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Corticosteroids in management of anaphylaxis; a systematic review of evidence

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Anaphylaxis; emergency management; corticosteroids; prednisolone; allergy

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Abbreviations

ED, Emergency department; IgE, Immunoglobulin E; IL-6, Interleukin 6; IL-33, Interleukin 33; PAF, Platelet activating factor; RCT, Randomized control trial; TNF α , Tumor necrosis factor α .

Introduction

Anaphylaxis is a “serious, generalized or systemic acute immunologic reaction” that is “rapid in onset and that would be fatal or life threatening” (1-3). Based on available data from international studies, the life-time prevalence of anaphylaxis has been estimated at 0.05 to 2% (4), with an estimated incidence ranging from 10 to 20/100,000 population per year (5-7). The incidence of anaphylaxis is also reportedly increasing worldwide, particularly food-induced anaphylaxis (8,9).

Summary

As anaphylaxis is a medical emergency, there are no randomized controlled clinical trials on its emergency management. Therefore, current guidelines are mostly based on data from observational studies, animal and laboratory studies. Although epinephrine is the mainstay of recommended treatment, corticosteroids are also frequently used. This review evaluates the evidence on the use of corticosteroids in emergency management of anaphylaxis from published human and animal or laboratories studies. Thirty original research papers were found with 22 human studies and eight animal or laboratory studies. The average rate of corticosteroid use in emergency treatment was 67.99% (range 48% to 100%). Corticosteroids appear to reduce the length of hospital stay, but did not reduce revisits to the emergency department. There was no consensus on whether corticosteroids reduce biphasic anaphylactic reactions. None of the human studies had sufficient data to compare the response to treatment in different treatment groups (i.e. corticosteroids, epinephrine, antihistamines). Animal studies demonstrated that corticosteroids act through multiple mechanisms. These modulate gene expression, with effects becoming evident 4 to 24 hours after administration. A much quicker response has been detected within 5 to 30 minutes, through blockade of signal activation of glucocorticoid receptors independent of their genomic effects. Therefore, we conclude that there is no compelling evidence to support or oppose the use of corticosteroid in emergency treatment of anaphylaxis. However, based on the available data, it appears to be beneficial and there was no evidence of adverse outcomes related to the use of corticosteroids in emergency treatment of anaphylaxis.

Anaphylaxis is brought about by direct or indirect activation of mast cells. Anaphylaxis classically involves the skin (80%), respiratory (70%), gastrointestinal (30-45%), cardiovascular (10-45%) and central nervous (10-15%) systems (2,6,10-12). Symptoms generally appear suddenly, progress over minutes to hours and increase in severity. Although only one organ system may be initially involved, symptoms will typically progress to eventually involve at least two organ systems (13,14). The diagnosis of anaphylaxis relies heavily on clinical judgment due to

the lack of availability of rapid diagnostic tests (13,15). Therefore, given the heterogeneity of presentation, there are widespread concerns regarding under diagnosis, under reporting and inadequate treatment of anaphylaxis and non-adherence to management guidelines (16-18).

As anaphylaxis is a medical emergency, conducting randomised control trials (RCT) on treatment of anaphylaxis is practically and ethically problematic. A Cochrane review published in 2013 concluded that there is no evidence from high quality studies for the use of steroids in the emergency management of anaphylaxis (19). The evidence base underpinning the currently recommended first-line of treatment epinephrine is also based on observational studies and extrapolated from laboratory studies. Although not as robust as evidence from RCTs, a systematic analysis of published data on other treatment modalities in the emergency management of anaphylaxis could lead to beneficial inferences.

Pathogenesis of anaphylaxis

The underlying pathogenesis of classical anaphylaxis involves IgE, synthesized in response to exposure to an allergen, becoming fixed to a high affinity receptors for IgE (FcεRI receptors) on the surface membranes of mast cells and basophils (11). On re-exposure to the same allergen, receptor-bound IgE molecules aggregate and results in cell activation. Activation of multiple tyrosine kinases (i.e. Lyn, Syk, and Fyn) with both positive and negative regulatory responses on the signal transduction cascade (20) leads to calcium influx in to the cells leading to cell degranulation (21,22). Mast cells and basophils release preformed chemical mediators and those that are synthesized de novo. Preformed mediators include histamine, tryptase, carboxypeptidase A, and proteoglycans. Inflammatory cytokines, such as IL-6, IL-33 (21,22) and TNF-α, a late-phase mediator are also released from mast cells as preformed mediators. Downstream activation of phospholipase A2 (PLA2), cyclooxygenases and lipoxygenases, leads to the production of arachidonic acid metabolites such as leukotrienes, prostaglandins and platelet activating factor (PAF). Furthermore, IgE enhances expression of FcεRI receptors on mast cells and basophils and increases the intensity of anaphylaxis.

In addition to this, non-IgE mediated mechanisms have also been implicated in anaphylaxis. IgG mediated anaphylaxis has been reported due to triggers such as high molecular weight iron dextran, infusion of chimeric and therapeutic monoclonal antibodies such as infliximab (23,24). Hemodialysis, liposomal drugs, polyethylene glycols and heparin contaminated with over sulfated chondroitin sulfate have been reported to cause complement-mediated anaphylaxis by generation of kallikrein, bradykinin, and complement protein-derived anaphylatoxins C3a and C5a (25). Factor XII and the coagulation system are also

postulated to be involved. There could also be a direct activation of the innate immune system triggering anaphylaxis as in peanut allergies (26). Idiopathic anaphylaxis has been described in some individuals in whom FcεRI receptors may aggregate through autoimmune mechanisms (27). The mechanism by which certain triggers such as exercise, cold air or water exposure and radiation induce anaphylaxis is not yet fully understood. Irrespective of the initiating mechanism, mast cells and basophils play a central role in mediating an anaphylactic reaction. The release of cellular mediators leads to end-organ responses in the skin, respiratory tract, cardio-vascular system, gastrointestinal tract and perhaps the nervous system (14). Most anaphylactic reactions are uniphasic. However, additional patterns of reactions have been described i.e. delayed onset, biphasic reactions and protracted or persistent reactions (28). In biphasic reactions, the initial reaction is followed by a relatively symptom-free period and the symptoms recur, often in a more severe and refractory to therapy form (29). The exact mechanism involved in each of these patterns is not yet fully understood.

Guidelines on treatment of anaphylaxis

Currently, the recommended mainstay of therapy in the event of an anaphylaxis is epinephrine given either intramuscularly or intravenously in specialist settings (14,30-33). It counters most of the pathophysiological processes giving rise to anaphylaxis (11,34). Second-line treatments include corticosteroids, H1- and H2-antihistamines, and bronchodilators. Unlike with epinephrine, there are differing recommendations regarding the use of glucocorticoids and other additional therapies in emergency management of anaphylaxis (35). Recommended emergency treatment of anaphylaxis according to recent guidelines is summarized in **table 1**.

The American (13) guidelines state that there is no place for glucocorticoids in emergency management of anaphylaxis. The British (36), European (30), Australasian (33), Canadian (37) and the World Allergy Organisation (31) guidelines recommend glucocorticoids as a second-line / adjuvant therapy after initial treatment with adrenaline and acknowledge the lack of robust evidence to support this practice (13,30,31,38). The recommended type, route, dose and duration of therapy of glucocorticoids are also varied. Prednisolone, methylprednisolone, dexamethasone and hydrocortisone administered orally, intravenously or intramuscularly are advocated in different guidelines (35). These are given as a single dose or continued for few days as a short course after the initial event (2,35,39).

Mechanism of action of glucocorticoids in anaphylaxis

The use of glucocorticoids in anaphylaxis is supported by the logical deduction of how the mechanism of action of glucocor-

Table 1 - Summary guidelines on emergency management of anaphylaxis.

Organisation, country, year of publication, reference	First line of therapy, route	Glucocorticoids				Other therapies
		Recommendation	Type	Route, dose	Dose	
World Allergy Organization, 2015	Adrenaline, IM	To prevent biphasic reactions. No effect on initial symptoms	Not given	Not given	Not given	Second or third-line: H1 antihistamines, H2-antihistamines
Australasian Society of Clinical Immunology and Allergy (ASCIA), Australia, 2015	Adrenaline, IM	Adjuvant	Prednisolone	Oral	1 mg/kg, maximum 50 mg daily	Glucagon, metaraminol, vasopressin
European Academy of Allergy and Clinical Immunology, 2014	Adrenaline, IM	Third-line	Not given Budesonide	Systemic, Nebulized	Not given Not given	Second-line: inhaled short-acting beta-2 agonists; Third-line: Oral H1- (and H2)-antihistamines
American Academy of Allergy, Asthma and Immunology (AAAAI) and the American College of Allergy, Asthma and Immunology (ACAAI), USA, 2014	Adrenaline, IM	Adjuvant, not effective in the acute management of anaphylaxis	Methylprednisolone	Intravenous	1 to 2 mg/kg	glucagon, b-agonist, H1 and/or H2 antihistamines
			Prednisone	Oral	1 mg/kg, up to 50 mg	
Canadian Paediatric Society, Canada, 2010	Adrenaline, IM	Second-line	Prednisone	Oral	1 mg/kg PO (maximum 75 mg)	second-line agents: cetirizine, diphenhydramine, ranitidine, salbutamol, glucagon
			Methylprednisolone	Intravenously	1 mg/kg IV (maximum 125 mg)	
Working Group of the Resuscitation Council, UK, 2008	Adrenaline, IM	Second-line	Hydrocortisone	Slow intravenous or intramuscular	200 mg	Adjuvant therapies: salbutamol (inhaled or IV), ipratropium (inhaled), aminophylline (IV) or magnesium (IV). When initial resuscitation with adrenaline and fluids has not been successful: noradrenaline, vasopressin, metaraminol and glucagon

ticoids counteracts the pathophysiological processes in anaphylaxis. It is also drawn from evidence of their efficacy in treatment of diseases mediated by similar immunological responses such as asthma (40,41). An illustration of the mechanism of glucocorticoids in anaphylaxis is given in **figure 1**.

Glucocorticoids are potent inhibitors of inflammatory processes and potent anti-allergic compounds reducing the number, maturation and activation of mast cell, which play a central role in anaphylaxis (42-44). They act through modulation of gene expression, and therefore require 4 to 6 hours for the effects to manifest (45). These anti-inflammatory effects are mediated by direct binding of the glucocorticoid / glucocorticoid receptor complex to specific elements in the promoter region of genes, or by interacting with other transcription factors such as the activating protein-1 or nuclear factor-kappa B (40,41,46). For example, in the mast cells, glucocorticoids down-regulate transcription of pro-inflammatory molecules such as cytokines,

chemokines, arachidonic acid metabolites and directly regulate multiple signaling and adaptor molecules (47). These genomic effects of glucocorticoids are relatively delayed with the maximal effect being detected at 2 hours (48). Thus, seemingly little evidence supports their use in emergency management of anaphylaxis, as many aspects of glucocorticoids action were initially thought to be both time and transcription-dependent.

However, recent studies have shown that glucocorticoids also exert rapid non-genomic effects, which can be non-specific or specific (10,49,50), brought about by membrane interactions at high concentration (51) or mediated by interactions with intracellular receptors or membrane-bound receptors (52,53). In some cases, these are thought to be mediated through the classical steroid receptor that functions as a ligand-activated transcription factor. Two animal studies have shown that glucocorticoids have detectable inhibitory effects on anaphylaxis within 5 to 30 minutes, mostly through blockade of these glucocorticoid

Figure 1 - Pathogenesis of anaphylaxis and mechanism of action of glucocorticoids.

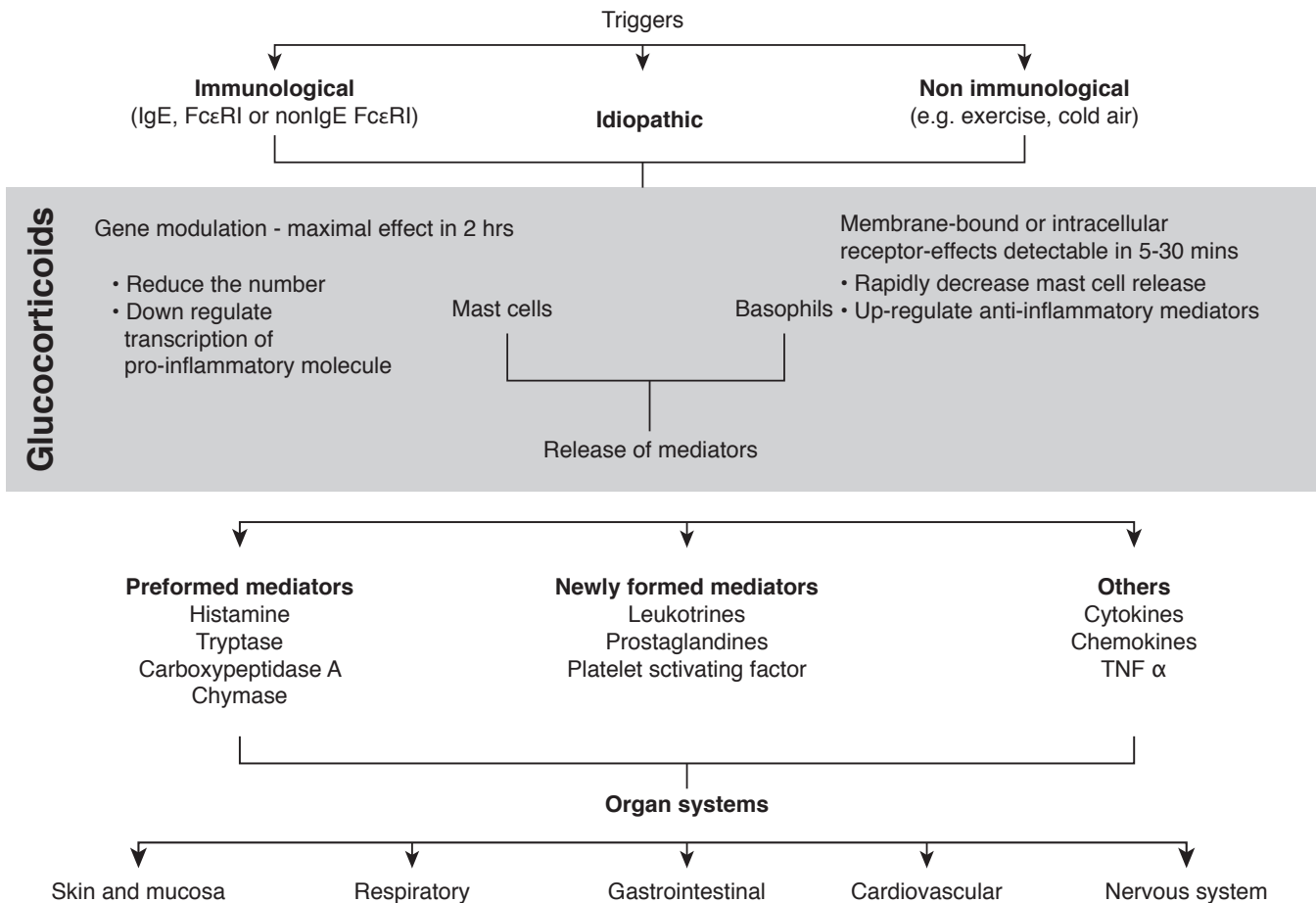
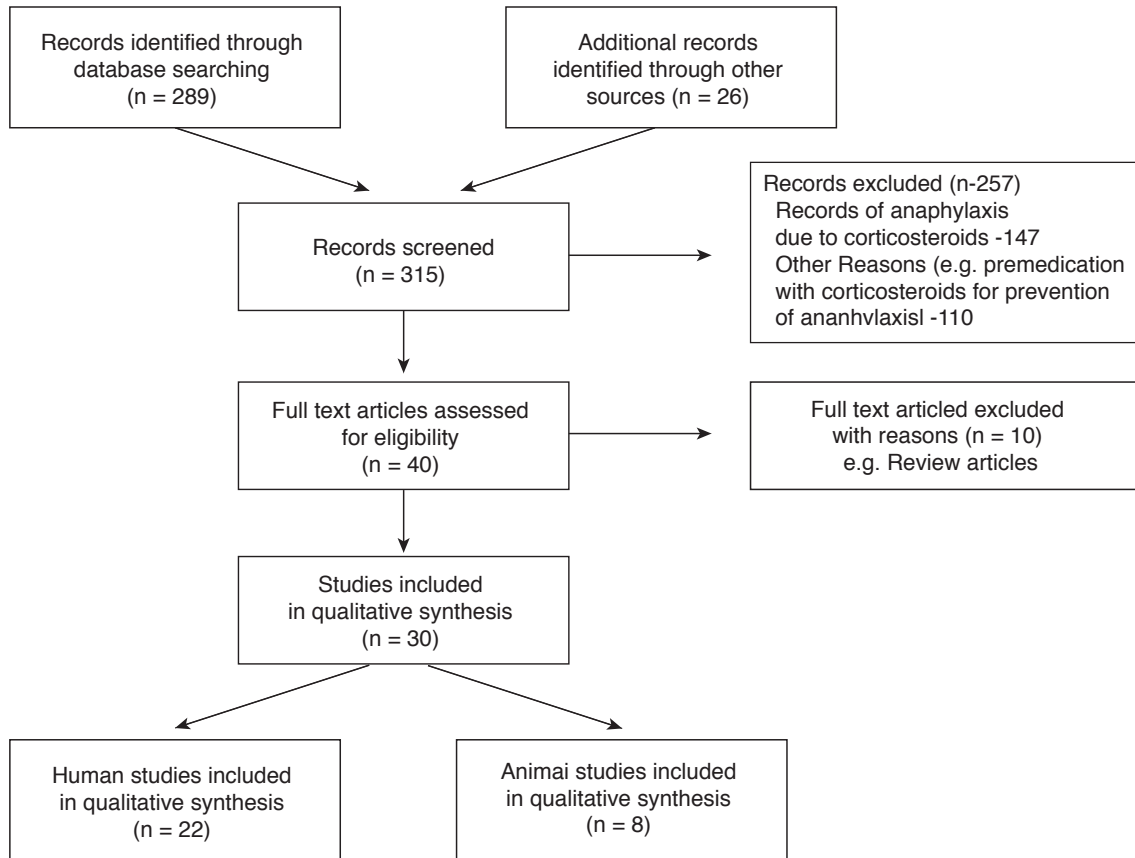


Figure 2 - Summary of search strategy and results.

receptors (52,53). There are diverse rapid effects of glucocorticoids, ranging from activation of adenylyl cyclase, mitogen-activated protein kinases (MAPKs), guanosine triphosphate-binding proteins, and protein kinase C (54). Glucocorticoids rapidly decrease histamine release from the mast cell surface and up-regulate anti-inflammatory mediators (46,49,55).

