JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Evaluation of intestinal parasites in patients with chronic spontaneous urticaria in a territory hospital in Turkey

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Abstract

Introduction: Chronic spontaneous urticaria (CSU) which develops without a known stimulation is defined as the occurrence of spontaneous wheals, angioedema or both for longer than six weeks. Infections, autoimmunity, food intolerance and internal parasitic infections are supposed to be underlying causes of CSU. The aim of this study was to evaluate the intestinal parasites in children and adult patients diagnosed as CSU, to determine the frequency of parasites in chronic urticaria, and to compare these patients with healthy demographic control groups.

Methodology: Seventy six children and 38 adult patients with CSU were examined in terms of parasitic infections. The patients whom parasites were detected received anti-parasitic therapy and the improvements in CSU symptoms were evaluated. Stool samples were examined with direct microscopic examination (native-lugol), stool concentration and trichrome staining methods.

Results: In pediatric patient group, 18.4% (n = 14) of the stool samples were positive for *Blastocystis* sp., 2.6% (n = 2), *Dientamoeba fragilis* and 1.3% (n = 1), *Giardia duodenalis*. In adult patient group, *Blastocystis* sp. was detected in 18.4% (n = 7) of the stool samples. Anti-parasitic therapy yielded substantial improvement in urticaria symptoms in 57.1% of pediatric and 60.0% of adult patients.

Conclusions: *Blastocystis* sp. and *D. fragilis* may play a role in chronic urticaria which seriously disrupts the patient's quality of life. Parasitic infections should not be neglected in patients with cutaneous manifestations.

Key words: *Blastocystis*; chronic spontaneous urticaria; *Dientamoeba fragilis*; intestinal parasite; protozoan infection.

J Infect Dev Ctries 2019; 13(10):927-932. doi:10.3855/jidc.11552

(Received 08 April 2019 – Accepted 12 August 2019)

Introduction

Urticaria, which is also known as hives, is a common dermatologic disorder characterized by erythematous, edematous and itchy plaques that involve skin and mucous membranes.Urticaria is classified as acute and chronic. Acute urticaria, which lasts less than six weeks is commonly triggered by medications, foods, infections, stress, or insect venom [1,2]. Chronic urticaria (CU) is defined as the presence of recurrent urticaria, with or without angioedema, for a period of six weeks or longer. Chronic urticaria is classified as urticaria which is triggered by specific stimulants or developed without stimulants which is named as chronic spontaneous urticaria (CSU). The incidence of CSU is between 0.5% and 1.5% in the population. The cause of CSU cannot be defined in about 90% of the cases. Autoimmunity, food intolerance and infections such as intestinal parasitic infections (IPI) are suggested to be some of the causes of CSU [3-7].

In 1949, a case report about CSU had been published which IPI had been blamed as the cause of CSU. In this report *G. duodenalis* and urticaria of unknown origin had been detected together after which IPI was successfully treated by the specific parasitic therapy [8]. Since then, the relation between CSU and IPI have been being studied [5].

Intestinal parasitic infections continue to be important as being a common health problem especially in developing countries. Low socioeconomic status, poor hygiene and crowded living conditions increase the risk of parasitic infections. It is estimated that IPI affects more than one billion people worldwilde. The

highly prevalent intestinal helmints have been reported as *Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Ancylostoma duodenale and Strongyloides stercoralis,* intestinal protozoa as *Blastocystis sp., G. duodenalis, Dientamoeba fragilis* and *Entamoeba spp.* [5,9].

In recent years, *Blastocystis* is the most common intestinal protozoon found in human stool specimens but its clinical importance and pathogenicity is still controversial. *Blastocystis* has been found to be associated with symptoms such as abdominal pain, diarrhea, nausea, vomiting, bloating, anorexia and urticaria, as can be detected in people without any gastrointestinal complaints [10].

In this research, we aimed to evaluate the association between CSU and IPI in pediatric and adult patients, and to compare the incidence of parasitic infections with similar demographic healthy control groups. We also examined the improvement of CSU symptoms after anti-parasitic treatment.

Methodology

Patient Selection

Patients with known possible causes of chronic urticaria like; suspicious drug usage and signs of infection or patients with high stress were excluded from the study. At last, 76 pediatric and 38 adult CSU patients were included in the study without known causes and having only urticarial plaques persistent more than six weeks. Exclusion criteria were acute spontaneous urticaria, chronic spontaneous urticaria of known cause and other types of urticaria. The study group was composed of patients referred to pediatric allergy and dermatology clinics due to chronic urticaria. As control group, 34 adult and 109 pediatric individuals were enrolled the study who does not have acute or chronic illnesses or CSU.

