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" *Evaluating The Correlation between Level of Serum Interleukin-9 and Disease Activity in Chronic Spontaneous Urticaria Patients* "

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ABSTRACT:

Background: Chronic spontaneous urticaria (CSU) is a common dermatological disease, estimated to occur in 0.5% to 5% of the general population, with an annual incidence rate of approximately 1.4%.

Chronic spontaneous urticaria is diagnosed when the patient has wheals with or without angioedema for longer than six weeks. The patient's symptoms may be caused by known factors (such as mast cell-activating autoantibodies) or unknown factors.

Aim of the work: Our study aimed to measure the association between IL-9 and CSU severity, in order to help improving knowledge about CSU management and prognosis.

Patients & methods: A case-control study was carried out from September 2024 to March 2025 at the dermatology outpatient clinics of Mansoura University, from people enrolled in the hospital registry. A total of 43 patients diagnosed with chronic spontaneous urticaria who met the inclusion criteria were enrolled in the study. Additionally, 43 age- and sex-matched healthy controls were included. Venous blood samples were collected from both the case and control groups, and serum IL-9 levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Disease activity in the case group was assessed using the Urticaria Activity Score over seven days (UAS7). Statistical analysis included correlation tests, odds ratio calculations, and subgroup comparisons.

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Results: The study findings reported that there was an association between the median of serum IL-9 among the studied group. Regarding the area under curve for serum IL-9 in differentiating between cases versus control group is excellent with the best detected cut off point is 115.48 pg/ml, yielding sensitivity 81.4%, specificity 83.7% and total accuracy 82.6%. The odds of elevated IL-9 among CSU cases were 8.75 times higher than in controls (95% CI: 3.15–24.27). Regarding the relation between Serum IL-9 and Urticaria activity score-7 among studied cases, there was no statistically significant difference between the two groups under study. (P>0.05). However, subgroup analysis revealed higher IL-9 levels in patients with moderate to severe disease versus those with mild activity.

Conclusion: We conclude that According to the analysis, CSU patients' IL-9 levels were statistically significantly higher than those of healthy controls. These findings suggest IL-9 may play a contributory role in CSU pathogenesis and could be considered a potential biomarker of disease activity. The results were critically compared with similar research that was just focused on CSU to put these findings into context.

Keywords: Chronic spontaneous urticaria, IL-9, Interleukin-9, Disease Activity, Cytokines, UAS7, Biomarker

Introduction

Urticaria is a clinical condition characterized by the appearance of wheals (hives), angioedema, or a combination of both. Wheals are defined as circumscribed, erythematous, or pale, non-pitting, edematous plaques that are typically pruritic and transient, with individual lesions resolving within 24 hours. Angioedema involves deeper swelling of the dermis or subcutaneous tissues and is often painful rather than pruritic, persisting for 2–3 days **(Zuberbier et al., 2021; Dinulos, 2021)**.

Urticaria can be classified based on its duration and etiology. Acute urticaria (AU) lasts for ≤6 weeks and is often triggered by infections, medications, or food allergies. Chronic urticaria, which persists for >6 weeks, is further subdivided into chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CIndU). CSU is characterized by the spontaneous appearance of wheals and/or angioedema without identifiable triggers, although factors such as stress or infections may exacerbate symptoms. In contrast, CIndU is triggered by specific stimuli, such as cold, pressure, or heat **(Kolkhir, Giménez-Arnau, et al., 2022)**.

In many cases, periods of years or even decades elapse before a patient's CSU is fully controlled by responding to treatment or achieving spontaneous remission, meaning these patients suffer for long time. Moreover, the cutaneous signs and symptoms markedly impact patients' quality of life and have an enormous socioeconomic impact **(Maurer, Pavel Kolkhir, et al., 2024).**

In CSU, mast cells are more susceptible to degranulation and release greater amounts of histamine than those from healthy individuals. This heightened histamine release drives the hallmark symptoms of urticaria, such as vasodilation, increased vascular permeability, and stimulation of sensory nerves, resulting in wheal formation and itching. Upon activation, mast cells also secrete other pro-inflammatory mediators-including leukotrienes, prostaglandins, and cytokines-which further amplify skin inflammation **(Folci et al., 2020; Hide & Kaplan, 2022).**