The lack of concrete evidence and the ethical and practical difficulties in conducting randomised control trials on acute management anaphylaxis is challenging for researchers and clinicians alike. This paper aims to review and systematically document the evidence on the use of corticosteroids in emergency treatment of anaphylaxis and identifies clinical research priorities.

Search strategy

Searched libraries included Pubmed / Medline from inception to March 2016. Additional references were found through cross-references from articles and reviews. The key words used included anaphylaxis, allergy, hypersensitivity, corticosteroids, glucocorticoids, steroids, dexamethasone, hydrocortisone, pred-

nisolone. Human and animal studies on glucocorticoids for management of allergy or anaphylaxis in peer-review journals were included. The search was refined by language (English).

Results

The original search found 289 articles in PubMed (**figure 2**). Twenty-six additional articles were identified through other sources. However, 147 of these were found to be on anaphylaxis due to corticosteroids. There were 28 relevant original research papers reviewed with 22 human studies and 8 animal or laboratory studies. The human studies included 19 retrospective cohort studies, one cross sectional descriptive study and two prospective cohort studies.

Use of corticosteroids in anaphylaxis: human studies

The relevant papers on human studies are summarised in **table 2**. The rate of corticosteroid use in emergency treatment of anaphylaxis varied from 48% to 100% with an average of 67.99%.

Biphasic anaphylactic reactions were reported in approximately 2.2% to 8.7% of patients reporting to emergency departments (56,57). A study conducted in a tertiary care hospital in Canada has found that those who develop biphasic reactions are less likely to have received epinephrine or corticosteroids during emergency management (58). However, three other studies have not found a significant difference in emergency treatment

with either epinephrine or steroids in those with or without biphasic reactions (56,59). There is some evidence suggesting that the length of hospital stay tends to be shorter in those treated with corticosteroids for anaphylaxis (60). However, it has not been found to reduce the revisits to the emergency department with anaphylaxis or other unrelated causes (61). None of the human studies had sufficient data to comment on the response

Table 2 - Use of corticosteroids in anaphylaxis; human studies.

Author, country, year	Study group	Study type	Percentage treated with steroids	Study objective	Findings relevant to corticosteroids	Study weakness
Jiang et al., China, 2016	1,952 cases of anaphylaxis reported in 907	Retrospective cohort study	72	Epidemiology, clinical features, possible triggers, treatment practices	Mild / moderate and severe reactions equally received corticosteroids (P = 0.118)	There was no comparison of the outcome of different treatment modalities
Michelson KA et al., USA, 2015	5203 patients (aged 1 month to 18 years) presenting to emergency departments at 35 hospitals	Retrospective cohort study	75.9	Association between glucocorticoid treatment and length of hospital stay and parenteral epinephrine beyond the first hospital day	Children receiving glucocorticoids were less likely to have prolonged length of hospital stay Glucocorticoid administration was associated with lower odds of epinephrine administration beyond the first hospital day Glucocorticoid use was not associated with less revisits to the emergency department	
Grunau BE et al., Canada, 2015	2701 patient with a discharge diagnosis code of "allergic reaction". Patients younger than 17 years of age have been excluded	Retrospective cohort study	48	Number of subsequent allergy-related ED visits within 7 days, all cause mortality, the number of clinically important biphasic reactions	Steroid administration does not prevent emergency department recidivism within 7 days	Data from only 2 urban centers has been included
Ko BS et al., Korea, 2015	655 patients with anaphylaxis admitted to the emergency department of a tertiary teaching hospital	Retrospective cohort study	100	Epidemiology, management, disposition, and clinical out-comes	Overall 87.2% were treated with methylprednisolone and 12.8% were treated with hydrocortisone Biphasic reaction occurred in 2.2% of the patients treated with steroids	Patients who have not been treated with steroids have been excluded from the study.

Author, country, year	Study group	Study type	Percentage treated with steroids	Study objective	Findings relevant to corticosteroids	Study weakness
Asia Y et al., Canada, 2014	37,730 patients with anaphylaxis visiting the emergency department of a tertiary care center	Retrospective cohort study	68.1	Demographic characteristics, suspected triggers and management of anaphylaxis	Higher percentage of severe reactions (71.4%) are treated with steroids compared to mild (50.0%) and moderate (67.8%) reactions.	The outcome of treatment is not assessed
Worm et al., Germany, 2014	3333 cases of anaphylaxis over a 3 year period from 10 European countries	Retrospective record analysis of one line data registry	60.4	Epidemiology, symptomatology, triggers, treatment	No additional data provided on steroid use or outcomes	There was no comparison of the outcome of different treatment modalities
Manuyakorn W et al., Thailand, 2013	160 children (aged 3 months to 18 years) diagnosed with anaphylaxis	Retrospective cohort study	93	Epidemiology, symptomatology, treatment practices	92.3% of patients with uniphasic reactions and 93.3% of patients with biphasic reactions are treated with systemic corticosteroids ($P > 0.05$)	Retrospective records analysis. The outcome of treatment is not stated
Rappo et al. Australia, 2013	34 cases anaphylaxis due to tick bite presenting an emergency department	Retrospective cohort study	97%	Epidemiology, symptomatology, course of illness, treatment	71% were discharged on oral prednisolone. One (4.1%) of them developed a biphasic reaction	Retrospective medical record review with a small sample size. There is no comparison of the outcome among the treatment groups
Vezir et al., Turkey, 2013	96 patients presenting to ED	Prospective cohort study	80.2	Epidemiology, symptomatology, triggers, treatment	Only 44.4% received epinephrine	
Hompes S. et al., Germany, 2011	1281 anaphylactic reactions voluntarily reported through an online registry	Cross sectional descriptive study	85	Demography, symptomatology, course of anaphylaxis, treatment	Corticosteroids were given intravenously in 50%, orally in 29%, and through rectal application in 6%.	
Hoffer et al., Israel, 2011	92 children admitted to a single medical center with the diagnosis of anaphylaxis	Retrospective cohort study	85	Epidemiology, symptomatology, course of illness, laboratory findings, treatment, concurrent illnesses	Only 72% were treated with epinephrine 75% received antihistamines	The outcome of the different treatment is not analyzed
Orhan et al., Turkey, 2011	224 cases of anaphylaxis reported in 137 children	Retrospective cohort study	83.5	Epidemiology, symptomatology, course of illness, treatment practices	Only 32.3% received epinephrine	
Sole et al., Brazil, 2011	634 patients from 15 Latin American countries and Portugal with severe allergic reactions	Cross sectional descriptive study	80.5	Epidemiology, symptomatology, triggers, course of illness, treatment, outcome		There is no comparison of outcome of different treatment

Author, country, year	Study group	Study type	Percentage treated with steroids	Study objective	Findings relevant to corticosteroids	Study weakness
Russell et al., USA, 2010	103 patients with the diagnosis of anaphylaxis visiting the emergency department	Retrospective descriptive study	79	Epidemiology, symptomatology, triggers, course of illness, treatment, outcome	Only 56% received intramuscular epinephrine	
De Swert et al., Belgium, 2008	64 cases of anaphylaxis	Prospective cohort study	45.6	Epidemiology, clinical features, triggers, treatment practices, outcome	Only 9.1% received epinephrine	Small sample size
Ellis AK & Day JH, Canada, 2007	134 patients with anaphylaxis (in-patients and outpatients) at a tertiary care center	Retrospective cohort study		Presence of biphasic reaction, course of illness, treatment	Biphasic reactors received less epinephrine and tended to receive less corticosteroid	
Gaeta et al., USA, 2007	12.4 million allergy-related emergency department visits with 1% being coded as anaphylaxis over a 9 year period	Retrospective cohort study	50	Epidemiology, symptomatology, triggers, treatment	Corticosteroid use increased during the study period (22% to 50%; $P < 0.001$) Epinephrine use was infrequent and declining (19% to 7%; $P = 0.04$)	
Clark et al., USA, 2005	617 with anaphylaxis presenting to the ED with in a one year period	Retrospective cohort study	49	Epidemiology, symptomatology, triggers, treatment	Only 7% were treated with epinephrine	
Clark et al., USA, 2004	A random sample of 678 charts of patients who presented with food allergy	Retrospective cohort study	48	Epidemiology, symptomatology, triggers, treatment	Only 16% were treated with epinephrine	
Brown et., Australia, 2001	162 patients with acute allergic reactions and 142 patients with anaphylaxis	Retrospective cohort study	78	Epidemiology, clinical features, possible triggers, treatment practices, outcome	Only 40% received epinephrine	A retrospective record analysis
Lee JM & Greenes DS, USA, 2000	106 inpatient (108 anaphylaxis episodes)	Retrospective cohort study		Symptomatology, course of illness, treatment	Patients with or without biphasic reactions did not differ significantly in the incidence of initial epinephrine use, initial steroid use	
Stewart & Ewan, 1996	9 patients with anaphylaxis admitted to ED	Retrospective cohort study	77.8	Epidemiology, symptomatology, triggers, treatment practices, outcome		A patient record analysis with a small sample size

to treatment in different treatment groups (i.e. corticosteroids, epinephrine, antihistamines).

Use of corticosteroids in anaphylaxis; animal and laboratory studies

The findings of the relevant papers are summarized in **table 3**. The animal models used included guinea pigs (62-64) and mice (48,53,65,66). One other study was conducted using A549 human adenocarcinoma cell line (52). Most investigated the effect of pretreatment with glucocorticoids. The postulated mechanisms of action include action through modulation of gene expression occurring 4 to 24 hours after treatment with glucocorticoids (48,65) and blockade of signal activation of glucocorticoid receptors independent of its genomic effects (52,53). The latter response has been detectable within 5 to 30 minutes (52,53). Interestingly, prednisolone has been found to inhibit passive cutaneous anaphylaxis in a biphasic manner at 8-12 and 24 hours (48).

Discussion

Anaphylaxis is an acute immunologic reaction due to direct or indirect activation of mast cells. Prompt definitive management can be life saving, and any delays may result in a fatal outcome. Irrespective of the trigger, management of anaphylaxis is the same for all patients. Although epinephrine is clearly recommended as the first line treatment for management of anaphylaxis, human studies reviewed here revealed that usage of epinephrine either during pre-hospital or emergency care varies widely from 7-70% (12). This could be due to over-prudent hesitancy to administer epinephrine with the fear of serious adverse effects. Conversely, there is a lower threshold to initiate glucocorticoid therapy in patients with anaphylaxis. Despite the lack of any strong recommendations, 45-97% of the patients receive glucocorticoids in emergency management of anaphylaxis (16,39,67,68). Some multicenter trials have demonstrated that corticosteroids are still being administered as the first-line therapy instead of epinephrine (17,18,39).

When considering the pathogenesis of anaphylaxis and the mechanism of action of glucocorticoids, it becomes evident that glucocorticoids have a theoretical benefit in treatment of anaphylaxis. For many years, glucocorticoids were thought to act through nuclear receptors by modulating gene expression, hence having delayed onset of action. However, recent advancements have demonstrated that glucocorticoids induce a rapid anti-inflammatory effect by a non-genomic mechanism, acting through membrane-bound or cytosolic receptors. Laboratory studies have demonstrated a detectable response in as little as 5 minutes from the point of administration.

Most cases of anaphylaxis resolved after initial treatment. One to 20% will develop a biphasic reaction, a delayed recurrence of illness occurring hours after improvement of the symptoms (59,61,69). There are multiple factors that have been described to be associated with biphasic reactions including under-treatment and delay in treatment (58,59). The World Allergy Organisation recommends the administration of glucocorticoids to prevent such biphasic reactions, and states that there are no or minimal effects on initial symptoms (31). This is supported by laboratory studies, which demonstrate that prednisolone inhibits passive cutaneous anaphylaxis in a biphasic manner. However, except for one retrospective cohort study, none of the other human studies found that glucocorticoids lower the incidence or prevents the progression of symptoms.

Although observational studies reveal that those with more severe reactions are more likely to be given corticosteroids, there is no conclusive evidence to infer that early administration of corticosteroids prevents progression of symptoms (56,70). Additional benefits such as shortening the length of hospital stay and reducing the need to repeated epinephrine injections have also been attributed to glucocorticoid therapy in anaphylaxis. A major limitation in the human studies included in this review is the lack of data on the outcome of patients by the treatment received in the emergency department to make a meaningful comparison between treatment groups.

Conclusions

Corticosteroids are often used in the management of anaphylaxis and sometimes used as a first-line therapy instead of adrenaline, despite the lack of compelling evidence and guidelines recommending their use only as an adjuvant therapy. There are no randomised or quasi-randomised trials providing support to this practice. Nevertheless, the pathophysiological basis of anaphylaxis and the mechanism of action of glucocorticoids, particularly the recent evidence of the rapid non-genomic effects provide a rational basis for using corticosteroids in the emergency treatment of anaphylaxis. However, there is no concrete evidence to support or oppose the use of corticosteroids in emergency management of anaphylaxis, particularly as short-term use of glucocorticoids is seldom associated with serious adverse effects (71). Therefore, based on the animal / laboratory studies and human studies reviewed, we conclude that use of glucocorticoids along with administration of epinephrine in the emergency management of anaphylaxis is rational and may be beneficial. Although RCT may not being plausible, more evidence is needed on treatment and treatment outcomes through at least prospective cohort studies. These could provide valid data to evaluate the definitive place of glucocorticoids in the emergency management of anaphylaxis.

Table 3 - Use of corticosteroids in anaphylaxis; animal studies.

