Original Article

Role of traditional Chinese medicine in the regulation of inflammatory mediators in paediatric asthma

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Abstract

Background: In many nations, there has been an increase in the prevalence of allergic diseases, many of which lack therapies for recovery. In addition, there are concerns regarding the use of conventional medicine, particularly corticosteroids, in children. Accordingly, in the case of asthma, many chronic allergic patients are looking at complementary and alternative drugs to treat their condition.

Objective: The study intended to observe the impact of traditional Chinese medicine (TCM) prescriptions children with asthma, with a specific focus on their impact on the molecular mechanism of asthma.

Method: A total number of 167 children with symptom of asthma were included in the study and were randomly allocated to two groups – those who received traditional Chinese medicine (TCM, the TCM group, n = 90) and those who were given salbutamol plus montelukast (SM, the SM group, n = 77). Different TCM treatments were given to patients in the TCM group, while salbutamol and montelukast were given to the SM group. The appropriate treatment was given to both groups for 12 weeks. The patient who are getting TCM treatment had 42 cases, and the SM group had 35 cases. Real-time quantitative fluorescent PCR detects interleukin 10 and interleukin 17 (IL-10 and IL17 respectively) and matrix metal-oproteinase 9 commonly known as MMP-9 expression levels including monocyte blood transforming growth factor 1 (MBTGF-β1). A diagnosis of IL-10 or IL-17 or, MMP-9 as well as MBTGF- β1 is monitored in peripheral blood which were treated before and after consecutively.

Results: MBTGF-β1-RNA decreased among the SM group children after the treatment Whereas after treatment no major variances in MMP-9 among TCM and the SM children group were noted (0.05>0.05). The amount of IL-10 including IL-17 and MMP-9 expressively declined (p = 0.01, 0.04 and 0.03 respectively) in the TCM group. On the other hand, MMP-9 and MBTGF- β1 levels expressively declined in the SM group following therapy. The IL-10 as well as IL-17, and MBTGF-β1 and MMP-9 values in the two groups were not significantly different (> 0.05). The differences between IL-17 and c-ACT scores in the Chinese medicine group and SM group were adversely correlated.

Conclusions: Traditional Chinese Medicine has controlling consequence on secretions of childhood asthma inflammatory mediators and help in asthma management immune mechanism. [*Ethiop. J. Health Dev.* 2020; 34(4):000-000]

Key words: Paediatric asthma, traditional Chinese medicine, children, Chinese herbal medicine

Introduction

Paediatric asthma is a significant public health problem. For centuries, TCM played a important part in treating the diseases not only within China but also in other countries in Asia, where it is usually used a monotherapy or combined medicine. TCM is one of the oldest health practices on earth.

TCM has a specific theory, diagnosis and treatment (1), where the main modes of treatment are acupuncture and Chinese herbal medicines (CHMs). Nevertheless, the situation in China, the role of TCM as conventional medicine in the USA and other Western countries is relatively undeveloped. Even so, TCM and CHM therapies have been introduced in Western medical centers in the USA (2,3), and patients undergo TCM therapy provided primarily by licensed non-hospital-based TCM practitioners. CHMs are considered nutritional supplements in compliance with US law. The Center for Supplementary and Alternative Medicine was founded by the National Institutes of Health (NIHC) in 1997 which is a part of bigger nexus of traditional medicines (4,5).

Chronic inflammation of the airways involves bronchial asthma. It is characterized by eosinophils' participation cells. Asthma has recently increased incidence all around the world where mortality study showed its pathogenesis. (6-7). An important part of development also plays major impact on Asthma bronchial. TCM for treatment of asthma has good results outcome among children. TCM applied in in children with asthma, treated under various conditions during the disease's phases and symptoms whereas the mechanism is empirical(8).

TCM has been used to treat airway disorders, including bronchial asthma, for many centuries. For a certain proportion of asthmatic patients, clinical findings by both modern doctors and Chinese doctors suggest a certain degree of efficacy (9). It is well known that ephedrine ($C_{10}H_{15}NO$), which is extensively used in asthma as a bronchodilator, acting as central nervous system stimulant, was extracted from Ma Huang (*Ephedra sinica*), and has been used for centuries in TCM to treat acute asthma (10). Ma Huang is commonly used in contemporary medicine for treating bronchial asthma now a days. In addition, some pure compounds, including antagonists in Platelet Activating Factor (PAF), with an anti-inflammatory effect, were isolated from herbs used in TCM, and used to treat bronchial asthma (11-13). It is important to examine the clinical effectiveness of TCM using empirical methods of modern medicine and to explore the mechanisms through which it functions.

