"AUTOLOGOUS SERUM SKIN TEST (ASST) IN CHRONIC IDIOPATHIC URTICARIA AND AUTOIMMUNE URTICARIA: A HOSPITAL-BASED, CROSS-SECTIONAL STUDY."

Submitted by

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Under the guidance of

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LIST OF ABBREVIATIONS

AST	-	Autologous serum therapy
ASST	-	Autologous serum skin test
CD	-	Cluster of differentiation
CAU	-	Chronic autoimmune urticaria
CRP	-	C-reactive protein
CsA	-	Ciclosporin A
CSU	-	Chronic spontaneous urticaria
CU	-	Chronic urticaria
ESR	-	erythrocyte sedimentation rate
HT	-	Hashimoto thyroiditis
HRA	-	Histamine release assay
MHC	-	Major histocompatibility complex
NSAIDs	-	Non-steroidal anti-inflammatory drugs
PAF	-	Platelet activating factor
RAST	-	Radio allergosorbent test
SLE	-	Systemic lupus erythematosus
UTSS	-	Urticaria total severity score
UV	-	Urticaria vasculiti
WBC	-	White blood cells

ABSTRACT

Background

Chronic urticaria is defined as urticaria persisting daily for more than six weeks and most patients are considered to be chronic spontaneous urticaria (CSU) in whom management is most challenging and frustrating therapeutically to a dermatologist. A significant number of patients with chronic spontaneous urticaria (CSU) demonstrate an abnormal type I reaction, immediate wheal and flare response, to intra-dermal injected autologous serum leading to concept of autoimmune urticaria.

Objectives

To estimate the prevalence of autoimmune urticaria using autologous serum skin test (ASST).

Methods

It was a hospital based prospective study. Sixty five patients with chronic urticaria and chronic spontaneous urticaria attending the Dermatology out-patient department of a tertiary care hospital were included in this study. Detailed history with respect to duration, frequency, atopy, any treatment received, presence of angioedema, urticaria total severity score (UTSS) were recorded. Test was done by injecting patients own serum intradermally and checking for a wheal and flare response after 30 minutes, if yes patient was considered chronic autoimmune urticaria.

Results

Among 65 chronic urticaria patients enrolled in the study 26 (40%) showed positivity to ASST. Severity of the disease based on UTSS, divided into three groups: mild (0-6), moderate (7–12), and severe (13-18) and their relation to the positivity of ASST was significant (*p* value 0.02). Also did presence of angioedema among ASST positive patients showed a significance (*p* value <.001)

Conclusion

Autologous serum skin test is a simple, bedside, cost-effective clinical test which can detect the presence of autoimmunity in patients with CSU who have no distinctive clinical features differentiating them from chronic autoimmune urticaria patients. A positive ASST has been associated strongly with severity of the disease and associated angioedema. One important advantage of testing is to promote more tailored prognostic counselling and the earlier use of immunosuppressive therapies in patients diagnosed CAU.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FLOWCHARTS

INTRODUCTION

Urticaria is defined as development of evanescent itchy wheal and flare type skin lesions and/or angioedema.[1] The word urticaria is derived from the latin word urtica, which means “nettle”, which are tooth-leaved plants covered with hairs capable of secreting a stinging fluid that immediately affects the skin on contact.[2]

Chronic urticaria is defined as wide spread short lived wheals occurring almost daily for at least 6 weeks with symptoms present at least 3 times weekly.[3] It is a common disorder affecting at least 0.1% of the population.[4] This condition poses a great challenge to the treating physician in its extremely severe form.[4] About 70% of patients of chronic urticaria are diagnosed as Chronic Idiopathic Urticaria (CIU), which is synonymous with chronic spontaneous urticaria (CSU), in whom no cause could be detected.[5] Mast cell degranulation is of central importance in the pathogenesis of chronic urticaria.[4] Among the CSU patients, 40-50% develop immediate wheal and flare response to intradermal autologous injection of serum leading to a disease entity called autoimmune urticaria (AIU).[4]

There is increasing acceptance that histamine releasing autoantibodies define an autoimmune etiology in AIU patients.[6] These patients might have circulating functional autoantibodies (histamine releasing IgG autoantibodies) against the high affinity IgE receptor on mast cells and basophils or less commonly against IgE itself (5-10%).[7] Based on immunoblot analysis most of the antireceptor antibodies are among IgG1 and IgG3 subclasses and rarely IgG4.[6]

The gold standard method for detecting these functional auto-antibodies in patient's serum currently is Basophil Histamine Release Assay. But to standardize this assay is difficult because it requires fresh basophils from healthy donors, it is time consuming procedure, needs expertise and setup.[8] Autologous serum skin test (ASST) is the most feasible *in vivo* clinical test for the detecting the histamine-releasing activity of basophils. This test has sensitivity and specificity of 70% and 80% respectively.[4] Identification of autoimmune urticaria may pave way to the treating clinician to use immunomodulatory drugs in patients with impaired quality of life and severe disease unresponsive to first line therapy with antihistamines.[9]

In the recent literatures, autohemotherapy is being regarded as specific and potentially curative therapeutic option in AIU subgroup of patients.[10] A study conducted by Staubach *et al,*[10] reported the efficacy of autologous whole blood (AWB) therapy in AIU patients. In an attempt to refine the AWB therapy, a study by Bajaj *et al,*[11] tried autologous serum therapy (AST) by removing cellular components of the blood and injecting just the serum.

OBJECTIVE OF THE STUDY

i. To estimate the prevalence of autoimmune urticaria using autologous serum skin test (ASST)

REVIEW OF LITERATURE

Urticaria is a heterogeneous group of diseases with many subtypes.[12] This condition affects 15%–30% of total population.[13] Almost all types of urticaria present with a common and distinctive clinical pattern, i.e., the development of itchy wheal and flare type skin lesions with or without angioedema.[12] Wheals are lesions ranging from a few millimeters to several centimeters in diameter, may become confluent and form larger plaques.[14]

A wheal consists of three typical features:

- It is characterized by a central swelling of variable size, almost invariably surrounded by a reflex erythema.
- It is associated with itching or sometimes a burning sensation.
- It has a fleeting nature, with the skin returning to its normal appearance, usually within 1–24 h. Sometimes wheals resolve even more quickly.[15]

Angioedema is characterized by:

- A sudden, pronounced erythematous or skin-colored swelling of the lower dermis and subcutis with frequent involvement below mucous membranes and
- Sometimes pain rather than itching and frequent involvement below mucous membranes. Its resolution is slower than that for wheals and can take up to 72 h.[15]

Individual wheals normally, last less than 24 hours, although there are exceptions. Wheals of the physical urticaria (delayed pressure) may individually last for as long as 48

hours and the wheals of urticarial vasculitis (UV) by definition should last more than 24 hours.[14]

Under clinical classification of the urticaria shown in table 1, chronic urticaria (CU) is a subset of ordinary urticaria and is defined as continuous urticarial activity persisting for more than 6 weeks.[16] Chronic urticaria is more common in adults, affecting mainly middle-aged women, and is rare in children and adolescents.[2]

Chronic idiopathic urticaria, which is synonymous with chronic spontaneous urticaria (CSU), is a sub-type of CU in whom possible etiological factors could not be elicited.[4] In few patients, one or more of the following most common causes of CSU can be found: (i) autoreactivity (ii) infection, and (iii) intolerance.[16] Patients suffering from CSU are highly affected by the disease and the impairment of quality of life should be assessed routinely.[12]

Some patients with CSU may have an autoimmune rather than idiopathic etiology giving rise to the term chronic autoimmune urticaria (CAU).[17] And these subset patients account for 30%-50% of CSU.[18]

Table 1: Clinical classification of urticaria[15, 17]

Ordinary urticaria	Acute (up to 6 weeks of continuous activity) Chronic (6 weeks or more activity) Idiopathic/spontaneous Episodic (acute intermittent or recurrent activity)
Physical urticarias (reproducibly induced by the same physical stimulus)	Mechanical -Delayed pressure urticaria -Symptomatic dermographism -Vibratory angioedema Thermal -Cholinergic urticaria -Cold contact urticaria -Localized heat urticaria Others -Aquagenic urticaria -Solar urticaria -Exercise-induced anaphylaxis
Angioedema without weals	Idiopathic Drug-induced C1 esterase inhibitor deficiency
Contact urticaria	
Urticarial vasculitis	
Autoinflammatory syndromes	Hereditary -Cryopyrin-associated periodic syndromes (CIASI mutations) Acquired -Schnitzler syndrome
Immunological	Autoimmune (autoantibodies against FcεRI or IgE) -Allergic (IgE-mediated type I hypersensitivity reactions) -Immune complex (UV) -Complement-dependent (C1 esterase inhibitor deficiency)
Non-immunological	Direct mast cell–releasing agents- Aspirin, NSAIDS and dietary pseudoallergens Angiotensin-converting enzyme inhibitors

Pathophysiology

Urticaria is a mast-cell-driven disease. Histamine and other mediators, such as platelet-activating factor (PAF) and cytokines are released from activated mast cells.[3] These key pathophysiological events are predominant at the very onset and the released cellular contents prime the immediate phase of inflammation, which progresses to a complex interplay of chemokine receptors, and adhesion molecules that regulate vasoactivity and specific kinetics of cellular infiltration, ultimately evolving into a lymphocyte and granulocyte mediated hypersensitivity reaction, evident as urticarial wheals.[16] These cells are found in high numbers throughout the body and in many locations, such as the skin, subdermis, and mucosal surfaces. When mast cells are activated, there is a rapid release of histamine, leukotriene C4, and prostaglandin D2. The release of these mediators leads to vasodilatation and subcutaneous and intradermal leakage of plasma from postcapillary venules, which in turn lead to pruritis.[19]

The mast-cell activating signals in urticaria are ill-defined and likely to be heterogeneous and diverse.[1] In angioedema, similar changes occur primarily in the lower dermis and the subcutis. Skin affected by wheals virtually always exhibits upregulation of endothelial cell adhesion molecules and a mixed inflammatory perivascular infiltrate of variable intensity, consisting of neutrophils and/or eosinophils, macrophages, and T cells, but without vessel-wall necrosis, which is a hallmark at UV.[15] In some subtypes of urticaria, up-regulation of adhesion molecules and altered cytokine expression are also seen in uninvolved skin. These findings underline the complex nature of the pathogenesis

of urticaria, which has many features in addition to the release of histamine from dermal mast cells.[12]

Vasoactive mediators released from dermal mast cells play a key role in the pathogenesis of CSU. Despite the presence of other mediators (eicosanoids, cytokines, and proteases), histamine is the most prominent and acts on H1 receptors (85%) and H2 receptors (15%) in the skin. While histamine binding to H1 receptors leads to pruritus (by stimulation of C fibers), binding to receptors on postcapillary venules induces vasodilation, increased vascular permeability and edema (mediated in part through nitric oxide).[20]

Most patients with CU appear to have an idiopathic disorder, but in a consistent proportion of cases an autoimmune (CAU) has been suggested, since they have circulating immunoglobulin G (IgG) directed against the alpha (α) subunit of the high affinity immunoglobulin E (IgE) receptor (anti FcεRIα) and less frequently IgG anti-IgE antibodies. The IgG predominantly being IgG1 and IgG3.[16] This to be consistent with complement dependence of the reaction, because IgG1 and IgG3 fix the classical complement pathway.