Author, Country, Year	Animal model	Study objective	Findings
Obiri et al., USA, 2011	PEP +/- mice	Effect of glucocorticoids in PEST-domain-enriched tyrosine phosphatase (PEP) in bone marrow derived mast cells	Glucocorticoid increased PEP expression in mast cells and only partially inhibited anaphylaxis. Glucocorticoid potently inhibited anaphylaxis when combined with the PEP inhibitor
Croxtall et al., 2000	A549 human adenocarcinoma cell line	Assess the rapidity changes induced by dexamethasone and the mechanism of action	Dexamethasone, inhibits the activation of AA release by EGF by a mechanism without the involvement of the suppression of cPLA2 expression. Blockade of signal activation by dexamethasone was detectable within 5 to 10 mins. The dose-dependence of this inhibitory effect of dexamethasone was the same at 5 min and 3 h. The rapid effect of glucocorticoids is mediated by occupation of glucocorticoid receptor
Miura et al., Japan, 1992	Rats with PCA and cutaneous reactions caused by histamine serotonin and leukotriene C4 elicited at the same time	Inhibitory mechanisms of glucocorticoids in immediate hypersensitivity reactions - passive cutaneous anaphylaxis (PCA) mediated by IgE antibodies and cutaneous reactions caused by histamine serotonin and leukotriene C4	Hydrocortisone, prednisolone and dexamethasone, inhibited all these reactions significantly. Hydrocortisone given 1-4 and 12 hours inhibited PCA significantly. Maximum inhibition observed at 2 hours. Maximum inhibitions of histamine, serotonin and LTC4-induced cutaneous reactions observed at 2 hours. Prednisolone inhibited PCA biphasically at 24 and 8-12 hours. Dexamethasone inhibited PCA persistently between 2 to 12 hours and maximally at 4 hours
Rong & Zhao-Gui, Changsha, 1989	Guinea pigs heart	The protective effect of H1 & H2 antagonists, adenosine and hydrocortisone on cardiac anaphylaxis	Hydrocortisone delayed the onset of arrhythmias and significantly reduced the duration of arrhythmias. When histamine receptor antagonists are used with hydrocortisone, a good protective effect can be achieved
Guhlmann et al., Germany, 1989	Guinea pigs suffering from anaphylactic shock		There was a lack of effect of dexamethasone on anaphylactic LTC4 generation in vivo
Inagaki et al., Japan, 1987	Rats injected with <i>Ascaris suum</i> extract serum	Effect of hydrocortisone, prednisolone and dexamethasone on IgE antibody-mediated homologous passive cutaneous anaphylaxis (PCA)	Injection sites were evaluated 30 minutes after injection. Glucocorticoids inhibited the PCA dose-dependently. They also inhibited the skin reactions caused by histamine, serotonin and LTC4 and reduced vascular permeability
King et al., USA, 1984	Outbred Wistar rats	Effect of glucocorticoids on intestinal anaphylaxis in the rat	Manifestations of anaphylaxis were abolished in rats previously treated with corticosteroids 48 and 24 before This was associated with depletion of RMCP-II and of MMC from the intestinal mucosa detectable at 4-24 hours after treatment
Andersson & Brattsand, Sweden, 1982	Guinea-pigs sensitized to two ovalbumin regimens	Effect of budesonide and hydrocortisone on histamine release from anaphylactically-shocked chopped lung fragments	Budesonide pretreatment reduced the capacity of anaphylactically-challenged chopped lung tissue to release histamine

References

- Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol.* 2004;113(5):832-6.
- Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease / Food Allergy and Anaphylaxis Network symposium. *Ann Emerg Med.* 2006;47(4):373-80.
- Sampson HB, Burks AW. Adverse reactions to foods. In: Middleton's allergy: principles and practice. Adkinson NF Jr B, Busse WW, Holgate ST, Lemanske RF Jr, Simons FER, editor. Mosby Elsevier Inc. 2009;1139-67.
- Lieberman P, Camargo CA, Jr., Bohlke K, Jick H, Miller RL, Sheikh A, et al. Epidemiology of anaphylaxis: findings of the American College of Allergy, Asthma and Immunology Epidemiology of Anaphylaxis Working Group. *Ann Allergy Asthma Immunol.* 2006;97(5):596-602.
- Bohlke K, Davis RL, DeStefano F, Marcy SM, Braun MM, Thompson RS, et al. Epidemiology of anaphylaxis among children and adolescents enrolled in a health maintenance organization. *J Allergy Clin Immunol.* 2004;113(3):536-42.
- Yocum MW, Butterfield JH, Klein JS, Volcheck GW, Schroeder DR, Silverstein MD. Epidemiology of anaphylaxis in Olmsted County: A population-based study. *J Allergy Clin Immunol.* 1999;104(2 Pt 1):452-6.
- Mullins RJ. Anaphylaxis: risk factors for recurrence. *Clin Exp Allergy.* 2003;33(8):1033-40.
- Simons FE, Arduzzo LR, Bilo MB, Cardona V, Ebisawa M, El-Gamal YM, et al. International consensus on (ICON) anaphylaxis. *World Allergy Organ J.* 2014;7(1):9.
- Decker WW, Campbell RL, Manivannan V, Luke A, St Sauver JL, Weaver A, et al. The etiology and incidence of anaphylaxis in Rochester, Minnesota: a report from the Rochester Epidemiology Project. *J Allergy Clin Immunol.* 2008;122(6):1161-5.
- Duval D, Durant S, Homo-Delarche F. Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. *Biochim Biophys Acta.* 1983;737(3-4):409-42.
- Simons FE. Anaphylaxis. *J Allergy Clin Immunol.* 2010;125(2 Suppl 2):S161-81.
- Gaspar A, Santos N, Piedade S, Santa-Marta C, Pires G, Sampaio G, et al. One-year survey of paediatric anaphylaxis in an allergy department. *Eur Ann Allergy Clin Immunol.* 2015;47(6):197-205.
- Campbell RL, Li JT, Nicklas RA, Sadosty AT, Members of the Joint Task F, Practice Parameter W. Emergency department diagnosis and treatment of anaphylaxis: a practice parameter. *Ann Allergy Asthma Immunol.* 2014;113(6):599-608.
- Simons FE. Anaphylaxis: Recent advances in assessment and treatment. *J Allergy Clin Immunol.* 2009;124(4):625-36; quiz 37-8.
- Simons FE, Arduzzo LR, Bilo MB, El-Gamal YM, Ledford DK, Ring J, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J.* 2011;4(2):13-37.
- Gaeta TJ, Clark S, Pelletier AJ, Camargo CA. National study of US emergency department visits for acute allergic reactions, 1993 to 2004. *Ann Allergy Asthma Immunol.* 2007;98(4):360-5.
- Clark S, Long AA, Gaeta TJ, Camargo CA, Jr. Multicenter study of emergency department visits for insect sting allergies. *J Allergy Clin Immunol.* 2005;116(3):643-9.
- Clark S, Bock SA, Gaeta TJ, Brenner BE, Cydulka RK, Camargo CA, et al. Multicenter study of emergency department visits for food allergies. *J Allergy Clin Immunol.* 2004;113(2):347-52.
- Choo KJ, Simons FE, Sheikh A. Glucocorticoids for the treatment of anaphylaxis. *Evid Based Child Health.* 2013;8(4):1276-94.
- Samant SA, Campbell RL, Li JT. Anaphylaxis: diagnostic criteria and epidemiology. *Allergy Asthma Proc.* 2013;34(2):115-9.
- Peavy RD, Metcalfe DD. Understanding the mechanisms of anaphylaxis. *Curr Opin Allergy Clin Immunol.* 2008;8(4):310-5.
- Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. *J Allergy Clin Immunol.* 2009;124(4):639-46; quiz 47-8.
- Cheifetz A, Smedley M, Martin S, Reiter M, Leone G, Mayer L, et al. The incidence and management of infusion reactions to infliximab: a large center experience. *Am J Gastroenterol.* 2003;98(6):1315-24.
- Zanoni G, Puccetti A, Dolcino M, Simone R, Peretti A, Ferro A, et al. Dextran-specific IgG response in hypersensitivity reactions to measles-mumps-rubella vaccine. *J Allergy Clin Immunol.* 2008;122(6):1233-5.
- Kishimoto TK, Viswanathan K, Ganguly T, Elankumaran S, Smith S, Pelzer K, et al. Contaminated heparin associated with adverse clinical events and activation of the contact system. *N Engl J Med.* 2008;358(23):2457-67.
- Khodoun M, Strait R, Orekov T, Hogan S, Karasuyama H, Herbert DR, et al. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol.* 2009;123(2):342-51.
- Lenchner K, Grammer LC. A current review of idiopathic anaphylaxis. *Curr Opin Allergy Clin Immunol.* 2003;3(4):305-11.
- Limb SL, Starke PR, Lee CE, Chowdhury BA. Delayed onset and protracted progression of anaphylaxis after omalizumab administration in patients with asthma. *J Allergy Clin Immunol.* 2007;120(6):1378-81.
- Stark BJ, Sullivan TJ. Biphasic and protracted anaphylaxis. *J Allergy Clin Immunol.* 1986;78(1 Pt 1):76-83.
- Muraro A, Roberts G, Worm M, Bilo MB, Brockow K, Fernandez Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy.* 2014;69(8):1026-45.
- Simons FE, Ebisawa M, Sanchez-Borges M, Thong BY, Worm M, Tanno LK, et al. 2015 update of the evidence base: World Allergy Organization anaphylaxis guidelines. *World Allergy Organ J.* 2015;8(1):32.
- Lieberman P, Nicklas RA, Oppenheimer J, Kemp SF, Lang DM, Bernstein DI, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. *J Allergy Clin Immunol.* 2010;126(3):477-80e1-42.
- Vale S, Smith J, Said M, Mullins RJ, Loh R. ASCIA guidelines for prevention of anaphylaxis in schools, pre-schools and childcare: 2015 update. *J Paediatr Child Health.* 2015;51(10):949-54.
- Perino A, Galimberti M, Bilo MB, Asero R, Pezzuto F, Guidelines ACfUoAiA. Use of adrenaline in allergy. *Eur Ann Allergy Clin Immunol.* 2008;40(2):35-52.
- Alrasbi M, Sheikh A. Comparison of international guidelines for the emergency medical management of anaphylaxis. *Allergy.* 2007;62(8):838-41.
- Burton C, Worth A. UK Resuscitation Council guidelines on emergency treatment of anaphylactic reactions: a primary care perspective. *Prim Care Respir J.* 2008;17(2):60-1.

37. Wasserman S, Chad Z, Francoeur MJ, Small P, Stark D, Vander Leek TK, et al. Management of anaphylaxis in primary care: Canadian expert consensus recommendations. *Allergy*. 2010;65(9):1082-92.
38. Soar J, Pumphrey R, Cant A, Clarke S, Corbett A, Dawson P, et al. Emergency treatment of anaphylactic reactions - guidelines for healthcare providers. *Resuscitation*. 2008;77(2):157-69.
39. Russell S, Monroe K, Losek JD. Anaphylaxis management in the pediatric emergency department: opportunities for improvement. *Pediatr Emerg Care*. 2010;26(2):71-6.
40. van der Velden VH. Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma. *Mediators Inflamm*. 1998;7(4):229-37.
41. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *N Engl J Med*. 2005;353(16):1711-23.
42. Rider LG, Hirasawa N, Santini F, Beaven MA. Activation of the mitogen-activated protein kinase cascade is suppressed by low concentrations of dexamethasone in mast cells. *J Immunol*. 1996;157(6):2374-80.
43. Eklund KK, Humphries DE, Xia Z, Ghildyal N, Friend DS, Gross V, et al. Glucocorticoids inhibit the cytokine-induced proliferation of mast cells, the high affinity IgE receptor-mediated expression of TNF-alpha, and the IL-10-induced expression of chymases. *J Immunol*. 1997;158(9):4373-80.
44. Finotto S, Mekori YA, Metcalfe DD. Glucocorticoids decrease tissue mast cell number by reducing the production of the c-kit ligand, stem cell factor, by resident cells: in vitro and in vivo evidence in murine systems. *J Clin Invest*. 1997;99(7):1721-8.
45. Krishnan JA, Davis SQ, Naureckas ET, Gibson P, Rowe BH. An umbrella review: corticosteroid therapy for adults with acute asthma. *Am J Med*. 2009;122(11):977-91.
46. Oppong E, Flink N, Cato AC. Molecular mechanisms of glucocorticoid action in mast cells. *Mol Cell Endocrinol*. 2013;380(1-2):119-26.
47. Heiman AS, Crews FT. Hydrocortisone selectively inhibits IgE-dependent arachidonic acid release from rat peritoneal mast cells. *Prostaglandins*. 1984;27(2):335-43.
48. Miura T, Inagaki N, Yoshida K, Nakajima T, Nagai H, Koda A. Mechanisms for glucocorticoid inhibition of immediate hypersensitivity reactions in rats. *Jpn J Pharmacol*. 1992;59(1):77-87.
49. Zhou J, Li M, Sheng CQ, Liu L, Li Z, Wang Y, et al. A novel strategy for development of glucocorticoids through non-genomic mechanism. *Cell Mol Life Sci*. 2011;68(8):1405-14.
50. Losel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol*. 2003;4(1):46-56.
51. Buttgereit F, Straub RH, Wehling M, Burmester GR. Glucocorticoids in the treatment of rheumatic diseases: an update on the mechanisms of action. *Arthritis Rheum*. 2004;50(11):3408-17.
52. Croxtall JD, Choudhury Q, Flower RJ. Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *Br J Pharmacol*. 2000;130(2):289-98.
53. Inagaki N, Miura T, Nagai H, Koda A. Inhibitory effects of glucocorticoids on increased vascular permeability caused by passive cutaneous anaphylaxis and some chemical mediators in rats. *Jpn J Pharmacol*. 1988;46(2):189-92.
54. Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE*. 2002;2002(138):re9.
55. Liu C, Zhou J, Zhang LD, Wang YX, Kang ZM, Chen YZ, et al. Rapid inhibitory effect of corticosterone on histamine release from rat peritoneal mast cells. *Horm Metab Res*. 2007;39(4):273-7.
56. Manuyakorn W, Benjaponpitak S, Kamchaisatian W, Vilaiyuk S, Sasisakulporn C, Jotikasthira W. Pediatric anaphylaxis: triggers, clinical features, and treatment in a tertiary-care hospital. *Asian Pac J Allergy Immunol*. 2015;33(4):281-8.
57. Ko BS, Kim WY, Ryoo SM, Ahn S, Sohn CH, Seo DW, et al. Biphasic reactions in patients with anaphylaxis treated with corticosteroids. *Ann Allergy Asthma Immunol*. 2015;115(4):312-6.
58. Ellis AK, Day JH. Incidence and characteristics of biphasic anaphylaxis: a prospective evaluation of 103 patients. *Ann Allergy Asthma Immunol*. 2007;98(1):64-9.
59. Lee JM, Greenes DS. Biphasic anaphylactic reactions in pediatrics. *Pediatrics*. 2000;106(4):762-6.
60. Michelson KA, Monuteaux MC, Neuman MI. Glucocorticoids and Hospital Length of Stay for Children with Anaphylaxis: A Retrospective Study. *J Pediatr*. 2015;167(3):719-24 e1-3.
61. Grunau BE, Li J, Yi TW, Stenstrom R, Grafstein E, Wiens MO, et al. Incidence of clinically important biphasic reactions in emergency department patients with allergic reactions or anaphylaxis. *Ann Emerg Med*. 2014;63(6):736-44 e2.
62. Andersson P, Brattsand R. Protective effects of the glucocorticoid, budesonide, on lung anaphylaxis in actively sensitized guinea-pigs: inhibition of IgE-but not of IgG-mediated anaphylaxis. *Br J Pharmacol*. 1982;76(1):139-47.
63. Qiu R, Guo ZG. Protective effects of histamine H1 and H2 antagonists, adenosine and hydrocortisone on cardiac anaphylaxis. *Zhongguo Yao Li Xue Bao*. 1989;10(1):34-40.
64. Guhlmann A, Keppler A, Kastner S, Krieter H, Bruckner UB, Messmer K, et al. Prevention of endogenous leukotriene production during anaphylaxis in the guinea pig by an inhibitor of leukotriene biosynthesis (MK-886) but not by dexamethasone. *J Exp Med*. 1989;170(6):1905-18.
65. King SJ, Miller HR, Newlands GF, Woodbury RG. Depletion of mucosal mast cell protease by corticosteroids: effect on intestinal anaphylaxis in the rat. *Proc Natl Acad Sci USA*. 1985;82(4):1214-8.
66. Obiri DD, Flink N, Maier JV, Neeb A, Maddalo D, Thiele W, et al. PEST-domain-enriched tyrosine phosphatase and glucocorticoids as regulators of anaphylaxis in mice. *Allergy*. 2012;67(2):175-82.
67. Simons FE. Emergency treatment of anaphylaxis. *BMJ*. 2008;336(7654):1141-2.
68. Simons FE. Pharmacologic treatment of anaphylaxis: can the evidence base be strengthened? *Curr Opin Allergy Clin Immunol*. 2010;10(4):384-93.
69. Lieberman P. Biphasic anaphylactic reactions. *Ann Allergy Asthma Immunol*. 2005;95(3):217-26; quiz 26,58.
70. Asai Y, Yanishevsky Y, Clarke A, La Vieille S, Delaney JS, Alizadehfar R, et al. Rate, triggers, severity and management of anaphylaxis in adults treated in a Canadian emergency department. *Int Arch Allergy Immunol*. 2014;164(3):246-52.
71. Chrousos G, Pavlaki AN, Magiakou MA. Glucocorticoid Therapy and Adrenal Suppression. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, et al., editors. *Endotext*. South Dartmouth (MA)2000.