Local institute board approved this study.

Sample Collection and Laboratory Analyses

One or more consecutive fresh stool samples collected from the patient and control subjects were transferred to the laboratory in 30 minutes. Fresh and fixative-containing (10% formalin solution) stool samples were collected from each patient. After macroscopic examination, fresh stool samples were examined with direct microscopic examination (saline and iodine) and Wheatley's trichrome staining methods. Fixative-containing stool samples were concentrated with stool concentration method (Parasep® Fecal Parasite Concentrators, Apacor, USA) and sediments were examined as wet mount in saline and iodine for the presence of intestinal parasites.

Also, serum samples have been collected from the patient and control subjects. The ratio of eosinophils and total IgE levels were detected with automatized systems (Beckman Coulter LH 780©, USA and Siemens BN II©, Erlangen, Germany).

Anti-parasitic treatment

Metronidazole therapy for ten days were prescribed (250 mg orally three times daily for adults, 30 mg/kg/day for children) for the patients who had IPI diagnosis and parasitic investigation has been performed with the same method after the end of the therapy. Besides, the urticaria symptoms were re-questioned after anti-parasitic therapy. *Enterobius vermicularis*, which was detected in the control group, was treated with pyrantel pamoate (11 mg/kg maximum 1 g single dose - repeat dose after 2 weeks).

Statistical Analyses

Statistical analyses were performed by SPSS Statistical Analysis program (version 20.0). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine their statistically evaluated distribution. Descriptive analyses were presented using means and standard deviations for normally distributed variables. Fisher's exact test or chi-square analyses tests were performed for the categorical data and $p < 0.05$ was accepted as statistically significant.

Results

Demographic characteristics of the patients

The pediatric patients included in the study were between 1 to 18 years of age and 33 (43.4%) of them

Table 1. Age and gender distribution in patient and control groups.

Group	Female (n)	Male (n)	Mean Age	p value
Child patients	43	33	10,16 ± 4,6	0.48
Child controls	56	53	9,2 ± 5,2	
Adult patients	24	14	40,3 ± 14,2	0.90
Adult controls	21	13	39,8 ± 13,8	

were male. Adult patients were between 19 to 69 years of age and 14 (36.8%) of them were male. The demographic data of the patient and control subjects were presented in Table 1. In terms of age and gender, statistically significant difference was not found between the patient and control groups

Detection of parasites

Intestinal parasites were identified by morphologically after microscopic examination and trichrome staining (Figure 1).

IPI detection rates

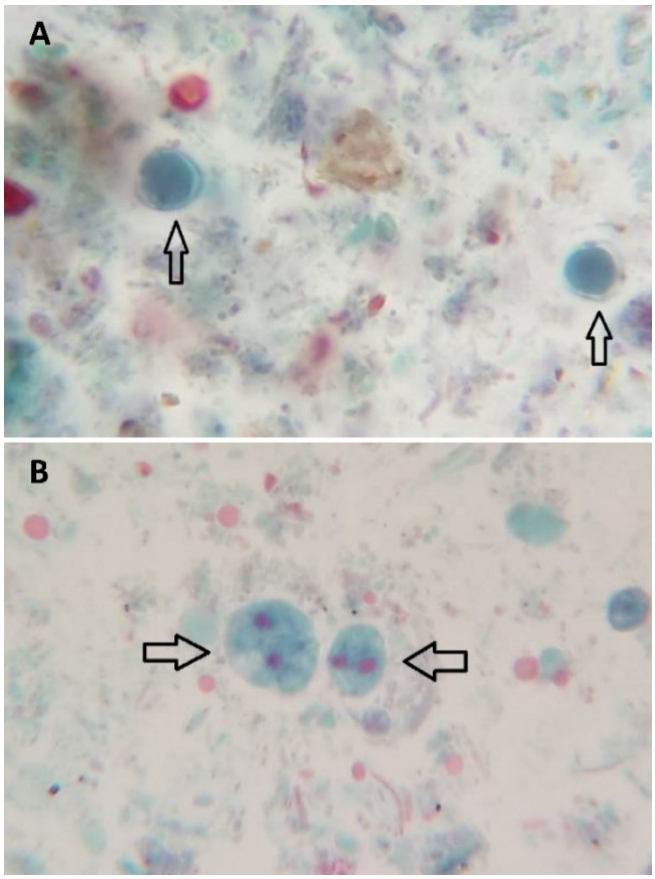
Intestinal parasites were detected in 22.3% (n = 17) and 14.6% (n = 16) of the children in pediatric patient and control groups, respectively. In adult subjects, intestinal parasites were detected in 18.4% (n = 7) and 8.8% (n = 3) of the patient and control groups, respectively. Although the incidence of intestinal parasites were higher in patient groups, the difference was not statistically significant in both children and adult patients when compared with their contols (p = 0.181, p = 0.376).