The activation of the classical complement pathway appears to be underlying requisite for the mast cell degranulation.[15] To initiate cell activation, the Fc portion of 2 IgG molecules in proximity must be able to bind to 2 of the 6 globular heads of C1q. To achieve this, only one fragment antibody (Fab) of each of 2 adjacent IgG molecules needs to bind to adjacent α-subunits.[2] In this process the C5a is likely the cell activator and it is known to be a more effective cutaneous basophils and mast cells activator which is

shown in figure 1. The complement activation and the release of C5a results not only in augmented mast cells and basophils, but it is also a chemotactic for neutrophils, eosinophils, and monocytes, which is one of the factors that would distinguish this lesion from a typical allergen-induced cutaneous late-phase reaction.[21]

The autoantibodies in CAU are able to induce histamine release from mast cells and basophils via a direct cross-linking of adjacent IgE receptors or IgE itself. The activated mast cells and basophils will express the CD63, a member of the transmembrane-4 superfamily, which is a mast cell and basophils activation marker as a result of the fusion between intracytoplasmic granules and the plasma membrane.[15] Another marker on activated mast cells and basophils, which is more specific, is CD203c (ectonucleotid pyrophosphatase/phosphodiesterase) is an ectoenzyme expressed on activated basophils, mast cells in response to cross-linking of the FcεRIα receptors and their CD34+ progenitor cells in peripheral blood.[22]

There is association of CAU with several other autoimmune diseases. The CAU may have association with Hashimoto's thyroiditis (less association with Graves disease) in 5-10% of CAU, since there is anti-thyroperoxidase antibodies (anti-TPO antibody) segregate with the presence of antibodies to the IgE receptor (or to IgE).[17] The celiac disease may also be related to CAU since they share the same major histocompability complex (MHC) alleles.[2] Infection by several microbes, such as *Helicobacter pylori, streptococcus, staphylococcus, yersenia,* hepatitis A and B virus, and larvae of *Anasakis simplex* (cephalopods parasite commonly found in fish) may also induce CAU through the molecular mimicry mechanism between their antigens and host proteins.[23]

CAU has been reported with a number of other systemic conditions, many of which have a complement-mediated or immunologic basis, including specific complement component deficiencies; cryoglobulinemia; serum sickness or other immune-complex mediated processes; connective tissue diseases, such as systemic lupus erythematosus (SLE) and juvenile rheumatoid arthritis; neoplasms (particularly lymphoreticular malignancy and lymphoproliferative disorders); and other endocrine disorders or hormonal therapies (eg, ovarian tumors and oral contraceptive use, respectively).[24]

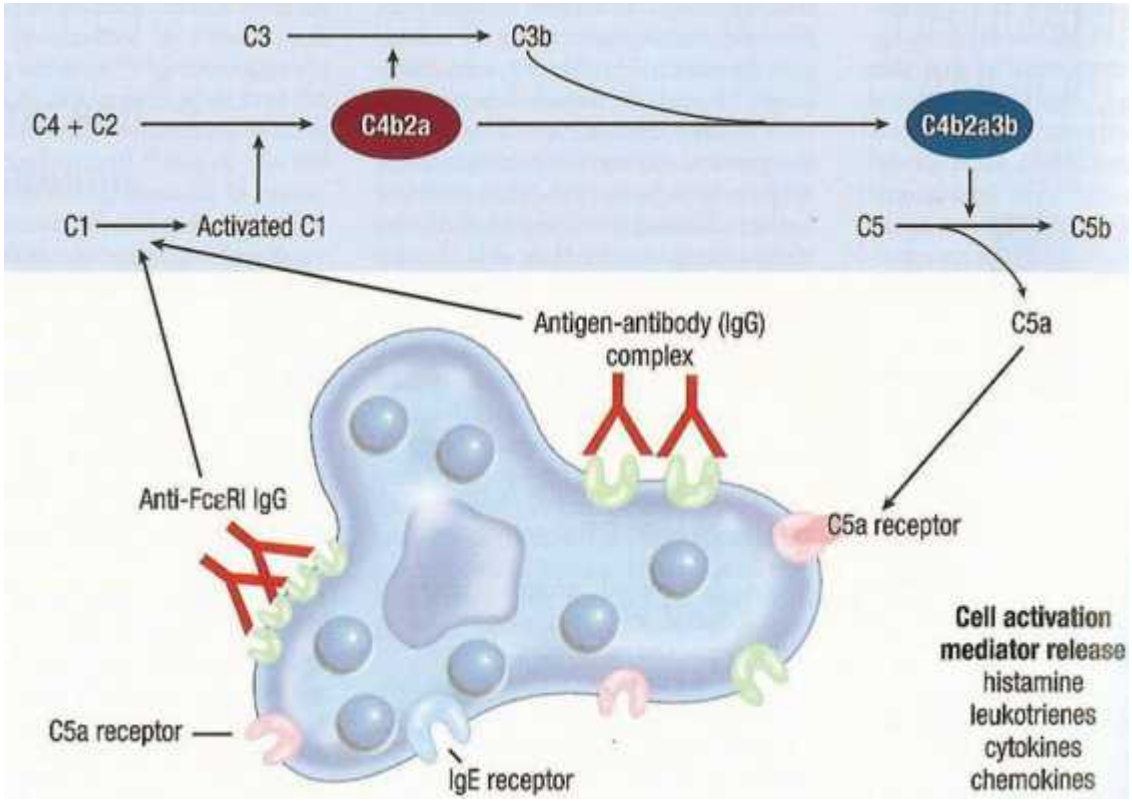


Figure 1: Diagrammatic representation of mast cell activation by cross-linking the IgE receptor, followed by complement activation and release of C5a[17]

Diagnosis

Patients are often able to identify a stimulus if the wheals occur 5 to 30 minutes after ingestion of a food or drug. If the wheals are short-lived or respond rapidly to over-the-counter antihistamines, patients do not typically seek medical care.[5] Patients whose wheals occur in the absence of an identifiable trigger and are recurrent in nature often come to the attention of physicians. The best initial approach to a patient with urticaria is a thorough history and physical examination shown in table 3.[15] History should include details of the wheals in relation to medications (including herbals, supplements), foods, physical triggers, infections, occupational exposures, insect bites, and contact exposures as well as a complete review of systems.[25] Physical examination should include at least examination of the skin, lymph nodes, eyes, joints, throat, neck, ears, lungs, heart, and abdomen to detect possible associated conditions. Often, no specific agent is found and the patient is treated symptomatically until they resolve spontaneously.[15]

Urticaria/angioedema may be associated with signs and symptoms in organs other than the skin, such as the pulmonary system (wheezing and cough), gastrointestinal system (vomiting and diarrhea), nervous system (dizziness and loss of consciousness), or cardiac system (changes in blood pressure or heart rate), can occur in patients with anaphylaxis. So evaluation of these organ systems is vital in treating the patients.[26]

For acute urticaria, skin testing or immunoassays to identify specific triggers for acute urticaria and angioedema can be helpful if an allergic cause is suggested by history. Skin testing in this scenario would usually be done after the resolution of acute urticaria

and after stopping of antihistamines. Though skin biopsy is not indicated in most cases of urticaria, it might occasionally be useful for differentiating this condition from other inflammatory disorders.[12]

Baseline laboratory investigations including a complete blood count (CBC) with differential, erythrocyte sedimentation rate (ESR), and/or C-reactive protein (CRP), liver enzyme, and thyroid-stimulating hormone measurement can be advised. In patients with CU with an unremarkable history and physical examination, baseline investigations might be appropriate to identify the infrequent or rare case in which CU is a manifestation of an underlying condition that might not be evident based on history or physical examination findings.[26] The initial patient evaluation should be focused to determine (through history and physical examination) whether the lesions that patients describe are consistent with CU. A painful or burning dysesthesia is not characteristic of CU and suggests the presence of cutaneous vasculitis.[27]

Individual urticarial lesions usually fade within 24 to 48 hours, but new lesions might be developing simultaneously at other skin sites. In contrast, vasculitis lesions are palpable and usually nonblanching, persisting for several days or more and often followed by residual hyperpigmented changes, although in some cases lesions might be more evanescent, similar to ordinary CU.[28] Angioedema typically appears as nonpruritic, brawny, nonpitting edema, typically without well-defined margins and without erythema.[24] The medical work-up of a patient with CU should be done, keeping in mind that CU is of undetermined cause in the majority of cases. After a thorough history and

physical examination, no diagnostic testing might be necessary for some patients with CU; however, limited routine laboratory testing can be performed to exclude underlying causes.[15]

In approximately 95% of patients with CU, neither the patient nor the physician can identify a specific ingested or contact allergen causing wheals. This is sometimes difficult for patients and physicians to accept. Therefore, an unnecessarily extensive, invasive, and expensive investigation is pursued without successfully identifying a specific culprit.[17] Included in a detailed physical examination should be exclusion of possible physical triggers. Physical urticarias can be demonstrated by various simple tests shown in table 2.

Table 2: Tests for physical urticarias[15, 19]

Physical Urticaria	Test	Interpretation
Cold-induced urticaria	Ice cube challenge	Wheals upon rewarming of the skin
Dermatographism	Fric test (gently stroking the skin)	Linear wheals
Pressure-induced urticaria	Applying pressure perpendicular to the skin	Observe for swellings 4 to 6 hours later
Aquagenic urticaria	Applying water to skin regardless of temperature	Wheals on immediate contact with water

Although foods and drugs are uncommon causes of acute urticaria, many patients are not satisfied until these are ruled out.[13] As in the evaluation of acute urticaria, patients must discontinue all unnecessary food supplements and drugs. Patients can then keep a food diary to identify suspect foods, which can then be eliminated. If the urticaria resolves, foods can be slowly reintroduced into the diet while monitoring for urticaria with the use of a food diary. This method rarely leads to identification of a specific trigger of CU in adults.[24]

The association between antithyroid antibodies (antimicrosomal and antithyroglobulin) that are most commonly seen in Hashimoto's thyroiditis (HT) and CU is particularly strong, although urticaria occurs only in a few patients with HT.[17] There are many reports, but no rigorous proof, that treatment of euthyroid urticaria patients who have antithyroid antibodies with L- thyroxine leads to resolution of the urticaria. In many of these cases, improvement of the urticaria appears to be coincidental. Nonetheless, some specialists do treat these patients with L-thyroxine.[17]

Additional tests, usually performed in specialty clinics, may be useful in patients with CU. Some allergists order immediate hypersensitivity skin tests or IgE RAST (Radio-allergosorbent test) tests for foods if the history is suggestive.[29] Approximately 40% of patients with CU have evidence of an autoimmune process that may contribute to their wheals. An in vitro test for antibodies to the α-subunit of the FcεRI can be ordered from specialized immunology laboratories. However, this test has not been approved by

the Food and Drug Administration, moreover are very expensive and not readily available.[30]

Autologous serum skin test (ASST)

Autoantibodies in patients' serum can be detected by serum induced histamine release from the basophils of healthy donors by ELISA or Western blot assay.[31] But neither Western blot nor ELISA can differentiate between functional histamine releasing autoantibody and nonfunctional autoantibody. Moreover, these tests are done only in some specialized centers and they are time consuming to perform.[32]

ASST is a rapid, reliable and *in vivo* test to distinguish between patients with and those without circulating functional autoantibodies to diagnose autoimmune urticaria and also to evaluate the effectiveness of immunomodulatory treatment.[33]

In this test, 0.05 ml (equivalent to 2 units on insulin syringe that has 1 ml marked as 40 units) of the patient's serum is injected intradermally over the volar aspect of forearm which is lesion free (therefore, autologous), with normal saline (control) in the middle keeping a gap of at least 5 cm between the two injection sites. If a wheal and flare develops 30 minutes after injecting serum and saline with a serum induced wheal this is thought to be due to an antibody to either FcεRIα or to IgE itself.[35] Despite being the most accessible and useful test for demonstrating endogenous vasoactive factors in patients' blood with chronic idiopathic urticaria, a positive ASST is not always diagnostic of autoimmune urticaria.[35] A positive test may reassure the patient, prevent further

anxiety, and avoid unnecessary testing to find an external cause. Sensitivity and specificity of ASST are at best 80% respectively.[36]

Azim *et al*,[4] conducted a study on 69 patients with CU and found that 34 patients (44.3%) had a known cause of urticaria whereas 35 (50.7%) had chronic idiopathic urticaria. Autologous Serum Skin test was positive in 15 (42.9%) patients with chronic idiopathic urticaria. These patients had severe urticaria of prolonged duration and more frequent attacks when compared patients with negative.

George *et al*,[8] conducted a prospective study on 100 patients with CU in whom 34% patients showed a positive ASST. The median duration of disease and severity were significantly higher in patients with positive ASST.

Godse conducted a study on 45 patients suffering from CU in which positivity for ASST was seen in 12 patients (26.67%) which is relatively less than reports from western literature.[6]

Fusari *et al*,[37] performed the test on 82 patients with CU to find out the incidence of positive Autologous Serum Skin Test. The prevalence of positive ASST was 46.6% and prevalence was higher in females (73%).

In a study conducted by Emek *et al*,[38] to compare efficacy between ASST and autologous plasma skin test in patients with CU found that ASST had a higher sensitivity and accuracy.

In another study by Kulthanan *et al,*[39] 24.7% of chronic idiopathic urticaria showed positive ASST. Comparison between the positive and negative ASST showed no significance of the severity and duration of the disease.

