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Research paper

Association analysis of traditional Uighur medicine differential syndrome typing with biochemical parameters in serum and lesional tissue fluid of vitiligo patients

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ABSTRACT

Introduction: Traditional Uighur medicine humoral (Hilit) theory recognises six different syndrome types of vitiligo. Levels of cytokines, hormones and neurotransmitters relevant to vitiligo were measured in patients diagnosed according to Uighur medicine criteria, in order to identify any association between biochemical parameters and the six syndromes identified by traditional differential diagnosis.
Methods: Using ELISA, interferon-gamma (IFN-gamma), soluble intercellular adhesion molecule 1 (sICAM-1), tyrosine enzyme antibody IgG (IgG), 5-hydroxytryptamine (5-HT), cortisol and norepinephrine (NE) levels were measured in peripheral blood serum of 90 healthy adults, and in serum and skin lesional tissue fluid of 450 vitiligo patients. Large sample multi-angle correlation analysis was used to compare data between patients and non-vitiligo suffers and between patients in the six vitiligo syndrome groups.
Results: Analysis of biochemical parameters showed that serum levels of sICAM-1, IgG and especially IFN-gamma, were higher in patients than in controls, whereas cortisol levels were lower (IFN-gamma, $P=0.017$; sICAM-1, $P=0.000$; IgG, $P=0.025$; cortisol, $P=0.000$). IFN-gamma, cortisol, IgG and NE levels were lower in serum than in skin lesional tissue fluid, whereas 5-HT was higher in serum (IFN-gamma, $P=0.000$; cortisol, $P=0.000$; IgG, $P=0.034$; NE, $P=0.000$; 5-HT, $P=0.000$). There were no differences in serum indicators between syndromes but in skin lesional tissue fluid, IFN-gamma, 5-HT and IgG levels showed significant differences between types,
Conclusion: Increased levels of IFN-gamma are associated with the development of vitiligo. The traditional Uighur dialectical typing of vitiligo into six different syndromes is supported by changes in levels of cytokines in skin lesional tissue fluid, but not serum.
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1. Introduction

Vitiligo is a disease of acquired depigmentation of the skin and mucous membranes and its incidence is rising. Its pathogenesis is unclear but recent research shows that oxidative stress, endocrine, immune and nervous mechanisms are involved in its development. It is also very difficult to treat, and resistance to most types of therapy is often encountered [1–6]. According to traditional Uighur medicine theory, which is similar in many respects to Unani Medicine, there are four Hilit (humors): Balgham (phlegm), Kan (blood), Sapra (yellow bile) and Savda (black bile) [7,8], and disease arises when the balance and quality of these is disrupted. Vitiligo is considered to result from an imbalance in the Balgham (phlegm) humor and six 'abnormal' types of Balgham humor or syndromes are recognized, which can be differentiated clinically by Uighur doctors. They are known as Tatlik (sweet), Shor (salty), Kirtak (bitter), Tamsiz (tasteless), Chuchumal (acid) and Gajsiman (chalky), and are considered to produce pathological and physiological changes in the skin which cause or aggravate vitiligo. Kirtak Balgham type vitiligo is the most common [9,10]. In Western

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medicine, vitiligo is categorized mainly as either segmental vitiligo (SV) or non-segmental vitiligo (NSV). In NSV, patches may occur over wide areas or localized to a particular area. NSV subclasses include generalized (or common) vitiligo with widely distributed areas of depigmentation, universal vitiligo where depigmentation encompasses most of the body and other more localized types. Segmental vitiligo is most often unilateral and spreads much more rapidly than NSV, although it is more stable and not associated with other auto-immune diseases [1–3]. There are other mixed, rare and undetermined types, and the classification of vitiligo is not ideal: therefore looking at it from other perspectives, such as differential diagnosis used in traditional medicine, may be useful in refining the classification and targeting treatment.

Evidence is accumulating to suggest that vitiligo is closely associated with changes in levels of endocrine, immune and other neurotransmitters, and also cytokines [4,5]. In this study, levels of IFN-gamma, soluble intercellular adhesion molecule 1 (sICAM-1), 5-hydroxytryptamine (5-HT), cortisol, tyrosine enzyme antibody IgG (IgG), norepinephrine (noradrenaline, NE) were measured in peripheral blood and lesional tissue fluid from the six types of vitiligo as differentiated by traditional Uighur clinical diagnostic criteria. Large sample multi-angle analysis was used to identify any association between the six abnormal Balgham syndrome types of vitiligo and various biochemical parameters in 450 patients with vitiligo, and compared with 90 non-vitiligo sufferers as a control, to explore the association between biochemical parameters and Uighur syndrome type. This may help to improve treatment and reduce the recurrence of vitiligo by targeting treatment more specifically, according to sub-type.