The central pathophysiological hallmark of CSU is the inappropriate degranulation of cutaneous mast cells, leading to the release of a diverse array of pro-inflammatory mediators. These include histamine, proteases, prostaglandin D2 (PGD2), leukotrienes (LTC4, LTD4, LTE4), and cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-8 **(Maurer, Zuberbier, et al., 2021; Dinulos, 2021 pp.176-181).**

Th2 cytokines, including IL-9, are central to CSU pathogenesis, promoting IgE synthesis and eosinophilic inflammation **(Krzysztof Gomułka et al., 2024).** IL-9 supports mast cell proliferation and histamine release, contributing to CSU chronicity. It is suggested to be elevated in antihistamine responders, suggesting a nuanced role in disease modulation **(Bhatia et al., 2024).**

CSU is recognized as a primarily autoimmune disorder mediated through distinct immunological pathways. The condition is classified into type I autoimmunity, characterized by IgE autoantibodies against self-antigens like TPO and IL-24 that typically respond to antihistamines, and type IIb autoimmunity, driven by IgG autoantibodies targeting FcεRI or IgE, which leads to more severe, treatment-resistant disease. Additional rare variants include type IIa involving cytolytic IgG mechanisms, and mixed endotypes combining features of both major subtypes **(Kolkhir et al., 2017).** Exploring the association between these endotypes and treatment outcomes may provide valuable insights into predicting the therapeutic response to omalizumab and support the development of personalized management strategies **(Chen, Ou, et al., 2024).**

Low total IgE is more common in CSU-type II autoimmunity (CSU^{aiTIIb}) and is associated with poor response to omalizumab treatment. Elevated IgE against self (e.g., TPO, IL-24, dsDNA) suggests type I autoimmunity **(Metz et al., 2021).**

The management of chronic urticaria typically begins with **H1 antihistamines**, which are the cornerstone of treatment. However, these medications do not completely clear the urticaria **(Zuberbier et al., 2021)**. Second-generation H1-antihistamines are favored over first-generation agents due to their selectivity for peripheral H1 receptors, resulting in fewer side effects **(Kam Lun Hon et al., 2021).**

When antihistamines are insufficient, the second-line treatment is **omalizumab**, an anti-IgE antibody. Despite this, approximately 70% of patients with antihistamine-refractory CSU fail to achieve complete disease control with omalizumab therapy. In clinical trials, omalizumab demonstrated complete response rates (UAS7=0) ranging from 33.7% to 44.0% at week 12. For patients who still do not achieve adequate control, **cyclosporine A** is the recommended third-line treatment. Novel therapies are critically

needed for chronic spontaneous urticaria (CSU) as many patients, especially those with autoimmune CSU, remain inadequately controlled with current H1-antihistamines and omalizumab **(Pavel Kolkhir et al., 2025)**.

In April 2025, the U.S. Food and Drug Administration (FDA) approved dupilumab (Dupixent; Regeneron and Sanofi) for the treatment of CSU in patients aged 12 years and older who have not achieved adequate disease control with H1 antihistamine treatment **(Andrus & Maddi, 2025).**

The management of chronic urticaria (CU) presents significant hurdles, such as delays in diagnosis, poor treatment compliance, and limited availability of newer therapies. Misdiagnosis is frequent due to the condition's heterogeneous symptoms and the absence of definitive diagnostic markers (**Emek Kocatürk & Grattan, 2019).**

2- Patients & methods

According to a type of case-control study, 43 patients diagnosed with chronic spontaneous urticaria met the inclusion criteria and were enrolled. Additionally, 43 healthy controls, matched for age group and sex distribution, participated in the study. Patients and controls were recruited from dermatology outpatient clinics at Mansoura University who enrolled in a hospital registry from September 2024 to March 2025 after providing written informed consent. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of Port Said Faculty of Medicine (code ERN: MED (1/5/2024) s.no (162) DRM818_007).