Table 3: Table showing detailed history taking in a case of chronic urticaria[15]

1.	Time of onset of disease
2.	Frequency/duration of and provoking factors for wheals
3.	Diurnal variation
4.	Occurrence in relation to weekends, holidays, and foreign travel
5.	Shape, size, distribution of wheals and associated angioedema
6.	Associated subjective symptoms of lesions, for example itch, pain
7.	Family and personal history regarding urticaria, atopy
8.	Previous or current allergies, infections, internal diseases, or other possible causes
9.	Psychosomatic and psychiatric diseases
10.	Surgical implantations and events during surgery, for example after local anesthesia
11.	Gastric/intestinal problems
12.	Induction by physical agents or exercise
13.	Use of drugs (e.g., non-steroidal anti-inflammatory drugs (NSAIDs), injections, immunizations, hormones, laxatives, suppositories, ear and eye drops, and alternative remedies)
14.	Observed correlation to food
15.	Relationship to the menstrual cycle
16.	Smoking habits (especially use of perfumed tobacco products or cannabis)
17.	Type of work, Hobbies
18.	Stress, Quality of life related to urticaria and emotional impact
19.	Previous therapy and response to therapy
20.	Previous diagnostic procedures/results

Management

While the classification of different subtypes is important in view of the diagnostic approach, the therapeutic approach is universal and based on the same principles as in other mast-cell-dependent diseases in the field of allergy: (i) elimination/avoidance of the cause or trigger/stimulus, (ii) symptomatic pharmacological treatment by reducing mast cell mediator release and/or the effect of these mediators at the target organ, and (iii) inducing tolerance.[15]

Identification and elimination/avoidance of the stimulus

With the use of this therapeutic approach, an exact diagnosis is a basic prerequisite. Identifying the cause of urticaria is not, however, easily possible in most cases. If remission following elimination of the suspected agent occurs, only recurrence of symptoms in a double-blind provocation test will provide definitive proof of its causative nature because spontaneous remission of urticaria might also occur incidentally in parallel with, but not because of, the elimination of a suspected cause or trigger.[34]

Drugs

When such agents are suspected in the course of diagnosis, they should be omitted entirely or substituted by another class of agents if indispensable. Drugs causing nonallergic hypersensitivity reactions (the prototypes being NSAID) cannot only elicit, but can also aggravate pre-existing CSU, so that elimination in the latter case will only improve symptoms in some patients.[15]

Physical stimuli

Avoidance of physical stimuli for the treatment of physical urticaria is desirable, but not always simple. Detailed information about the physical properties of the respective stimulus should make the patient sufficiently knowledgeable to recognize and control exposure in normal daily life.[20] Thus, for instance, it is important in delayed pressure urticaria/angioedema and in symptomatic dermographism to point out that pressure is defined as force per area and that simple measures, such as broadening of the handle of heavy bags for pressure urticaria or reducing friction in case of symptomatic dermographism/urticaria factitia, may already be helpful in the prevention of symptoms.[20] Similar considerations hold for cold urticaria where the impact of the chill factor in cold winds needs to be remembered. For solar urticaria, the exact identification of the range of eliciting wavelengths may be important for the appropriate selection of sunscreens or for the selection of light bulbs with an UV-A filter.[5] However, in many patients, the threshold for the relevant physical trigger is low and total avoidance of symptoms is virtually impossible.[15] Severe dermographic urticaria is sometimes confused with CSU because seemingly spontaneous wheals are observed where even loose fitting clothing rubs on the patient's skin or unintentional scratching by patients readily develop wheals on that area.[5]

Eradication of infectious agents and treatment of inflammatory processes

CSU is often reported to be associated with a variety of inflammatory or infectious diseases. This is regarded as significant in some instances, but some studies show conflicting results and have methodological weaknesses.[23] These infections, which

should be treated appropriately, include those of the gastrointestinal tract, such as *H. pylori* or bacterial infections of the nasopharynx. Intestinal candidiasis was regarded as a highly important underlying cause of CSU, but more recent findings fail to support a significant causative role.[13] Apart from infectious diseases, chronic inflammatory processes due to diverse other diseases have been identified as potentially causative for CSU. This holds particularly for gastritis, reflux esophagitis, or inflammation of the bile duct or gall bladder. However, similar to infections, it is not easily possible to determine whether any of these are relevant causes of CSU.[14]

Reduction of functional autoantibodies

Treatment for CSU by direct reduction of functional autoantibodies by plasmapheresis, has been shown to be of temporary benefit in individual. Due to high costs, this therapy is suggested for autoantibody-positive CSU patients who are unresponsive to all other forms of treatment.[40]

Dietary management

IgE-mediated food allergy is rarely the underlying cause of CSU. If identified, the specific food allergens need to be omitted as far as possible. In a subgroup of CSU patients, pseudoallergic reactions (non-IgE-mediated hypersensitivity reactions) to naturally occurring food ingredients and in some cases to food additives have been observed.[26] Similar to drugs, pseudoallergens can both elicit and aggravate CSU. In these cases, a diet containing only low levels of natural as well as artificial food pseudoallergens should be instituted and maintained for a prolonged period, at least 3–6

months.[15] In pseudoallergy, a diet must often be maintained for a minimum of 3 weeks before beneficial effects are observed. However, it should be pointed out that success rates may vary considerably due to regional differences in food and dietary habits.[15]

Inducing tolerance

Inducing tolerance can be useful in some subtypes of urticaria. Examples are cold urticaria, cholinergic urticaria, and solar urticaria, where even a rush therapy with UV-A has been proven to be effective within 3 days. However, tolerance induction is only lasting for a few days; thus, a consistent daily exposure to the stimulus just at threshold level is required.[5]

Symptomatic pharmacological treatment

The main option in therapies are aimed at symptomatic relief is to reduce the effect of mast cell mediators such as histamine, PAF, and others on the target organs. Many symptoms of urticaria are mediated primarily by the actions of histamine on H1-receptors located on endothelial cells (the wheal) and on sensory nerves (neurogenic flare and pruritus). Thus, continuous treatment with H1-antihistamines is of eminent importance in the treatment for urticaria.[2]

Antihistamines are inverse agonists with preferential affinity for the active state of the histamine H1-receptor and stabilize it in this conformation, shifting the equilibrium toward the inactive state. However, in some cases, especially of CSU, other mast cell mediators (PAF, leukotrienes, cytokines) are also involved and a pronounced cellular

infiltrate including basophils, lymphocytes, and eosinophils may be observed.[19] These may respond completely to a brief burst of corticosteroid and may be relatively refractory to antihistamines.[18]

The older first-generation antihistamines have pronounced anticholinergic effects and sedative actions on the central nervous system (CNS), which last longer than 12 hours, whereas the antipruritic effects last only for 4–6 hours.[15] Consequently, many interactions have been described for these sedating antihistamines with alcohol and drugs affecting the CNS, such as analgesics, hypnotics, sedatives, and mood-elevating drugs.[13] In addition, first-generation antihistamines can interfere with rapid eye movement sleep and impact on learning and performance. So it is strongly recommended not to use first-generation antihistamines any longer in allergy both for adults and especially children.[20] This recommendation is based on strong evidence regarding potential serious side-effects of old sedating antihistamines and the availability of modern second- generation antihistamines, which not only lack these side-effects but also have a higher efficacy and duration of action.[15]

The side-effects of first-generation H1 antihistamines are most pronounced in promethazine, diphenhydramine, ketotifen, and chlorpheniramine and are well understood. They penetrate the blood–brain barrier, bind to H1 receptors in the CNS, and interfere with the neurotransmitter effects of histamine.[24] Impairment is particularly prominent during multitasking and performance of complex sensorimotor tasks such as driving. Old first-generation H1-antihistamines are a particular concern in the elderly in

whom they increase the risk of impaired cognition, inattention, disorganized speech, altered consciousness, and falls.[24]

The development of modern second-generation antihistamines led to drugs which are minimally or not sedating and free of anticholinergic effects. However, two of the earlier modern second-generation drugs, astemizole and terfenadine, which were essentially pro-drugs requiring hepatic metabolism to become fully active, had cardiotoxic effects if this metabolism was blocked by concomitant administration of ketoconazole or erythromycin. These two drugs are no longer available.[28]

Further progress with regard to drug safety was achieved by the development of the newer modern second-generation antihistamines cetirizine (metabolite of hydroxyzine), loratadine, and fexofenadine, some of which are mostly nonsedating metabolites of earlier sedative antihistamines.[28] More recently, acrivastine, azelastine, bepotastine, bilastine, desloratadine, the active metabolite of loratadine, ebastine, epinastine, levocetirizine, the active enantiomer of cetirizine, mequitanzine, mizolastine, olopatadine, and rupatadine have been added to the list of modern second-generation antihistamines.[15] Many of these antihistamines have not been appropriately studied in urticaria, and there are considerable clinical differences between them. Only seven of them (cetirizine, desloratadine, fexofenadine, levocetirizine, loratadine, rupatadine, and bilastine) have been tested in detail in urticaria. Taken together, modern second generation antihistamines should be considered as the first-line symptomatic treatment for urticaria because of their good safety profile.[15]

There are numerous studies showing the benefit of a higher dosage of antihistamines in individual patients corroborating earlier studies which came to the same conclusion employing first-generation antihistamines. This has been verified in studies using up to fourfold higher than recommended doses of bilastine, cetirizine, desloratadine, levocetirizine, fexofenadine, and rupatadine.[20] Furthermore, a recent study showed the benefit of using desloratadine and levocetirizine at doses up to fourfold higher than the recommended dose in the majority of patients. In summary, these studies suggest that the majority of patients with urticaria not responding to single dose will profit from up-dosing of antihistamines. Modern second-generation antihistamines at licensed doses are first-line treatment in urticaria, and updosing is second-line treatment.[28]

Therapeutic options for antihistamines refractory patients

Omalizumab (anti-IgE) has now been shown to be very effective in the treatment for CSU, both in case reports and case series as well as in double-blind placebo-controlled studies in antihistamine refractory selected patients.[41] Omalizumab has also been reported to be effective in cholinergic urticaria, cold urticaria, solar urticaria, heat urticaria, symptomatic dermographism, and delayed pressure urticaria. Omalizumab is effective already in doses from 150 to 300 mg per month, often independently from total serum IgE.[41]

Ciclosporin A (CsA) also has a moderate, direct effect on mast cell mediator release. CsA has now been shown to be effective in double-blind placebo-controlled studies and is the only agent of this type to inhibit basophil histamine release.[18] Efficacy

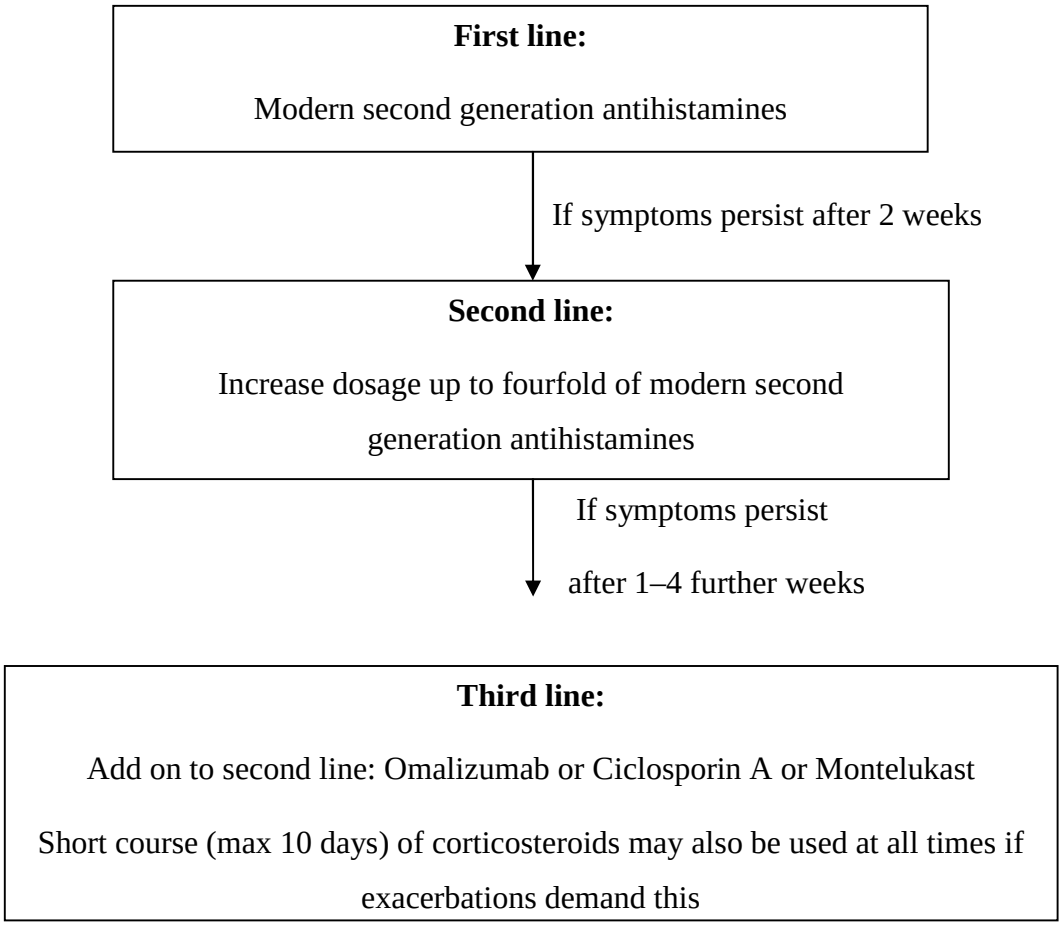
of CsA in combination with a modern second-generation H1-antihistamine has been shown to be effective few trials, but this drug cannot be recommended as standard treatment due to a high incidence of adverse effects.[15] It is recommended only for patients with severe disease refractory to any dose of antihistamine, but CsA has a far better risk/benefit ratio compared with longterm use of steroids.[18]

