahashi T, Soeda S, Jimbo M, Yoshida-Komiya H, Fujimori K. Hydrosalpinx in a premenarcheal 14-year-old girl with the proliferation of smooth muscle cells in the isthmus of the fallopian tube: A case report. *J Obstet Gynaecol Res*. 2023 Feb;49(2):769-774. doi: 10.1111/jog.15481. Epub 2022 Oct 28. PMID: 36305392.
- Soyama H, Okuguchi S, Yoshida T, Taniguchi F. Unilateral Partial Absence of the Fallopian Tube in a Female Patient With Infertility: A Case Report and Literature Review. *Cureus*. 2022 Dec 28;14(12):e33057. doi: 10.7759/cureus.33057. PMID: 36721610; PMCID: PMC9883063.
- Kenta, Iwasaki, Nana, et al. Suppressive effect of everolimus on IL-2, IL-10, IL-21, and IFN γ levels: Implications for the successful minimization of calcineurin inhibitor use in transplantation. *Ther Drug Monit* 2019;41(3):371-375.
- Akarawit J, Karanrat S. Effect of Premedication Hyoscine-N-Butylbromide before Hysterosalpingography for Diagnosis of Proximal Tubal Obstruction in Infertile Women: a Randomized Double-Blind Controlled Trial. *J Minim Invasive Gynecol* 2018; 26(1):110-116.
- Teixeira S C, Silva R J, Lopes-Maria J B, et al. Transforming growth factor (TGF)- β 1 and interferon (IFN)- γ differentially regulate ICAM-1 expression and adhesion of *Toxoplasma gondii* to human trophoblast (BeWo) and uterine cervical (HeLa) cells. *Acta Trop* 2021;224:10611.
- Devadoss D, Daly G, Manevski M, et al. A long noncoding RNA antisense to ICAM-1 is involved in allergic asthma associated hyperreactive response of airway epithelial cells. *Mucosal Immunol* 2021;14(3):630-639.
- Karki R, Sharma B R, Tuladhar S, et al. Synergism of TNF- α and IFN- γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell* 2020;184(1):149-168.
- Zhao M Z, Guo X, Sun B, et al. HA of H1N1 enhanced the expression of ICAM-1 and IL-6 in HUVECs and pathological injury in the lungs in mice. *Gene*, 2021; 801:145854.
- LI Xiang, DING Yang, LU Jian-jun. The predictive value of serum INF- γ and ICAM-1 combined with clinical features in patients with tubal infertility after double mirror treatment. *J Guizhou Med University* 2021; 46(5):567-572.
- Jiang Long-long, QING Song. Clinical Effect and Safety of Chitosan for Preventing Postoperative Intestinal Adhesion in 40 Cases. *China Pharmaceut* 2018; 27(4):29-31.