Brief Report

To Evaluate the Role and Relevance of Cytokines IL-17, IL-18, IL-23 and TNF-α and Their Correlation with Disease Severity in Chronic Urticaria

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Abstract

Introduction: The basic event in the pathogenesis of urticaria is inappropriate activation and degranulation of dermal mast cells. Cytokines are soluble polypeptide mediators that play a key role in immunological, inflammatory and reparative host responses including chronic urticaria. **Objective:** The aim of this study was to evaluate the role and relevance of cytokines interleukin-17 (IL-17), interleukin-18(IL-18), interleukin-23(IL-23) and tumor necrosis factor-alpha (TNF-α) and their correlation with disease severity in patients with chronic urticaria. **Materials and Methods:** A prospective cross-sectional study was conducted to measure the serum concentration of IL-17, IL-18, IL-23 and TNF-α in 50 chronic urticaria patients and in 30 healthy controls. Disease activity was assessed by using urticaria activity score (UAS). **Results:** Serum concentration of IL-17, IL-18, IL-23 and TNF-α were significantly higher during the acute episode in chronic urticaria patients as compared with the healthy control subjects (mean: 1.84 ± 0.81 vs 0.03 ± 0.02 pg/ml; $P < 0.001$, 501.41 ± 208.98 vs 218.39 ± 39.83 pg/ml; $P < 0.001$; 25.57 ± 10.79 vs 0.15 ± 0.14 pg/ml, $P < 0.001$; and 455.54 ± 253.54 vs 8.498 ± 3.644 pg/ml, $P < 0.001$, respectively). There was a significant positive correlation between serum levels of IL-17, IL-18, IL-23 and TNF-α and severity of disease. **Conclusion:** The serum levels of IL-17, IL-18, IL-23 and TNF-α were raised in patients of chronic urticaria and positively correlated with the severity of urticaria.

Keywords: *Cytokines, severity, urticaria*

Introduction

Chronic spontaneous urticaria (CSU) is a complex, systemic disease with a multifactorial etiopathogenesis associated with autoimmune and inflammatory phenomena.[1-3] The basic event in the pathogenesis of urticaria is inappropriate activation and degranulation of dermal mast cells. The released cellular contents mediate the immediate phase of inflammation with various proinflammatory mediators, cytokines, chemokines and adhesion molecules regulating vasoactivity and cellular infiltration. The role of cytokines released by T helper type 1 (Th1) cells (Interleukin-2 [IL-2], Interferon gamma [IFN-γ], and tumor necrosis factor-alpha [TNF-α]) and Th2 cells (IL-4, IL-5, and IL-13) as a pro-inflammatory response for killing intracellular parasites and perpetuating autoimmune responses, respectively, is well- established. TNF-α is secreted by T- cells, natural killer (NK) cells, monocytes, macrophages, mast cells, basophils, eosinophils, keratinocytes, and fibroblasts. Th17 cells secrete IL-17, IL-17F, and IL-22, which are involved in autoimmunity. The differentiation of IL-17 producing CD4+ T cells requires a novel set of transcription factors that do not overlap with those factors required for Th1 or Th2 cells including signal transducer and activator of transcription 3 (Stat3), retinoic acid receptor- related orphan receptor γ (RORγ) and nuclear factor κ light-chain-enhancer of activated B (NF- κB) cells, which reinforce IL-17A and IL-17F producing Th17 cells as a new T-cell lineage.[4] IL-17 is mainly secreted by Th17 cells, innate immune cells, γδ T cells, invariant natural killer T (iNKT) cells and natural killer cells.[5] The differentiation of Th17 cells from naïve CD4+ T cells is dependent on signals from IL-6 and TGF-β, while maintenance of this lineage requires IL-23 and IL-21.[6] The combined action of IL-17A or IL-17F with other cytokines such as TNF-α, IL-1β and IFN-γ synergistically augments the pro-inflammatory responses

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from various target cells.[7] Although these proteins play an important role in host defense, excessive activation of this pathway contributes to autoimmunity.[8] In our study, to have a better understanding of "cytokine milieu" by the secretory Th17 cells in patients of chronic urticaria, we measured the levels of IL-17, IL-18, IL-23, and TNF-α as well found their significance in the severity of chronic urticaria.

Materials and Methods

A prospective cross-sectional study was approved by the research ethics committee of the institute. It was carried out in allergy-clinic of the Department of Dermatology and Department of Biochemistry at PGIMER Dr. Ram Manohar Lohia Hospital, New Delhi. Written informed consent was obtained from all the patients before their participation in the study.