Materials and methods

Invention project: The research consisted of a controlled, single-blind randomized study carried out at the University Affiliated Huangdao District Hospital, Qingdao City, Shandong Province, China. Standard treatments for children with asthma (salbutamol and

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montelukast) were used as positive controls to determine the clinical effectiveness of TCM. Random numbering method was used and sample size were calculated according to the medication given to them in a random way. All the subjects who gave written consent and wanted to give veins were chosen. They were separated into two groups based on a random numbering method. Student's T-test were used as there were two groups in consideration. Usually the t-Test depending on the groups consists of either single population or multiple populations. The formula for the student t-test is given below.

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{(s^2(\frac{1}{n_1} + \frac{1}{n_2}))}}$$

Where t is the t-value and X_1 and X_2 are the means of the two groups being compared. S^2 is the pooled standard error of the two groups, and n1 and n2 are the number of observations in each of the groups.

Criteria for selection: This research fulfils the investigative standards set in 2008, based on the Chinese Medical Association Paediatric Bronchial Asthma Standards for the Respiratory Group (14). Traditional Chinese health administration has defined TCM syndrome based on the 'TCM syndrome diagnosis and clinical effectiveness criteria' (15). The study consisted of children between 3 and 12 years old. Children were selected for inclusion in the present study, according to their severity of asthma diagnosis criteria. Asthma attacks are classified as the following according to Chinese medicine differentiation syndromes: a type of mucus-generated heat that prevents lung or cold liquid from staying in the lungs, lung and spleen diseases and renal failure. Patients with congenital, cardiovascular and cancer diseases were excluded.

Subjects: 167 subjects with asthma were chosen and divided in SM children group (n=77) and TCM children group (traditional Chinese medicine) (n=90), on the basis of a random numbering method, between March 2018 and December 2019. All the children have to sign a written consent (in case of minor by parents) to donate samples and then assigned the samples to various groups by chance. Fortyy-two TCM patients were treated before and 35 MS patients received venous blood treatment later on. In the TCM group, 28 were males and 14 females, between age of 5.0±1.5 years and an average illness duration of 8.2±6.3 months. In the SM children group, 20 were males and 15 females. Their average age was 5±1.2 years and their average disease duration was 7.6±6.5 months. In terms of age, gender and illness, there was no significant variance between the two groups.

Interventions: In the TCM group, patients with acute asthma attacks were given a 'Shegan' mixture consisting of 9grams of ephedrine, 9 grams of almonds, 6 grams of China Bela Ganda of, 9 grams of Indian rib grass, 9grams of yellow wedge from Chinese root tuber of the plant Pseudostellaria heterophylla and 9 grams of wild wedge from Chinese mountains commonly known as Cang Er Zi. Patients took 10ml of the blend three times a day for seven days. The medicine was accustomed slowly to the TCM group, and CS results during the four-week and eight-week follow-up period respectively.

Patients in the TCM group with persistent asthma were given the Shegan mixture and 'Astragalus kidney tonic'. The tonic combination was treated with 9 grams of astragalus, 9 grams of actylode, 15 grams of Chinese yam, 9 grams of Pseudostellaria heterophyll, 12 grams of coconut, and 9 grams of Indian medicinal mulberry. Astragalus kidney tonic enhances the immune system and reduces inflammation. It is also used as an antihistamine and to treat heart conditions, as well as disorders of the kidney. Additional herbs are added or removed depending on the symptoms. For example, for nasal congestion and sneezing, magnolia and dogwood are included; for hyperhidrosis, ephedrine and floating weed are added. The therapy lasted 12 weeks. Herbs used complied with *Chinese Pharmacopoeia* quality standards (2005) (16).

