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Original Article

Effects of Gushen Antai decoction on serum homocysteine and anticardiolipin antibodies levels in patients with unexplained recurrent spontaneous abortion

Minhuan Ye[1], Runyi Fang[1], Simin Lun[1], Jianhua Fang[2]

[1]Department of Obstetrics and Gynecology, Houjie Hospital, Dongguan, Guangdong Province, China; [2]Department of Ultrasound, Houjie Hospital, Dongguan, Guangdong Province, China

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Abstract: Objective: To investigate the effects of heparin combined with Gushen Antai Decoction on the levels of homocysteine (Hcy) and anticardiolipin antibodies (ACA) in patients with unexplained recurrent spontaneous abortion (URSA). Methods: A total of 100 patients with URSA who were admitted to our hospital were enrolled in this study. According to the treatment options, they were divided into a control group (CG) (N=50) and an observation group (OG) (N=50). The CG was given heparin combined with strategies that prevent miscarriage, and the OG was additionally treated with Gushen Antai decoction. The changes in serum ACA, Hcy and Th1/Th2 cytokines were observed and compared between the two groups, and the clinical treatment efficacy of the two groups was recorded. Results: The OG showed an increased total response rate (86.00%), compared with the 58.00% of the CG (X^2=6.146, P<0.05). After treatment, the OG showed improved levels of IL-12 and IL-10 compared with the CG (P<0.05); and the OG showed decreased ACA and Hcy levels after treatment compared with the CG (P<0.05); as well as levels of FIB, DD, and PAI-1 in the OG were significantly improved compared with the CG (P<0.05). Conclusion: Gushen Antai Decoction combined with heparin therapy can effectively improve the response rate of treatment of URSA, balance ratio of Th1/Th2 cells, and reduce the levels of ACA and Hcy. This combined method can effectively increase the pregnancy success rate and is worthy of clinical application.

Keywords: Unexplained recurrent spontaneous abortion, anticardiolipin antibody, Gushen Antai decoction, heparin therapy

Introduction

Recurrent spontaneous abortion (RSA) is defined as 2-3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period. In recent years, the incidence rate of RSA in China is as high as 1%-2% in reproductive-age women, which seriously affects women's life and health [1, 2]. Many factors have been described as having an association with RSA. It is generally believed that genital infection, immune function abnormality, genetics and endocrine factors are involved. However, more than 50% of reproductive-age women have RSA for unknown reasons, and this is clinically called unexplained recurrent spontaneous abortion (URSA). It is clinically refractory infertility, and an inherited prothrombotic state is related to biochemical pregnancy loss [3]. Western medicine mainly uses heparin to treat this inherited prothrombotic state. However, heparin alone can cause side effects such as osteoporosis, bleeding, and allergies. In recent years, traditional Chinese medicine (TCM) combined with Western medicine has made some progress in the treatment of RSA [4]. TCM indicates that kidney deficiency and blood stasis are the main causes of miscarriage. Studies have found that low-dose prednisone or aspirin is not an effective treatment for URSA [5]. Chinese herbs combined with Western medicine are currently the main research direction for clinical treatment of URSA. As a traditional Chinese prescription, Gushen Antai Decoction has become more and more prominent in clinical application in recent years with the increasing clinical emphasis on TCM treatment. An experimental study in rats with kidney deficiency induced abortion found that the rats with abortion after modeling had obvious immune

dysfunction, and the application of Gushen Antai Decoction significantly improved the immune dysfunction in the rats [6]. It was found in another study that the application of Gushen Antai pills combined with progesterone significantly reduced the serum level of CA125 in patients with possible early abortion due to kidney deficiency [7]. ACA and Hcy have been confirmed to have a significant clinical correlation with a variety of pregnancy outcomes [8]. This study included 100 URSA patients and divided them into an observation group (OG) and a control group (CG) according to the treatment regimen, and compared the effects of two regimens on ACA, Hcy and Th1/Th2 cytokines in patients with URSA.