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The link between chronic spontaneous urticaria and metabolic syndrome

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KEY WORDS

cardiovascular risk; chronic urticaria; chronic spontaneous urticaria; metabolic syndrome; obesity

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Summary

Metabolic syndrome (MS) is a cluster of risk factors for cardiovascular disease and is considered a chronic low-level systemic inflammatory condition. Recent preliminary findings have shown an increased prevalence of MS among patients with chronic urticaria (CU) as compared to controls, with a particularly higher prevalence detected in patients with uncontrolled CU. Chronic spontaneous urticaria (CSU) appears to share some pathomechanisms with MS, including a pro-inflammatory state, increased oxidative stress, alterations in adipokine profile and activation of the coagulation system. Further studies are needed to assess the association of MS and its components with CU/CSU and to obtain more precise information regarding epidemiological aspects, clinical significance and implications. The aim of this review is to present the most relevant literature data on the link between CU/CSU and MS.

Introduction

Chronic spontaneous urticaria

Chronic urticaria (CU) is characterized by recurrent wheals, angioedema, or both for at least six weeks. CU can be distinguished into two main groups depending on whether the lesions occur spontaneously [chronic spontaneous urticaria (CSU)] or are induced by specific physical-environmental stimuli [chronic inducible urticaria (CIndU)] (1).

Various aetiological factors and mechanisms have been implicated in the development of CSU, but a specific cause is unidentifiable in most cases. Disease pathogenesis remains incompletely understood, although an autoimmune basis is increasingly being recognized based on the presence of functional histamine-releasing autoantibodies in a subset of patients (2,3). The autologous serum skin test (ASST) is a simple screening tool able to detect autore-

activity related to the presence of circulating histamine-releasing factors of any type, and not only of functional autoantibodies (1). Current evidence supports the possible contribution of other pathomechanisms, including the dysregulation of intracellular signalling pathways in basophils and mast cells, an abnormal innate immunity response, and the simultaneous activation of inflammatory response and coagulation system (2-4). With regard to the last aspect in more detail, significantly increased circulating levels of inflammatory markers [such as C-reactive protein (CRP) and interleukin (IL)-6], fibrin degradation products and D-dimer have been associated with the active phase of CSU, in correlation with disease severity (5). The activation of coagulation in CSU is likely to take place through the involvement of eosinophils and tissue factor pathway with thrombin generation and increased vascular permeability (6). An elevated oxidative stress level was demonstrated in patients with CSU (7-9) in parallel with systemic inflammation (10).

Nevertheless, the various mechanisms proposed so far individually cannot explain all the cases of CSU. CSU can be considered a heterogeneous multifactorial condition resulting from a complex interplay between the different pathogenic pathways and/or consisting of various subgroups driven by different pathomechanisms.

Metabolic syndrome

Metabolic syndrome (MS) is estimated to affect approximately 20-25% of the adult population and is a cluster of risk factors for cardiovascular disease, including atherogenic dyslipidaemia, glucose intolerance, arterial hypertension and central obesity. In particular, this syndrome has been found to cause a 5-fold increase in the risk of type 2 diabetes mellitus and 2-fold increase in the risk of cardiovascular disease over the next 5 to 10 years (11). Among the various definitions of MS, the most widely accepted and used are those developed by the World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII), the American Association for Clinical Endocrinology (AACE) and the International Diabetes Federation (IDF) (12,13). Insulin resistance is a required criterion for diagnosis in the WHO definition. According to the NCEP-ATP III 2001 definition, MS can be diagnosed in the presence of three or more of the following criteria: waist circumference ≥ 102 cm in men or ≥ 88 cm in women; triglyceride level ≥ 150 mg/dl; high-density lipoprotein (HDL) cholesterol level < 40 mg/dl in men or < 50 mg/dl in women; blood pressure $\geq 130/85$ mmHg; and fasting plasma glucose level ≥ 100 mg/dl (14).

In addition, the syndrome encompasses a systemic prothrombotic and pro-inflammatory state as relevant features with endothelial dysfunction and hypercoagulability (12), and can be considered a chronic low-level systemic inflammatory condition. Elevated circulating cytokines and acute-phase reactants, and abnormalities in clotting and antifibrinolytic factors have been found, such as high levels of IL-1, IL-6, tumor necrosis factor (TNF)-alpha, CRP, fibrinogen, tissue factor, factor VII, and plasminogen activator inhibitor (PAI)-1. Adipose tissue plays a crucial role in conditioning the prothrombotic risk, as well as systemic inflammation, through an altered secretion of adipokines that enhance and sustain the inflammatory response and the hepatic production of CRP (12,15).

The role of oxidative stress in MS components, as well as in the onset of MS-related cardiovascular complications, has been remarked (16). A linear increase in the concentration of the thrombotic marker D-dimer was detected in MS, in correlation with chronic inflammation, oxidative stress and altered hemorheology (17).

Methods

Considering the above-mentioned premises outlining possible shared inflammatory pathways between CSU/CU and MS, we reviewed the available literature on the association and interaction between such conditions. PubMed searches were conducted using the keywords “chronic urticaria” or “chronic spontaneous urticaria”, and “metabolic syndrome” and single MS components. The search was also extended to cover other relevant topics, such as “adipokines” and “cardiovascular risk”. Articles concerning heterogeneous patient populations without proper selection of CU or CSU patients (e.g. subjects with unspecified urticaria or dermatitis / urticaria) were excluded.

Association with MS or MS components

The link between CU and MS has been highlighted by a cross-sectional study carried out on 131 Korean patients with CU (18). MS, diagnosed using the NCEP-ATP III definition, was present in 29.8% of patients and 17.8% of subjects in the control group, giving rise to a statistically significant difference. Comparison of each component of MS between patients and controls showed a statistically higher frequency of central obesity, hypertriglyceridemia and hyperglycemia in CU patients. Compared with CU patients without MS, those with MS were found to be significantly older and more often males, to have more frequent negative ASST results, less frequent angioedema and higher urticaria activity score. Therefore, the presence of MS was more strictly associated with nonautoreactive form and correlated with disease severity but not with angioedema. Logistic regression analysis revealed that the presence of MS was an independent predictor of uncontrolled CU defined by the absence of symptom control after 3 months of treatment. Moreover, patients with CU and MS had higher serum levels of eosinophil cationic protein, TNF-alpha, and complement factors C3 and C4.

An investigation on multiple epidemiological features of CSU was conducted using the Health Search IMS Longitudinal Patient Database (HSD), that contains electronic medical records of patients aged ≥ 15 years with at least 1 year of medical history registered by 700 selected Italian general practitioners (19). In this population-based study, the risk of CSU was shown to be significantly increased (adjusted hazard ratio, 1.40; 95% confidence interval, 1.17-1.67) in the presence of obesity (ICD-9-CM code 278.0 or body mass index ≥ 30).

Rogala et al. (20) described the association between impaired glucose tolerance and recurrent angioedema without wheals. The authors noted that fasting plasma glucose levels, random blood glucose levels and oral glucose tolerance testing values were significantly higher in patients with angioedema alone as compared to CSU patients.

A relationship between CU and serum lipids and fatty acids was previously suggested by Kobayashi (21), who hypothesized the role of omega-6 and omega-3 series of polyunsaturated fatty acids and lipid peroxidation as mediators in CU.

The association between hyperlipidemia and CU has recently been evaluated by a case-control study using a population-based dataset in Taiwan, and involving 9,798 adults with CU and 9,798 sex- and age-matched controls (22). These subjects were examined for whether they had received a prior diagnosis of hyperlipidemia (pure hypercholesterolemia; pure hypertriglyceridemia; mixed hyperlipidemia; hyperchylomicronemia; other and unspecified hyperlipidemia). Compared to patients without CU, CU patients independently had a 1.65-fold (95% confidence interval, 1.55-1.76; $p < 0.001$) increased risk of having a prior diagnosis of hyperlipidemia, after adjusting for relevant covariates. The analysis of patients with atopic dermatitis matched by sex, age group and index year disclosed that atopic dermatitis was not associated with a previous hyperlipidemia diagnosis, ruling out a relationship of this diagnosis with inflammatory skin diseases in general.

A prospective study in a cohort of 228 consecutive adults with moderate to severe CSU revealed that blood hypertension was associated with extended duration of CSU and influenced disease remission (23). In particular, persistence of CSU after 5 years was still detected in 74% and 54% of CSU patients with and without hypertension, respectively.

The association between CSU and hypertension was examined by a retrospective cohort study of 2,460 patients with CSU and 9,840 age-, sex-, and index year-matched control patients, using the National Health Insurance of Taiwan database (24). The median follow-up periods were 7.13 years and 7.20 years for the CSU cohort and for the control group, respectively. Patients with CSU were found to have a 1.37-fold (95% confidence interval, 1.22-1.53) greater risk of developing subsequent hypertension than the non-CSU cohort after adjusting for sex, age, comorbidities, and non-sedating antihistamine use.

Role of adipokines

The association between CSU and MS as a whole or individual components is very interesting, and might support the existence of shared factors and/or mediators. Systemic inflammation is a phenomenon common for MS and CSU acting as a possible link between the two conditions.

In this context, the role of obesity appears to be particularly relevant.

In recent years, visceral adipose tissue, previously thought to be an inert tissue, has been shown to be an active secretory organ and a source of adipokines crucially involved in immunity and inflammation (25,26). As a consequence, a connection between obesity and autoimmunity has been hypothesized and

appears to be corroborated by several findings. Obesity may in fact contribute to the onset and progression of various autoimmune conditions. A systematic review disclosed that obesity significantly increases the risk of rheumatoid arthritis, multiple sclerosis, psoriasis and psoriatic arthritis, and worsens the course of rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, psoriasis and psoriatic arthritis (26).

Adipokines have been linked to the pathogenesis of MS and its comorbidities through their effects on vascular function and inflammation. Human mast cells may be also a direct target of adipokine activity (27,28).

Systemic inflammation generated during obesity is characterized by an oversecretion of inflammatory markers such as CRP, IL-6, IL-1beta, TNF-alpha, leptin and visfatin, with a simultaneous hyposecretion of anti-inflammatory and anti-atherogenic substances (12,15,29).

Trinh et al. recently described an imbalance in pro- and anti-inflammatory adipokines in CU. They assessed serum levels of adiponectin, leptin, lipocalin-2 (LCN2), IL-10, IL-6, and TNF-alpha in 191 CU patients and 89 healthy controls (30). In CU patients compared to controls, mean levels of serum LCN2, TNF-alpha, IL-6, and IL-10 were significantly higher, and adiponectin levels were significantly lower.

Adiponectin is one of the most abundant peptide hormones derived from adipose tissue and has anti-inflammatory and anti-oxidative properties. Adiponectin acts as a key regulator of innate immune system and progression of inflammation and metabolic disorders. This protein downregulates the expression and release of a number of pro-inflammatory immune mediators, plays a major role in glucose and lipid metabolism and prevents development of vascular changes. Hypoadiponectinemia is associated with a prothrombotic state, atherosclerosis, obesity, and MS (31-33).

LCN2, also known as neutrophil gelatinase-associated lipocalin (NGAL), is a secreted glycoprotein that belongs to the lipocalin family of proteins that transport small hydrophobic ligands, such as steroid hormones, lipids and retinoids, and has recently been characterized as a member of the adipokines superfamily (34). This adipokine plays a role in innate immunity and acute phase response to infection, and has been reported to have roles in the induction of apoptosis in hematopoietic cells, modulation of oxidative stress and inflammation, and metabolic homeostasis. LCN2 has been investigated as a diagnostic and prognostic biomarker in numerous pathological conditions, such as cancer, tissue injury, inflammation and autoimmunity (34,35). Recently, LCN2 has also been proposed as a possible biomarker for disease activity and clinical response to antihistamine therapy in CU. In particular, in the study performed by Trinh et al. (30), elevated levels of LCN2 in CU patients were inversely correlated with urticarial activity score, while LCN2 was significantly increased in patients with responsive CU compared to those with CU resistant to antihistamines.

Assessment of cardiovascular risk

Several immune-mediated inflammatory disorders, including rheumatoid arthritis, systemic lupus erythematosus, psoriatic disease and intestinal bowel diseases, have been associated with an elevated cardiovascular burden (25,36-39). The pathophysiological basis of such an increased risk is not completely understood, but MS and obesity in particular, with a dysregulated secretion of pro-inflammatory adipokines, could be major contributing features. Low-grade inflammation, that occurs in both CSU and MS, plays an important role in atherosclerosis.

In a nationwide Danish cohort using prospectively collected administrative data, the assessment of cardiovascular risk was performed in 2,215 patients with CU and 977 with CIndU (40). Patients were adults who received a first time diagnosis between 1997 and 2012, and were matched with healthy controls according to a 1:30 ratio. After adjustment for potential confounding factors, there were not significantly higher risks of myocardial infarction, ischaemic stroke, cardiovascular death, or major adverse cardiovascular events in the total population of patients with CU compared to controls. Similarly, no increased risk in any of the above-mentioned cardiovascular outcomes was seen in patients with CIndU, as well as in those with CSU.

Moreover, in patients with long-lasting moderate-severe CSU compared to healthy subjects, Grzanka et al. did not find any increase of circulating levels of matrix GLA protein, a biomarker of arterial calcification that is known to be overexpressed in patients with atherosclerosis (41).

Conclusions

Preliminary evidence from the limited data currently available seems to support the association between CU/CSU and MS. The presence of MS was shown to be associated with nonauto-reactive forms and to act as an independent predictor of severe and uncontrolled disease, suggesting the possible involvement of the low-grade inflammatory status related to MS in perpetuating and exacerbating the inflammatory processes underlying CSU pathogenesis. CSU appears to share some pathobiological pathways with MS, including a pro-inflammatory state, increased oxidative stress, alterations in adipokine profile and activation of the coagulation system.

The role of obesity appears to be of particular interest. It has been reported that the serum levels of mast cell-derived tryptase and the number of adipose tissue mast cells are increased in obese patients and high fat diet-fed mice, respectively (42,43). Mast cells in adipose tissue have been proposed to contribute to the pathophysiology of obesity and diabetes and related systemic inflammation (44).

MS and some components in particular (e.g. obesity) might also affect the response to the pharmacotherapy of CSU. Further

research is needed to assess the association with MS and the practical implications in terms of prognosis and treatment response among CSU patients. The influence of drugs used to manage MS components on CSU severity and course should also be defined.

While CU has been associated with MS components in recent reports, there appears to be no increased prevalence of cardiovascular disease among CU patients, possibly because of the relatively short disease duration, that is unable to confer a relevant arteriosclerotic risk following sustained low-level inflammation, unlike other chronic long-standing skin conditions, such as psoriasis (40).

Conflict of interest

During the last three years, G.A. Vena has been a speaker and/or an advisory board member for Novartis Farma and Pfizer, and N. Cassano has been a scientific consultant for Leo Pharma, Novartis Farma, and Pfizer.

