

Egyptian Dermatology Online Journal

Volume 5 Number 1

Interleukin-18 Correlates with Disease Severity in Chronic Autoimmune Urticaria

Samia A Ibrahim* and Naglaa A khalifa

Egyptian Dermatology Online Journal 5 (1): 1, June 2009

Pathology, Zagazeg University.

Accepted for publication in May 28th, 2009.

Abstract:

Background: Autoimmune urticaria represents a relatively recent diagnostic advance since the discovery of histamine releasing autoantibodies against IgE and IgE receptors. Interleukin-18 (IL-18) is an immuno-regulatory cytokine and has roles in both allergic and autoimmune disorders.

Aim: The aim of this work was to measure IL-18 in patients with chronic urticaria (CU) to find a correlation between IL-18 levels, clinical disease severity, autologous serum skin test (Asst) and basophil histamine releasing antibodies.

Patients and methods: thirty two patients with CU and sixteen healthy control subjects were included in this study. The disease was classified according to the number and the size of wheals into mild, moderate and severe. All the patients and the controls underwent (Asst) to detect histamine releasing antibodies, basophil histamine release (BHR) assay. IL-18 levels were measured and (BHR) were assayed in the sera.

Results: In this study, the mean ages of the patients \pm standard mean of error (SME) was 42.6 ± 2.6 years. The number of patients according to the severity of the disease was, mild=17, moderate=11 and severe=4 patients. The mean \pm SME of IL-18 levels in pg/ml was 251.25 ± 19.69 in patients vs 215.20 ± 28.41 in healthy control with a non significant difference ($P=0.29$). When Asst assessment was done, 50% of patients were scored Asst +ve and 50% were scored Asst -ve. BHR assays were +ve in (56.25%) of Asst +ve patients and in only (12.50%) of Asst -ve patients and the mean percentage histamine release was 15.26 ± 5.6 and 6.10 ± 0.6 in both groups respectively showing a significant difference, $P=0.042$. IL-18 levels did not differ significantly between Asst +ve (243.75 ± 29.93 vs 258.75 ± 26.40 in Asst -ve patients, $P=0.71$). When IL-18 levels were compared in the groups of patients which were classified according to Asst and BHR, (Asst +ve, BHR +ve and -ve * Asst-ve, BHR +ve and -ve), a non significant differences were found, but when its levels compared with

percentage in histamine release, a significant relation was detected in Asst +ve, BHR +ve. ($r=0.07$, value more than 0.001 is significant). When patients with CU were grouped according to the severity of the disease, the highest serum IL-18 levels were detected in patients with severe disease with a significant +ve correlation in Asst +ve ($P=0.04$) and a non significant correlation in Asst -ve ($P=0.07$).

Conclusion: IL-18 may play an important role in immune activation in patients with CU.

Introduction:

Urticaria is a vascular reaction pattern affecting one fifth of the population, sometime or the other in their lives. The primary lesions of urticaria are wheals that appear suddenly as red, itchy, circumscribed areas of dermal oedema, lasting for few hours and then fade away to reappear in the same or other sites [1]. Chronic urticaria is defined as recurrence of wheals for at least six weeks with or without angioedema. In at least 30% of the patients, the urticaria is chronic, and may severely worsen the quality of life [2].

The basic mechanism for the urticaria is degranulation of tissue mast cells and blood basophils (either immunological, non immunological or idiopathic) [3]. Pin pointing to the cause of chronic urticaria may be challenging or impossible because of the many and varied triggers. In only 25% of the cases the cause is known [3]. The remainder is labelled chronic idiopathic urticaria. Autoimmune urticaria represents a relatively recent diagnostic advance and this condition is now believed to account for as many as 40% of patients with chronic idiopathic urticaria [4]. It has been evolved as evidence for histamine releasing autoantibodies and their relationship to the disease activity has accrued and allowed the differentiation between chronic autoimmune and chronic idiopathic urticaria [5,6].