Systemic corticosteroids are used at doses between 20 and 50 mg/day for acute urticaria and acute exacerbations of CSU. A short course of oral corticosteroids, that is, treatment for a maximum of up to 10 days may be helpful to reduce disease duration/activity. There is a strong recommendation against the long-term use of corticosteroids due to its long term side-effects. Nevertheless, well designed randomized clinical trials are lacking.[34]

Before changing to an alternative therapy, it is recommended to wait for 1–4 weeks to allow full effectiveness of the antihistamines. As the severity of urticaria may fluctuate, and as spontaneous remission may occur at any time, it is also recommended to re-evaluate the necessity for continued or alternative drug treatment every 3–6 months.[15]

Phototherapy has been successfully used in mastocytosis and is helpful in treatment-resistant patients with this condition. For the treatment of CSU and symptomatic dermographism, UV-A, PUVA, and UV-B treatment for 1–3 months can be added to antihistamine treatment.[28]

Flowchart 1: Treatment algorithm[15]



METHODOLOGY

SOURCE OF DATA:

A “hospital-based, cross-sectional study of autologous serum skin test (ASST) in chronic idiopathic urticaria and autoimmune urticaria” was conducted in all cases of chronic urticaria attending out-patient clinic of Department of Dermatology, Venereology and Leprosy at BLDE University’s Shri B.M. PATIL Medical College, Hospital and Research Center, Vijayapur, Karnataka. A total of 65 patients were recruited into the study which was conducted between November 2013 to July 2015.

METHOD OF COLLECTION OF DATA:

All male and female patients suffering from chronic urticaria are included in the study based on the following inclusion and exclusion criteria:

Inclusion criteria:

- All cases of chronic idiopathic urticaria not responding to conventional anti-histamines.
- Age 18-60 years

Exclusion criteria:

- Age less than 18 and more than 60 years
- All known causes of urticaria
- Urticarial vasculitis
- Pregnant and lactating mothers
- Failure to discontinue drugs prior to the test

PROCEDURE:

A detailed history along with physical examination is conducted with special emphasis on the following:

1. Frequency and duration of attacks.
2. History of angioedema and difficulty in swallowing / breathing.
3. Details of occupation
4. History of known thyroid conditions or any other co-morbidities.
5. History of any drug intake specially NSAIDS.
6. History of taking treatment for urticaria (if so details of the medication)
7. Precipitating factors (infections, exposure to cold, exercise, vibration, dental caries)
8. Presence of worms in stool.
9. Family history of atopy.

AUTOLOGOUS SERUM SKIN TEST

Material required

1. Insulin or tuberculin syringes (2)
2. Sterile 5cc syringes (2)
3. Tourniquet
4. Red plain tube for blood collection (1)
5. Normal saline 0.9% 10 ml ampoule (1)
6. Centrifuge machine
7. Skin marker

Antihistamines are withdrawn 48 hours before the procedure and corticosteroids, doxepin for 3 weeks and immunosuppressives 4 weeks before the study.

Patient's blood, 3cc, is drawn with a sterile syringe and put in a red plain tube. The tube is let to stand for 30 to 45 minutes to allow the clotting to happen. Then the clotted blood is centrifuged at spin of 2000 rpm to separate the serum which is floating on top of the clotted cells.

Test is performed by injecting 0.05ml of patient's serum intradermally into the left forearm 2 inches below the anterior cubital crease which is lesion free and 0.9% normal saline as control 2 inches below the test. After 30 minutes, wheal formed at each injection site is measured. In few patients the reading are taken after 45 minutes to 1 hour expecting a delayed reaction to the serum. A positive ASST is defined as serum induced wheal and flare of 1.5mm or more than the saline induced.

Disease assessment

In all the patients severity of the disease was assessed using urticaria total severity score. Six separate parameters of disease activity and severity were recorded on a 0-3 point scale. Based on these, a 0-18 total severity score (TSS) was generated and overall disease severity classified as clear (TSS = 0), mild (TSS 1-6), moderate (TSS 7-12) or severe (TSS 13-18).

Table 4: Urticaria total severity score

Parameter	0	1	2	3
Number of wheals	None	</= 10	11-50	>50
Size of wheals	None	< 1 cm	1-3cm	>3 cm
Intensity of pruritus	None	Mild	Moderate	Severe
Duration of persistence	None	<1 h	1-12 h	>12h
Frequency of appearance	None	<once or once/week	2-3 times a week	Daily/ almost daily
Frequency of antihistamine use	None	<once or once/week	2-3 times a week	Daily/ almost daily

INVESTIGATIONS

Following laboratory investigations will be carried out for all patients.

- Haemoglobin
- Total WBC count
- Differential count
- Peripheral smear

Following tests will be done wherever necessary

- Liver function test
- Thyroid function test

STATISTICAL ANALYSIS:

Clinico-epidemiological data collected from the patients were calculated with mean +/- standard deviation (SD). Mann-Whitney test has been used to calculate median of duration and frequency among positive and negative test. Chi square test has been used to check association between angioedema and test interpretation. Fishers exact test has been used to verify association between atopy and test interpretation.

ETHICAL CLEARANCE:

Institutional ethical committee clearance was taken for the study.

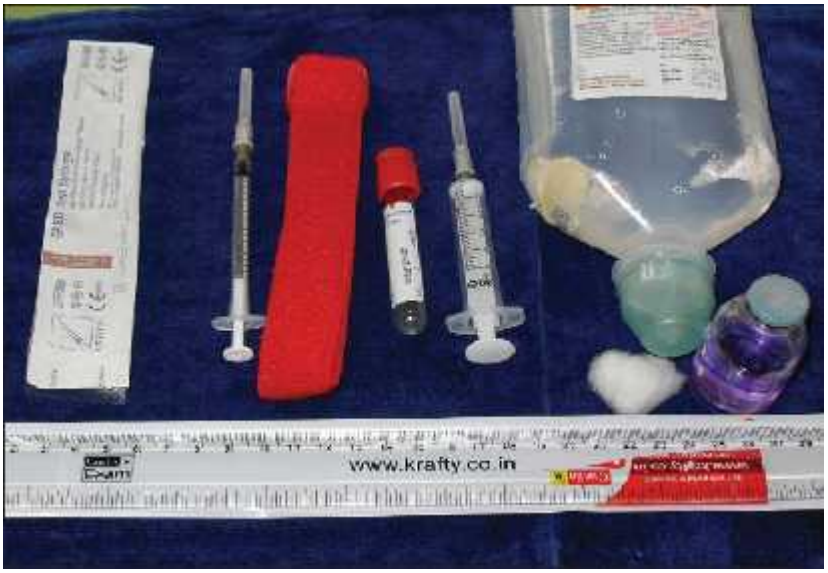


Figure 2: Materials used in the autologous serum skin test

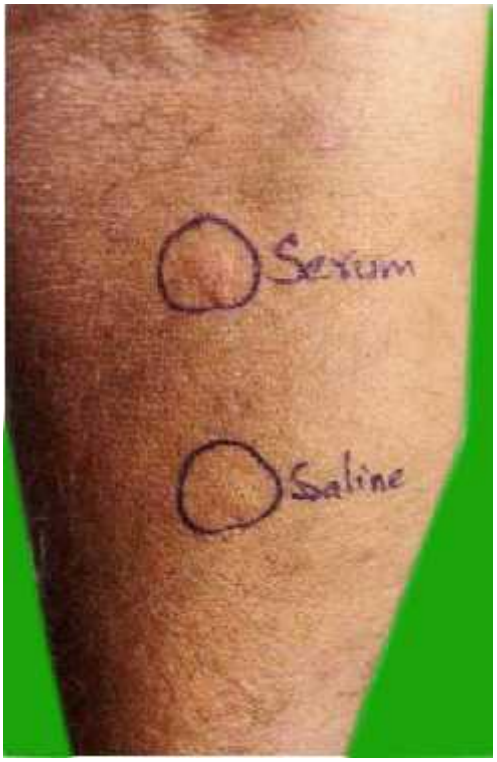


Figure 3: Patient showing positive ASST with serum induced wheal 1.5mm greater than control

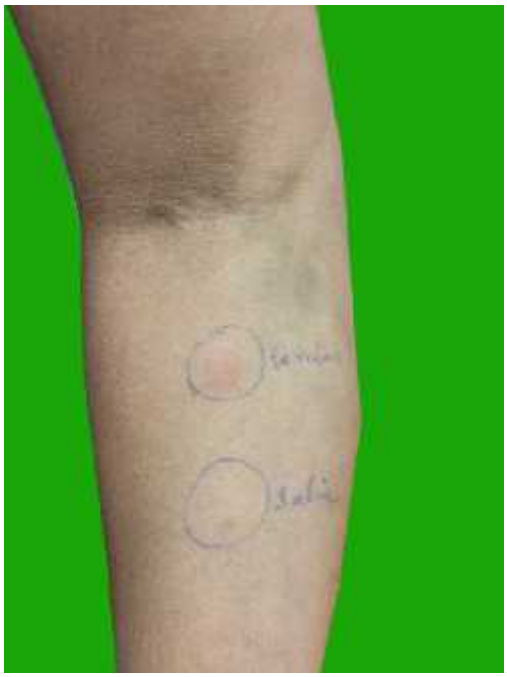


Figure 4: Another patient showing positive ASST

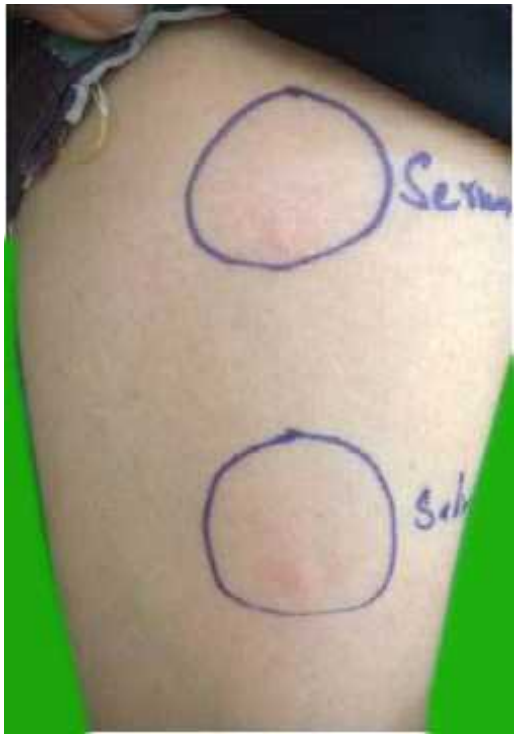


Figure 5: Patient showing negative ASST with serum and saline induced wheal approximately same

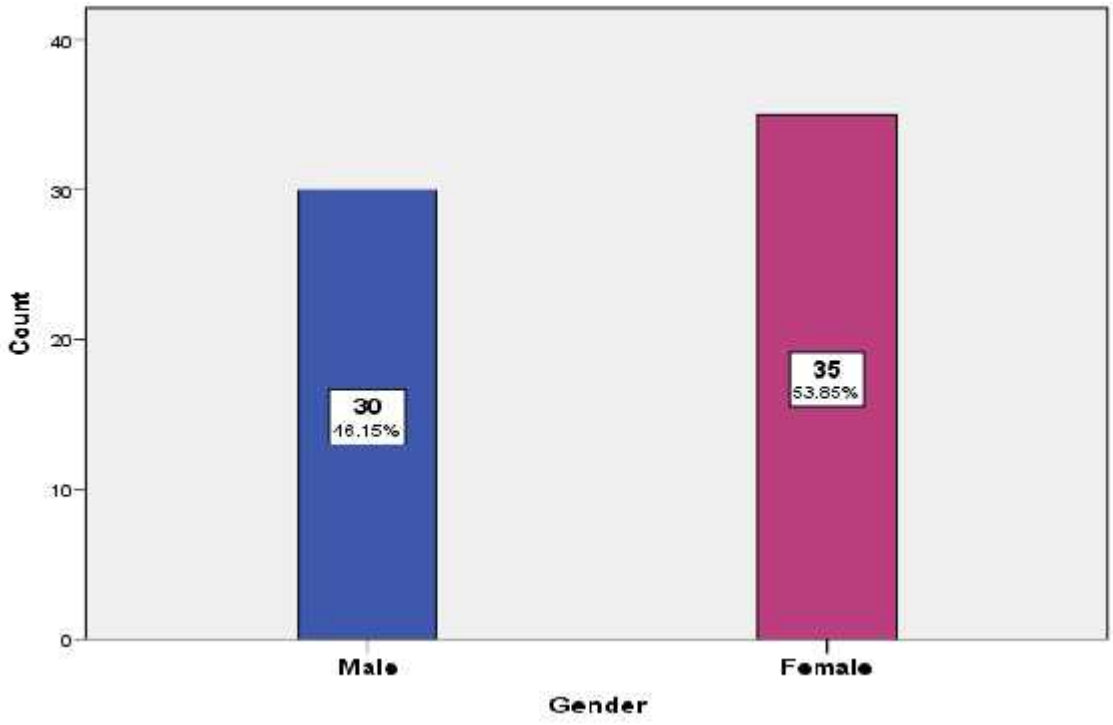
RESULTS

A hospital-based, cross-sectional study was conducted between November 2013 to July 2015. A total of 65 patients were recruited into the study.