References

1. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW et al. The EAACI/GAALEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*. 2014;69(7):868-87.
2. Najib U, Sheikh J. The spectrum of chronic urticaria. *Allergy Asthma Proc*. 2009;30(1):1-10.
3. Altman K, Chang C. Pathogenic intracellular and autoimmune mechanisms in urticaria and angioedema. *Clin Rev Allergy Immunol*. 2013;45(1):47-62.
4. Jain S. Pathogenesis of chronic urticaria: an overview. *Dermatol Res Pract*. 2014;2014:674709.
5. Kolkhir P, Andre F, Church MK, Maurer M, Metz M. Potential blood biomarkers in chronic spontaneous urticaria. *Clin Exp Allergy*. 2017;47(1):19-36.
6. Cugno M, Asero R, Tedeschi A, Lazzari R, Marzano AV. Inflammation and coagulation in urticaria and angioedema. *Curr Vasc Pharmacol*. 2012;10(5):653-8.
7. Raho G, Cassano N, D'Argento V, Vena GA, Zanotti F. Over-expression of Mn-superoxide dismutase as a marker of oxidative stress in lesional skin of chronic idiopathic urticaria. *Clin Exp Dermatol*. 2003;28(3):318-20.
8. Cassano N, Raho G, Filieri M, D'Argento V, Amoroso A, Filotico R, Vena GA. Influence of desloratadine on oxidative stress markers in patients with chronic idiopathic urticaria. *Int J Dermatol*. 2006;45(4):394-6.
9. Akbas A, Kilinc F, Sener S, Aktaş A, Baran P, Ergin M. Investigation of thiol-disulphide balance in patients with acute urticaria and chronic spontaneous urticaria. *Cutan Ocul Toxicol*. 2017;36(3):205-10.
10. Rajappa M, Chandrashekar L, Sundar I, Munisamy M, Ananthanarayanan PH, Thappa DM, Toi PCh. Platelet oxidative stress and systemic inflammation in chronic spontaneous urticaria. *Clin Chem Lab Med*. 2013;51(9):1789-94.
11. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint inter-

- im statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120(16):1640-5.
12. Kaur J. A comprehensive review on metabolic syndrome. *Cardiol Res Pract*. 2014;2014:943162.
 13. O'Neill S, O'Driscoll L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes Rev*. 2015;16(1):1-12.
 14. Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III): Executive summary of the third report of the national cholesterol education program (NCEP) JAMA 2001;285(19):2486-97.
 15. Matsuzawa Y. The metabolic syndrome and adipocytokines. *Expert Rev Clin Immunol*. 2007;3(1):39-46.
 16. Bonomini F, Rodella LF, Rezzani R. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis*. 2015;6(2):109-20.
 17. Gyawali P, Richards RS. Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome. *Redox Rep*. 2015;20(3):139-44.
 18. Ye Y-M, Jin H-J, Hwang E-K et al. Co-existence of chronic urticaria and metabolic syndrome: Clinical implications. *Acta Derm Venereol*. 2012; 93(2):156-60.
 19. Lapi F, Cassano N, Pegoraro V, Cataldo N, Heiman F, Cricelli I, Levi M, Colombo D, Zagni E, Cricelli C, Vena GA. Epidemiology of chronic spontaneous urticaria: results from a nationwide, population-based study in Italy. *Br J Dermatol*. 2016;174(5):996-1004.
 20. Rogala B, Bozek A, Glück J, Rymarczyk B, Jarzab J, Maurer M. Coexistence of angioedema alone with impaired glucose tolerance. *Int Arch Allergy Immunol*. 2014;165(4):265-9.
 21. Kobayashi S. Investigation of the roles of the substances in serum lipids and their constitutive fatty acids in chronic urticaria. *J Dermatol*. 1989;16(3):196-206.
 22. Chung SD, Wang KH, Tsai MC, Lin HC, Chen CH. Hyperlipidemia is associated with chronic urticaria: A population-based study. *PLoS One* 2016;11(3):e0150304.
 23. Nebiolo F, Bergia R, Bommarito L, Bugiani M, Heffler E, Carosso A, Castiglioni G, Guida G, Badiu I, Pizzimenti S, Mietta S, Ferrero N, Rolla G. Effect of arterial hypertension on chronic urticaria duration. *Ann Allergy Asthma Immunol*. 2009;103(5):407-10.
 24. Chang HW, Cheng HM, Yen HR, Hsu CY, Lee YC, Chiang JH, Sun MF. Association between chronic idiopathic urticaria and hypertension: A population-based retrospective cohort study. *Ann Allergy Asthma Immunol*. 2016;116(6):554-8.
 25. Abella V, Scotece M, Conde J, López V, Lazzaro V, Pino J, Gómez-Reino JJ, Gualillo O. Adipokines, metabolic syndrome and rheumatic diseases. *J Immunol Res*. 2014;2014:343746.
 26. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: Not a passive bystander. *Autoimmun Rev*. 2014;13(9):981-1000.
 27. Sismanopoulos N, Delivanis DA, Mavrommati D, Hatzigelaki E, Conti P, Theoharides TC. Do mast cells link obesity and asthma? *Allergy*. 2013;68(1):8-15.
 28. Taideman J, Pérez-Novo CA, Rottiers I, Ferdinande L, Waeytens A, De Colvenaer V, Bachert C, Demetter P, Waelput W, Braet K, Cuvelier CA. Human mast cells express leptin and leptin receptors. *Histochem Cell Biol*. 2009;131(6):703-11.
 29. Debnath M, Agrawal S, Agrawal A, Dubey GP. Metaflammatory responses during obesity: Pathomechanism and treatment. *Obes Res Clin Pract*. 2016;10(2):103-13.
 30. Trinh HK, Pham DL, Ban GY, Lee HY, Park HS, Ye YM. Altered systemic adipokines in patients with chronic urticaria. *Int Arch Allergy Immunol*. 2016;171(2):102-10.
 31. Fisman EZ, Tenenbaum A. Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? *Cardiovasc Diabetol*. 2014;13:103.
 32. Esfahani M, Movahedian A, Baranchi M, Goodarzi MT. Adiponectin: an adipokine with protective features against metabolic syndrome. *Iran J Basic Med Sci*. 2015;18(5):430-42.
 33. Luo Y, Liu M. Adiponectin: a versatile player of innate immunity. *J Mol Cell Biol*. 2016;8(2):120-8.
 34. Abella V, Scotece M, Conde J, Gómez R, Lois A, Pino J, Gómez-Reino JJ, Lago F, Mobasher A, Gualillo O. The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. *Biomarkers*. 2015;20(8):565-71.
 35. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. *Biochim Biophys Acta*. 2012;1826(1):129-69.
 36. Vena GA, Vestita M, Cassano N. Psoriasis and cardiovascular disease. *Dermatol Ther*. 2010;23(2):144-51.
 37. Frieder J, Ryan C. Psoriasis and cardiovascular disorders. *G Ital Dermatol Venereol*. 2016;151(6):678-93.
 38. Husni ME. Comorbidities in psoriatic arthritis. *Rheum Dis Clin North Am*. 2015;41(4):677-98.
 39. Michalak A, Mosińska P, Fichna J. Common links between metabolic syndrome and inflammatory bowel disease: Current overview and future perspectives. *Pharmacol Rep*. 2016;68(4):837-46.
 40. Egeberg A, Kofoed K, Gislason GH, Vestergaard C, Thyssen JP. Cardiovascular risk is not increased in patients with chronic urticaria: A retrospective population-based cohort study. *Acta Derm Venereol*. 2017;97(2):261-2.
 41. Grzanka A, Machura E, Misiolek M, Polaniak R, Kasperski J, Kasperska-Zajac A. Systemic inflammatory response and calcification markers in patients with long lasting moderate-severe chronic spontaneous urticaria. *Eur J Dermatol*. 2015;25(1):26-8.
 42. Liu J, Divoux A, Sun J, Zhang J, Clément K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med*. 2009;15(8):940-5.
 43. Moreno M, Puig J, Serrano M, Moreno-Navarrete JM, Ortega F, Ricart W, Fernandez-Real JM. Circulating tryptase as a marker for subclinical atherosclerosis in obese subjects. *PLoS One*. 2014;9(5):e0097014.
 44. Divoux A, Moutel S, Poitou C, Lacasa D, Veyrie N, Aissat A, Arock M, Guerre-Millo M, Clément K. Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. *J Clin Endocrinol Metab*. 2012;97(9):E1677-85.

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Assessment of allergen-specific IgE by immunoblotting method in atopic dermatitis

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Summary

Background and Objectives. Stimulating the immune system by exposure to various allergens to produce specific IgE has a significant role in the pathogenesis of atopic dermatitis. Identifying disease-causing allergens, prevention of exposure to those allergens, and immunotherapy will play an important role in the treatment of Atopic Disease. The purpose of this study was to determine the common allergens of northwest of Iran in patients with atopic dermatitis that are resistant to treatment. **Materials and methods.** In this descriptive-analytical study, serum levels of total IgE and frequency of specific IgE were measured by Immunoblotting against 20 common allergens in 150 cases of patients with atopic dermatitis, attending to dermatology and asthma and allergy clinics from 2010 to 2011. The control group consisted of individuals who had been clinically healthy. **Results.** In the 90% of patients that were included in this study, total IgE levels were higher than in healthy people with mean serum levels of total IgE 227.51 ± 103 IU/ml. 136 patients (90.6%) had specific IgE for at least one allergen. The frequency of positive allergens among the patients who were included in this study were 53.34%, 26.8%, and 19.56% respectively in plants and fungus allergens group, animal allergens group and food allergens group. After avoiding of the allergens (which they had been sensitized to), 60% of patients were cured with immune therapy, and total IgE serum levels in the control group were not increased. **Conclusion.** Identifying the abundant allergens such as cultivated rye, Timothy grass, house dust mite, birch, cat, horse, potato, dog, egg white, cow milk, in order to advise patients to avoid them or to do immunotherapy and desensitization, is useful in this area.

Introduction

Atopic dermatitis as a chronic inflammatory disease is characterized by papulovesicular and eczematous eruptions, which are recurrent and itchy. Because of severe eczema and nerve crushing itch, most of these patients are disabled in doing chores, typically have difficulties in their social relationships, and might also face some familial and mental problems as well.

This disease involves face, trunk and extensor surfaces in neonates and infants. However, the flexor surfaces of the extremities are in-

involved in adults and juveniles (1). It is associated with activation of different immunological and inflammatory pathways, and it leads to increasing of T lymphocytes, Langerhans cells (APC) stimulation, and overproduction of specific IgE by B cells. Exposure to different allergens, and subsequently stimulation of Langerhans cells and Ag presentation by them to Th2 cells, causes the release of IL4, IL5, IL9, IL10, IL13, which results in B cell proliferation and consequent increasing of the production of specific IgE. Specific IgE plays an important role in the allergic reactions of people who

are sensitive to the allergens, and can also exacerbate symptoms and stimulate the immune system more and more (2).

The overall prevalence rate is estimated about 10-15.6%, and it is about 20% for children (3). In an investigation that has been done in the United States, the prevalence of AD for school-age children has been estimated about 17% (4). In another investigation, the prevalence of atopic dermatitis in years before 1960 was reported about 2-3%; however, this parameter in 1960-70 has been increased up to 9-12% (5).

Half of the children with AD before age 7 have shown asthma or rhinitis (2). About 80% of the children with AD have shown inflammatory diseases, such as allergic rhinitis and asthma (6). Allergens have a major role in stimulating the immune system and causing the disease (7).

There are newer methods to measure the serum levels of specific IgE. It is typically measured by some methods such as Radioallergosorbent test (RAST) and Enzyme immunoassay test (EIA) (7). Specific IgE antibodies show diagnostic values against various allergens, and previous studies reported that the correlation of specific IgE level with the disease severity is more significant than the total IgE level (5).

Warner et al (1997) conducted a study on 817 children showing atopic dermatitis diagnose. They showed a direct relationship between increased total IgE level and the severity of the disease, by measuring total IgE and specific IgE (8).

In 2004, Perry et al studied the relationship between the specific IgE levels using the CAP_RAST and oral food challenge tests. Based on the test results on 391 children, they concluded that the measurement of specific IgE in milk, egg, peanut, wheat, soybean and use of oral food challenge tests is considered an effective method to determine the causes of the allergic disease and AD determination (9).

More recently, Hon et al in a study on 117 children below 18 years investigated to find whether any age-specific IgE levels in children with AD is associated with more severe symptoms or not. They have shown that high levels of age-specific IgE are associated with the development and severity of AD (10).

Almost in all of the previous works, the specific antibodies have been measured by some old methods and only in certain allergens in patients with AD. In this study, we measured the serum level of specific IgE for several allergens by immunoblotting, in order to identify the primary allergens that cause AD in the patients. We aim to measure the specific IgE level of twenty allergens in patients with atopic dermatitis who are resistant to the treatment by an immunoblotting method, in order to identify the prevalent allergens which are responsible for the disease.

Materials and methods

In this descriptive-analytical study, we aimed to determine total and specific serum level of IgE caused by twenty most fre-

quent allergens in 150 AD patients diagnosed, by using the Hanifin-Rajka criteria in order to evaluate the importance and frequency of that allergen. 150 healthy people subject to total IgE measurement, and 92 healthy individuals for specific IgE, were selected as the control groups. The study was approved by the ethical committee of Tabriz University of Medical Sciences.

Sampling

According to the standard principles, informed consent was obtained from all AD patients. Pregnant and breastfeeding women, and people with other diseases, were excluded from the study. Control groups included people who did not have AD and had been diagnosed healthy by a dermatologist. Samples were selected from atopic patients that referred to the dermatology clinics and asthma clinics in East Azerbaijan of Iran. The standardized questionnaire which includes the demographic information, personal or family history, disease duration, clinical symptoms, was filled by the patients. Serum samples from all patients prior to the treatment were taken in order to measure total IgE and specific IgE. Before analyzing total and specific IgE in serum, control and patient samples were stored at -80°C.

ELISA test

Total and specific IgE measured according to the manufacturer's instructions of kit (Monobind Inc, Lake Forest, USA). We measured serum levels with the predefined standard steps in the manual (i.e., 0, 10, 50, 100, 200, and 500). According to this investigation, we acquired the normal serum range, about 0 to 46 IU/mL, and 0 to 75 IU/mL, for infants (< 2) and adults respectively.

Immunoblotting test

We selected 20 allergens, including food allergens (egg whites, milk, fish, flowers, wheat, rice, soybeans, nuts, carrots, potatoes and apples), plant and fungal allergens (Timothy grass, cultivated rye, birch, sagebrush, Cladosporium, and Alternaria), animals (cats, horses, dogs, house dust mite). For the determination of specific IgE against allergens, the German business kit (Euroimmun) was used. In this method, patients' serum is tested directly and based on the manufacturer's instructions in the manual. Strips of blotting after staining are scanned and then analyzed with the Euroimmun's special software, **figure 1**.

At the same time, other discussed items, including some demographic information (age, gender, and occupation), primary and secondary standards and clinical symptoms of atopic dermatitis, were considered and evaluated. We used a descriptive statistical method to investigate the mean values, their standard deviations and evaluate the correlation among different factors that we have mentioned earlier. The analysis has been conducted by using the

Figure 1- Immunoblotting strip of Atopic patients.

	Concentration	Class	0	1	2	3	4	5	6
Timothy grass	1,4 kU/l								
Cultivated rye	19 kU/l								
Birch	0,35 kU/l								
Mugwort	0,35 kU/l								
Dermatophagoides pter.	< 0,35 kU/l								
Cat	< 0,35 kU/l								
Dog	< 0,35 kU/l								
Horse	< 0,35 kU/l								
Cladosporium herbarum	< 0,35 kU/l								
Alternaria alternata	< 0,35 kU/l								
Egg white	< 0,35 kU/l								
Cow milk	< 0,35 kU/l								
Codfish	< 0,35 kU/l								
Wheat flour	0,70 kU/l								
Rice	0,70 kU/l								
Soya bean	0,70 kU/l								
Hazelnut	0,58 kU/l								
Carrot	1,4 kU/l								
Potato	11,5 kU/l								
Apple	0,70 kU/l								
CCD maker	0,47 kU/l								

Statistical Product and Service Solutions (SPSS) software version 13.0, which is a powerful numerical tool for statistical studies. The standard chi-square test and Fisher's exact test are applied to the obtained data, to estimate the probability of exceeding.

Results

The mean age of 150 selected patients was 30.2 ± 14.7 years. Patients were classified into three sub-groups: 0-2 years old, i.e. infants (8.6%), 2-12 years old, i.e. children (11.4%), and 12 years and older, i.e. adults (80.0%). 51.3% of patients were male and 48.7% of patients were female. This study indicated that 100% of the youngest sub-group (i.e. infants) including itching considered as the most important factor in the Hanifin-Rajka criteria. The involved areas were face, neck, and the extensor areas, but flexor areas were not involved in any of the infants. Children and adult patients showed itching symptoms in these areas, as follows: 90.0% in flexor areas, 9.3% in the face, 3.1% in the neck, and 18.5% in the extensor areas. History of patients showed that 24 cases (16.0%) had experienced allergic diseases (asthma, allergic rhinitis) and 11 atopic allergy patients, including allergic rhinitis disease too (7.3%). The frequency of each sub-criterion is listed in **table 1**. Mean of serum levels of total IgE was 227.5 ± 103.0 . The mean of total IgE levels in men and

women was respectively 233.8 ± 97.1 , and 221.6 ± 111.5 . T-test results showed that there are no statistically significant differences between men and women in terms of total IgE levels ($p = 0.74$). Although, we must note the highly scattered serum levels around the mean values, and take into account this as we interpret these results. The mean value of the serum for the total IgE was 55.0 ± 15.5 in the control group. This suggests that 90% of the patients had total IgE levels higher than the mean serum value in the normal group. We report that 91% of the patients had specific IgE for at least one allergen. The patients, as mean to 3.06 ± 2.31 of the total of 20 tested allergens, had allergen-specific IgE. We found that the most abundant allergens related to AD are cultivated rye (48.6%), Timothy grass (42.6%), house dust mites (22.7%) dog (16.7%), birch (11.3%), potato (11.3%), horse (10%), hazelnut (10%), cat (10%), soybean (10%), sagebrush (10%). In contrast, we found that apple, carrot, fish, wheat, rice, Cladosporium herbarum, cow milk, egg white, Alternaria had a low frequency (**table 2**). The frequency of tested allergens by gender classification is presented in **table 2**. Cat allergens were significantly more frequent in women ($p = 0.02$). Alternaria was significantly more common in men than women ($p = 0.042$). The frequency of tested allergens in patients in plants and fungi group, animal group and food group was 54.34%, 26.08%, and 19.56%, respectively.