Inclusion criteria:

For the cases group:

1) Age ranges from 18 to 50 years.
2) Both sexes were included.
3) Chronic spontaneous urticaria patients who have recurrent wheals, angioedema, or both for more than 6 weeks (about 1 and a half months).

For controls group:

1) Age ranges from 18 to 50 years
2) Both sexes were included.
3) Healthy individuals do not experience urticaria, nor have they experienced an attack of wheals or angioedema before.

Exclusion criteria:

1) Patients younger than 18 or older than 50 years.
2) Patients with acute urticaria (urticarial attacks within less than 6 weeks).
3) Patients with inducible urticaria, angiotensin converting enzyme inhibitor-induced angioedema, anaphylaxis, hereditary angioedema, and urticarial vasculitis.
4) Patients with active, untreated atopic dermatitis (acute exacerbation of atopy or atopic diathesis).
5) Patients on any vaccine regimen (e.g., COVID-19 vaccine).
6) Patients who received systemic treatment modalities for chronic spontaneous urticaria.

7) Patients on suspected inducing medication such as: non-steroidal anti-inflammatory drug.
8) pregnancy or lactation.
9) Severe uncontrolled chronic illnesses, e.g., diabetes mellitus, underlying hepatic and renal disease, and malignancy.
10) Patients with active chest diseases, e.g., asthma, chronic obstructive lung disease.
11) Untreated or uncontrolled thyroid disorders.
12) Untreated or uncontrolled Autoimmune diseases, e.g., systemic lupus erythematosus, dermatomyositis.
13) Auto-inflammatory diseases, especially syndromes associated with urticarial lesions.
14) Patient on immunosuppressive drugs, e.g., systemic steroid.

Methods of assessment

Regarding cases group, all data were collected as follows:

1- Full history including (age, disease duration, smoking, comorbidities, current treatment, and previous medical history).

2- Complete general and dermatological examination.

3- At the time of the first presentation, the severity of urticarial attacks had been assessed using the Urticaria Activity Score (UAS) shown in Table (1).

4- Blood samples (3 cm of venous blood) had been withdrawn from each candidate.

Medication history

We have selected the patients who stopped the medications 2 weeks before the visit to the clinic (i.e. if someone complains about NSAIDs from inflammatory diseases and has already stopped the treatment since the last 2 weeks before the first visit).

Table (1): Urticaria activity score (Yashdeep Singh Pathania et al., 2019)

Score	Wheals	Pruritus
Urticaria activity score (UAS)		
0	None	None
1	Mild (<20 wheals/ 24 h)	Mild (present but not troublesome)
2	Moderate (20–50 wheals/24 h)	Moderate (troublesome but does not interfere with sleep)
3	Severe (>50 wheals/ 24 h)	Severe (sufficiently troublesome to interfere with normal daily activity and sleep)

Laboratory testing:

Blood sampling

A 3 ml venous blood sample was collected from all patients and controls and delivered into plain tubes. Samples were centrifuged to get the sera stored at −20°C until analysis.

Principle

IL-9 concentrations were tested using Develop Human Interleukin 9 (IL9) enzyme-linked immunosorbent assay (ELISA) kits (Catalog No: DLR-IL9-Hu) according to the manufacturer's instructions.

• Measurement of serum IL-9 by DLR-IL9-Hu ready-to-use ELISA kit.

The kit employed a sandwich enzyme-linked immunoassay technique for quantitatively measuring IL-9 in human serum, plasma, tissue homogenates, cell culture supernates, cell lysates, or other biological fluids in vitro. The microtiter plate included in the kit was pre-coated with a specific antibody to IL-9. Following this, standards or test samples were added to the suitable microtiter plate wells with a biotin-conjugated antibody preparation specific to IL-9. Subsequently, Avidin conjugated to Horseradish Peroxidase (HRP) was added and incubated. Upon adding the TMB substrate, a color change occurred in wells containing IL-9, biotin-conjugated antibody, and enzyme-conjugated Avidin. The enzyme-substrate reaction was terminated by the addition of sulphuric acid, and the color change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentrations of Interleukin-9 in the samples were calculated through a standard curve. A standard curve was constructed by plotting the O.D. for all standards against their known concentrations. Interleukin-9 concentrations in the samples were expressed as ng/ ml and then converted to pg/ml by multiplying the results by 1000.