Gender distribution:

Among 65 patients, 30 were males (46.15%), and 35 were females (53.85%). There was female preponderance as shown in figure 6.

Figure 6: Gender distribution of patients with chronic urticaria



Age distribution

The age of the patients ranged between 17 years to 62 years, with mean (± SD) age value of 34.57 (± 11.957) years. It is observed that high peak of patients were in early 20's. There was no statistical significance between age and disease. Figure 7 presents the age distribution of the patients.

Figure 7: Age distribution of patients with chronic urticaria

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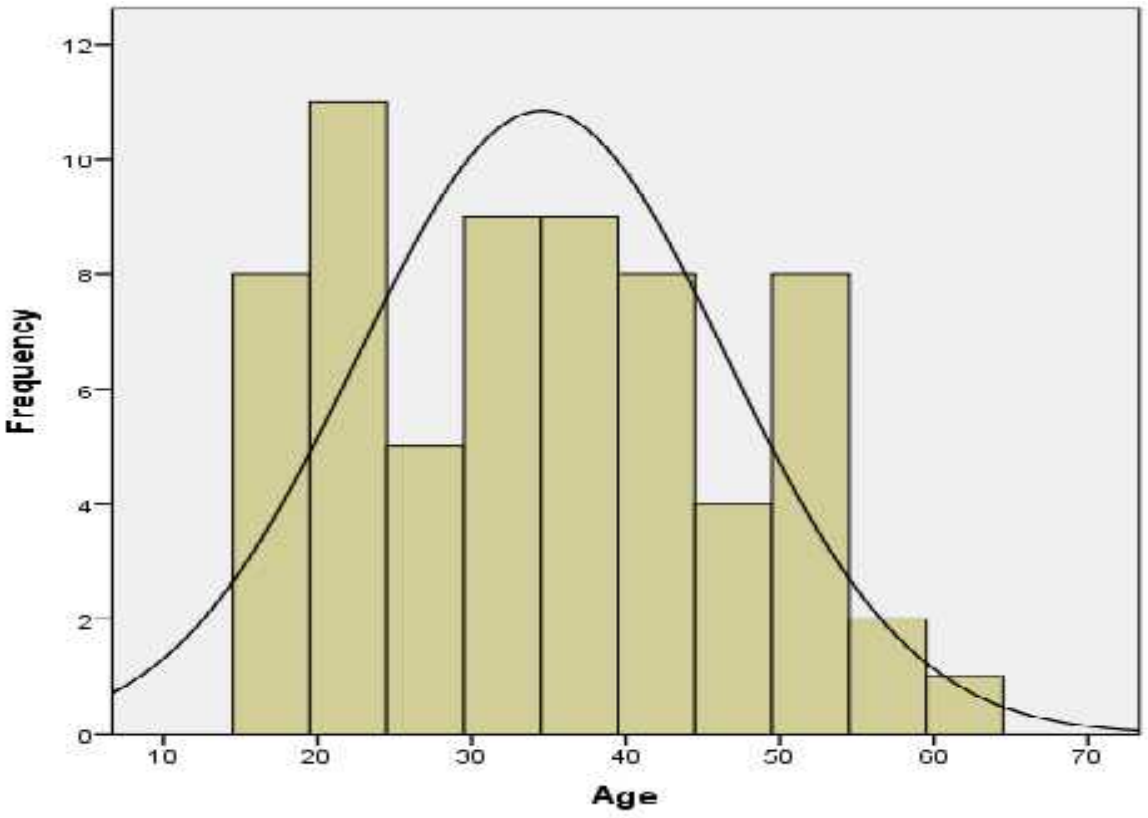
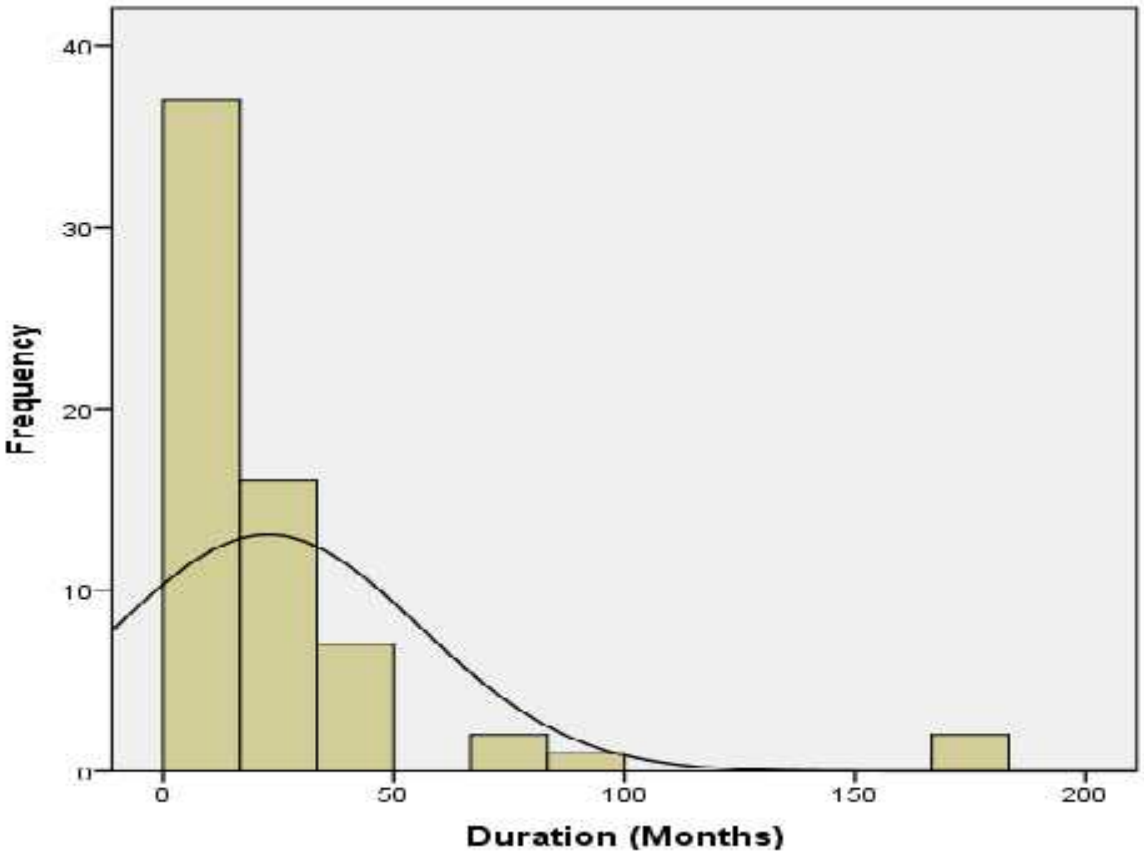


Duration of the disease

Duration of the disease ranged from, with a minimum of 2 months to a maximum of 180 months (25 years) in 1 patient. Mean duration (± SD) of the disease was 22.74 (± 33.20) months. Figure 8 shows the duration of the disease among the study patients.

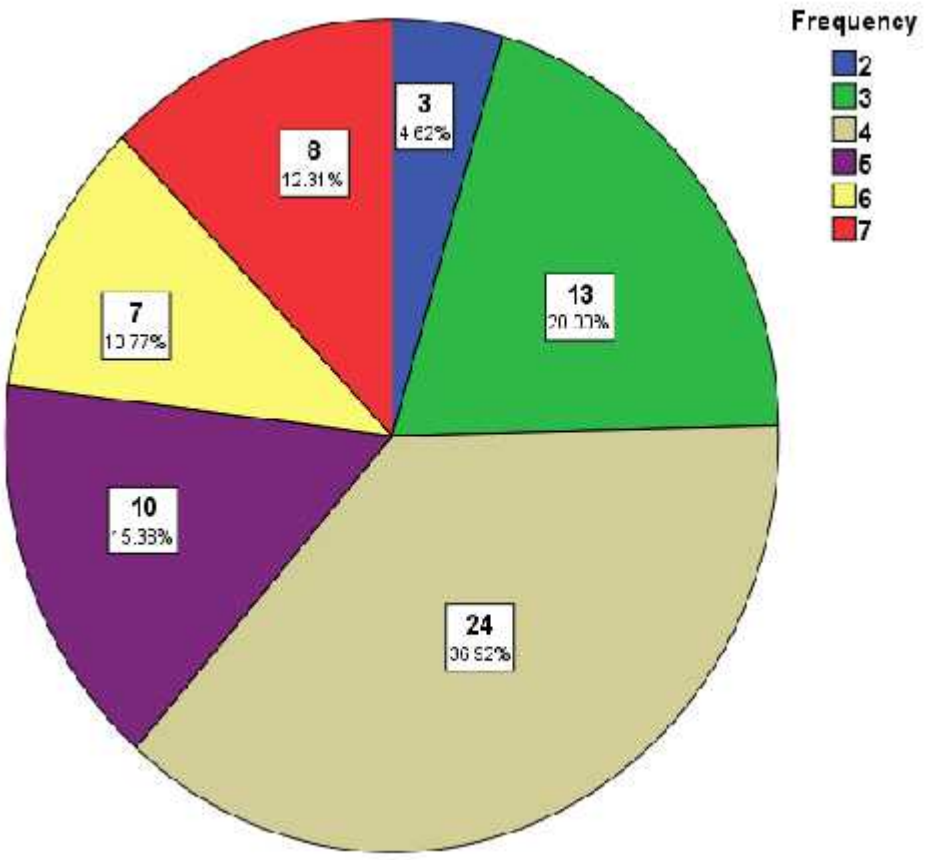
Figure 8: Duration of the disease



Frequency of the disease

Frequency of the disease when calculated (days per week) showed that maximum number of patients, 24 (36.92%) had disease for 4 days per week. Frequency of the disease in the remaining patients were, 13 (20%) had disease for 3 days per week, 10 (15.38%) had disease for 5 days per week, 8 (12.31%) had disease for 7 days per week, 7 (10.77%) had for 6 days per week and very few, 3 (4.62%), had disease to a minimum of 2 days per week. Frequency of the disease has been shown in figure 9.