Fifty patients with chronic urticaria of age above the age of 12 years having a disease duration of more than 6 weeks and 30 healthy volunteers were taken as controls, as per the convenience and statistician advice. Patients were graded as mild, moderate, and severe based on the urticaria activity score (UAS) as per the EAACI/GALEN/EDF/WAO guidelines.[9] Patients of acute urticaria (duration of urticaria <6 weeks), physical urticaria, urticarial vasculitis, systemic or autoimmune disorders, on any immunosuppressant drugs, children less than 12 years, and pregnant and lactating women were excluded.

TNF-α, IL-17, IL-18 and IL-23 were measured quantitatively from the collected serum samples by the automated microplate processor EVOLIS Twin Plus system for sandwich enzyme-linked immunosorbent assay (ELISA) in each patient and control. Anti-nuclear antibodies (ANA), rheumatoid factor (RF), thyroid autoantibodies-thyroperoxidase (anti- TPO), serum total IgE, and C-reactive protein (CRP) were assessed by ELISA. Thyroid function test (TFT) was assessed by chemiluminescence (Vitros Eci, Jhonson and Jhonson) and anti-streptolysin antibodies (ASO) by latex agglutination method. Hepatitis serology (anti HCV and HbsAg), complete haemogram and erythrocyte sedimentation (ESR), liver and kidney function tests, routine and microscopic examination of urine and stool examination for ova and cyst were done for each of the patients.

Statistical analysis

Quantitative data were presented as mean ± standard deviation (SD). For normally distributed data, means of 3 groups, one-way analysis of variance (ANOVA) followed by *Post Hoc* Multiple Comparisons test and for skewed data the Kruskal-Wallis test followed by Mann-Whitney test for two groups were applied. For Categorical variables, the number and percentage were calculated and Chi-square test or Fisher's exact, whichever was appropriate, was applied. The Spearman's rank correlation coefficient was applied to see correlation between different variables. $P < 0.05$ was considered as statically significant. We used the Statistical Package for the Social Sciences (SPSS) software version 17.

Results

Fifty patients with chronic Urticaria and 30 healthy controls were included for in the study. Among chronic urticaria patients (50), 19 (38%) were males and 31 (62%) females, and in the control group (30), 11 (37%) were males and 19 (63%) were females. The UAS ranged from 0-6. [Table 1] In our study, the minimum UAS was 2, seen in 3 (6%) patients and the maximum score was 6, observed in 8 (16%) patients. Thirty-nine (78%) patients had UAS of 3-5 [Table 2].

Twenty- four (48%) patients and 31 (62%) patients had raised absolute Eosinophil Count (488) and immunoglobin E (IgE) levels (344IU/ml), respectively. Fifteen of them had both raised AEC and IgE levels. The mean serum levels of TNF-α in chronic urticaria patients were 455.54 ± 253.54 pg/ml whereas in healthy controls they were 8.498 ± 3.644 pg/ml ($P < 0.05$). The serum levels of IL-17 in patients of chronic urticaria were 1.84 ± 0.81 pg/ml while in healthy controls were 0.03 ± 0.02 pg/ml ($P < 0.05$). The mean serum levels of IL-18 in chronic urticaria were 501.41 ± 208.98 pg/ml and median levels were 436.53 with 25th-75th percentile 353.45-658.53 whereas, healthy controls had mean of 218.39 ± 39.83 and median value 211.96 with 25th-75th percentile being 199.95-234.62 ($P < 0.05$). The mean serum levels of IL-23 in chronic urticaria patients and healthy controls were 25.57 ± 10.79 pg/ml and 0.15 ± 0.14 pg/ml ($P < 0.05$) respectively [Table 3].

Table 1: Age and sex distribution among cases and controls

	Age group	Mean±SD	Sex		*P*
			Female	Male	
Cases	21-50	33.84±6.816	31	19	0.68
Control	21-50	33.20±7.014	17	13	

SD=Standard deviation

Table 2: Number of wheals, the intensity of pruritus, and UAS

Number of wheals/24 h	Frequency	Intensity of pruritus	Number of cases	UAS 0-6	Number of cases
<20	20	Mild	5	1	0
20-50	17	Moderate	27	2	3
>50	13	Severe	18	3	13
Total	50	Total	50	4	17
				5	9
				6	8

UAS=Urticaria activity score

In our study, the positive correlation coefficient was found between UAS and AEC (0.765) and UAS and serum IgE (0.842). A Positive correlation between levels of IL-17, IL-18, IL-23, and TNF-α; and UAS was found. Correlation coefficient for TNF-α was 0.88, IL-17 was 0.854, 0.866 for IL-18, and 0.861 for IL-23 [Table 4].