2.5 Statistical analysis

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 26 for Windows® (IBMSPSS Inc., Chicago, IL, USA). Qualitative variables were described as number and percentage, while quantitative variables were presented as mean ± SD or median (range) according to normality. The appropriate statistical tests were used according to the nature of the data. AP<0.05 was considered statistically significant.

3- Results

A case-control study has been conducted at the dermatology outpatient clinics-Mansoura University hospitals. A total of 86 candidates were enrolled, who had presented to Dermatology clinic at Mansoura university hospital. The candidates were classified into 2 groups, Group A: CSU group (n=43 patients). Group B: The Control group (n=43 healthy patients) and well matching baseline clinical characteristics to the study group.

Table (2) shows that mean age of cases is 36.12±11.15 years versus 36.19 ± 10.98 years for control group. Females represent 74.4% versus males 25.6% for cases and control group, respectively. No statistically significant difference was identified between cases versus control groups as regards all sociodemographic characters including the following: age, marital status, occupation, and smoking.

Table (2) comparison of sociodemographic characters between studied groups

Variables		Case group	Control group	Test		P-value
		No. = 43	No. = 43			
Age	Mean ± SD	36.12±11.15	36.19±10.98	t test	0.029	> 0.05
Sex	Male	11 (25.6%)	11 (25.6%)	Chi-square test	-	-
	Female	32 (74.4%)	32 (74.4%)			
Marital status	Single	8 (18.6%)	6 (14%)		0.341	> 0.05
	Married	35 (81.4%)	37 (86%)			
Occupation	Not-working	29 (67.4%)	34 (79.1%)		1.48	> 0.05
	Working	14 (32.6%)	9 (20.9%)			
Smoking	Non-smoker	40 (93%)	40 (93%)		-	-
	Smoker	3 (7%)	3 (7%)			

t: Student t test, Chi-Square test.

Table (3) and figure (1) illustrates that 46.5% of studied cases have severe degree of activity, 9.3% moderate, and 44.2% of mild activity.

Table (3) Urticaria activity score 7 among studied cases

Urticaria activity score 7	N	%
Mild	19	44.2
Moderate	4	9.3
Severe	20	46.5

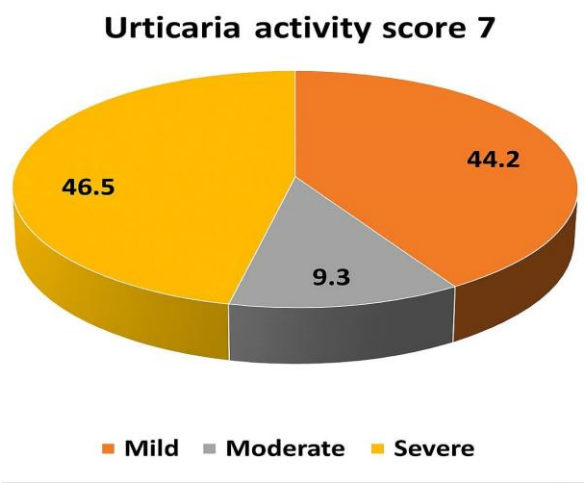

Figure (1): Urticaria activity score 7 among studied cases. N=43

Table (4): illustrates that median serum IL-9 is 440.62 pg/ml ranging from 20.3 to 31200 pg/ml for cases group versus 41.17 ranging from 1 to 569.88 pg/ml for control group, with statistically significant difference between them.

Table (4): comparison of serum IL-9 between studied groups

	Cases group N=43	**Control group N=43**	**Test of significance**	**P-value**
Serum IL-9 level (pg/ml) Median (min-max)	440.62 (20.3-31200)	41.17 (1.0-569.88)	Z =6.35	<0.001*

Z: Mann Whitney U test, *statistically significant.