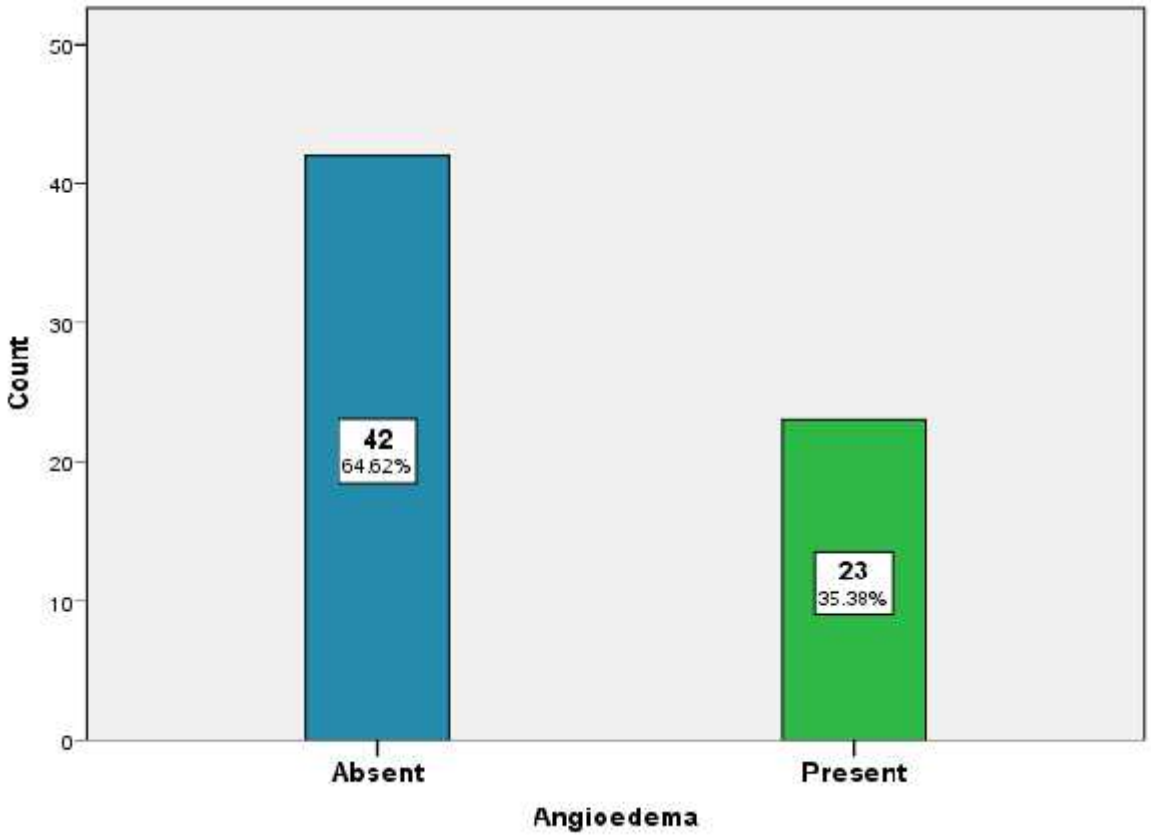
Figure 9: Frequency of the disease



Angioedema

Among the 65 patients, 23 (35.38%) had associated angioedema along with urticaria and the remaining 42 (64.62%) patients did not have angioedema. Associated presence of angioedema is shown in figure 10.

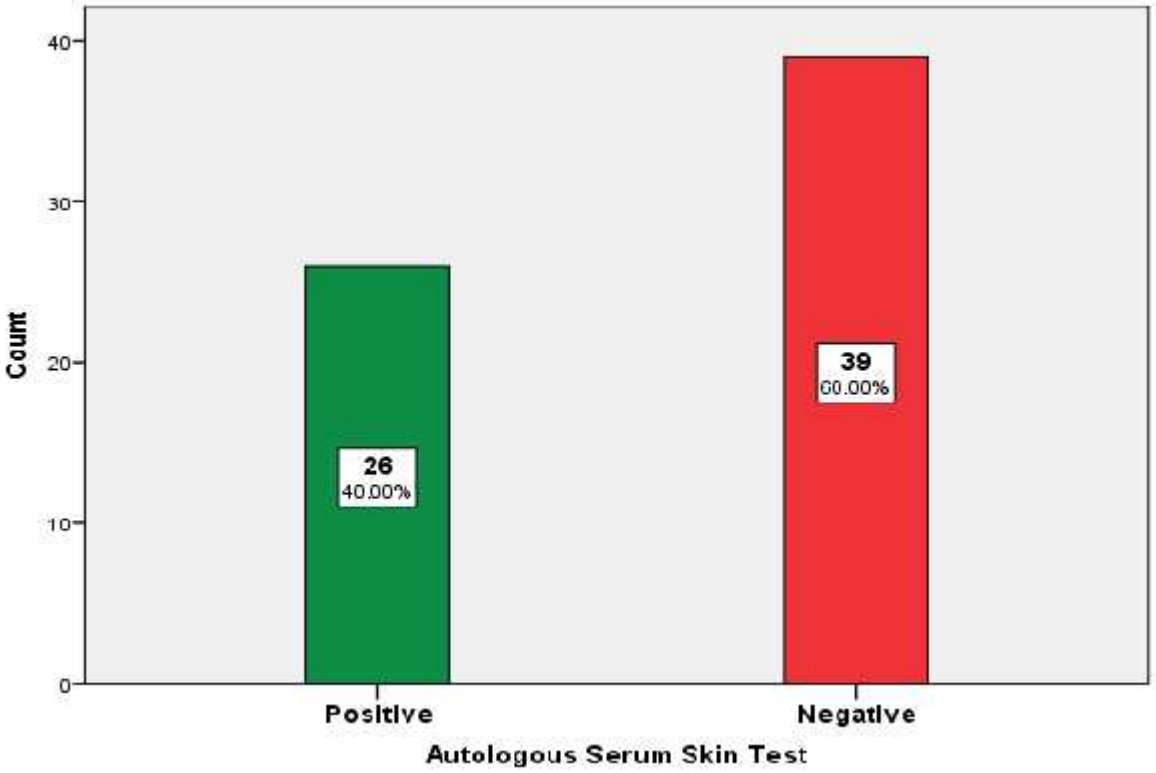
Figure 10: Associated angioedema among patients



Autologous serum skin test (ASST)

Twenty six (40%) patients showed positivity to ASST designating them as chronic autoimmune urticaria and 39 patients (60%) had negative interpretation to the test and were diagnosed as chronic spontaneous urticaria. Interpretation of the test results are shown in the figure 11.

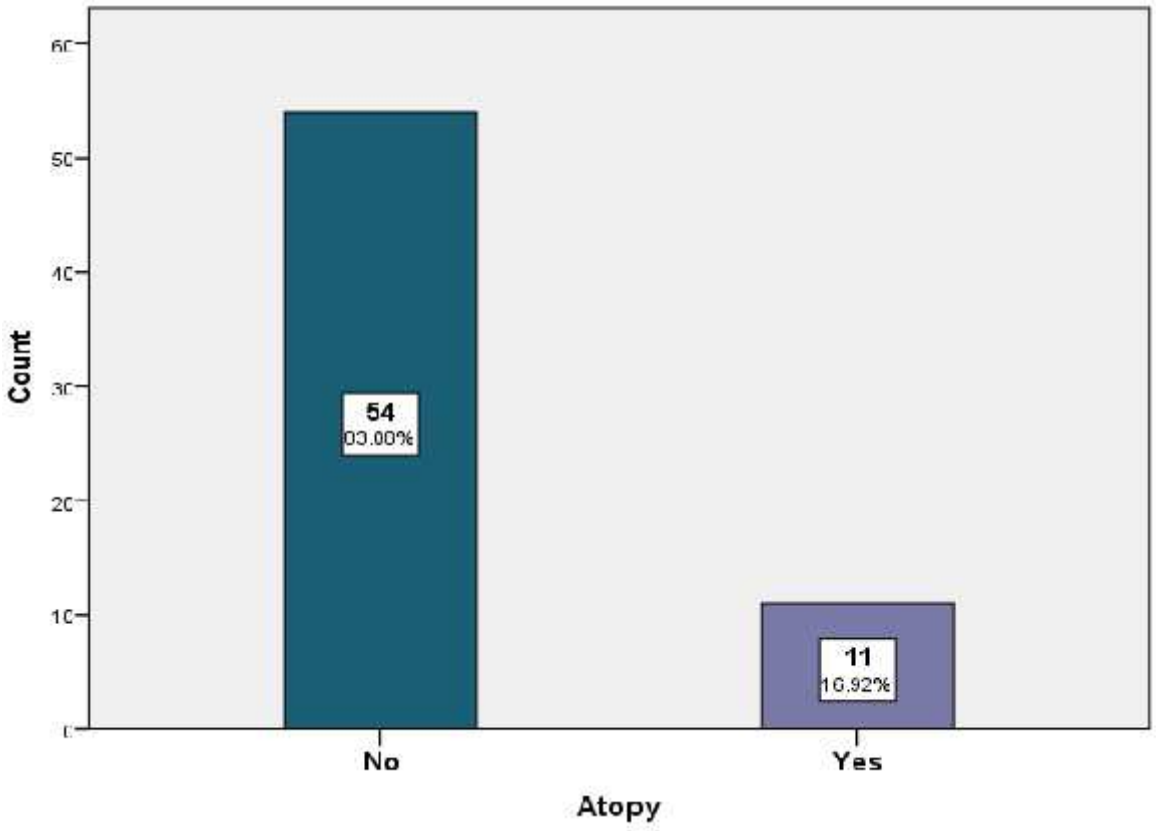
Figure 11: Autologous serum skin test (ASST) interpretation



Atopy

When assessed for atopy among the study subjects only 11 (16.92%) patients had atopy as personal or family history and majority of the patients, 54 (83.08%) gave no history of atopy. Figure 12 shows presence or absence of atopy among the patients.

Figure 12: History of atopy

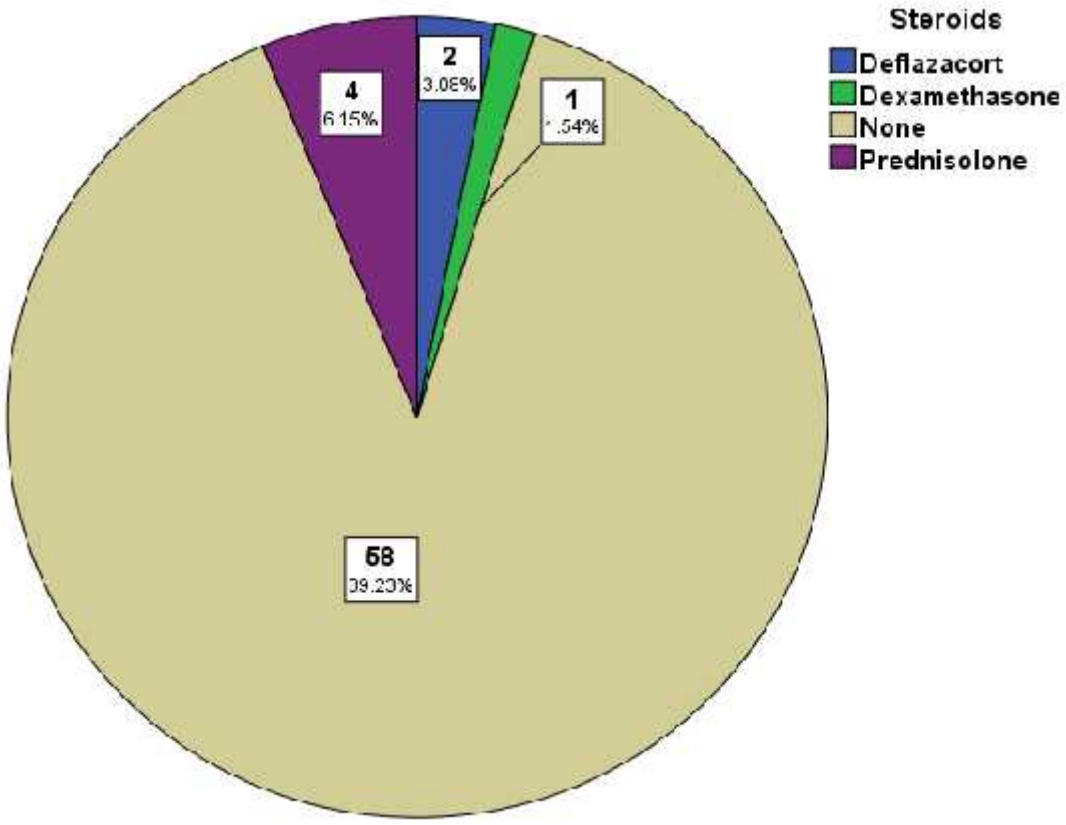


Drugs used in the management of urticaria

Use of steroids for the treatment of urticaria

Oral steroids like prednisolone, dexamethasone and deflazacort were taken by 4 (6.15%), 2 (3.06%) and 1 (1.54%) patients respectively. A bulk of patients gave no history of steroid use. Steroid use has been portrayed in figure 13.