Table 1 - Distribution of patients with minor symptoms of atopic dermatitis.

Minor symptoms	Frequency %	Minor symptoms	Frequency %
Familial allergy background	24 (16)	Itching in body	150 (100)
Allergic rhinitis experiencing	51 (84)	Itching of the upper limbs	75 (50)
Associated Atopy with allergic rhinitis	11 (7.3)	Itching of the upper limbs	45 (80)
Disorders in digestive system	6 (4)	Itching in all over the body	30 (20)
Hives	6 (4)	Throat itching	6 (4)
Red rash	99 (66)	Ear itching	11 (7.3)
Eczema	135 (90)	Eye itching	9 (6)
Increased Total IgE	135 (90)		
Skin dryness	81 (54)		

Table 2 - Distribution of sensitivity to any of the tested allergens in patients with atopic dermatitis.

Allergens	Sum of patients (%)	Women (%)	Men (%)
Timothygrass	64 (42.6)	34 (46.5)	30 (38.9)
Cultivated rye	78 (48.6)	39 (53.4)	34 (44.1)
Birch	17 (11.33)	5 (6)	12 (5.5)
Sagebrush	15 (10)	7 (9)	8 (10.4)
Dermatophagoides pteronyssinus (house dust mite)	34 (22.7)	26 (35.6)	8 (10.4)
Cat	15 (10)	11 (15)	4 (5)
Dog	25 (16.7)	17 (23.2)	8 (10.4)
Horse	15 (10)	7 (9)	8 (10.4)
Cladosporium herbarum	9 (6)	5 (6)	4 (5)
Alternaria alternate	12 (8)	4 (5)	8 (10.4)
Egg white	13 (8.7)	8 (10.9)	5 (6.4)
Cow milk	13 (8.7)	10 (13.6)	3 (3.8)
Fish	4 (4.2)	4 (5)	0 (0)
Wheat	13 (8.7)	6 (%)	7 (%)
Rice	12 (8)	3 (%)	9 (%)
Soybean	15 (10)	9 (12.3)	6 (7.7)
Hazelnut	15 (10)	7 (9)	8 (10.4)
Carrot	11 (7.3)	5 (6)	6 (7.7)
Potato	17 (11.3)	12 (16.4)	5 (6.4)
Apple	11 (7.3)	5 (6)	6 (7.7)
Total numbers	150	78 (48.7)	77 (51.3)

Discussion

We have assessed the common allergens of northwest of Iran in patients with atopic dermatitis that were resistant to treatment. Specific IgE antibodies have diagnostic values against various allergens, and a correlation between specific IgE level and disease severity was reported, and is more significant than total IgE level (8). The importance of identifying the presence of IgE antibody to any allergen in patients with atopic dermatitis, even in the absence of other clinical manifestations, could consist in allowing the prevention of accidental exposure and in educating in such prevention (11).

We have used Immunoblotting method in this study, while skin prick test is rapid and low cost, but has some limitations in children lower than 4 years old, and in the patients who are using antihistamines or suffering from serious atopic eczema. Specific IgE tests are more reliable and reproducible for the entire patients, without side effect risks, but 1 to 2 days are needed to reach to the result. ImmunoCAP Immuno-Solid Phase Allergy Chip could assess multiple allergen components on one slide, providing more detailed information than single IgE testing, but its clinical effectiveness and cost-effectiveness need to be more investigated (12,13).

Our results show that atopic dermatitis can happen at any age, but it generally appears during childhood and infancy; it includes periods of exacerbations and improvement, but recovery will be achieved with increasing in age. These results are in agreement with the previous works (1,7,14). Kulthanan et al conducted a similar study in Thailand with a focus on the clinical symptoms of patients with AD. They reported an average age of the patients as 34.1 ± 11.7 years. According to the several previous studies the prevalence of atopic dermatitis is approximately considered 13-47% in adults (15,16).

We used the age categorization suggested by Mgeladze et al to classify our patients and control groups, which were mostly adults (51.3% male and 48.7% female) (17). In this study, primary and secondary criteria of itching were observed in all patients and 100% of cases were in consistent with the previous studies (18). According to various studies, compared to other methods the Hanifin-Rajka criteria for diagnosing of atopic dermatitis comprise the same high sensitivity and specificity of detection, and we also used these criteria in this study (19). Involved areas in atopic dermatitis in this study were: flexor in 90% of patients, face in 9.25% of patients, neck in 3.12% of patients and extensor areas in 8.5% of the patients. The study by Kulthanan et al in Thailand showed that characteristic morphology of disease was clear in 73.5% of patients. They reported the involved areas as the flexor areas, neck, extensor areas, and face in 72.1%, 27%, 18.5%, and 18.1% of patients, respectively (15,16). These percentages are slightly lower in comparison to the results that we presented in the previous section.

From another point of view, the difference in the percentage of involved areas in the adults by this work and Kulthanan et al

might also be affected by the difference in the regions that the studies are conducted in, where people have different lifestyles. In the evaluation of minor criteria, we observed that the incidence of itching, skin dryness, itching of upper limbs, itching of lower extremities, hives, eczema, maculopapular rash, eye itching, were respectively 100%, 54%, 50%, 30%, 4%, 90%, 46%, 6% among the patients. In many respects, there are similarities between different studies in the prevalence of minor criteria, and the differences can be attributed to the genetic and ethnic differences. In a study in Sweden by Bohme, due to the dry and cold weather, 100% of patients had dry skin (20). While a study by Wahab in Bangladesh, with warm and humid weather, showed that 43.8% of patients have dry skin (21). Kulthanan et al in Thailand studied clinical symptoms of 56 patients with atopic dermatitis and showed that patients have minor symptoms such as itching during sweating (67.3%), skin dryness (67.3%), nonspecific dermatitis of the hands and feet (34.7%), frequent conjunctivitis (20.2%), skin infection (4.1%). Keratoconus was not seen in any cases (15,16). The current study showed similar results as Kulthanan and colleagues.

135 (90%) of cases in our patient group had higher serum levels of total IgE than normal, and average of total IgE serum levels, regardless of age and gender, was 227.5 ± 103.0 . According to the previous studies, approximately 60-80% of patients have increased levels of total IgE than the general population (1, 3, 10, 15, 20). Results from this study on the serum levels of total IgE are in agreement with the previous studies. Most frequent allergens were related to cultivated rye (48.6%), Timothy grass (42.6%), house dust mites (22.7%), dog (16.7%), birch (11.3%), potato (11.3%), horse (10%), hazelnut (10%), cat (10%), soybean (10%), sagebrush (10%).

AhmadiAfshar et al (2008) investigated on the allergic patients with AD. They reported the frequency of observed allergens in Zanjan-Iran as follows: grass pollen 38.5 to 41%, weed pollen 21-27%, olive trees 22%, ash 20%, house dust mite 16%, cockroach 14.5%, *Aspergillus* 11.5%, *Alternaria* (7.5%) (22).

Worm and colleagues (2005) conducted a study on 111 adult patients in Germany through the Prick test. They showed allergic reactions to birch (55.6%), grass pollen (44.4%), dog (40.7%), potato (37%), Cat (33.3%), carrot (29.6%), hazelnut (29.6%), sesame (25.9%), house dust mite (22.2%), wheat flour (22.2%), apple (22.2%), parsley (22.2%), sagebrush (18.5%), barley flour (14.8%), soybean (11.1%), egg, cow milk and poppy seed each 7.4%, peanut, pig meat, muscle meat, latex and *Alternaria* 3.7%, *Cladosporium*, crab and a special type of fish have not been found in any of the patients (23).

Celakovska and colleagues in Paraguay studied 120 adult patients with AD using two methods: Atopy patch test and Evaluation of specific IgE with EIA method. Their investigations showed that the allergy frequency observed with both methods

was as follows: wheat and soybeans, eggs, peanuts, cow's milk 4%, 8%, 13%, 1%, respectively (24). Moreover, Hon and co-workers in a study of 85 children with atopic dermatitis (assessed by EIA) in China observed the food allergy occurrence in their patient population as follow: shrimp 54%, egg whites 43%, wheat 42%, peanuts 41%, tomatoes 38%, milk 34%, fish 30%, vea land orange 20% (25).

In summarizing the results of other studies, it seems that prevalence of food allergens in children is more than in adults, and about 35% or 1/3 of children with severe atopic dermatitis have increased the level of IgE against food allergens. It is mostly believed that the lack of resistance evolution in children is the responsible factor which by growing to adulthood 80% of studied group did not show the food allergy. While the pollen and respiratory materials are more allergic in adults, the food allergens have less importance in them (23). In this study, 80% of investigated patients were adult and pollen and respiratory allergen (house dust mite, cat and fungi allergens) considered as an allergen and the food allergen could be considered as the less importance factor.

We emphasise the conclusion made by Worn investigation in Germany (23) and Celakovska et al in Paraguay (24) conducted on adults' allergies. They concluded that the frequency of plant and respiratory allergen in adults is higher than in the younger population. We found that the most frequent allergens are pollen of rye and Timothy grass. We conclude that the three most effective methods for the treatment of AD that we found are 1 - avoiding contact with the mentioned factors in pollen seasons (i.e. spring and early summer), 2 - increasing the drug dose in treatment, and particularly, 3 - the use of immunotherapy.

We found food allergy to potato is the most frequent observed allergen in food allergens group in the adult group, and that is consistent with Worn et al conclusion on the German patients. There are two possible explanations for that. The first idea is that the rate of potato consumption is high in both countries (i.e. Iran and Germany). In fact, Germany is ranked as the 7th in the world's potato consumer list and Iran is the 10th one. To test this idea, we need to conduct further studies on the low potato consumption rate countries such as African countries. The second possibility is that adults are allergic to potato in general. The answer to this question is open and that begs for further studies globally.

In contrast, cow milk, egg, soybean, and wheat are the most frequent observed allergen in food allergens group in children according to this study, which is consistent with some previous works but not with all. On the other hand, cow milk is the most widely consumed baby food in Iran. This suggests that the most frequent allergen in children might be related to the food behavior and eating style of the region of study.

Racial and ethnic differences may have an important role too. For instance, Benedict et al studied 2,084 children in

12 European countries (26). They have reported a variety of allergic patterns among those countries, and have concluded that the food allergens are generally more important in AD than inhaled allergens. According to Benedict's et al analysis, the most important inhaled allergens are house dust mite, cat, grass pollen, and *Alternaria*, in order from high to low, and our analysis also supports their conclusion. In this study, house dust mite, cat, horse, and dog also had a high frequency, that necessary training to contact prevention with animals and health measures to combat the mites is useful in these regions. Patch test methods that have been used to identify allergens have undoubtedly a decisive role; as of Owczarek et al, they showed that the method used will make the difference in the prevalence of allergen (27). In this study we used immunoblotting, that is one of the newest methods for measurement of specific IgE. During a search of the Internet and of different similar sources of information, blot immunoassay method for the study of the spectrum of allergens was not found. As is evident, the distribution pattern of allergens in atopic dermatitis patients is highly variable, and the reason for this difference can be relevant to the differences between races, genetic, patch test methods, other different laboratory methods, vegetation and different eating habits, as this is proof of the necessity for detection of particular allergens in that particular region for the treatment of atopic dermatitis in the same area. In this study, frequency of allergens in patients was as follow: plants and fungi group 54.34%, animal group 26.8% food groups 19.56%. These results confirm the potential effect of inhaled allergens and plant allergens in patients with atopic dermatitis.

Conclusions

In this study, we used an immunoblotting technique, which is one of the most recent methods for the measurement of specific IgE, and the current study is the first on this topic which has used this technique. We believe that using this technique can revolutionize the identification of allergens, and significantly improve the currently presented treatment procedures to a higher level of quality and efficiency.

This is the first study in Azerbaijan which has focused on AD with the newest method that we have used. Our results would significantly improve the treatment procedure in Azerbaijan-Iran by focusing on the most frequent allergens in this region.

Based on the findings of this study, common allergens in East Azerbaijan, including cultivated rye, Timothy grass, house dust mite, *Alternaria*, cat, *Cladosporium*, horse, birch, potato, dog, were determined. With regard these findings, we can recommend to avoiding these allergens for atopic patients, and to use patient-specific allergy immunotherapy for treatments.

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References

1. Burns T, Breathnach S, Cox N, Griffiths C. Rook. Textbook of Dermatology. 4 Volume Set: Wiley;2010.
2. Bologna J, Jorizzo JL, Rapini RP. Dermatology. Mosby/Elsevier; 2008.
3. Ou L-S, Huang J-L. Cellular aspects of atopic dermatitis. *Clinical reviews in allergy & immunology*. 2007;33(3):191-8.
4. Borirakchanyavat K, Kurban AK. Atopic dermatitis. *Clinics in dermatology*. 2000;18(6):649-55. Epub 2001/02/15.
5. Kim T-E, Park S-W, Cho N-Y, Yong T, Nahm B, Lee S, et al. Quantitative measurement of serum allergen-specific IgE on protein chip. *Experimental and molecular medicine*. 2002;34(2):152-8.
6. Boguniewicz M, Schmid-Grendelmeier P, Leung DY. Atopic dermatitis. *The Journal of allergy and clinical immunology*. 2006;118(1):40-3. Epub 2006/07/04.
7. Sampson HA. The evaluation and management of food allergy in atopic dermatitis. *Clinics in dermatology*. 2003;21(3):183-92.
8. Warner J. Determinants of total and specific IgE in infants with atopic dermatitis. *Pediatric Allergy and Immunology*. 1997;8(4):177-84.
9. Perry TT, Matsui EC, Kay Conover-Walker M, Wood RA. The relationship of allergen-specific IgE levels and oral food challenge outcome. *Journal of allergy and clinical immunology*. 2004;114(1):144-9.
10. Hon KLE, Lam McA, Leung Tf, Wong Ky, Chow Cm, Fok Tf, et al. Are age-specific high serum IgE levels associated with worse symptomatology in children with atopic dermatitis? *International journal of dermatology*. 2007;46(12):1258-62.
11. Pádua I, Moreira A, Moreira P, Barros R. Food allergy: practical approach on education and accidental exposure prevention. *European annals of allergy and clinical immunology*. 2016;48(5):174.
12. Brand PL. Allergy diagnosis: pros and cons of different tests, indications and limitations. *Breathe*. 2007;3(4):345-9.
13. Westwood M, Ramaekers B, Lang S, Armstrong N, Noake C, de Kock S, Joore M, Severens J, Kleijnen J. ImmunoCAP® ISAC and Microtest for multiplex allergen testing in people with difficult to manage allergic disease: a systematic review and cost analysis. *Health Technology Assessment (Winchester, England)*. 2016;20(67):1.
14. Wakamori T, Katoh N, Hirano S, Kishimoto S, Ozasa K. Atopic dermatitis, dry skin and serum IgE in children in a community in Japan. *International archives of allergy and immunology*. 2009;149(2):103-10.
15. Kulthanan K, Samutrapong P, Jiamton S, Tuchinda P. Adult-onset atopic dermatitis: a cross-sectional study of natural history and clinical manifestation. *Asian Pacific Journal of Allergy and Immunology*. 2010;25(4):207-14.
16. Kulthanan K, Boochangkool K, Tuchinda P, Chularojanamontri L. Clinical features of the extrinsic and intrinsic types of adult-onset atopic dermatitis. *Asia Pacific Allergy*. 2011;1(2):80.
17. Mgeladze N, Karseladze R, Zhorzholiani L, Karseladze E. Clinical-epidemiological characteristics of atopic dermatitis in children of junior school level in Tbilisi. *Georgian medical news*. 2009(167):68-71.
18. Sharma L. Diagnostic clinical features of atopic dermatitis. *Indian Journal of Dermatology, Venereology, and Leprology*. 2001;67(1):25.
19. De D, Kanwar A, Handa S. Comparative efficacy of Hanifin and Rajka's criteria and the UK working party's diagnostic criteria in diagnosis of atopic dermatitis in a hospital setting in North India. *Journal of the European Academy of Dermatology and Venereology*. 2006;20(7):853-9.
20. Böhme M, Svensson Å, Kull I, Wahlgren C-F. Hanifin's and Rajka's minor criteria for atopic dermatitis: which do 2-year-olds exhibit? *Journal of the American Academy of Dermatology*. 2000;43(5):785-92.
21. Wahab M, Rahman M, Khondker L, Hawlader A, Ali A, Hafiz M, et al. Minor criteria for atopic dermatitis in children. *Mymensingh medical journal: MMJ*. 2011;20(3):419-24.
22. Ahmadi A, Sepehri S, Mosavinasab N, Torabi Z. Determination of Allergens Prevalence In allergic patients in Zanjan by Skin Prick Test. *Journal of Zanjan University of Medical Sciences*. 2008(54):25.
23. Worm M, Forschner K, Lee H-H, Roehr CC, Edenharter G, Niggemann B, et al. Frequency of atopic dermatitis and relevance of food allergy in adults in Germany. *Acta dermato-venereologica*. 2006;86(2).
24. Celakovska J, Vaneckova J, Ettlerova K, Ettler K, Bukac J. The role of atopy patch test in diagnosis of food allergy in atopic eczema/dermatitis syndrome in patients over 14 years of age. *Acta medica (Hradec Kralove) / Universitas Carolina, Facultas Medica Hradec Kralove*. 2010;53(2):101-8. Epub 2010/08/03.
25. Hon KL, Chan IHS, Chow CM, Wang SS, Lam CWK, Ng PC, et al. Specific IgE of common foods in Chinese children with eczema. *Pediatric Allergy and Immunology*. 2011;22(1-Part-I):50-3.
26. De Benedictis F, Franceschini F, Hill D, Naspitz C, Simons F, Wahn U, et al. The allergic sensitization in infants with atopic eczema from different countries. *Allergy*. 2009;64(2):295-303.
27. Owczarek W, Paluchowska E, Targowski T. [The relation of atopy patch tests with immunoglobulin E. Atopy patch tests with aeroallergens results dependence with the concentration of specific class E antibodies assay level assessment]. *Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego*. 2009;26(151):35-9.