Among the SM children, who has severe asthma treated first with bronchodilators, 2 to 4mg daily, for seven consecutive days. Leukotriene receptor and montelukast sodium were received by patients in clinical remission at a dose of 4-5mg per night for three months. Salbutamol and montelukast were given to patients with persistent asthma. Antibiotics or aerosol therapy were also directed in cases of severe bacterial contamination and breathless.

Products: The amount of IL-10 and IL-17 including MMP-9 and MBTGF-β1 with marginal blood and mRNA countenance changed 12 weeks before and after treatment.

C-ACT: The childhood Asthma Control Test (c-ACT) is an extensively used method of perceiving poorly controlled asthma in children. This c-ACT test comprises seven queries, separated into two portions: parents of the child were asked the first four questions. Based on the condition of the child, the doctor answers the remaining three questions. Scores from 23 to 27 are considered a good check for a total of 27 points. Partial control 20-22; and poor asthma control 0-19. Experts are strictly responsible for the quality of this test. Although in this research this C-ACT has not been applied but it is useful to add in this manuscript to let the audience understand and detect asthma in children.

Collection and treatment of specimens: Samples from the peripheral blood (2ml) of children with asthma were collected and placed in an ethylenediaminetetraacetic acid (EDTA) anticoagulation tube. At 2,500 rpm for 15 min, the plasma was removed and frozen for further analysis for 5 minutes. The Ficoll method were used for the separation of peripheral blood mononuclear cells (PBMCs).

Rt-PCR

Total mRNA was also removed with the help of TRIzol RNA substance removal. The Primer Premier 5.0 (PRE-Primer Sequence) and Amplicon Size are shown in Table 1 which is standard notation of DNA sequences from 5' to 3'. Real-time quantifiable Reverse Transcript Polymerase Chain reaction (qRT-PCR) were conducted on the Mastercycler ep realplex Real-time PCR System (Eppendorf AG, Hamburg, Germany) by means of SYBR green premix Ex Taq. The process was as follows: initially denatured for 20 minutes at 95ºC; desaturated for 30 seconds at 95ºC, 30 seconds at 54-55ºC, then extended for 40 seconds at 44ºC. The comparative quantity of the objective gene mRNA to GAPDH was normalized.

Table 1: **Primer sequence and amplicon dimensions**

Genes name	Primer sequences (5' to 3')	Amplicon dimensions (bp)
GAPDH-F	CTCTCTGCCTCCTCCTGTTCGAC	68
GAPDH-R	TGAGCGATGTGGCTCGGCT	
IL-10-F	GATGCCTTCCAGCAGAGTGAA	106
IL-10-F	GCAACCCAAGGTAACCCTTAAA	
IL-17A-F	GCTGATCGGGAACGTGGACTA	122
IL-17A-R	CCCACCGGACACCAGTATCTT	
TGFβ-F	TGGTGGGAAACCCACAACGAA	115
TGFβ-R	GAGCAACCACGGGTTCAGGTA	
MMP9-F	CATCGTTCCACCGGACTCAAA	182
MMP9-R	AAACCGAAGTTGGAACCACGA	

ELISA Test

Enzyme-linked immunosorbent assay (ELISA) were done to understand the total circulating levels of PBMC to see they had a significant effect on the extrapolated values. The marker was associated with the provided kit prepared by RayBiotech, Inc. (Peachtree Corners, Georgia, USA). Centrifugation and sample anticoagulation were used to get the plasma. Use the double antibody kit for IL-17, MMP-9, IL-10, and MBTGF-β1measurement according to the instructions in the kit. The kit was provided by RayBiotech, Inc, USA, and the optical density (OD) value was detected at 450nm using a microplate reader. The standard IL-17 and IL-10 and MMP-9 and MBTGF-β1 dilution solutions were prepared.

Statistical analysis

In order to establish baseline equivalents and differences between the two groups, the demographic data were analysed. The continuous variables are expressed as average ± standard deviation (StdDev) and estimated by means of Student's t-test, in accordance with the normal distribution. The misplaced data were evaluated using the t-test following the protocol conversion. The correlation analysis of the typically disseminated variables was analysed by means of Spearman's correlation coefficient. Every statistic remained examined by means of SPSS software version 22. The statistically significant value of 0.05<0.05 is considered. T-test was employed.