IgG class autoantibodies against IgE and/or the high affinity IgE receptors have been demonstrated in the serum of patients with CU [7]. Autologous serum skin test is a screening test for histamine releasing autoantibodies [5,6]. Thyroid diseases and other autoimmune diseases have been reported to be associated with CU [5]. Biopsies of lesions of CU reveal a perivascular accumulation of eosinophils, mast cells, activated CD4 lymphocytes, consisting of Th1 and Th2 subtypes and neutrophils suggesting a role for lymphocyte-mast cell activation in the patho-mechanism of CU [8].

Interleukin-18 (IL-18) is an immuno regulatory cytokine. It is produced by monocytes / macrophages and dendritic cells in its active form [9]. Keratinocytes, Langerhans cells, B cells and other epithelial cells throughout the body produce IL -18. It exerts its effects on the immune system through activation of the T helper lymphocytes (Th1, Th2) responses depending upon the cytokine environment [10]. Thus, IL-18 may play a role in autoimmune and allergic disorders characterized by Th1 and Th2 responses respectively [11]. The biological effects of IL-18 are mainly due to the production of IFN γ [12].

Few studies have been undertaken to explore the role of IL-18 in (CU) [13]. In this study, serum IL - 18 will be measured and its possible relation to histamine releasing autoantibodies and the severity of the disease will be studied.

Patients and Methods :

Thirty two adult patients (20 females and 12 males) with chronic urticaria were chosen randomly from the Allergy and Immunity and Skin Departments of Zagazeg University Hospitals, during the period from January to June 2008. Their ages ranged from (16-65 years). The diagnosis was dependant on the recurrence of wheals with or without angioedema for more than 6 weeks. In all the patients, the known causes of chronic or recurrent urticarias were ruled out by careful history taking and by the proper investigations as CBC, ESR, ANA, ALT, AST, kidney function tests, and urine and stool analysis. Patients with physical urticarias or other chronic inflammatory diseases including atopic dermatitis were also excluded from the study. Sixteen gender matched and age matched healthy subjects were used as a control group. This group included 4 males and 12 females with age ranging from 16 to 62 years. Patients and control subjects signed informed consents and patients agreed to discontinue systemic antihistamines 5 days prior to the date of testing. Disease activity was estimated according to the number of wheals present at the time when the serum samples for thirty two patients were collected [14]: 1 to 10 small (< 3cm in diameter) wheals = grade 1 or mild; 10 to 50 small wheals or 1 to 10 large wheals = grade 2 or moderate; > 50 small wheals or > 10 large wheals = grade 3 or severe; and virtually covered with wheals = very severe or grade 4.

All the patients and control subjects underwent the following tests:-

- Autologous serum skin test (Asst): This test was performed by an intradermal injection of 0.05ml of fresh autologous serum and read after 30 minutes. We used a normal saline solution (0.9% NaCl sol) injected intradermally as a negative control and histamine solution 1mg/ml for the skin prick test as a positive control. Wheals 1.5 mm greater than that produced by the normal saline were considered positive [5].
- Basophil Histamine Release (BHR) assay: In this test, leucocyte suspensions from three normal blood donors were separated by dextran and stimulated to histamine release on challenge with an optimum dose (10µg/ml) of goat poly clonal anti-human IgE and was showing a 30% net release. (Sigma Chemical Co. St. Louis, Mo, USA). Histamine concentration was measured by an automated fluorimetric method. A 5% net release cut off value was used. One assay per serum was performed [15] to detect histamine releasing activity.
- Serum IL-18 concentration was measured by using a sandwich enzyme immunoassay with sensitivity of 12.5pg/ml (MBL. Medical and Biological Laboratories, Nagoya, Japan), according to Asero et al. 2007.

Statistical Analysis: -

Mann - Whitney U test, Kruskal - Wallis test with Dunn's multiple comparisons test, Fisher's exact test and Spearman rank correlation test were used in the study. P values < 0.05 were considered significant. All the results were expressed in the form of mean \pm SME.

Results:

In this study, thirty two patients with CU (their mean ages was 42.6 ± 2.6 years) and sixteen healthy age and sex matched control subjects (their mean ages was 43.5 ± 3.8 years) were enrolled. The patients grades according to the severity of the disease were mild=17, moderate=11 and severe=4 patients. IL-18 concentrations in patients with CU was 251.25 ± 19.69 Pg /ml and its levels in normal control subjects 215.20 ± 28.41 Pg /ml showed a non significant difference as in **Table 1**.