Table (5) demonstrates that the area under curve for serum IL-9 in differentiating between cases versus control group is excellent. The best detected cut off point is 115.48 pg/ml yielding sensitivity 81.4%, specificity 83.7% and total accuracy 82.6%.

Table (5): ROC curve of serum IL-9 in differentiating between cases versus control group

	AUC (95%CI)	**P value**	**Cut off point**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
Serum IL9(Pg/ml)	0.897 (0.8330.961)	<0.001*	≥115.48	81.4	83.7	81.8	83.3	82.6

AUC: Area under curve, PPV: Positive predictive value, NPV: Negative

Figure (2) shows a forest plot of the odds ratio (OR) and 95% confidence interval (CI) for elevated serum IL-9 in CSU patients compared to controls, considering that cut off value of high IL-9 level when level is ≥115.48 pg/ml. CSU patients exhibited significantly higher odds of elevated IL-9 compared to healthy controls (OR = 22.5, 95% CI: 7.37–68.68).

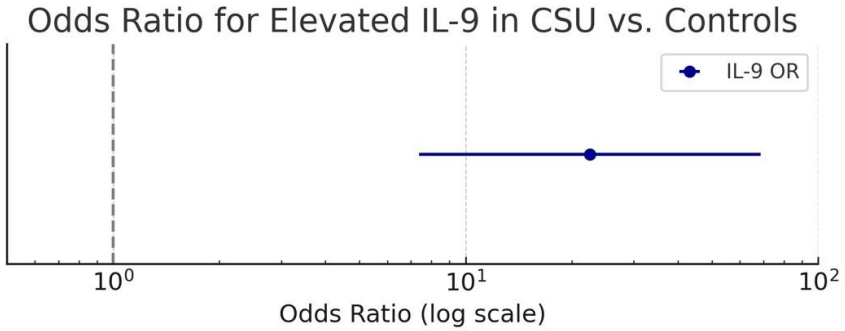

Figure (2): Forest plot of the odds ratio (OR) and 95% confidence interval (CI) for elevated serum IL-9 (≥115.48 pg/ml) in CSU patients compared to controls.

Table (6) shows no statistically significant association between UAS 7 and serum IL9 (P=0.111). Median Serum IL-9 is 776.52 pg/ml among cases with mild activity, 115.48 pg/ml for cases with moderate activity and 333.23 pg/ml for cases with severe activity.

Table (6) relation between Serum IL-9 and Urticaria activity score 7 among studied cases

Urticaria activity score 7	Serum IL-9(Pg/ml) Median (min-max)	Test of significance	P value
Mild	709.94(65.86-26100)	Kw=6.0 P=0.111	P >0.05
Moderate	115.48(39.86-20100)		
Severe	333.23(20.3-31200)		

KW: Kruskal Wallis test

Table (7) illustrates no statistically significant correlation between serum IL-9 and age (p=0.254), duration (p=0.861) and UAS-7 (P=0.370).

Table (7): correlation between serum IL-9 and age, disease duration and activity score.

		Serum IL-9 level (pg/ml)
Age (years)	r	-.178
	P value	.254
	N	43
Duration (years)	r	.028
	P value	.861
	N	43
UAS-7	r	-.140
	P value	.370
	N	43

r: Spearman correlation coefficient

Table (8) shows statistically significant association between smoking and IL-9 with higher median value of IL9 was detected among smokers than non-smokers (20100 versus 366.81 pg/ml, respectively).

Table (8): relation between Serum IL-9(Pg/ml) and sociodemographic characters among studied cases.

Sociodemographic characters		Serum IL-9(Pg/ml) Median (min-max)	Test of significance	P value
Age / years	<25 25-39 40-50 >50	507.88(20.3-31200) 704.94(114.4-26100) 235.21(39.86-31200) 120.47(88.4-468.5)	Kw=3.93 P=0.269	P >0.05
Sex	Male Female	2724.6(20.3-26100) 366.81(39.86-31200)	Z=1.17 P=0.242	P >0.05
Marital status	Single Married	298.09(20.3-20100) 440.62(39.86-31200)	Z=1.22 P=0.224	P >0.05
Occupation	Not working Working	468.5(39.86-31200) 379.79(20.3-31200)	Z=0.505 P=0.613	P >0.05
Smoking	Non-smoker Smoker	366.81(20.3-31200) 20100(2724.6-24200)	Z=2.15 P=0.032*	P <0.05

Z: Mann Whitney U test, KW: Kruskal Wallis test *statistically significant

Table (9) shows no statistically significant correlation between disease severity and any of the tested laboratory parameters (p > 0.05).