Materials and methods

Baseline data

A total of 100 patients with URSA who were admitted to our hospital from October 2018 to October 2019, were aged 25-38 years with an average age (33.17±8.29) years, with 3-8 miscarriages, with an average of (4.16±1.35) occurrences. According to treatment options, they were divided into the OG of 50 cases and the CG of 50 cases. The OG had an average age of (34.05±7.11) years and an average of (3.88±8.42) miscarriages; the CG had an average age of (35.74±7.42) years and had an average of (4.27±1.34) miscarriages. This study has been approved by the Ethics Committee of Houjie Hospital. All study participants provided written informed consent before participating in the study.

Inclusion and exclusion

Inclusion criteria: patients with 3 times of spontaneous miscarriage; with no abnormalities in chromosomes and reproductive organs; with normal blood glucose, normal sex hormones and thyroid function; negative results for gonococcus and chlamydia. Exclusion criteria: those with patients with miscarriages caused by immunity, infection, heredity, endocrine and genital tract malformations.

Treatment regimen

The CG was treated with western medicine and heparin therapy, including aspirin enteric-coated tablets, progesterone injection, estradiol, dydrogesterone, and fenmetone, which were administrated until 12 weeks of pregnancy; low molecular weight heparin was administered 4100 IU/time via Qd subcutaneous injection until 16 weeks of gestation. The OG was treated with Gushen Antai decoction in addition to the treatment option of the CG, including Angelica 10 g, Rehmannia glutinosa 15 g, Rehmannia glutinosa Libosch 15 g, Chinese yam 12 g, Codonopsis 15 g, Ejiao 10 g, wolfberry 10 g, licorice 6 g, Stir-fried atractylodes rhizome 12 g, eucommia 15 g and Dipsacus 15 g, which was prepared and consumed for 16 weeks of pregnancy. During treatment, liver function, coagulation function and platelet count were reviewed every week in the two groups. If abnormalities occurred, a dosage of low-molecular-weight heparin was reduced or withdrawn. Those with elevated transaminase level were given liver-protecting drugs.

Evaluation criteria

No response: heavy vaginal bleeding, fetal death or miscarriage. Improvement: improved vaginal bleeding and other clinical symptoms, with normal pregnancy. Cure: no vaginal bleeding, healthy fetus with success pregnancy. The total response rate = cure rate + improvement rate.

Outcome measurement

The clinical efficacy of two groups before and after treatment was compared; 2 ml of cubital venous blood was collected for serum separation and measured using enzyme-linked immunosorbent assay. The changes of Th1 and Th2 cytokines before and after treatment in the two groups were compared. The serum Hcy and ACA levels were compared between the two groups before and after treatment. ACA was detected by enzyme-linked immunosorbent assay, and Hcy was determined by high performance liquid chromatography; and the levels of PT, TT, DD, FIB and PAI-1 were recorded. The ACA results are expressed in BI, BI = sample absorbance (A)/standard sample (A). If BI of sample > average BI of controls ±2 times of standard deviation, then ACA results are positive.

Statistical method

The SPSS 22.0 was used for data analysis, the measurement data was measured by t-test, and the count data was measured by chi-

Table 1. Comparison of general clinical data in the two groups (x ± s)/[n (%)]

General data		Observation group (n=50)	Control group (n=50)	t/X^2	*P*
Gender	M	27	28	0.547	0.362
	F	23	22		
Average age (year)		45.98±4.33	46.01±4.29	0.036	0.987
Average weight (kg)		64.29±3.91	64.34±3.89	0.066	0.917
Average course of disease (year)		2.89±0.28	2.93±0.18	0.871	0.317
Educational level	University and above	11	16	0.781	0.334
	Senior high school	26	24		
	Junior high school and below	13	10		

Table 2. Comparison of response rate of the two groups after treatment [n (%)]

Group	n	Cure	Improvement	No response	Total response rate
Observation group	50	28 (56.00)	15 (30.00)	7 (14.00)	43 (86.00)
Control group	50	15 (30.00)	14 (28.00)	21 (42.00)	29 (58.00)
X^2					6.146
P					<0.05