Number	Mean \pm SME	Range	Median	t	p
Cases No =32	251.25 \pm 19.69	75-525	217.5	1.05	0.29
Control No =16	215.20 \pm 28.41	70-400	220	Non significant	

SME: standard mean of error.

Table 1 :Comparison between serum IL-18 concentrations(pg/ml) in patients with chronic urticaria and control .

Regarding the results of Asst, 16 patients (50%) were scored +ve for Asst and 16 patients (50%) were scored Asst - ve. IL-18 concentration did not differ significantly as in **Table 2** between Asst +ve (243.75 ± 29.93) Pg /ml and Asst -ve (258.75 ± 26.40) groups. BHR was +v in 9 (56.25%), Asst +ve and in only 2(12.50%) Asst - ve patients with CU.

	Number of cases	Percent	Mean \pm SME	Range	Median	t	p
Asst positive	16	50%	243.75 \pm 29.95	75-525	197.5	0.31	0.71
Asst negative	16	50%	258.75 \pm 26.40	125-485	225	Non significant	

Asst: Autologous serum skin test, SME: standard mean of error.

Table 2: Comparison between serum IL-18 concentrations(pg/ml) in both Asst positive and Asst negative patients with chronic urticaria.

The mean \pm SME percentage histamine release in both groups were 15.56 ± 5.60 and 6.10 ± 0.60 respectively showing a significant difference as in **Table 3**.

	Number of cases	Mean \pm SME	Range	Median	t	p
BHR +ve in Asst positive	9	15.56 \pm 5.60	7-23.80	16.6	2.33	0.042
BHR -ve in Asst negative	2	6.10 \pm 0.60	5.70-6.30	6.1	Significant	

Asst: autologous serum skin test, SME: standard mean of error.

Table 3: Percentage Histamine release in both Asst positive and Asst negative patients with chronic urticaria

When percentages histamine release in Asst +ve patients with +ve BHR were compared with serum IL-18 concentrations, a significant relation was present ($r=0.77$, value more than 0.001 was significant). When IL-18 levels compared in patients with CU grouped according to Asst and BHR (Asst +ve, BHR +ve and -ve * Asst -ve, BHR +ve and -ve), a non significant differences were found as in **Table 4**, but when its levels correlated with the disease severity, the highest serum IL-18 levels were detected in patients with severe disease with a significant +ve correlation in Asst +ve and a non significant correlation in Asst -ve group as in **Fig.1**

	Number of cases	Percent %	Mean \pm SME	Range	Median	t	p
Asst +ve BHR+ve	9	56.25	241.74 \pm 36.93	75-405	195	0.07	0.93
Asst +ve BHR -ve	7	43.75	246.42 \pm 52.76	140-525	200	Non significant	
Asst -ve BHR -ve	14	87.5	265.33 \pm 29.93	125-485	245	0.4	0.5
Asst -ve BHR+ve	2	12.5	212.51 \pm 2.47	210-215	212.5	Non significant	

BHR: basophil histamine release, SME: standard mean of error.

Table 4: Comparison of serum IL-18 concentrations according to Asst and BHR positive and negative in patients with chronic urticaria.

