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Original Article

Transient positivity of anti-tissue transglutaminase IgA autoantibody in febrile children: a case-control study

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ABSTRACT

Background. Fever is a physiological response activated by integrative interactions between the neuronal and immune systems. The association of fever with the development of autoantibodies against various self-antigens is controversial. We here evaluated if fever was associated with increased levels of anti-tissue transglutaminase (tTG) IgA autoreactive antibodies in children.

Methods. This was a case-control study performed the Amir-Al-Momenin Hospital of Zabol City from January to December 2018. Febrile children (N=135) and apparently healthy counterparts (N=135) were included. Total IgA and anti-tTG IgA were measured by ELISA.

Results. From 270 children evaluated, 144 (53.6%) and 126 (46.4%) were males and females, respectively. The mean age was 4.7 ± 2.6 years. The mean total IgA titer was 208 ± 100 mg/dl, and the mean anti-tTG IgA titer was 15.9 ± 68 mg/dl. There was a significant difference in the mean titer of anti-tTG IgA between apparently healthy controls (1.97 ± 1.12 mg/dl) and febrile children (30.2 ± 94.9 mg/dl, p=0.002). Positivity for anti-tTG IgA was observed in 16 (11.8%) out of 135 febrile children while no subject in the control group had positive results. One out of the 16 positive cases showed persistent elevated levels after fever disappearance. On biopsy examination, this child was confirmed to have celiac disease.

Conclusions. We showed that fever can trigger the production of anti-tTG IgA autoantibody in children. It is recommended for pediatricians to be vigilant in interpreting anti-tTG IgA results during fever episodes and repeat positive cases after the cease of fever. It is also recommended to reassess anti-tTG IgA seropositivity in other clinical settings in future studies.

Key words: fever, tissue transglutaminase, autoantibody, immune system, celiac disease.

Fever is a physiological response activated by integrative interactions between the neuronal and immune systems.[1] Fever is in fact a regulated boost in the core body temperature to combat microorganisms and inhibit their growth within human tissues and cells. In this manner, fever is considered to be a major factor affecting physio-pathological features and the clinical course of human infectious diseases.[1]

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Overall, fever is a multifactorial adaptive response intercalating with various body systems. The role of immune system in promoting and regulating fever upon infections has been highlighted.[2] Immune components including immune cells and various cytokines are bridges linking immunological reactions to the nervous system to induce fever. These two systems act in a highly intercalated manner to promote a vigilant response against stresses and potential environmental threats.[3]

The cytokines involved in regulating the interaction between immune and nervous systems are known as "endogenous pyrogens". A wide variety of inflammatory cytokines

including tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and interferons can play a role as pyrogens. The main function of these mediators in neuronal system is to induce the production of prostaglandins which subsequently lead to hyperthermia and fever. However, induction of fever by pyrogens has been suggested to involve other parallel mechanisms which are poorly understood. The role of immune system receptors such as toll-like receptors has been suggested in these processes.[4,5] Nevertheless, these inflammatory cytokines also exert vast influences on immune system function.[6,7] Accordingly, fever induced inflammatory cytokines may promote exaggerated immune response leading to detrimental effects against self-tissues and autoimmunity.[8,9] Nonetheless, the association of fever with the development of autoantibodies against many self-antigens is unknown. In the present study, we evaluated if fever is associated with increased levels of anti-tissue transglutaminase (tTG) IgA autoreactive antibodies in febrile children.

Material and Methods

This case control study was performed in the Amir-Al-Momenin Hospital of Zabol City from January to December 2018. All children fulfilling our inclusion criteria were considered as the study population. Control subjects were recruited from age- and sex-matched apparently healthy children visiting the clinic for periodical checkups. The study was approved by the local Ethics Committee in Research of Zabol University of Medical Sciences (20th November 2018, Code: Ir.Zbmu.Rec.1397.115). Informed consent was acquired from the children's parents.

Inclusion and exclusion criteria

Children hospitalized in the pediatric ward of the hospital due to fever were included in the case group. Those with a previous diagnosis of celiac disease, a family history of celiac disease or other autoimmune diseases, and patients under treatment with anti-inflammatory drugs were excluded from both the case and control groups.

Sample size

The sample size was calculated based on the below formula.

$$n = (Z_{\alpha/2} + Z_{\beta})^2 * (p_1(1-p_1)+p_2(1-p_2)) / (p_1-p_2)^2$$

In this equation, $Z_{\alpha/2}$ (1.96) represented the coefficient of significance threshold ($p<0.05$). The Z_{β} was the coefficient related to the power of study (80%) and considered as 0.84. P1 and P2 represented the expected frequencies of positivity for anti-tTG IgA in the case and control groups (2% and 4%, respectively). Accordingly, the sample size for each group was obtained (N = 135).