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Hydroxychloroquine in the treatment of anti-histamine refractory chronic spontaneous urticaria, randomized single-blinded placebo-controlled trial and an open label comparison study

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KEY WORDS

chronic urticaria; anti-histamine refractory; hydroxychloroquine; randomized controlled trial; leukotriene receptor antagonist; montelukast

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Summary

Background. The management of anti-histamine refractory chronic spontaneous urticaria (CSU) has poorly defined therapeutic options. Some patients with CSU respond poorly to a fourfold increase in dosage of H1-anti-histamines treatment. **Aim.** The objective of this study was to determine the effect of an adjunct treatment of hydroxychloroquine (HCQ) on remission rate and reduction of urticarial symptoms. **Methods.** Sixty subjects with anti-histamine refractory CSU were randomly assigned to 400 mg of HCQ daily or placebo for 12 weeks in a single blind placebo controlled trial. In a second follow up trial, non-remission subjects were offered open-label HCQ in the placebo group or a leukotriene receptor antagonist (LTRA) in the HCQ group for 12 weeks. All subjects took 4 H1-anti-histamines tablets throughout the study. The endpoints measured were the urticarial symptom score (USS) and dermatology life quality index (DLQI). **Results.** Forty-eight patients (24 HCQ, 24 placebos) completed the randomized trial medication. Five of 24 on HCQ treatment but none on placebo had a remission at 12 weeks ($P = 0.01$). There was a low proportion of therapeutic failures occurred with 12-week HCQ treatment ($n = 5$) compared with placebo ($n = 14$, $P = 0.001$). After 12 weeks, USS and DLQI significantly improved in HCQ group over the placebo group. Forty non-remission subjects completed an open-label HCQ ($n = 22$) or LTRA ($n = 18$) comparison study. The remission rates on HCQ and LTRA were 22.72% and 5.55% at 12 weeks. However, no significant difference between the two groups in the therapeutic responses was observed. The mean USS on HCQ significantly decreased compared to the LTRA group, but there was no significant difference in DLQI. The adverse events reported were minimal and there were no subjects who discontinued the trial. **Conclusions.** This study suggests that HCQ is clinically effective as an adjunct treatment for CSU.

Introduction

The H1-antihistamines remain the first line of symptomatic treatment for chronic spontaneous urticaria (CSU). CSU typically responds to H1-anti-histamines therapy and the dosing can be increased to four-fold in cases of non-response (1). In

spite of the use of anti-histamines, some patients with CSU remain refractory to this therapy. An immunomodulatory drug may be added as an adjunct treatment to control urticaria symptoms, which include leukotriene receptor antagonist (LTRA), cyclosporine A and omalizumab (1).

Hydroxychloroquine (HCQ) is considered a disease-modifying anti-rheumatic drug, which is very well tolerated and inexpensive, and serious side effects are rare. However, its mechanism of action in the treatment of CSU is unknown, and the reports of the efficacy of HCQ treatment in CSU are limited. We designed a single-blind placebo-controlled randomized trial to evaluate the efficacy and safety of HCQ in patients with CSU, who did not respond to a four-fold increasing in dosing of H1-anti-histamines drugs. We also conducted a follow up open-label comparison study, to assess the efficacy of HCQ compared to LTRA to assess efficacy on the remission rate in the patients with CSU.

Materials and methods

Sixty adult patients who were diagnosed with CSU and did not respond to 4 tablets of H1-anti-histamines for 4 weeks at Allergy Clinic, Phramongkutklo Hospital, were invited to participate in this study. The exclusion criteria for this study included inducible urticaria and urticarial vasculitis. After a 2 week washout period, the subjects were randomly assigned to receive HCQ 400 mg/day or placebo adjunct treatment with 4 tablets of H1-anti-histamines drug for 12 weeks. Matched placebo pills were prepared by Department of pharmacy, Phramongkutklo Hospital. Only the patients were blinded. No increase in any other treatment for CSU was permitted for the duration of the study. The subjects with urticarial symptoms at the end of the study were recruited to an open-label comparison study and followed by a 2-week washout period. HCQ at a dose of 400 mg/day was administered in the former placebo group and montelukast (LTRA) at a dose of 10 mg/day was administered in the former HCQ-treated group for 12 weeks. This study was approved by the Institutional Review Board, Royal Thai Army Medical Department. All the subjects provided informed writ-

ten consent. Subject recruitment commenced in August 2010 and finished in September 2013.

Clinical evaluation, ophthalmic examination as well as the following investigations: erythrocyte sedimentation rate (ESR), thyroid autoantibodies, antinuclear antibody (ANA) and autologous serum skin test (ASST), were performed at baseline. The primary endpoint was remission rate. The secondary endpoints were urticarial responses that were assessed by the urticarial symptom score (USS) and dermatology life quality index (DLQI) (2,3). The response to treatment was assessed subjectively and recorded as remission (completely cured and discontinue medication at least 2 weeks), improved (urticaria symptoms improved during the treatment, and therefore reduce anti-histamine tablets but could not discontinue medication) or unchanged (urticarial symptoms still occurred). In follow-up visits symptoms and adverse events were assessed after taking HCQ every 2 weeks. The subjects had a second eye examination at the end of the study.

To compare baseline clinical characteristics between two groups, continuous data were analyzed by unpaired T-Test and others data were analyzed by Fisher's Exact Test. Comparison of remission rate and urticaria status was performed by Chi square test. Mann-Whitney *U* test was used to determine UAS and DLQI between groups. Data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). Significant levels were set at $p < 0.05$.

Results

A total of 55 subjects were randomized, 28 subjects in the HCQ group and 27 subjects in the placebo group (**figure 1**). Seven subjects did not complete the study, 4 subjects in the HCQ group and 3 subjects in placebo group. The baseline characteris-

Figure 1 - Schematic of study design.

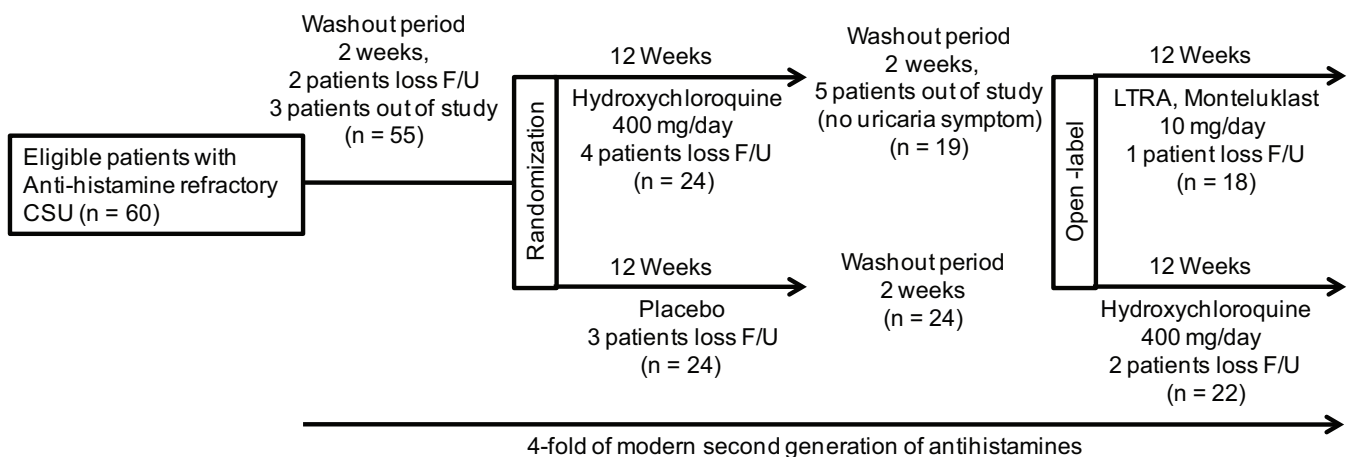
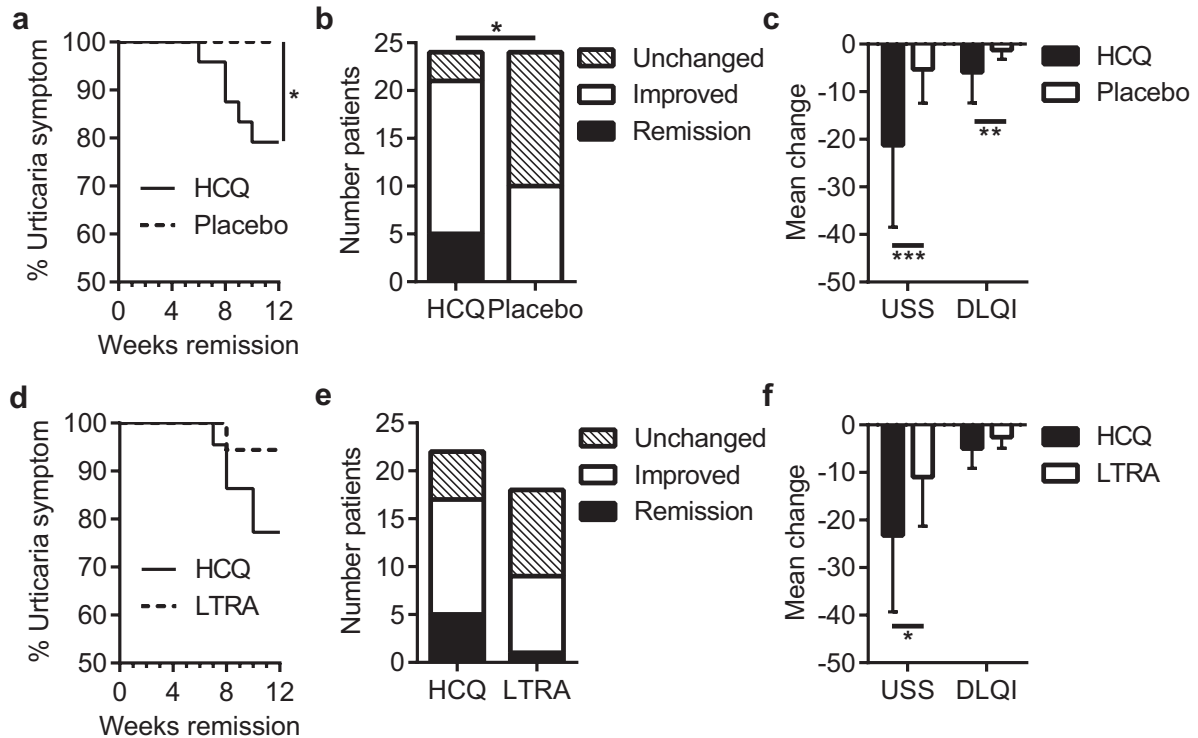


Table 1 - Baseline clinical characteristics of the subjects randomized to hydroxychloroquine (HCQ) or placebo.

	HCQ (n = 24) n (%)	Placebo (n = 24) n (%)	p-value
Sex, female	21 (87.50)	20 (83.33)	1.00
Age (Years, Mean ± SD)	33.00 ± 12.11	33.95 ± 11.91	0.78
Duration (Weeks, Mean ± SD)	21.71 ± 19.53	24.291 ± 23.41	0.68
ESR (Mean ± SD)	18.29 ± 12.49	17.62 ± 6.80	0.81
Thyroid autoantibodies, positive	6 (25.00)	10 (41.66)	0.22
ANA, positive	10 (41.66)	11 (45.83)	0.77
ASST, positive	19 (79.16)	20 (83.33)	1.00
USS-baseline (Mean ± SD)	45.54 ± 11.25	43.42 ± 12.26	0.53
DLQI-baseline (Mean ± SD)	10.96 ± 6.68	9.75 ± 5.19	0.48

ESR, erythrocyte sedimentation rate; ANA, antinuclear antibody ASST, Autologous Serum Skin Test; USS, urticaria symptom score; DLQI, dermatology life quality index.

Figure 2 - Time to urticarial remission (a), proportion of urticarial responses (b) and reduction in urticarial symptom score (USS) and dermatology life quality index (DLQI) (c) in patients randomized to hydroxychloroquine (HCQ) or placebo. Inpatients with unchanged urticarial symptoms were followed by open-label HCQ or leukotriene receptor antagonist (LTRA). Time to urticarial remission (d), proportion of urticarial responses (e) and reduction in USS and DLQI (f) in patients treated with HCQ or LTRA. F/U, follow-up, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



tics of the two groups are shown in **table 1**. There was no significant difference in these baseline characteristics between the two groups. Five of 19 subjects (20.83%) on HCQ but none on placebo achieved remission at 12 weeks ($p = 0.01$) (**figure 2a**). Sixteen subjects in the HCQ group and 10 subjects in the placebo group revealed clinical improvement. There was a significantly lower proportion of therapeutic failures in the subjects treated with HCQ, $n = 5$ than in those who received placebo, $n = 14$ ($p = 0.001$) (**figure 2b**). After 12 weeks, the severity score was significantly improved by HCQ treatment ($p = 0.0001$), and these patients experienced significant improvement in DLQI scores with HCQ compared with the placebo ($p = 0.004$) (**figure 2c**). In a follow up study, forty-three subjects with urticarial symptoms, 24 subjects with HCQ and 19 subjects with LTRA continued in the open-label comparison study. Three subjects did not complete the study, 2 subjects in the HCQ group and 1 subject in the LTRA group. After the 2-week washout period, the mean USS scores (38.54 ± 12.30 and 34.77 ± 10.90) and DLQI scores (8.68 ± 3.51 and 7.83 ± 2.72) between the HCQ and LTRA group were not significantly different. The HCQ group showed a higher rate of remission, 22.72% compared to the LTRA group, 5.55%, although significance was not reached (**figure 2d**). The difference between HCQ and LTRA in the urticarial responses was not statistically significant either (**figure 2e**). However, a significantly greater reduction in symptom score was observed at 12 weeks in the HCQ group compared to LTRA group ($p = 0.01$) (**figure 2f**). Five subjects of the total 46 (10.86%) who were HCQ-treated reported adverse events. One subject had a problem with a severe headache at the first week of taking HCQ. Four subjects felt their skin were darker. However, none of the subjects complained about eye problem and all of the subjects had passed their eye examination at the end of the study. One subject in the placebo group had dizziness and two subjects reported a gastrointestinal disturbance. No subject in LTRA group was reported as an adverse event.