Table (9): correlation between disease severity & the laboratory results. (n=43)

		Disease severity (UAS)			P value
		Mild (n=19)	Moderate (n=4)	Severe (n=20)	
H-pylori stool-Ag	Negative	18 (94.7)	2 (50)	16 (80)	**0.153***
	Positive	1 (5.3)	2 (50)	4 (20)	
Total IgE	Normal	15 (78.9)	3 (75)	11 (55)	**0.375**
	Abnormal	4 (21.1)	1 (25)	9 (45)	
CBC	Normal	19 (100)	3 (75)	17 (85)	**0.274**
	Abnormal	0 (0)	1 (25)	3 (15)	
Urine analysis	Normal	17 (89.5)	4 (100)	17 (85)	**0.407**
	Abnormal	2 (10.5)	0 (0)	3 (15)	
Stool analysis	Normal	17 (89.5)	4 (100)	17 (85)	**0.407**
	Abnormal	2 (10.5)	0 (0)	3 (15)	
ESR	Normal	19 (100)	4 (100)	18 (90)	**0.491**
	Abnormal	0 (0)	0 (0)	2 (10)	
Ferrtin	Normal	19 (100)	4 (100)	16 (80)	**0.182**
	Abnormal	0 (0)	0 (0)	4 (20)	

*P-value>0.05 = not statistically significant. By Chi-square test.

4- Discussion:

CSU is a common dermatological disorder. Many cytokines and chemokines are involved in its inflammatory process, and their corresponding intracellular signaling cascades have been recognized. These insights have guided the development of novel therapeutic strategies, highlighting a renewed focus on the pathogenesis of CSU in clinical research. Advancing the understanding of CSU pathogenesis and its associated biomarkers is essential for enabling a more targeted and potentially curative approach to treatment development **(Hide & Kaplan, 2022).**

This study investigated the relationship between serum Interleukin-9 (IL-9) concentrations and disease activity among patients diagnosed with CSU at the Dermatology clinic at Mansoura University Hospital. The data analysis demonstrated a statistically significant elevation of IL-9 levels in CSU patients relative to healthy controls. To interpret these results, the findings were critically compared with analogous studies exclusively focused on CSU.

Demographics data

Our study recruited 43 patients with CSU and 43 healthy controls. The mean age in the CSU group was 36.12 ± 11.15 years, with a male-to-female ratio of 3:1. In comparison, Bhatia et al. (2024) examined a larger sample of 95 CSU patients and 42 controls, reporting a slightly lower mean age of 33.77 ± 8.07 years and a female-predominant gender distribution (male-to-female ratio of 1:1.41). The observed discrepancies in demographic profiles between the two studies may reflect population-specific differences in health-seeking behavior, occupational or environmental exposures, or underlying demographic characteristics.

In terms of atopic predisposition, only five patients in the present study reported a history of atopy, whereas **Bhatia et al. (2024)** documented a notably higher number—23 patients—with atopic conditions such as asthma and allergic rhinitis. This difference may be attributable to geographical, environmental, or genetic variations influencing immune response patterns in different populations. **Chen, Yang, et al. (2024)** highlighted the importance of atopic background in CSU pathogenesis, demonstrating that individuals with atopic disorders tend to exhibit increased levels of Th2- and Th9-associated cytokines, including interleukin-9 (IL-9). These elevated cytokine levels suggest a skewed immune profile in atopic individuals that may exacerbate CSU severity and chronicity, positioning IL-9 as a potentially relevant mediator in such cases.