Distribution of intestinal parasites

The distribution of intestinal parasites in pediatric patient group were 13 *Blastocystis* sp. (17.1%), 2 *D. fragilis* (2.6%), 1 *G.duodenalis* (1.3%) and 1 *Blastocystis* sp. and *Entamoeba coli* (1.3%). The distribution of intestinal parasites in pediatric control group were 11 *Blastocystis* sp. (10.1%), 1 *D. fragilis* (0.9%), 1 *G. duodenalis* (0.9%), 1 *E. coli* (0.9%), 1 *Blastocystis* sp., 1 *E. coli* and 1 *Enterobius vermicularis* (0.9%) (Table 2). Although the incidence of *Blastocystis* sp. in pediatric patient group were higher when compared with control group, the difference was not statistically significant (p = 0.155).

In adult subjects, *Blastocystis* sp. were detected in 18.4% (n = 7) and 8.8% (n = 3) of patient and control groups, respectively. Although *Blastocystis* sp. incidence had two times higher incidence in adult patient group, it was not statistically significant (p = 0.376).

Figure 1. A) *Blastocystis* sp. B) *Dientamoeba fragilis* stained with trichrome (×1000).



Eosinophil ratios and IgE levels

The median value of eosinophil percentages in peripheral blood smears were 2% (0.7-3.6%) in the pediatric patient group and 1.6% (0.7-2.3%) in the control group. Eosinophil percentages were found to be different between the pediatric patient and control groups (p = 0,046). Total IgE median value was 98.5

Table 2. The distribution of intestinal parasites in adult and children groups.

Parasite	Child patients	Child controls	p value	Adult patients	Adult controls	p value
Blastocystis sp.	17.1% (n = 13)	10.1% (n = 11)	0.16	18.4% (n = 7)	8.8% (n = 3)	0.23
D. fragilis	2.6% (n = 2)	0.9% (n = 1)	0.36	0	0	-
G. duodenalis	1.3% (n = 1)	0.9% (n = 1)	0.79	0	0	-
E. coli	0% (n = 0)	0.9% (n = 1)	-	0	0	-
Blastocystis spp. and *E. coli*	1.3% (n = 1)	0.9% (n = 1)	0.79	0	0	-
E. vermicularis	0% (n = 0)	0.9% (n = 1)	-	0	0	-
Parasite not found	77.6% (n = 59)	85.3% (n = 93)	0.17	81.6% (n = 31)	91.2% (n = 31)	0.23

IU/mL (28-247 IU/mL) in the pediatric patient group and 31.5 IU/mL (15.1-65.6 IU/mL) in the control group (Table 3). Total IgE levels were found to be significantly different between pediatric patient and control groups ($p < 0,001$).

The median value of eosinophil percentages was 1.5% (0.9-2.2%) in the adult patient group and 1.9% (1.3-2.3%) in the control group. No difference was found between adult patients and control groups ($p = 0.2$). Total IgE median value was 107 IU/mL (24.6-277 IU/mL) in the adult patient group and 32.2 IU/mL (19.5-54.6 IU/mL) in the control group (Table 3). Total IgE levels were significantly higher in adult CU patients when compared with controls ($p = 0.002$).

Reassessment of patients after anti-parasitic treatment

In pediatric patient group, a total of 14 children who had been diagnosed with parasitic infection were treated for ten days with metronidazole. Urticaria symptoms ceased in 57.1% of these patients. In adult patient group, five patients diagnosed with parasitic infection received metronidazole treatment and urticarial symptoms ceased in 60%.

Discussion

Chronic spontaneous urticaria is a chronic disease, which worsens the quality of life, disturbs daily activities and sleep pattern because of pruritus and causes emotional disorders and social isolation [11,12]. Several studies indicate that CU has a significant impact on patient's psychological state. Especially in pediatric patients with CU, itching and aesthetic aspect may cause anxiety and depression [13]. CU also causes economic burden because of long lasting therapies. Therefore, it is very important to lay out the cause of CSU to apply an appropriate and efficient therapy [14].

Autoimmunity, stress, pseudo allergens, inflammation and chronic underlying infections have been found associated with CSU [15]. Particularly parasitic infections are defined as one of the causes of urticaria especially in developing countries [16]. The type and frequency of intestinal parasites varies among different regions of the world. Low socioeconomic status and education levels, climate and environmental factors influence parasitic diversity and numbers. In Turkey, in general population the incidence of intestinal parasites varies between 2.7-57.8% in several studies [17-19]. In the present study, the prevalence of intestinal parasites in healthy adult and pediatric individuals were 8.8% and 14.6% respectively. It should be noted that these results were obtained from a single stool examination for most of the subjects with limited diagnostic methods which may lead to underestimation of intestinal parasite prevalence.