Results

In all IL-17, MMP-9, IL-10, and MBTGF-β1 we compared expression levels of mRNA. Not all-important changes in the TCM and SM groups (0.05>0.05) were observed after therapy (see Table 2) as they have various timeframe according to the individual body reaction given by Std-Dev. In case of SM children group of patients, the appearance of MBTGF-β1 mRNA declined. Subsequently treatment with TCM, there were no noteworthy alteration in IL-10, IL-17, MBTGF-β1and MMP-9 mRNA expression (Table 3).

Table 2: **Evaluation of mRNA expressions of** IL-17, MMP-9, IL-10, and MBTGF-β1**in the children groups (mean ± standard deviation)**

Variables	**TCM group (n = 42)**			**SM group (n = 35)**		
	Before treatment	**After treatment**	**p value**	**Before treatment**	**After treatment**	**p value**
IL-17	5.1±7.9	7.9±7.3	0.41	4.1±6.5	8.5±10.9	0.21
MBTGF-*β*1	195.4±93.9	169.9±84.0	0.51	226.5±129.5	121.2±61.2	0.01
MMP-9	2.3±3.4	3.1±4.1	0.99	1.5±2.9	1.5±3.5	0.91
IL-10	0.6±0.2	0.5±0.5	0.59	0.4±0.6	0.6±0.69	0.59

Assessment of marginal blood levels of the IL-17, IL-10 including MBTGF-β1 as well as MMP-9 were performed. But after treatment no major differences was observed in case of peripheral blood levels amongst the TCM children group and SM groups (treatment > 0.05) at IL-17, IL-10, MBTGF-β1 and MMP-9 levels. Following therapy, the TCM children group (decline = 0.01, 0.04 and 0.03, respectively), levels of IL-17, IL-10, and MMP-9 were expressively reduced. It was also observed the same trend in SM group children, after treatment, IL-17, MMP-9 and MBTGF-β1 levels (see Table 4) were significantly reduced.

Table 3: **Evaluation of peripheral blood of** IL-17, MMP-9, IL-10, and MBTGF-β1**in children groups (pg/mL, MMP-9ng/mL, mean ± SD)**

Variables	**Before treatment**			**After treatment**		
	TCM group (n = 42)	**SM group (n = 35)**	**p value**	**TCM group (n = 42)**	**SM group (n = 35)**	**p value**
IL-17	1,910.8±1860.5	1,840.8±1550.5	0.99	1,070.5±685.7	920.2±445.3	0.55
MBTGF-*β*1	999.5±905.1	1,510.2±990.8	0.03	1,075.3±725.1	630.6±392.5	0.05
MMP-9	380.5±115.5	420.2±172.3	0.51	305.4±70.2	325.1±95.5	0.40
IL-10	40.3±59.9	20.6±10.9	0.07	10.4±6.0	15.0±12.2	0.42

After 12 weeks of treatment, the value of c-ACT in the TCM children group was significantly greater than the previous one (22.5±1.9, 16.5±1.9, compared with < 0.001). In the SM group, after 12 weeks of treatment, the value of c-ACT significantly increased (after 22.1±2.0 compared to p<0.001 before 17.0±2.2). The difference between IL-17 and the c-ACT scores in the TCM group including SM children was negatively related. Alteration in c-ACT scores in the two groups in relation to IL-10, MBTGF-β1 and MMP-9 levels (see Tables 5) was not known.

Table 4: **Relationship between changes of c-ACT and t levels of IL-17, IL-10, MBTGF-β1, and MMP-9 in the TCM children's group**

Variables	d IL-10	d IL-17	d MMP-9	d ACT	d MBTGF-β1
d IL-10	1.0000				
d IL-17	-0.9000	1.1000			
d MMP-9	-0.2000	-0.1000	1.0000		
d-ACT	0.8721	-0.9737	0.2052	1.0000	
d MBTGF-β1	-0.6190	0.5010	-0.4100	-0.5642	1.0000

Table 5: **Relationship between changes of c-ACT and t levels of IL-17, IL-10, MBTGF-β1, and MMP-9 in the SM children**

Variables	d IL-10	d IL-17	d MMP-9	d ACT	d MBTGF-β1
d IL-10	1.0000				
d IL-17	0.1120	1.0000			
d MMP-9	0.4762	0.0238	1.0000		
d ACT	-0.4514	-0.7123	-0.2629	1.0000	
d MBTGF-β1	0.4049	0.0080	-0.2391	-0.1529	1.0000