The course of CSU in this study was predominantly progressive, with 65% of patients experiencing gradual worsening of symptoms. The average disease duration was three years (36 months), suggesting a more chronic or persistent disease pattern among participants. In contrast, **Bhatia et al. (2024)** reported a mean disease duration of 20.82 ± 16.57 months, indicating a relatively shorter course. This variation may be linked to differences in disease recognition, access to specialized care, or treatment strategies across regions. It may also reflect differences in inclusion criteria, patient selection, or follow-up timing. Longer disease duration in this cohort could contribute

to higher cytokine levels due to prolonged immune activation, which may help explain variations in immunological markers such as IL-9 across studies.

Diagnostic Utility of IL-9

In our study, serum IL-9 levels were significantly elevated in patients with chronic spontaneous urticaria (CSU) compared to healthy controls, with median levels of 440.62 pg/mL in the CSU group versus 41.17 pg/mL in controls ($P < 0.001$). A receiver operating characteristic (ROC) curve analysis determined a cutoff point of 115.48 pg/mL, which yielded an area under the curve (AUC) of 0.897, indicating excellent diagnostic accuracy. At this threshold, IL-9 showed a sensitivity of 81.4% and specificity of 83.7%. Moreover, CSU patients had 22.5 times greater odds of elevated IL-9 than controls (95% CI: 7.37–68.68), demonstrating a strong and statistically significant association. These findings suggest that IL-9 may be a useful biomarker in determining disease chronicity and its progressive course.

Previous studies support these results. **G Ciprandi et al. (2012)**, in an Italian cohort of 157 individuals, also found significantly higher IL-9 levels in patients with spontaneous urticaria compared to healthy volunteers ($P = 0.0156$), with median levels of 10.07 pg/mL in urticaria patients versus 2.29 pg/mL in controls ($P < 0.0001$).

Feng et al. (2020) further confirmed the role of IL-9 in CSU pathogenesis through both clinical and experimental approaches. In CSU patient skin samples, IL-9 mRNA and protein expression were significantly upregulated compared to controls ($P < 0.05$). Additionally, in a mouse model, IL-9 overexpression led to increased pruritus behaviors, eosinophilic infiltration, and elevated inflammatory cytokine expression, suggesting that IL-9 contributes to CSU progression via the JAK/STAT signaling pathway.

However, several studies have reported conflicting findings regarding IL-9's diagnostic utility. **Bhatia et al. (2024)** found no significant difference in serum IL-9 levels between CSU patients (mean: 1,607 ± 1,182.5 pg/mL) and healthy controls (1,838.70 ± 929.89 pg/mL; $P = 0.082$), and they noted that IL-9 had lower diagnostic accuracy than C5a. **Zheng et al. (2017)** also reported no significant difference in IL-9 levels between CSU patients and controls ($P > 0.05$), although higher IL-9 levels were observed in patients with acute spontaneous urticaria (ASU).

Similarly, **Metz et al. (2013)** failed to identify elevated IL-9 levels in CSU patients or any significant association with disease activity scores (UAS7), suggesting limited value of IL-9 as a marker for CSU. These discrepancies may be attributed to variations in sample size, geographic and ethnic factors, disease severity, and duration across studies.

Further contributing to inconsistencies are methodological differences, such as assay type and statistical analysis approaches. For instance, while this study and **Ciprandi et al. (2012)** used ELISA for IL-9 quantification, different kits with variable sensitivity and specificity could have been employed. **Bhatia et al. (2024)** also used ELISA but reported results as mean ± SD, which can be skewed by outliers in cytokine data, whereas this study used medians, a more robust measure for non-normally distributed data. **Feng et al. (2020)** used RT-qPCR and Western blot on skin tissue, and

Zheng et al. (2017) applied Luminex 200 for serum cytokines, further complicating direct comparisons.

Relationship Between IL-9 Levels and Disease Activity

This study investigated the potential correlation between serum interleukin-9 (IL-9) levels and disease severity in chronic spontaneous urticaria (CSU), as measured by the Urticaria Activity Score over 7 days (UAS7). Although IL-9 was initially hypothesized to be associated with disease activity, no statistically significant correlation was observed (P = 0.111). Median IL-9 levels across severity groups were as follows: mild (776.52 pg/ml), moderate (115.48 pg/ ml), and severe (333.23 pg/ml), with no significant trend (P = 0.370).