In a prospective study held in Thailand, in 92 pediatric patients with CSU, parasitic infections were diagnosed in 5.4% of the patients and *Blastocystis* sp., *D. fragilis* and *G. duodenalis* were detected [20]. In another study from the same country, the incidence of IPI and the distribution of the parasites were similar with the previous study [21]. Besides, in a study of 211 pediatric patients with CSU held in our country, the rate of IPI was 10% and the most prevalent parasites detected were *Blastocystis* sp., *G. duodenalis*, *D. fragilis* and *E. vermicularis* in row with decreasing order [16]. In another study held in Turkey, parasitic infections had been detected in 38.8% of the CSU patients and 11.1% of control group of which the difference between the groups was statistically significant. In this study, *Blastocystis* sp. and *G. duodenalis* were the most common detected parasites [22]. In another study with 55 CSU patients, *Blastocystis* sp. were detected in 20% of the patients and 11.6% of the controls and the difference between the groups were found statistically significant [23]. In our study, IPI were determined in 18.4% and 22.3% of the adult and pediatric CSU patients, respectively and *Blastocystis* sp. was the most prevalent parasite similar to other mentioned reports. Although the rate of IPI in CSU patients were higher than the control group, the difference was not statistically significant which may be due to the number of patients enrolled the study.

Some studies report that urticaria may disappear in the patients with parasitic infection after specific treatment for the parasite [14]. In a study, 16 CSU patients with IPI were treated with anti-parasitic therapy and 43.7% of them had regression in urticaria symptoms [23]. In other local studies, CSU patients

Table 3. Total IgE and eosinophil median values in pediatric and adult groups.

	Child patients	Child controls	p value	Adult patients	Adult controls	p value
Median value of total IgE	98.5 IU/mL (28-247 IU/mL)	31.5 IU/mL (15.1-65.6 IU/mL)	p < 0,001	107 IU/mL (24.6-277 IU/mL)	32.2 IU/mL (19.5-54.6 IU/mL)	p = 0.002
Median value of eosinophil percentage	2% (0.7-3.6%)	1.6% (0.7-2.3%)	p = 0,046	1.5% (0.9-2.2%)	1.9% (1.3-2.3%)	p = 0.2

received anti-parasitic treatment, 47.6% and 42% of the subjects had regression in urticaria symptoms respectively [16,22]. In our study, symptoms of CSU ceased in 57.1% of the pediatric group and 60% of the adult group. The difference between treatment successes in these studies above may be related to the population differences or the level and period of the disease like convalescence period of which, higher success rates may be achieved. So double-blind placebo studies are needed to confirm these results.

When all studies are evaluated, CSU patients have higher intestinal parasite rates when compared with relevant control groups and among these, the most prevalent parasite was *Blastocystis* sp. After effective anti-parasitic therapy, in more than half of the patients urticaria symptoms ceased.

Blastocystis is one of the most prevalent intestinal protozoa reported in developing countries as well as developed countries. Despite of the increased number of studies, the pathogenicity of *Blastocystis* on human health is still controversial. In recent years, it is found that *Blastocystis* could be a marker of healthy gut which is an interesting discovery [24]. Although most of *Blastocystis* infections are asymptomatic, some patients represent dermatological symptoms. Some reports indicate that *Blastocystis* may be associated with chronic urticaria [25]. Our findings support that CSU patients may have higher prevalence of *Blastocystis* sp. than control groups.

D. fragilis is another protozoan which is commonly seen worldwide. Up to now, *D. fragilis* has been related to gastrointestinal symptoms mostly. The relation of *D. fragilis* with urticaria has been reported in only two cases [26,27]. In our study, two *D. fragilis* patients did not have any gastrointestinal symptoms and the symptoms of urticaria ceased after specific treatment.

Conclusion

In CSU patients with or without gastrointestinal symptoms, intestinal parasites must be investigated and if detected, anti-parasitic therapy must be implemented to alleviate the symptoms, increase the quality of life, and prevent economic burden.

Acknowledgements

This work was approved by Local Institute Board of Health Science University, Ankara Education and Research Hospital, Turkey.

This study was presented as a poster presentation in the 4. National Clinical Microbiology Congress, 08-12 October 2017, Antalya, Turkey.

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Conflict of interests: No conflict of interests is declared.