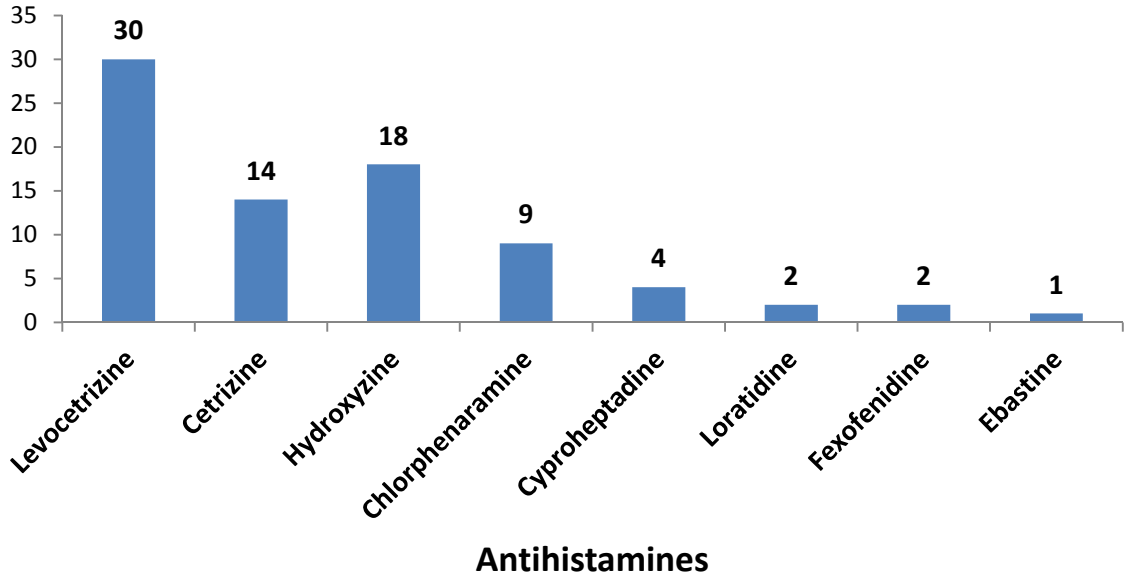
Figure 13: Use of steroids



Use of antihistamines

Array of sedating and non-sedating antihistamines were used by patients. Non-sedating antihistamines like levocetrizine, cetrizine, loratidine, fexofenidine and ebastine were taken by 30, 14, 2, 2 and 1 patients respectively. Sedating antihistamines like hydroxyzine, chlorphenaramine and cyproheptadine were taken by 18, 9 and 4 patients correspondingly. Antihistamines use has been depicted in figure 14.

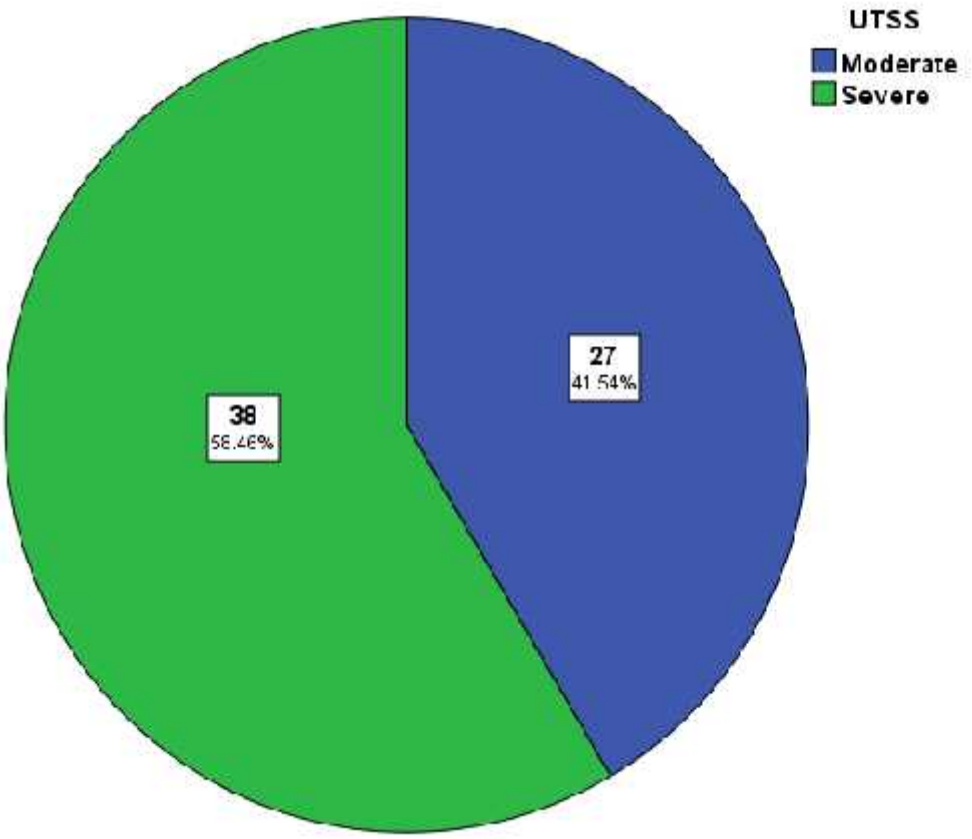
Figure 14: Use of antihistamines



Urticaria total severity score (UTSS)

On grading the severity of the disease by UTSS, 27 (41.54%) patients had moderate urticaria with score between 7-12 and majority, 38 (58.46%) patients had severe urticaria with score between 13-18. Severity of the disease has been shown in figure 15.

Figure 15: Urticaria total severity score (UTSS)



Association between autologous serum skin test (ASST) with duration of the disease, angioedema, urticaria total severity score (UTSS) and atopy

The mean disease duration (±SD) was 33.20 months. According to the duration of disease in the patients, it did not influence the positivity of ASST (*p* value 0.49).

The severity score calculated using UTSS was divided into three groups: mild (1-6), moderate (7-12), and severe (13-18) and their relation to the positivity of ASST was statistically significant (*p* value 0.02) which is shown in table 5.

Table 5: Association between UTSS and ASST

		UTSS			***p* value**
		Moderate n (%)	**Severe** n (%)	**Total** n (%)	
Test	**Negative**	21 (53.8%)	18 (46.2%)	39 (100%)	0.02 (significant)
	Positive	6 (23.1%)	20 (76.9%)	26 (100%)	
	Total	27 (41.5%)	38 (58.5%)	65 (100%)	

Eleven out of 65 patients had atopy in either personal or family history and its relation to ASST positivity had no significance (*p* value 0.503).

Among the 65 patients, history of angioedema was present at anytime in 23 (35.4%) patients during the course of the disease. Sixteen (61.5%) and 7 (17.9%) patients had angioedema among who showed positive and negative ASST respectively. The association between presence angioedema and ASST has been illustrated in table 6.

Table 6: Association between angioedema and autologous serum skin test

		Angioedema			
		Absent n (%)	**Present n (%)**	**Total**	***p* value**
Test	**Negative**	32 (82.1%)	7 (17.9%)	39 (100%)	<.001 (significant)
	Positive	10 (38.5%)	16 (61.5%)	26 (100.0%)	
	Total	42 (64.6%)	23 (35.4%)	65 (100.0%)	

DISCUSSION

Chronic urticaria is a highly disabling disorder, recognition of chronic autoimmune urticaria (CAU) as a distinct subgroup of CSU during the last decades has facilitated better understanding of unremitting CSU.[2] These patients have histamine-releasing autoantibodies and they usually need high doses of antihistamines and/or systemic corticosteroids during acute exacerbations and use of immunomodulatory drugs is necessary in CSU in antihistamine refractory chronic urticaria cases.[2]

The basophil histamine release assay is currently the "gold standard" for detecting functional autoantibodies in patients with chronic idiopathic urticaria. However, this bioassay is difficult to standardize because it requires fresh basophils from healthy donors and is time consuming.[16] Western blotting and other nonfunctional assays, including enzyme-linked immunosorbent assay (ELISA) and flow cytometry using chimeric cell lines expressing the human FcεRIα, may be useful for screening sera in the future but need to be validated. Hence, ASST is the only practicable test available to clinicians to detect autoimmune urticaria.[24]

The ASST has a sensitivity of 70% and a specificity of 80% when read as a pink serum-induced wheal 1.5 mm or greater than an adjacent normal saline control injection at 30 minutes. A positive test is suggestive but not diagnostic of an autoimmune basis for patient's urticaria. Confirmation is needed by *in vitro* testing of the patient's serum for anti-FcεRIα or anti-IgE autoantibodies.[35]

Patients with autoimmune autoantibodies have no distinctive diagnostic clinical features. Autoimmune and non-autoimmune cases are indistinguishable clinically and histologically. However, they do tend to have more severe urticaria.[15]

The present study has evaluated patients with CSU by autologous serum skin testing and compared the clinical features of patients with positive and negative ASST results and found the incidence of autoimmune urticaria to be 40% based on ASST which is on par with reports from western literatures or studies. This test can be done by a dermatologist to determine whether chronic spontaneous urticaria is autoimmune in origin. This is especially important from a management viewpoint since immunosuppressive therapies may be tried if conventional approaches of management are unsuccessful.

Zweiman *et al*,[13] reported basophil histamine releasing activity in 30% of 70 chronic urticaria sera while Tong *et al*,[21] found that 52% of 50 chronic urticaria sera released histamine from basophils.

In addition, removal of autoantibodies by plasmapheresis has been shown to produce clinical improvement in patients with CSU.[34] The clinical features of patients with CSU were defined in several studies before the identification of autoantibodies. Now it is known that there are at least two subsets of patients with CSU those with and without autoantibodies.[34]

In the present study, different clinical parameters were studied to evaluate any proposed relationship between these parameters and the positivity of the ASST. These parameters are as follows: age of patients at presentation, urticaria total severity score, duration of the disease in months, frequency of attacks per week, gender of the patients, interference of the disease with daily activities, presence of angioedema, presence of family history of chronic urticaria, presence of personal and/or family history of atopy, and autoimmune diseases.

The mean duration of disease in this study was 22.5 and 22.6 months for ASST positive and negative patients respectively, which was almost similar in both the groups and was not statistically significant. Though patients with a positive ASST had more frequent attacks in a week but did not show statistical significance compared to the frequency of attacks in ASST negative group.

George *et al*,[8] conducted a prospective study on 100 patients with chronic urticaria in which 34% patients showed a positive response to Autologous Serum Skin Test. The median duration of disease and severity were significantly higher in patients with positive Autologous Serum Skin test.

A study done by Sabroe *et al*,[31] concluded that patients with autoantibodies in their sera have more severe attacks according to frequency and duration. This is in concordance with present study based on UTSS which included number and size of wheals, intensity of pruritus, frequency and duration of the disease and antihistamine use. Severe disease with a UTSS of 13-18 was observed more in ASST positive patients.

Azim *et al*,[4] conducted a study Autologous Serum Skin test was positive in 15 (42.9%) patients with chronic idiopathic urticaria who had more severe urticaria of prolonged duration and more frequent attacks when compared patients with negative.

Godse conducted a study on 45 patients suffering from chronic urticaria.12 patients (26.67%) showed a positive result Autologous Serum Skin test. All of them had severe disease in the form of recurrent wheals and itching.[6]

In the present study angioedema occurred in 23 out of 65 patients and showed statistical significance ($p < .001$). In a similar study done by Sabroe *et al*,[31] angioedema occurred in 93 out of 107 cases but there was no significant difference between ASST-positive or -negative patients in distribution of angioedema.

In a study by Krupashankar *et al*,[9] ASST was positive in 58.75% and negative in 41.25% of the patients, respectively. Out of 33 patients with history of angioedema, 9 (27.3%) patients were in ASST negative group and 24 were in positive group, this was statistically significant.

Autoimmune diseases like thyroid disease, vitiligo, diabetes mellitus, pernicious anemia and rheumatoid arthritis were reported more commonly in patients with chronic autoimmune urticaria although none of the patients in this study had any clinical signs or history of autoimmune diseases.[17]

The association of chronic urticaria with thyroid autoimmunity has been studied by Leznoff *et al*,[17] and it has been postulated that thyroid autoimmunity may play a role in the pathogenesis of chronic urticaria and angioedema. However, in contrast with previous studies, present study did not find any difference in the incidence of thyroid disease. This is likely to be because of less number of patients included in the present study or TFT and thyroid autoantibodies were not routinely measured for all patients.

Fusari *et al*,[37] found that ASST was positive in 86.7% and 8% of patients with and without HT, respectively ($p < 0.001$).

In our setting, ASST was the only available test for the diagnosis of autoimmune urticaria. It is a simple, inexpensive, semi-invasive and easy-to-perform test which can be done and recorded by the dermatologist himself to determine whether the patient's CSU is autoimmune in origin. As conventional approaches of management may be unsuccessful, ASST is especially important from the therapeutic point of view as it can help the dermatologist to commit himself to initiate immunosuppressive therapy in such patients.

Immunomodulatory drugs, while their use is not justified in CSU (except in anti-histamine refractory chronic urticaria cases), are therapeutic benefit in recalcitrant to therapy autoimmune urticaria patients having significantly impaired quality life.[7]

A positive ASST has been associated with prolonged disease that is poorly responsive to regular antihistamines. One important advantage of testing is to promote more tailored prognostic counseling and the early appropriate use of immunosuppressive drugs.

CONCLUSION

Chronic autoimmune urticaria is in patients who may present with transient eruption of itchy, erythematous, edematous swellings of the dermis, which lasts more than six weeks. They have circulating IgG directed against α-subunit of the high affinity IgE receptor or anti-FcεRIα and less frequently IgG anti-IgE antibodies. Detailed history is of utmost importance, especially related to the duration, frequency and angioedema.

The gold standard for detecting clinically, relevant to autoantibodies to FcεRI is the functional *in vitro* donor basophil histamine release assay (HRA) which is very difficult to perform and requires special equipment, expertise and setup.