These findings are consistent with those of **Zheng et al. (2017),** who also reported no association between IL-9 levels and CSU severity. Similarly, **Metz et al. (2013)** failed to find a significant correlation between IL-9 and disease activity assessed via UAS7, and visual stratification by disease severity (mild, moderate, severe) did not demonstrate clear differences.

Ciprandi et al. (2012) found significant differences in serum IL-9 levels among patients with acute spontaneous urticaria (ASU, 35.6%), CSU (33.8%), and healthy controls (30.6%) (P < 0.0001, Kruskal–Wallis test). However, no significant correlation between IL-9 levels and symptom severity was detected within these groups, despite a significant difference between ASU and CSU groups (P = 0.0001).

Bhatia et al. (2024) reported a weak but positive correlation with statistical significance between IL-9 levels and baseline disease severity (ρ = 0.277, P = 0.007), highlighting variability in findings across studies.

Feng et al. (2020) demonstrated that IL-9 enhances mast cell survival and inflammatory cytokine expression, likely via the JAK/STAT signaling pathway. Although IL-9 may contribute to CSU pathogenesis through mast cell activation, its diagnostic utility is limited due to the lack of consistent correlation with disease severity. IL-9's role appears to be more associated with CSU susceptibility rather than progression, and its influence may be modulated by other dominant inflammatory mediators.

Serum IgE level, disease severity and IL-9 level

In this study, it has been found that 32.6% (14 cases) out of 43 cases patients had an elevated serum IgE level. It had no statistical significance between serum IgE & the serum IL-9 nor disease severity, as measured by the Urticaria Activity Score (UAS7). Specifically for Total IgE (Normal vs. Abnormal) and Serum IL-9, the P-value was 0.358, indicating no significant association.

Similarly, **Bhatia et al. (2024)** found no significant correlation between baseline disease severity and serum IgE (ρ = 0.07, P = 0.549). This indicates that higher or lower total IgE levels at baseline did not correspond to greater or lesser disease severity as measured by UAS7.As well, **Baek et al., (2014)** found no significant correlation between disease severity (as assessed by the Urticaria Activity Score or UAS) and total IgE levels in patients with chronic urticaria (r < 0.01, P > 0.05).

Influence of Smoking and Medical History on IL-9 Levels

This study identified a statistically significant relationship between smoking status and elevated IL-9 levels (p=0.032). No studies in the current literature explicitly report that smoking alters IL-9 levels or that IL-9 plays a prominent role in smoking-induced inflammation. Instead, as regarding other inflammatory markers rather than IL-9 and correlation to smoking, **Moos et al. (2024**) investigated the neutrophil-lymphocyte ratio (NLR), neutrophils (NEU), lymphocytes (LYM), and C-reactive protein (CRP) in CSU patients based on their smoking status. No statistical difference was found between smokers and non-smokers regarding the overall NEU, LYM, NLR, or CRP levels.

5- Conclusion and Recommendations:

We conclude that, according to the analysis, CSU patients' IL-9 levels were statistically significantly higher than those of healthy controls. To put these findings into context, the results were critically compared with similar research that was focused on CSU.

To sum up, finding a trustworthy biomarker to gauge the severity of the condition and the effectiveness of treatment is essential for managing chronic urticaria. In addition to improving our knowledge of the pathophysiology of urticaria, such a biomarker would help with disease activity monitoring, treatment efficacy prediction, and possibly future therapeutic opportunities.

6- Limitations

This investigation was limited by its relatively small population size and case-control study design. Future research should emphasize longitudinal study designs to assess temporal changes in IL-9 levels and their relationship with therapeutic responses. Additionally, pathophysiological studies are warranted to delineate the precise immunological pathways through which IL-9 modulates CSU pathogenesis, particularly concerning mast cell function and JAK/STAT signaling. Resolving contradictions among existing studies will require harmonized study protocols, larger multicentric cohorts, and advanced molecular profiling techniques.

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