Data acquisition

The demographic data and the past clinical history were obtained by interviewing parents. After that, blood samples (5 ml) were drawn from case and control children. The serum samples were separated in the hospital laboratory, and anti-tTG IgA and total IgA levels were determined using specific ELISA kits (Pars Azmoun Co, Iran).

Statistical analysis

SPSS 16 (Chicago Inc, USA) was used for statistical procedures. Shapiro-Wilk test was applied to screen the normal distribution. Independent sample Student t-test and Chi-square test were used for inferential statistics. P value <0.05 was considered as the statistical significance threshold.

Results

From 270 children evaluated, 144 (53.6%) and 126 (46.4%) were males and females, respectively. The mean age was 4.7 ± 2.6 years, and the mean weight was 19.7 ± 10.9 kg. Fever was the constant clinical finding in all the children in the case group. In 106 (78.5%) of the patients, fever was

accompanied with cough, and in 7 (5.1%) with diarrhea. Gastroenteritis and malnutrition each were observed in one patient. Recurrent fever was observed in 9 (6.6%). Mean hemoglobin level was 13.9 ± 1.9 g/dl, and the mean values of AST and ALT were 14.4 ± 2.1 IU/L and 11.8 ± 2.1 IU/L, respectively (Table I).

The mean total IgA value ranged from 7 to 677 mg/dl with the mean value of 208 ± 100 mg/dl. The mean anti-tTG IgA was obtained 15.9 ± 68 mg/dl. There was a significant difference in the mean titer of anti-tTG IgA between apparently healthy controls and febrile children (Table II). Based on the reference threshold, positivity for anti-tTG was noted in 16 out of 135 (11.8%) febrile while no subject in controls had positive results. From these 16 children, one case showed persistent elevated levels after fever disappearance (i.e. after discharge). On follow up biopsy examination, this child was confirmed to have celiac disease.

There were no significant differences comparing demographic or clinical variables between patients with positive or negative anti-tTG results except for the levels of alkaline phosphatase enzyme (p= 0.01, Table III).

Discussion

The main goal of the present study was to evaluate the levels of total IgA and autoreactive anti-tTG IgA antibodies in febrile children and compare them with healthy counterparts. Overall, 16 (11.8%) of 135 febrile children revealed positivity for anti-tTG IgA. One patient preserved positive results on follow-up which later was diagnosed with celiac disease based on intestinal biopsy examination. Therefore, the elevation of autoreactive anti-tTG IgA was temporary in most of the patients.

Although we found no similar studies assessing anti-tTG IgA autoantibodies in febrile children,

Table I. Demographic, clinical and laboratory features in febrile and non-febrile healthy children.

Parameters	Febrile (N=135)	Non-febrile (N=135)	P
Male/female, n/n (%/%)	66/69 (49.6/50.4)	77/58 (57.5/42.5)	0.13
Age (years)	5.8 ± 2.2	3.6 ± 2.4	<0.001
Weight (kg)	21.8 ± 11.6	17.5 ± 9.7	0.002
White blood cell (10^3/μl)	12.6 ± 1.7	13.6 ± 1.5	<0.001
Red blood cell (10^6/μl)	4.8 ± 0.3	4.8 ± 0.2	0.51
Hemoglobin (g/dl)	13.7 ± 2.6	14.1 ± 0.9	0.13
Hematocrit (%)	38.9 ± 1.9	40.1 ± 1.8	<0.001
Mean cell volume (fl)	79.5 ± 7.2	80.5 ± 2.1	0.11
Mean cell hemoglobin (pg)	28.8 ± 3.2	26.9 ± 0.6	<0.001
Platelet (10^3/μl)	356.8 ± 122.2	218.3 ± 61.7	0.005
Blood urea nitrogen (mg/dl)	14.1 ± 2.1	14.7 ± 1.3	0.21
Creatinine (mg/dl)	0.62 ± 0.79	0.54 ± 0.17	0.01
Aspartate aminotransferase (IU/L)	14.1 ± 2.7	14.8 ± 1.3	0.26
Alanine aminotransferase (IU/L)	11.6 ± 2.7	11.9 ± 1.4	<0.3
Alkaline phosphatase (IU/L)	139.7 ± 79.8	91.6 ± 45.9	0.73
Erythrocyte sedimentation rate (mm/h)	13.7 ± 5.2	13.4 ± 4.2	0.52

Table II. The mean titers of total IgA and anti-tTG IgA antibodies in febrile and non-febrile children.

Parameters	Febrile (N=135)	Non-febrile (N=135)	P
Total IgA (mg/dl)	211.5 ± 120.7 (range: 7-677)	204.5 ± 74.9 (range: 71-492)	0.59
Anti-tTG IgA (mg/dl)	30.2 ± 94.9 (range: 0.11-542)	1.97 ± 1.12 (range: 0.21-5.8)	0.002

tTG: tissue transglutaminase

Table III. Comparisons of demographic, clinical and laboratory features between anti-tTG IgA positive and negative patients among febrile children.