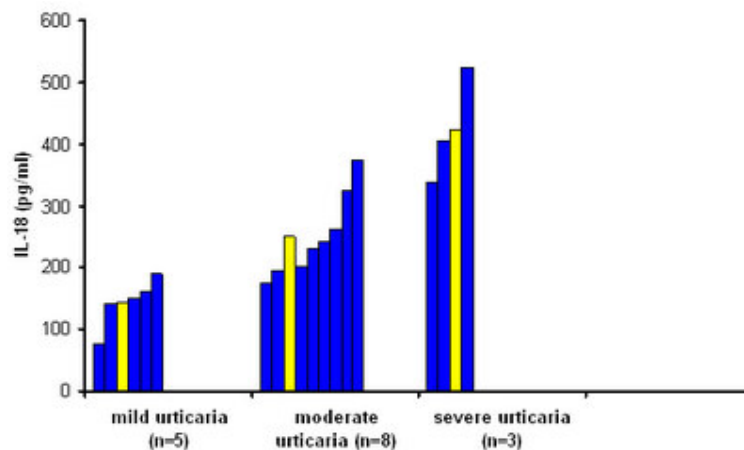


Fig 1a: Serum IL-18 levels in ASST-positive patients classified according to clinical severity score. Statistical analysis by Kruskal-Wallis Test showed that there was a significant difference between the 3 groups ($P= 0.04$). Post tests were performed by Dunn's multiple comparison tests. The yellow color represents the mean values, the blue color represents the original values.

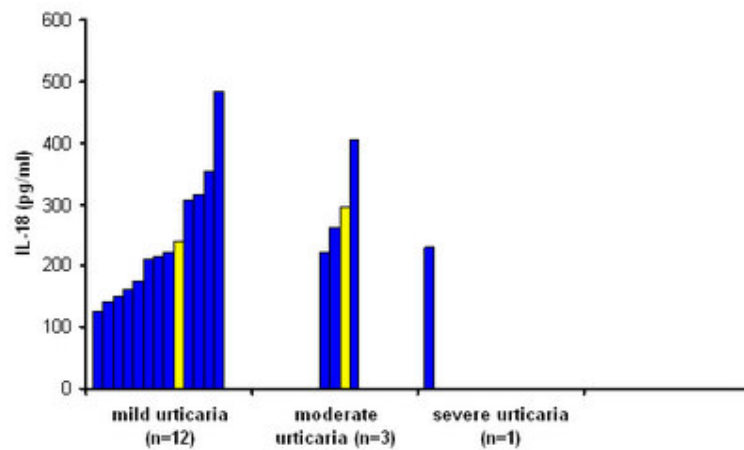


Fig 1b: Serum IL-18 levels in ASST-negative patients classified according to clinical severity score. No significant difference was found between the patient groups ($P=0.07$). The yellow color represents the mean values, the blue color represents the original values.

Discussion:

Urticaria is one of the most common dermatological problems that cause frustration to the patients and physicians. The impact on the quality of life that CU causes to the patients is so distressing, especially sleep disturbance, and can be difficult to treat [16]. CU is without a clear etiology in the majority of cases, but the basic mechanism is the degranulation of mast

cells and basophils with release of histamine and vasoactive mediators [3].

Interleukin-18 was measured in the serum of patients with CU in this study. Its level did not differ significantly between patients and healthy control subjects, and did not differ between Asst +ve and Asst -ve groups. This is in agreement with the previous results [13].

In the present study, when the patients with CU were grouped according to the severity of the disease, there was a positive correlation between serum IL-18 concentrations and the severity of the disease in Asst +ve patients and showed a tendency to correlate positively with BHR. These data suggested that IL-18 has been proposed to play a role in mast cell degranulation and histamine release as a direct factor. This may be through the production of IL-4 and increase in IgE level and histamine release by basophils [17].

In Asst -ve group, this correlation was not found. Other mechanisms than the direct effects of IL-18 were suggested [8,21].

Up to 50% of patients with CU included in this study were Asst +ve due to the presence of histamine releasing autoantibodies that is in agreement with the previous studies [4,5]. In only 9 out of 16 Asst +ve patients, BHR was +ve, detecting the presence of functioning histamine releasing autoantibodies [18,19]. In this group antibody mediated mast cell activation is involved [20].

It has been suggested that lymphocyte- mast cell activation may be a possible mechanism in the remaining of patients as evidenced by the presence of a perivascular accumulation of an infiltrate rich in CD4 lymphocytes, consisting of Th1 and Th2 cells in the biopsy of lesions from CU patients [8], or it may be due to activation of the extrinsic pathway of coagulation, with the production of thrombin. Thrombin is able to stimulate mast cell degranulation and C5a that enhances histamine release induced by Fc ϵ R autoantibodies [21,22].

In conclusion:

IL-18 may be involved in chronic urticaria and its level correlates with the disease severity in Asst +ve patients and shows a tendency to correlate with in vitro histamine release. This suggests that the immune system is strongly activated by IL-18 which may have some role as a direct factor.