Discussion

There are many studies concerning the immunopathogenesis of chronic urticaria. But the role of T cell subsets, apart from T helper Th1 and Th2 effector cells require further research work to validate their role in immunopathothenesis of CSU. More recently the Th1, Th2 paradigm has been updated to include a new subset called theTh17 cell. There are various associations that substantiate the role of autoimmunity in the pathogenesis of urticaria, like higher prevalence of thyroid autoantibodies, positive autologus serum skin test (ASST), identification of immunoglobulin G (IgG) directed against α-subunit of immunoglobulin E (IgE) receptor and presence of anti- IgE antibodies, association of human leukocyte antigen (HLA) -DR4 and HLA-DQ8[10] and therapeutic response to plasmapheresis and intravenous immunoglobulin (IVIG) in some patients of chronic urticaria. In our study, we found TNF-α levels to be significantly raised in chronic urticaria patients as has been reported in previous studies.[11,12] Earlier studies did not classify IL-17 producing CD4+ cells into Th1 or Th2 cells.[13-15] It was discovered that inducible T cell co-stimulator and IL-23 selectively regulate IL-17 producing T cells, which suggested that these cells are a separate helper cells subset.[16-18] IL-17 levels were significantly raised in our study and raised IL-17 levels also correlated with severity of urticaria in chronic urticaria patients, similar to the earlier studies by Iona G. Crisan *et al*.[11] and M.A. Atwa *et al*.[12] Interleukin-18 (IL-18) is an immuno regulatory cytokine. It is produced by monocytes/macrophages and dendritic cells in its active form. Keratinocytes, Langerhans cells, B cells and other epithelial cells throughout the body produce IL-18. IL-18 is a member of the IL-1 family of cytokines. It also has a role in the pathomechanism of chronic urticaria as seen by their raised levels and correlation with severity in chronic urticaria patients. Similar findings have also been observed by I.Puxeddu *et al*.[19] and Kurt E *et al*.[20] however Tedeschi *et al*.[21] and Roohi Rasool *et al*.[22] found no significant association. Interleukin-23 (IL-23), a member of the IL-12 cytokine family, is a heterodimeric cytokine composed of the IL-12p40 subunit, and with a novel p19 subunit.[23] IL-23 is mainly secreted by activated macrophages and dendritic cells (DCs) located in the peripheral tissues (skin, intestinal mucosa and lung). Serum concentrations of IL-23 were significantly higher in CSU patients than healthy controls in our study.

Cytokines secreted by Th1 and Th17 have important roles in pathomechanism of chronic urticaria. Th2 cytokines are mainly involved with acute urticaria which has drugs or parasitic infections or food allergy as the underlying cause. Thus, chronic urticaria is a state of chronic inflammation mediated by various cytokines and autoimmunity and Th1 cytokines might have a role in causation of chronic urticaria.

We observed in our study, that serum levels of TNF-α, IL-17, IL-18 and IL-23 were significantly raised in chronic urticaria patients as compared to healthy controls. Along with Th1 response, in chronic urticaria there is a Th17 response as indicated by increased IL-23 and IL-17 levels. On comparing UAS with serum cytokine levels, we found that a positive correlation existed between these cytokines and UAS. Thus the release of these cytokines in chronic urticaria is highly suggestive of tissue inflammation and immune dysregulation, involved in pathogenesis of urticaria. Therefore, patients not responding to antihistamines might respond to immunosuppressants and anti-inflammatory drugs. In addition to the serum levels, these cytokines should also be measured at the tissue level in future studies.

Table 3: Serum levels of TNF-α, IL-17, IL-18, IL-23, sr IgE, and AEC among cases and controls

Variables (pg/ml)	Cases (*n*=50)	Control (*n*=30)	*P*
Sr TNF-α	455.54±253.54	8.498±3.644	<0.05
Sr IL-17	1.84±0.81	0.03±0.02	<0.05
Sr IL-18	501.41±208.98	218.39±39.83	<0.05
Sr IL-23	25.57±10.79	0.15±0.14	<0.05
Sr IgE (mg/dl)	634.44±576.590	-	-
AEC	352.26±174.367	-	-

TNF-α = Tumor necrosis factor alpha, Sr=Serum, IL-17=Interleukin-17, IgE=Immunoglobulin E, ACE=Absolute eosinophil count

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not

Table 4: Correlation coefficient of cytokine levels, serum IgE, and AEC with UAS

	UAS	TNF-α	IL-17	IL-18	IL-23	Serum IgE	AEC
Correlation Coefficient	1.000	0.88	0.854	0.866	0.861	0.842	0.765

UAS=Urticaria activity score, TNF-α = Tumor necrosis factor alpha, IL-17=Interleukin-17, IgE=Serum immunoglobulin E, ACE=Absolute eosinophil count

be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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