Autologous serum skin test is a simple office based procedure to reveal autoimmune, though not diagnostic, cause in chronic spontaneous urticaria.

Out of 65 patients, 26 (40%) patients showed positivity to ASST diagnosing them as chronic autoimmune urticaria. A statistical significance was observed when compared between ASST and angioedema and severity of the disease (UTSS).

This study helped chronic autoimmune urticaria patients to be initiated on a vigorous management schedule of antihistamines and also autologous serum therapy. Also this test helps treating clinician to start immunotherapies like methotrexate and cyclocporine, biologics like omalizumab.

SUMMARY

A hospital based cross sectional study to assess the autoimmune status of chronic urticaria and chronic spontaneous urticaria using autologous serum skin test. Male and female patients, age ranging from 16-60years with history of urticaria persisting more than 6 weeks, having no definable underlying cause and patients who are not responding to antihistamines were recruited into the study. The taken patients were advised to stop antihistamines 2-3 days prior to test and were injected with 0.05ml of own serum (test) on the volar aspect of lesion free forearm 5 cms below the cubital crease and saline (control) 5 cms below the test site. After injecting patient is asked to wait for 30-45mins to check for wheal and flare reaction at test site which is 1.5mm more than that of control site.

Following are the salient observations of the study:

- Male to female ratio was 1:1.1.
- The mean (± SD) age of the study population was 34.57 (± 11.957) years.
- Mean duration (± SD) of the disease was 22.74 (± 33.20) months.
- The frequency of the disease ranged from a maximum of 7 days per week in 8 (12.81%) patients to a minimum of 3 days per week in 3 (4.62%) patients.
- Autologous serum skin test was positive in 26 (40%) patients.
- Angioedema was present in 42 (64.62%) patients which showed clinical significance with ASST positivity.
- Moderate and severe urticaria based on UTSS was observed in 38 (58.46%) and 27 (41.54%) patients respectively, which also showed statistical significance to ASST positivity.

- No test-related side-effects were noted in any of the patients.
- None of the patient's history revealed any autoimmune thyroid conditions or other autoimmune diseases.

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ANNEXURES

BIJAPUR-586 103.
OUTWARD
No. 5213
Date 14-11-13

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title Autologous Serum skin test (ASST) in Chronic idiopathic urticaria and autoimmune urticaria: A hospital based cross-sectional study.

Name of P.G. student Dr. Ajay Mujja,
Department of Dermatology

Name of Guide/Co-investigator Dr Arun. C. Inamadar.
Prof & HOD. of Dermatology

DR.TEJASWINI VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

SAMPLE INFORMED CONSENT FORM

B.L.D.E.U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,

VIJAYAPUR-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: AUTOLOGOUS SERUM SKIN TEST (ASST) IN CHRONIC IDIOPATHIC URTICARIA AND AUTOIMMUNE URTICARIA: A HOSPITAL-BASED, CROSS-SECTIONAL STUDY.

PG GUIDE **:** DR.ARUN C. INAMADAR

PG STUDENT **:** DR. AJAY MUJJA

PURPOSE OF RESEARCH:

I have been informed that this project will study the incidence of positive ASST among chronic idiopathic urticaria and autoimmune urticaria patients.

BENEFITS:

I understand that my participation in this study will help the investigator to understand the disease better and will help in the management of the disease.

PROCEDURE:

I understand that relevant history will be taken and I will undergo detailed clinical examination after which necessary investigation will be done whenever required.

RISK AND DISCOMFORTS:

I understand there is no risk involved and I will experience minimal pain during the procedures performed.

CONFIDENTIALITY:

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time concerned. The researcher is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that the researcher may terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician if this is appropriate.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient/ parent/relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Investigator / P. G. Guide Date

I confirm that …….(Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I will undergo, and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my participation as a subject in this research project.

_____ _____

Participant / guardian Date

_____ _____

Witness to signature Date

PROFORMA

SCHEME OF CASE TAKING

B.L.D.E.U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR.

Department of Dermatology, Venereology and Leprosy

SL.NO: Date:

Personal details

Name: Age/sex:

Father's/Husband's name:

Address:

Occupation: Phone no.

History:

Duration of disease	**Frequency of attacks**

Family history:

Atopy: yes/no

Similar complaints: yes/no

Personal history:

Smoking/Alcohol/ Tobacco/Betel nut: yes/no

Treatment history:

Anti-histamines:

Systemic steroids:

Antibiotics/NSAIDS:

Precipitating factors:

Infection:

Drugs:

Allergies:

Infestations:

Exposure to cold:

Exercise:

Vibration:

Dental caries:

General Physical Examination:

Weight-	Height-	Clubbing-
Pallor-	Cyanosis-	Lymphadenopathy-
Icterus -	Edema-	

Vital parameters:

Temperature:

Pulse rate:

Respiratory rate:

Blood pressure:

On examination:

Systemic examination:

Cardiovascular system:

Respiratory system:

Per abdomen:

Central nervous system:

Assessment of angioedema:

Swelling of lips/eye lids/ face:

Difficulty in swallowing:

Difficulty in breathing:

Diagnosis:

Urticaria total severity score

Parameter	**0**	**1**	**2**	**3**
Number of wheals	None	</= 10	11-50	>50
Size of wheals	None	M 1 cm	1-3cm	>3 cm
Intensity of pruritus	None	Mild	Moderate	Severe
Duration of persistence	None	<1 h	1-12 h	>12h
Frequency of appearance	None	<once or once/week	2-3 times a week	Daily/almost daily
Frequency of antihistamine use	None	<once or once/week	2-3 times a week	Daily/almost daily

Score:

Investigations:

Hb (gm %)	
Total WBC count (cells/cumm)	
Differential count (%) Neutrophils Lymphocytes Eosinophils Monocytes Basophils	
ESR(mm after 1st hr)	
Peripheral smear	

Interpretation of autologous serum skin test (ASST):

Size of serum wheal	
Size of saline control wheal	
Interpretation	

KEY TO MASTER CHART

-	-	Negative
+	-	Positive
A	-	Absent
F	-	Female
M	-	Male
n	-	None
N	-	No
N/D	-	No details
P	-	Present
Sl No	-	Serial Number
UTSS	-	Urticarial total severity score
Y	-	Yes

MASTER CHART

Sl No	Name	Age (years)	Sex	Duration (months)	Frequency (days/week)	Angioedema	UTSS	Test interpretation	Atopy	Treatment (antihistamines)	Treatment (steroids)
1	Avinash Rathod	20	M	5	7	A	17	-	N	N/D	n
2	Avinash Solapur	17	M	7	4	P	17	+	N	levocetrizine	n
3	Manjunath Mittnalli	27	M	6	5	A	17	-	N	cetrizine	n
4	Padmashree Metri	30	F	18	2	A	10	-	N	levocetrizine	n
5	Basamma Vahil	55	F	5	6	A	17	-	Y	levocetrizine	n
6	Chinnappa Mahamuni	19	M	4	4	P	16	-	N	levocetrizine, chlophinaramine	n
7	Ramachandra	22	M	6	4	P	12	-	N	levocetrizine, hydroxyzine	n
8	kamala sindagi	36	F	18	3	A	17	-	Y	levocetrizine, hydroxyzine	n
9	Saraswati Jainapur	45	F	180	7	A	17	-	N	levocetrizine, chlophinaramine	n
10	RukminiSsinde	23	F	8	3	A	11	-	N	N/D	n
11	Ramesh Padralagi	42	M	4	3	P	16	+	Y	levocetrizine, hydroxyzine, cyprohrptadine, loratidine	n
12	Siddayya	23	M	3	5	P	17	+	N	hydroxyzine, chlophinaramine	n
13	Akkawwa Dodmani	52	F	3	3	P	11	+	N	cetrizine	n
14	Sonali Supriya	45	F	3	4	A	15	+	N	N/D	n
15	Laxmi Bidri	48	F	5	3	P	11	-	N	N/D	n
16	Mahadevi	22	F	2	4	A	12	-	N	levocetrizine	n
17	vanitha panchal	18	F	12	7	P	17	-	N	N/D	n
18	Savitri kamble	38	F	7	4	P	12	+	N	levocetrizine	deflazacort
19	Muskan	38	F	3	2	A	14	+	N	hydroxyzine	prednisolone
20	Tejaswini Dinni	55	F	5	4	A	16	-	N	levocetrizine	n
21	Divya Naik	18	F	12	5	P	16	+	N	cetrizine	n
22	Shivanand	42	M	6	7	A	16	+	N	N/D	n
23	Lingayya Hiremath	32	M	180	7	P	16	+	N	N/D	dexamethasone
24	sajid mulla	17	M	48	6	A	14	-	N	N/D	n
25	dowleshwar priya	22	F	24	4	A	16	-	Y	hydroxyzine, loratidine	n
26	raju mothimat	32	M	4	2	A	10	-	Y	hydrxyzine	n
27	bharath mukkad	53	M	12	4	A	15	+	Y	levocetrizine	n
28	mahadevipadyaknur	26	F	6	3	P	10	+	N	levocetrizine, cetrizine	prednisolone
29	savitri hiregonda	35	F	24	5	P	12	+	N	levocetrizine	n
30	Sumangala Patil	18	F	12	7	P	15	+	N	levocetrizine	n

31	Amit Balappagoal	18	M	24	5	P	14	+	N	levocetrizine	n
32	santosh B Biradar	34	M	36	4	A	14	+	N	levocetrizine, hydroxyzine	n
33	Mahesh	52	M	72	4	A	14	+	N	cetrizine, hydroxyzine	n
34	Mallika	18	F	18	4	A	16	+	N	levocetrizine	n
35	Sanjay M	42	M	24	4	P	12	+	N	N/D	n
36	Yannappa lalappa basareddy	50	M	18	5	A	13	+	N	hydroxyzine, chlophinaramine	n
37	Malkappa	34	M	12	6	A	16	+	N	N/D	n
38	Vajravathi Mali	52	F	24	4	A	10	-	N	N/D	n
39	Prathibha Siddapagonda	38	F	72	4	A	12	+	N	levocetrizine, hydroxyzine	n
40	Anjali	41	F	36	4	P	16	-	Y	levocetrizine, hydroxyzine	n
41	Dareppa	26	M	18	4	A	10	-	N	levocetrizine	n
42	Ahmed	62	M	24	4	A	10	-	N	levocetrizine, chlophinaramine	prednisolone
43	kashibai	42	F	24	6	A	16	-	N	N/D	n
44	Anand Rathod	38	M	8	5	P	16	-	N	levocetrizine, hydroxyzine, fexofenidine	n
45	Shivappa Pattar	50	M	24	3	A	10	-	N	N/D	prednisolone
46	Drakshayani Biradar	40	F	8	3	A	10	-	N	N/D	n
47	Shreedevi Biradar	32	F	36	4	A	15	-	Y	levocetrizine, cetrizine	n
48	Ramesh Nategar	22	M	4	7	A	14	-	N	levocetrizine, hydroxyzine	n
49	Rudramari Shantappa Jogur	24	M	24	3	A	10	-	N	hydroxyzine	n
50	Malik Naaz	21	M	36	3	A	11	-	N	cetrizine	n
51	Neelamma	36	F	12	6	A	14	-	N	hydroxyzine, ebastine	n
52	Nazreen	30	F	3	4	P	15	+	Y	cetrizine	n
53	Basavaraj G Kalappagol	24	M	18	4	A	9	-	Y	cetrizine	n
54	Saina	28	F	12	3	A	11	-	Y	levocetrizine, cyproheptadine	n
55	Nilofer	24	F	7	5	A	9	-	N	cetrizine, chlophinaramine	n
56	Rekha uppar	34	F	12	6	P	16	+	N	cetrizine, chlophinaramine	n
57	Basamma Gami	40	F	24	5	A	9	-	N	fexofenidine, chlorphenaramine	n
58	Nyamagond Sunil	30	M	48	4	A	11	-	N	cetrizine	n
59	Ningamma Horti	50	F	6	3	A	10	-	N	levocetrizine, hydroxyzine	n
60	Rihana Begum	42	F	8	4	P	17	+	N	cyproheptadine, hydoxyzine	n
61	Kumar sankanavar	28	M	7	3	A	11	-	N	levocetrizine	n
62	Sangeeta Ramachandra Parande	38	F	3	7	A	16	-	N	levocetrizine, cyproheptadine	n
63	Sharan Bidri	48	M	12	5	P	13	+	N	hydroxyzine, cetrizine	n
64	Ambreesh Gudadini	36	M	84	4	P	15	-	N	levocetrizine	deflazacort
65	Madhuri Hanjagi	53	F	48	6	A	10	-	N	cetrizine	n