2. Materials and methods

2.1. Patient recruitment and basic characteristics

450 patients with vitiligo (232 male, 218 female), aged 18–65 years (average 34.3 ± 8.6), who were attending the Xinjiang Uighur Medicine Hospital from January 2011 to December 2012, were recruited into the study. All were receiving treatment at the Xinjiang hospital for traditional Uighur Medicine in Urumqi, either as in-patients or out-patients, and all were in the active stages of vitiligo diagnosed according to the inclusion criteria described below. 90 healthy adult volunteers (45 male, 45 female, average age 28.1 ± 3.2) were recruited as a control group. Gender and age were comparable to the patient group ($P > 0.05$), and although the average age of the patient group was older than the controls, there was no statistical difference between them. As body mass index (BMI) affects the inflammatory status in body, this was measured in both groups and determined to be 24.36 ± 2.89 in the controls and 25.49 ± in the patient group. The groups were therefore deemed to be comparable.

2.2. Inclusion and exclusion criteria

2.2.1. Inclusion criteria

Patients aged 18 to 65 years old who had been diagnosed with vitiligo in accordance with the standards set by the Guidelines for the Diagnosis and Treatment of Vitiligo in Uighur Medicine [11] and the Pigment Disease Study Group, Dermatology and Venereology Professional Committee, of the China Society of Integrated Traditional Chinese and Western Medicine [12], and who had given their informed consent.

2.2.2. Exclusion criteria

Patients aged below 18 or over 65 years; women who were pregnant, lactating or menopausal; patients with other autoimmune, endocrine, psychiatric, liver, kidney, cardiovascular and allergic diseases; patients taking hormonal, oral contraceptive, or immunosuppressive agents; those who had been on corticosteroid or photochemical therapy in the last month; patients who were allergic to at least 2 kinds of food or drugs.

2.3. Sample collection

2.3.1. Serum

5 ml venous blood from fasting patients and healthy subjects was collected between 8 and 9 am. Female blood was taken during the 3–9 days after the start of menstruation. Blood was allowed to stand at room temperature for 90 min and the serum separated by centrifugation at 2000 rpm for 5 min.

2.3.2. Lesional skin tissue fluid

Lesion tissue fluid was obtained using a vacuum absorber over the vitiligo lesional area of the skin, stored at −40 °C and measured within 1 month.

2.4. Biochemical tests

IFN-gamma, sICAM-1, 5-HT, cortisol, IgG and NE were measured using the appropriate Elisa kits (American R&D Company, Beijing) in accordance with the manufacturer' instructions. Anthos 2010 Microplate Reader analysis software (Zhengzhou Antu Experimental Instrument Co., Ltd.) was used to determine the results.

2.5. Statistics

SPSS 18.0 statistical software was used to compare the data from serum and lesional tissue fluid with that of the control group, using a non-parametric (Wilcoxon) test. Analysis of variance was carried out to explore any association between traditional Uighur medicine dialectical typing with data from serum and lesional skin tissue fluid. Test results in SPSS analysis are extreme values.

Table 1
Comparison of levels of serum parameter indicators in vitiligo patients and healthy volunteers.

Parameter	Mean concentration		Mann–Whitney test	
	Patient Serum	Healthy control serum	Value of Z	Value of P
IFN-gamma	56.86 ± 34.87	42.89 ± 7.35	2.390	0.017*
sICAM-1	59.11 ± 28.84	12.63 ± 3.96	11.249	0.000*
5-HT	253.98 ± 118.95	246.67 ± 50.13	1.762	0.078
Cortisol	11.72 ± 7.74	14.12 ± 4.67	3.491	0.000*
IgG	17.12 ± 9.23	13.85 ± 4.26	2.242	0.025*
NE	297.88 ± 189.23	294.06 ± 56.46	1.076	0.282

Note: In Mann–Whitney test Z value is calculated first, then according to Z value table P value is found. If $^*P < 0.05$, with significant difference.

Table 2
Comparison of levels of parameter indicators in serum and skin lesional tissue fluid of vitiligo patients.

Parameter	Mean concentration		Mann–Whitney test	
	Serum	Skin lesional tissue fluid	Value of Z	Value of P
IFN-gamma	57.15 ± 34.72	112.89 ± 28.41	10.512	0.000*
sICAM-1	59.11 ± 28.84	59.62 ± 17.89	1.499	0.134
5-HT	253.98 ± 118.95	121.88 ± 57.82	11.647	0.000*
Cortisol	11.72 ± 7.74	13.62 ± 5.40	3.467	0.001*
IgG	17.12 ± 9.23	18.36 ± 8.54	2.123	0.034*
NE	297.88 ± 189.23	347.57 ± 100.11	3.506	0.000*

Note: In Mann–Whitney test Z value is calculated first, then according to Z value table P value is found. If $^*P < 0.05$, with significant difference.

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