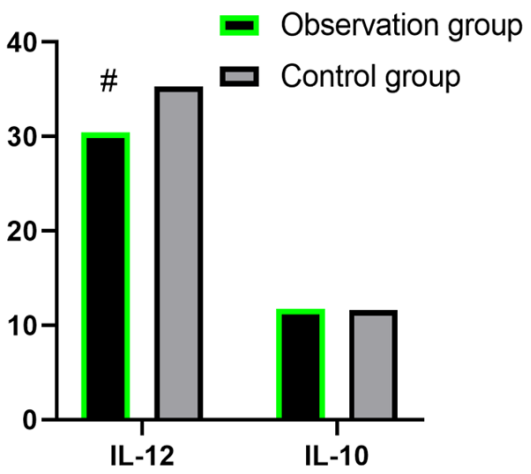

Figure 1. Comparison of the changes of IL-12 and IL-10 levels before and after treatment in the two groups. IL-12 in the observation group was 49.27±10.35 before treatment, 29.41±8.32 after treatment, and IL-12 before treatment in the control group was 50.26±12.09, after treatment it was 41.71±9.29, the difference between the two groups after treatment was statistically significant (t=7.209, P<0.05). The IL-10 level of the observation group was 11.85±1.64 before treatment, 15.52±2.22 after treatment, and the IL-10 level was 12.91±1.58 and 13.75±1.86 after treatment in control group. The difference between the two groups after treatment was significant (t=4.149, P<0.05). # indicates statistically significant difference compared with the control group.

squared test. P<0.05 indicates that the difference is significant.

Results

Comparison of general clinical data in the two groups

There was no significant difference in general clinical data such as gender, age, weight, education level, *etc*. between the two groups, which indicates the groups were comparable (P> 0.05, **Table 1**).

Comparison of response rate

The response rate in the OG was 86.00%, which is higher than 58.00% in the CG (P<0.05, **Table 2**).

Changes of Th1 and Th2 cytokines after treatment in the two groups

No significant difference in the levels of IL-12 and IL-10 between two groups before treatment were noted (P>0.05); they were better in the OG than in the CG after treatment (P<0.05, **Figure 1**).

Changes of Hcy and ACA levels before and after treatment in the two groups

Hcy levels of the two groups decreased significantly after treatment, and it was lower in the OG than in CG (P<0.05). After treatment, the OG showed decreased ACA level compared with the CG (P<0.05, **Tables 3**, **4**; **Figures 2**, **3**).

Table 3. Comparison of changes in Hcy levels before and after treatment (x ± s)

Group	n	Before treatment	After treatment	t	P
Observation group	50	19.34±4.29	10.63±3.39	11.427	<0.05
Control group	50	18.68±5.11	14.42±4.47	4.227	<0.05
t		0.699	4.671		
P		>0.05	<0.05		

Table 4. Comparison of changes in ACA levels before and after treatment [n (%)]

Group	n	Before treatment	After treatment	t	P
Observation group	50	16 (32.00)	5 (10.00)	7.648	<0.05
Control group	50	15 (30.00)	13 (26.00)	0.197	<0.05
t		0.001	5.297		
P		>0.05	<0.05		

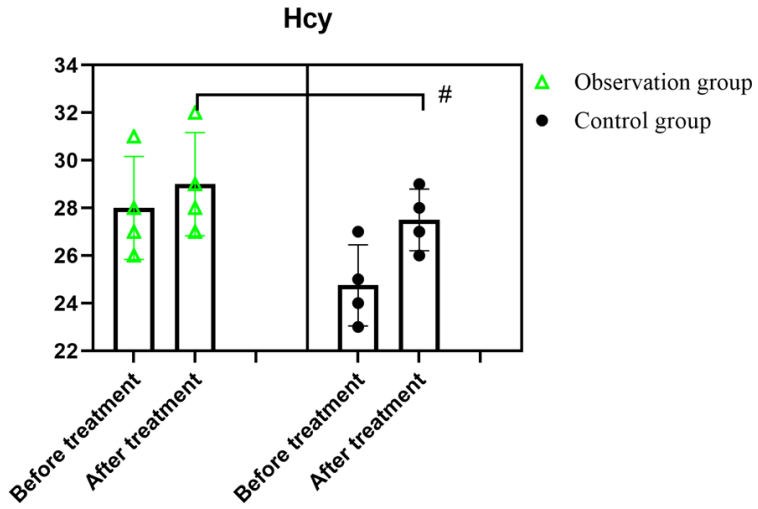

Figure 2. Observation of the changes in Hcy levels before and after treatment in the two groups. The Hcy level in the observation group after treatment significantly lower than before treatment (P<0.05). The Hcy level in the control group was significantly higher than that before improvement (P<0.05). The Hcy level of the observation group was significantly lower than that of the control group after treatment (P<0.05). # indicates statistically significant difference compared with the control group.