Recommendations:

As IL-18 may have some role in CU, in the future it can be inhibited by caspase-1 inhibitors [23] and IL-18 binding protein which is able to bind IL-18 with a high affinity and to inhibit IL-18 induced IFN- γ and IL-18 production [24] to treat some cases of autoimmune urticaria and other auto immune diseases, a subject that needs more researches.

References

1. Sharma B, Gera MB, Tivari I TH Chronic urticaria: Expanding the autoimmune kaleidoscope, MJAFI 2004; 60: 372.
2. Clive EH, Ruth A, Malcom W et al. Chronic urticaria .J Am. Acad. Dermatol. 2002; 46:

645.

3. Ring J, Brockow K, Ollert M et al. Antihistamines In Urticaria. *Clinical Exp Allergy* 1999; 29 (suppl. 1): 31.

4. Hein R .Chronic urticaria: impact of allergic inflammation. *Allergy* 2002; 57(1) (75): 19.

5. Sabroe R A, Gratten CA, Francis DM et al. The autologous serum skin test: A screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140: 446.

6. Gratten CE, The urticaria spectrum recognition of clinical pattern can help management. *Clin Exp Dermatol* 2004; 29: 217.

7. Ferrer M, Kint JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti FCER1 α (α subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998; 101: 672.

8. Ying S, Kikuchi Y, Meng Q et al. TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: comparison with the allergen induced late phase cutaneous reaction. *J Allergy Clin Immunol* 2002; 109: 694.

9. Nakanishi K, Yoshimoto T, Tsutsui H et al. Interleukin-18 regulates both Th1 and Th2 responses. *Ann Rev Immunol* 2001; 19: 423.

10. Boraschi D and Dinarello C. IL-18 in autoimmunity: review. *Eur Cytokine Netw* 2006; 17(4): 224.

11. Izakovicova Holla L. Interleukin in asthma and other allergies. *Clin Exp Allergy* 2003; 33: 1023.

12. Nakamura K, Okamura H, Wada M et al. Endotoxin -induced serum factor that stimulates gamma interferone production. *Infect. Immunol* 1989; 37: 590.

13. Tedeschi M, Lorini C. Suli and Asero R. Serum IL-18 in patients with chronic ordinary urticaria association with disease activity. *Clin Exp Dermatol* 2007; 32: 568

14. Sabroe RA, Fiebiger E, Francis DM et al. Classification of anti -FCER1 and anti IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; 110: 492.

15. Asero R, Tedeschi A , Lorini M et al. Chronic urticaria. Novel clinical and serological aspects. *Clin Exp Allergy* 2001; 31: 1105.

16. O'Donnell BF, Lawlor F, Simpson J et al. The impact of chronic urticaria in the quality of life indices. *Br J Dermatol* 1997; 136: 197.

17. Yoshimoto T, Tsutusi H, Tominaga K et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc Natl Acad Sci USA* 1999; 96: 13962.

18. Greaves MW. Chronic Urticaria. *J Allergy Clin Immunol* 2000; 105: 664.

19. Sabroe RA and Greaves MW. Chronic idiopathic urticaria with functional autoantibodies: 12 years on. *Br J Dermatol* 2006; 154: 813.
20. Boguniewicz M. The autoimmune nature of chronic urticaria. *Allergy Asthma Proc* 2008; 29: 433.
21. Asero R, Riboldi P, Tedeschi A et al. Severe Chronic Urticaria: A disease at a crossroad between autoimmunity and coagulation. *Autoimmunity Review* 2007; 7: 71.
22. Asero R, Tedeschi A, Riboldi P et al. Severe chronic urticaria is associated with elevated plasma levels of D-dimer. *Allergy* 2008; 63:176.
23. Linton SD. Caspase inhibitor, A pharmaceutical industry perspective. *Curr Top Med Chem* 2005; 5: 1697.
24. Novick D, Kim SH, Fantuzzi Get al. Interleukin -18 binding protein a novel modulator of Th1 cytokine response. *Immunity* 1999; 445: 338.

© 2009 Egyptian Dermatology Online Journal