Parameters	Anti-tTG IgA		p
	Positive (N=16)	Negative (N=119)	
Male/female, n/n (%/%)	10/6 (62.5/37.5)	56/63 (47.1/52.9)	0.2*
Gastric symptoms (Yes), n (%)	1 (7.1)	18 (15.1)	0.6*
Recurrence of fever (Yes), n (%)	0	9 (7.6)	0.47*
Age (years)	6.5 ± 1.7	5.8 ± 2.3	0.24
Weight (kg)	24.1 ± 12.2	21.5 ± 11.5	0.41
White blood cell (10^3/μl)	12.8 ± 1	12.5 ± 1.8	0.69
Red blood cell (10^6/μl)	4.8 ± 0.3	4.8 ± 0.3	0.92
Hemoglobin (g/dl)	15.6 ± 6.7	13.4 ± 0.8	0.23
Hematocrit (%)	40 ± 2	38.8 ± 1.8	0.42
Mean cell volume (fl)	80.7 ± 2.5	79.3 ± 7.7	0.47
Mean cell hemoglobin (pg)	29.5 ± 1	28.7 ± 3.4	0.35
Platelet (10^3/μl)	362 ± 90.2	356 ± 127.6	0.85
Blood urea nitrogen (mg/dl)	13.6 ± 2.7	14.1 ± 2	0.34
Creatinine (mg/dl)	0.58 ± 0.17	0.63 ± 85	0.68
Aspartate aminotransferase (IU/L)	14.5 ± 2.7	14 ± 2.7	0.52
Alanine aminotransferase (IU/L)	12.6 ± 2.3	11.4 ± 2.8	0.09
Alkaline phosphatase (IU/L)	95.4 ± 61	146.7 ± 80.4	0.01
Erythrocyte sedimentation rate (mm/h)	13.8 ± 3.3	13.6 ± 5.5	0.92

*; Fisher's exact test, tTG: tissue transglutaminase

evidence from other diseases support a link between fever and autoimmune reactions. In fact, inflammatory cytokines can trigger leukocytes and other immune cells to promote the synthesis of pyrogens.[10] In one study on children with a family history of diabetes mellitus, fever episodes (either associated or independent of infections) within the first year of life predicted autoimmunity against pancreatic cells.[9] In another study with patients with scrub typhus infection, ANA autoantibodies developed within one week after initiation of fever.[11] These reports support our findings regarding that fever can be a trigger for development of auto-antibodies; however, the clinical significance and persistency of various autoantibodies should be further scrutinized in various clinical conditions.

Among immune cells, neutrophils have been known as major contributors to inflammatory fever associated with various infections. Neutrophils exposed to 45°C express a higher activity for 5-lipoxygenase, an enzymes involved in leukotriene synthesis.[12] Although fever-like temperatures have been shown to prevent lipopolysaccharide-induced activation of NF-κB transcription factor[13,14], neutrophils can promote the production of autoantibodies (e.g. anti-neutrophil cytoplasmic autoantibodies) through NF-κB-independent signaling pathways as well.[15] On the other hand, fever-like temperatures were noted to activate NF-κB pathway in macrophages.[16] Fever range temperatures also induce T lymphocytes to produce stress proteins (such as heat-shock proteins-Hsp).[17,18] In particular, Hsp90 protein can regulate T lymphocytes trafficking in feverish conditions.[17] Following infection with type A streptococcus bacteria, an interaction between interleukin-1β-granulocyte-macrophage colony-stimulating factor resulted in the proliferation of CD4+ T helper 1 lymphocytes. These events have been

suggested to participate in autoimmunity observed in acute rheumatic fever following group A streptococcal infections.[19,20] The roles of new identified mediators such as complement factor 5a and platelet-activating factor in fever induced immunity is yet to be divulged.[21] In a reciprocal manner, autoimmunity may be itself a reason for fever.[22] This is further supported by some reports noting that autoimmunity[23,24] and lymphoproliferative disorders[25] can be associated with fever of unknown origin. Fever can also regulate toll-like receptor (TLR) signaling pathways,[16] cytoplasmic phospholipase 2,[26] intercellular adhesion molecule 1 (ICAM-1), and CCL21 chemokine.[27]

We could not explain an isolated significant decrease in ALP in patients with anti-tTG positivity accompanying normal levels of AST and ALT with no significant deviations in other tests. This observation may be a basis for checking the fluctuations of this enzyme in these patients in future studies.

We showed that fever can transiently trigger anti-tTG IgA autoantibody production in children which probably is promoted by modulating cellular and humoral immunities. Based on these findings, it is recommended to pediatricians to be vigilant in interpreting anti-tTG IgA results during fever episodes and repeat positive cases after the cease of fever. It is recommended to reassess the association of anti-tTG IgA seropositivity with fever in other clinical settings in future studies as well.

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