Changes of coagulation function in two groups

Two groups showed improved levels of FIB, DD, and PAI following treatment (P<0.05). After treatment, the OG showed decreased levels of FIB, DD and PAI compared with the CG (P< 0.05). Coagulation indexes PT and TT did not differ significantly before and after treatment (P>0.05, **Figure 4**).

Discussion

URSA is a refractory infertility, and with the continuous improvement of living standards, the incidence rate of URSA has been increasing every year [9]. During normal pregnancy, the fetus, as a semi-allograft, will not be rejected by immune suppression. However, when the mother-embryo immune tolerance mechanism is disturbed, it will cause immune attack-related miscarriage [10]. URSA is common in the first trimester, and studies have confirmed that a disorder of the maternal-embryo immune tolerance mechanism in some patients is the main cause of URSA [11, 12]. $CD4^+$ Th cells play an important role in maternal-fetal immunotolerance and can be divided into Th1 and Th2 cells, and the Th1 mediated primarily immune response is initiated by IL-2 and IL-12, etc., while IL-4 and IL-10 are Th2 type cytokines [13, 14]. Th1 cytokines are the main risk factors affecting embryonic development, implantation and fetal survival, while Th2 can inhibit Th1 cell-mediated activation of NK response, which can effectively prevent Th1 cells from harming embryos and improve pregnancy success rates [15, 16].

TCM indicates that unhealthy embryos and nonfatal maternal injury are the main factors that cause miscarriage. According to TCM, the most common reason for miscarriage is Qi deficiency within the kidney meridian. Other factors that may contribute include spleen Qi deficiency, weakness of the penetrating and directing vessels, and Qi sinking. The main treatment in TCM is to increase energy within the kidney channel, nourish blood, strengthen the spleen channel to prevent sinking, and consolidate the directing and

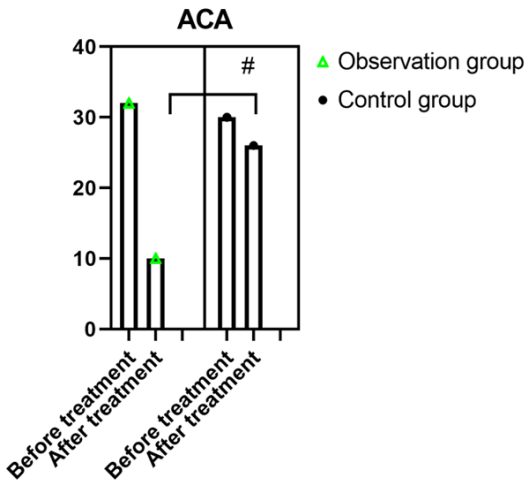

Figure 3. Comparison of the changes in ACA levels before and after treatment in the two groups. The ACA levels in the observation group after treatment was 10.00%, which was significantly lower than before treatment (32.00%) (P<0.05). The percentage after treatment in the control group was 26.00%, which was significantly lower than before treatment (P<0.05), but the degree of decline in ACA in the observation group was significantly lower than that of the control group (P<0.05). # indicates statistically significant difference compared with the control group.

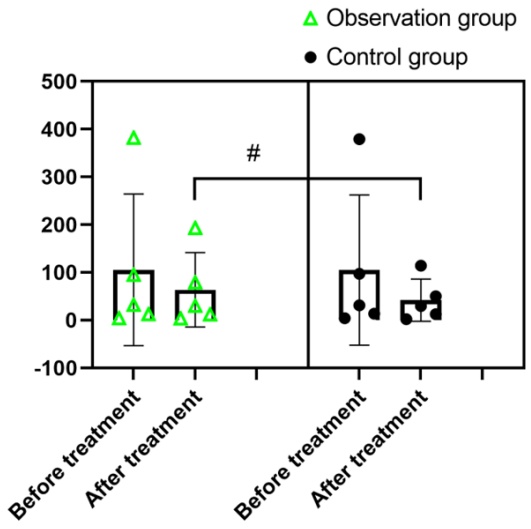

Figure 4. Changes of coagulation function indexes before and after treatment in the two groups. The levels of PT, TT, FIB, DD and PAI-1 in the observation group before treatment and the control group were not statistically different (P>0.05). The levels of DD and PAI-1 were significantly improved compared with the control group (P<0.05), but the difference in PT and TT levels between the two groups was not statistically significant (P>0.05). # indicates statistically significant difference compared with the control group.