Discussion

Asthma is a chronic inflammatory disorder that plays an important role in several cells and cytokines. We have adopted Chinese herbal formula for step therapy to inflammatory mediators and released cytokines, which deals with asthma attacks. Earlier studies show that asthma patients can be linked with Th1/Th2 imbalance by the pathogenesis of inflammation by the airway. The increase in Th2 cell number (IL-5, IL-4, and IL-10), which is a major element in the induction and maintenance of cellular inflames, leading to increased secretion of Th2 cells (17-18). In previous studies, we found many empirical prescriptions to regulate leukotriene and immune cell imbalances in asthma attacks on the expression of leukotriene receptors and observed the empirical prescriptions for treatment for asthma attacks in TCM (7,19). The most prevalent two types of receptor are CysLT1 and CysLT2. As we know CysLT1 receptor is generally expressed in annoyance, lung muscle cells and interstitials lungs macrophage blood leukocyte and eosinophils. Whereas the receptor CysLT2 is mostly found in placenta, heart, and in leukocytes of peripheral blood. It can efficiently control and enhance different lung function indicators (8).

In this study, we examined several additional asthma-related cytokines, including IL-17, IL-10, MBTGF-β1 and in MMP-9, for purpose in clarifying the immune mechanisms for TCM treatment for asthma. Previous studies showed that Shegan's mixture and ephedrine are extracted in TCM formulas. One specific medicine cannot reduce asthma. They are present in various forms and nomenclatures Early studies have shown that the belamcandae and the ephedrine can also help recovering patients' immune systems (preventing hypersensitivity responses efficiently) (19), reducing the severity of childhood asthmatic cough, and altering IL-10 serum levels.

IL-13 and IL-14:

Luo *et al.* (20) found MBTGF-β1 in the belamcandae and ephedrine decoction group to be reduced compared to the asthma model group of children studied, bronchial wall thickness and smooth muscle thickness was reduced, and inflammatory cell infiltration decreased. The IL-10 level reduced 12 weeks of therapy afterward with TCM, indicating that the secretion of IL-10 to control asthma attacks could reduce TCM, thereby inhibiting inflammation of the respiratory system. We found no difference in the serum concentration of MBTGF-β1 after 12 weeks of TCM therapy. The various result obtained in this experimental test as well as in different studies can be accredited to the various methods employed to enumerate inflammatory mediators' secretion stages.

Current studies show that asthma is also associated with Th17/Treg imbalances. In airway restructuring and inflammation by chemotaxis and neutrophil activation, IL-17 plays an important role (16). MMP-9 is also involved in the remodelling of airways through the destruction of collagen and promoting the migration of epithelium (21). Miao *et al.* (14) conclude that 'Zhike Pingchuan Mixture could reduce IL-17 and MMP-9 expression in lung tissues and progress asthma airway remodelling. In the empirical prescription of TCM, our study decreased peripheral blood levels of IL-17 and MMP-9 and indicated a controlling consequence on IL-17 and MMP secretion. C-ACT results were also recorded to assess the control of asthma. The results showed that after 12 weeks of TCM treatment, the c-ACT levels increased at a similar rate to our earlier studies (17-19). Liu *et al.* found (22), and this was confirmed by our research, that the results of c-ACT could well reflect clinical symptoms. The results also demonstrate that differences between IL-17 and change in c-ACT in the TCM group were negatively correlated. We assume that Chinese medicine has been proven to reduce IL-17 secretion, inhibiting the inflammation of the airways. Of particular interest, there has been no variation in Chinese treatment group IL-17, IL-10, and the MMP-.9 mRNA, and with all of these the serum levels have been reduced among all children. These results were not in line with our expectations.

Conclusions

Chinese medicine empirically prescribed may affect cytokine levels in the translation process. In this study we demonstrated that the TCM can efficiently regulate asthma occurrences and decrease peripheral blood amount of MMP--9, IL--10, IL--17 (23). TCM has been verified to have a controlling consequence on the secretions of certain childhood asthma inflammatory mediators and can help comprehend asthma management immune mechanisms.

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