penetrating vessels. In this study, Dipsacus in the Gushen Antai decoction invigorated the kidney, nourished the essence and solidified the body. Ejiao strengthens blood, stops bleeding, and improves the quality of vital fluids. Cuscuta is also capable of nourishing the kidney and essence. On this basis, yam, angelica, rehmannia glutinosa, wolfberry and atractylodes macrocephala were added to enhance the effects of strengthening the kidney and protecting the fetus [17-20].

The study showed that the response rate of the OG was significantly higher than that of the CG (P<0.05); Th1 and Th2 cytokines in the OG were significantly improved compared with the CG (P<0.05), indicating that Gushen Antai Decoction combined with heparin can improve the balance of Th1 and Th2 cells, increasing the response rate. The results of a controlled study of 80 patients with RSA have indicated that the conventional Western medicine treatment of RSA usually uses hormonal intervention, of which the short-term effect is obvious, although the patients also can experience more apparent side effects, while Gushen Antai Decoction is a traditional Chinese prescription with the effect of nourishing Yin and kidney, consolidating Chong Vessel and tranquilizing the fetus to prevent miscarriage, and the total effective rate is 95.00%, significantly higher than the 75.00% of Western medicine intervention [21], which is similar with the results of this study. It was found in clinical practice that the Hcy level is affected by nutrition and genetic factors. In normal pregnancy, Hcy levels will be significantly reduced. This level reduction is beneficial to fetal development. When the level of Hcy increases, it easily causes congenital heart disease and neurovascular malformation [22]. ACA can cause damage to vascular endothelial cells, and when ACA binds with phospholipid receptors it can activate platelet aggregation, resulting in the formation of thrombus in the placenta, thereby triggering spontaneous miscarriage [23]. A study on URSA and healthy pregnant women showed that the serum levels of ACA and Hcy in URSA subjects were significantly higher than those in healthy pregnant women, and Logistic multivariate regression analysis found that high levels of Hcy and ACA were independent risk factors for maternal abortion [24].

The present study found that the levels of ACA and Hcy in the OG were significantly lower than those in the CG after treatment ($P<0.05$), indicating that Gushen Antai Decoction combined with heparin can reduce the levels of ACA and Hcy in maternal serum and increase the pregnancy success rate. To analyze the mechanisms of action, it can be attributed to the following two reasons: (1) Gushen Antai Decoction has the function of nourishing Yin and tonifying kidney and consolidating Chong Vessel and tranquilizing the fetus to prevent miscarriage, which corresponds to the pathological mechanism of URSA, such as kidney deficiency, blood heat and weakness of qi and blood; (2) Most of the drugs in the Gushen Antai Decoction can exert estrogen-like effects, adjust or enhance the body's immunity, improve the body's endocrine system, and improve the intrauterine environment to achieve the effect of nourishing the kidney and tranquilizing fetus to prevent miscarriage [25].

In summary, Gushen Antai Decoction with heparin can reduce the levels of ACA and Hcy, and regulate the balance of Th1 and Th2 cytokines, which increases the success rate of pregnancy. The innovation of this study was to explore the clinical intervention effect of Gushen Antai Decoction on patients with URSA through grouping and comparison from the aspects of immune factors, inflammatory factors and blood coagulation indicators. The data are relatively detailed, which can provide a theoretical reference for follow-up treatment. The limitation of this study was that although the changes before and after treatment of various indicators were compared, no detailed analysis of their therapeutic mechanisms was carried out, which will be further explored in the next step.

Disclosure of conflict of interest

None.

Address correspondence to: Jianhua Fang, Department of Ultrasound, Houjie Hospital, No. 21 Hetian Avenue, Houjie Town, Dongguan, Guangdong Province, China. Tel: +86-13712228126; E-mail: jianhuafang100@163.com

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