Discussion

We reported that the adjunct HCQ treatment in addition to primary therapy with the fourfold dose of H1-anti-histamines is more effective than placebo and LTRA on remission rate, and in reducing the severity of CSU after 12 weeks. Moreover, no serious side effects were reported from this study. There has been only one placebo controlled trial previously by Reeves et al. reported in 2004 (4). They showed the efficacy of HCQ therapy in chronic autoimmune urticaria, where patients treated with HCQ achieved significantly more improvements in quality of life than the placebo group at 12 weeks. However, they did not report the remission rate in the HCQ group. In this study, we reported a low remission rate in the HCQ group which might be due to a high rate of positive ASST (autologous

serum skin tests) among subjects. Patients with the positive autologous skin test had a lower remission rate compared with those who had negative skin test (5,6). The rate of remission over 2 years in the negative, positive ASST, and both positive ASST and autologous plasma skin test (APST) groups, were 81.1%, 62.3% and 46.1%, respectively (6). Moreover, negative ASST had the chance to achieve remission more than positive ASST (OR, 3.97; 95% CI, 1.47-9.43; $p = 0.001$) (6). However, in the previous study, the subjects were not only treated with HCQ, they also received other third line therapy (eg, dapsone, cyclosporine and leukotriene antagonist). Low-dose cyclosporine A in the treatment of severe CSU achieved remission 26-68% (7,8). Vena GA et al reported, after 16 weeks, symptoms scores significantly improved with cyclosporine A treatment group more than placebo, but no patients achieved full remission, and 6% of patients led to discontinuation of treatment due to adverse events (9). The adverse side effects of cyclosporine A require constant monitoring, including its effects on blood pressure and renal function. Omalizumab (anti-IgE) is a very effective treatment for CSU, but the cost of the treatment is expensive for routine use in developing countries (10). The limitation of the study was a high incidence of withdraws, and we conducted the non-randomized controlled study to assess the efficacy of HCQ and LTRA.

In summary, HCQ was a useful short-term treatment for the patient with CSU who were refractory to a fourfold increase in dosage of H1-anti-histamines treatment. The benefits of HCQ include enhancing remission of patients, diminishing urticarial symptoms, and safety.

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References

1. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, Church MK, Ensina LF, Gimenez-Arnau A, Godse K, Goncalo M, Grattan C, Hebert J, Hide M, Kaplan A, Kapp A, Abdul Latiff AH, Mathelier-Fusade P, Metz M, Nast A, Saini SS, Sanchez-Borges M, Schmid-Grendelmeier P, Simons FE, Staubach P, Sussman G, Toubi E, Vena GA, Wedi B, Zhu XJ, Maurer M, European Academy of Allergy and Clinical Immunology, Global Allergy Asthma and COPD Strategy Committee, Global Allergy and Asthma European Network, European Dermatology Forum, World Allergy Organization. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*. 2014;69(7):868-87.
2. Jariwala SP, Moday H, de Asis ML, Fodeman J, Hudes G, de Vos G, Rosenstreich D. The Urticaria Severity Score: a sensitive questionnaire / index for monitoring response to therapy in patients with chronic urticaria. *Ann Allergy Asthma Immunol*. 2009;102(6):475-82.

3. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI) - a simple practical measure for routine clinical use. *Clin Exp Dermatol.* 1994;19(3):210-6.
4. Reeves GE, Boyle MJ, Bonfield J, Dobson P, Loewenthal M. Impact of hydroxychloroquine therapy on chronic urticaria: chronic autoimmune urticaria study and evaluation. *Intern Med J.* 2004;34(4):182-6.
5. Kocaturk E, Kavala M, Kural E, Sarigul S, Zindanci I. Autologous serum skin test vs autologous plasma skin test in patients with chronic urticaria: evaluation of reproducibility, sensitivity and specificity and relationship with disease activity, quality of life and anti-thyroid antibodies. *Eur J Dermatol.* 2011;21(3):339-43.
6. Boonpiyathad T, Sangasapaviliya A. Autologous serum and plasma skin test to predict 2-year outcome in chronic spontaneous urticaria. *Asia Pac Allergy.* 2016;6(4):226-235.
7. Toubi E, Blant A, Kessel A, Golan TD. Low-dose cyclosporin A in the treatment of severe chronic idiopathic urticaria. *Allergy.* 1997;52(3):312-6.
8. Grattan CE, O'Donnell BF, Francis DM, Niimi N, Barlow RJ, Seed PT, Kobza Black A, Greaves MW. Randomized double-blind study of cyclosporin in chronic 'idiopathic' urticaria. *Br J Dermatol.* 2000;143(2):365-72.
9. Vena GA, Cassano N, Colombo D, Peruzzi E, Pigatto P, Neo ISG. Cyclosporine in chronic idiopathic urticaria: a double-blind, randomized, placebo-controlled trial. *J Am Acad Dermatol.* 2006;55(4):705-9.
10. Maurer M, Rosen K, Hsieh HJ, Saini S, Grattan C, Gimenez-Arnau A, Agarwal S, Doyle R, Canvin J, Kaplan A, Casale T. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N Engl J Med.* 2013;368(10):924-35.

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Evaluation of asthma control in the pharmacy: an Italian cross-sectional study

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Summary

Background. In Western countries a large proportion of asthmatic patients remain uncontrolled, despite the availability of effective drugs. An involvement of pharmacies / pharmacists in asthma management has been suggested in guidelines, since this could provide a relevant support. **Objective.** The present cross-sectional study aimed at assessing the level of asthma control, by using ACT questionnaire, in the community pharmacies in the County of Verona, North East of Italy. **Methods.** A call for participation was sent by Verona Pharmacists' Association to all the pharmacies located in the Verona municipality. Patients with a medical prescription and an asthma exemption code were recruited in pharmacies. They were asked to fill the ACT questionnaire and to answer some additional questions on asthma treatment, smoke habits and comorbidities. **Results.** Thirty-seven community pharmacies recruited 239 patients. According to the ACT score, more than 50% of patients had a controlled asthma but 20% of them were totally uncontrolled and 12% were using oral steroid. Only 2.9% of patients had received an asthma action plan. Asthma was intermittent in 17.6% of patients, mild persistent in 13.8%, moderate persistent in 63.1% and severe in 5.4%. Discordance was observed between the self-perceived asthma control and objective parameters, when available. Of note, in the severe asthma group, most patients had an ACT > 20. **Conclusion.** This is the first Italian pharmacy-based study on asthma control. A better asthma control was recorded in this study in comparison with other trials, but about 50% of patients were insufficiently controlled. The community pharmacies can play a relevant role in the preliminary assessment of asthma control by using easy and not time consuming tools, such as ACT.

Introduction

Asthma is overall poorly controlled in Western countries (1,2) despite the availability of effective drugs (3). Many reasons may account for this finding, such as a low adherence to the treatment, observed in every chronic condition, the fear of systemic side effects related to the long term treatment with inhaled corticosteroids (ICS), the switch to Complementary Medicines, the inadequate knowledge of the disease (4-7). However, a relevant drawback is the limited time for consultation in the GP's office,

and the difficult access to in-hospital follow up. As reported in a recent Italian survey, the majority of patients receive no more than one visit / year, and spirometry is rarely performed (6,7). The Asthma Control Test (ACT™) is a standardized questionnaire, representing as a fast and easy tool for assessing asthma control in every setting (8). However, according to the available data, it is routinely used only by 20% of GPs and by 42% of specialists, mainly because it is considered time consuming (7). Though asthma is usually managed in medical settings, the involvement of community pharmacies could provide a significant

support, as demonstrated by the growing interest in this topic (9). Several pharmacy-based studies evaluating the control of asthma have been performed in Europe, but no similar studies have been carried out in Italy. Aim of this cross-sectional study was to assess the level of asthma control by the ACT administered in pharmacies in the county of Verona, in the North East of Italy.

Methods

Community pharmacies

Community pharmacists underwent a two-session seminar performed one month before starting the study, where information about bronchial asthma and about the study design were provided. Subsequently, a call for participation was sent by the Verona Pharmacists' Association to all the community pharmacies in Verona territory. The study lasted seven months, from February the 1st to July the 31th 2015, and was approved by Verona Pharmacists' Association Ethical Committee.

Patients. The only inclusion criterion of consenting patients was the presence of the payment exemption related to asthma diagnostic procedures and treatments (code 007 present on the medical prescription). In Italy, the exemption code is released to the patient by the National Health Service only to subjects provided with a diagnosis of bronchial asthma, relying on the clinical history and the positivity to metacholine test and/or bronchodilator test.

Evaluations

Asthma control was evaluated by ACTTM (8). The questionnaire was self-completed by the recruited patients on site in pharmacies. ACT is a validated questionnaire including five questions. The possible answers are scored from 5 (best) to 1 (worst). The final score ranges from 5 to 25, and the higher is the score the better is the asthma control: ACT = 25, totally controlled asthma; ACT = 20-24, well controlled; ACT < 20, insufficiently controlled; ACT < 15, uncontrolled asthma. The pharmacists were instructed to address patients to immediate referral to GP or specialist if the ACT score was below 15.

Some additional information was also collected together with the ACT: demography, work occupation, smoking habit, characteristics / doses of asthma medications, having an action plan. These data were reported by patients on a separate standard questionnaire. Asthma severity was deduced on the basis of the treatment used.

Results

Thirty-seven pharmacies (27% of all pharmacies in the Verona municipality) adhered to the observational study, and 239 patients were consecutively recruited. Among the responding sub-

jects there was a slight female prevalence (54%), the mean age of study population was 50.6 yrs (20% of subjects were retired), and 27.6% were older than 65 years. Their characteristics are summarized in **table 1**. According to the ACT score, the prevalence of uncontrolled asthma was 20%, and in other 26% of patients the control was insufficient (**table 2**). However, the majority of patients were under control, 11% being totally controlled (**figure 1**). According to ACT overall analysis, about one in five patients (19.7%) suffered from night awakenings and used short acting bronchodilators more than three times a week (18.8%). However, a discordance was observed between the ACT questions, exploring the self-perceived overall asthma control, and the other questions, investigating more objective parameters. For instance, among the patients marking with a low score the questions on night awakenings and short acting bronchodilators use, 10.6% declared a total asthma control (**table 2**). In the severe asthma group, all patients had an ACT score higher than 20 (**figure 2**). Asthma severity was evaluated on the basis of concomitant treatment, as per GINA recommendations (10). According to this criterion, asthma was intermittent in 17.6% of patients, mild persistent in 13.8%, moderate persistent in 63.1%, and severe in 5.4%, but 12% of patients were on regular oral steroids (thus severe by definition). Only 7 patients (2.9%) had received a written asthma action plan, to manage exacerbations (**table 2**). In the study population, 36% of patients were never smoker, 30% were current smokers, and the remaining 33% were ex smokers. The mean ACT of these three categories did not differ

Table 1 - Demographic data of patients.

SUBJECTS N	239 (100%)
MALE/FEMALE	110/129
MEAN AGE (SD)	50.8 (19.8)
AGE RANGE	4-88
SMOKING STATUS	
Current smoker (%)	72 (30.0%)
Ex-smoker (%)	79 (33.2%)
Never smoker (%)	88 (36.8%)
WORKING HABIT	
Retired	57 (23.8%)
Student	20 (8.4%)
Unemployed	3 (1.3%)
Employee	55 (23%)
Workers	78 (32,6%)
Other	26 (10,9%)

Table 2 - ACT results and clinical characteristics.

ACT SCORE	
ACT < 15	48 (20.1)
ACT 15-19	62 (25.9)
ACT 20-24	101 (42.3)
ACT = 25	28 (11.7)
Question 5 > 4 (self evaluation) ¹	5/47 (10.6%)
Question 3 < 3 (nocturnal awakenings)	47/239 (19.7%)
Question 4 < 4 (rescue medications)	45/239 (18.8%)
ACT SCORE ACCORDING TO SMOKE	
Current smoker	17.5 (± 3.82)
Never smoker	19.2 (± 4.90)
Ex smoker	19.1 (± 4.99)
CONCOMITANT RHINITIS	
HAVING A WRITTEN ACTION PLAN	7 (2.9%)
USE OF ORAL CS (burst or daily)	11 (4.7%)
MEDICATIONS	
ICS	18 (7.5%)
SABA	14 (5.9%)
ICS+LABA	147 (61,5%)
Leukotriene modifiers	15 (6.3 %)
ICS + Leukotriene modifiers	3 (1.3%)
ICS + LABA + Leukotriene modifiers	8 (3.4%)
SABA + Leukotriene modifiers	1 (0.4%)

¹Only those patients who had an ACT score < 15

significantly, being slightly lower among smokers. Concomitant rhinitis was reported in 17.7% of patients.

Discussion

The level of asthma control recorded in this Italian population sample is overall higher than that reported in recent studies carried out in medical settings in Italy (11,12), as well as in community pharmacies in other countries (table 3) 13-25. In fact, more than 50% of patients were controlled according to the ACT score, being a total control present in more than 10% of the whole population. This finding is indirectly confirmed by the low percentage of asthmatics with night awakenings or using frequently short acting beta agonists. However, among the patients marking with a low score the ACT questions on night awakenings and short acting bronchodilators use, 10.6% answered the last ACT question on the overall perceived control by marking the maximum score, and in the severe asthma group, most of patients had a total ACT score higher than 20. The discordance between the self-perceived asthma control and more objective parameters is a well-known phenomenon (26,27). For these reason, a careful and global evaluation should be part of the regular asthmatic patients' follow-up, and cannot be managed by the pharmacists only. However, the community pharmacies can play a relevant role in the preliminary assessment of asthma control and in advising the patient to the medical evaluation.

Non-atopic asthma seemed to be prevalent in our population, owing to the mean age over 50 years and to the low prevalence of concomitant rhinitis. Previous studies reported a lower control of asthma in comparison with the present one, but in those studies the number of adolescents and young adults

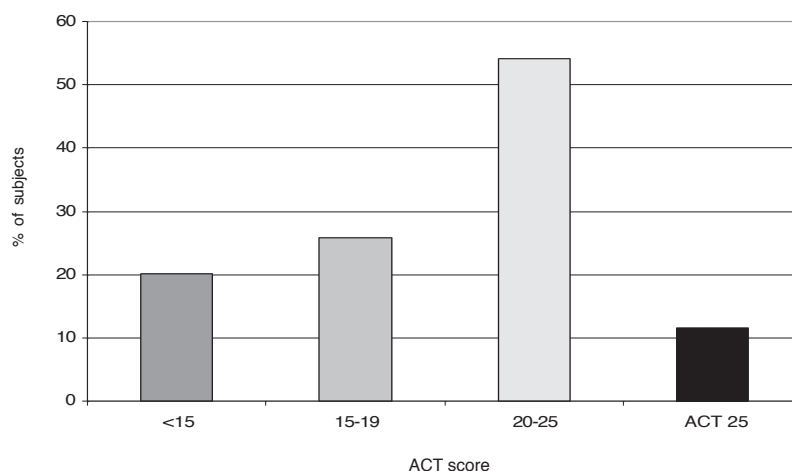
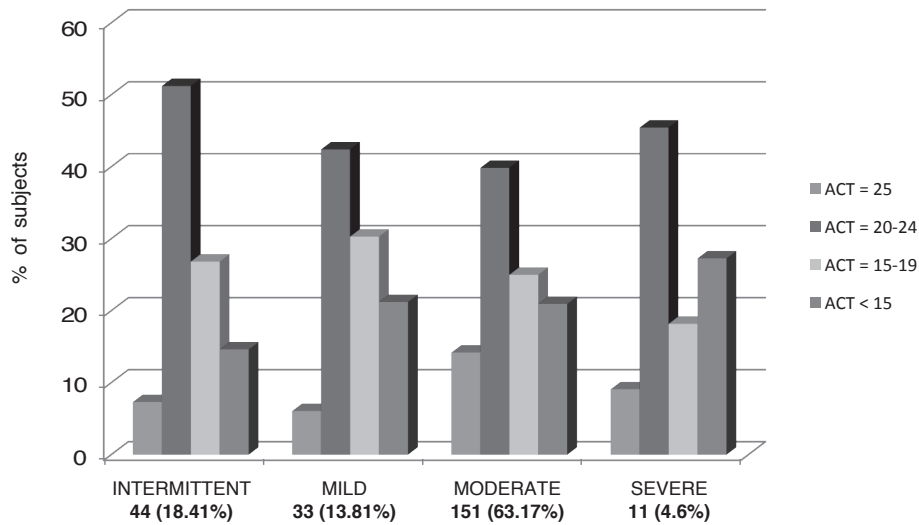
Figure 1 - ACT- based level of asthma control in the study population.

Figure 2 - ACT score in different asthma severity clusters.

was prevalent (13-25). Therefore, it is conceivable that a worse control of asthma is more common in younger ages. Moreover, the older age of our patients raised the problem of the differential diagnosis with COPD. However, we overcame this confounding factor selecting only patients with a diagnosis of asthma proved by pulmonary function test, and confirmed by

the exemption code. Of note, in our study population, around one out of three patients (27.6%) was older than 65 years. As highlighted by other reports (28,29) asthma in the elderly is not a rare disease, and its detection and management in that specific population deserves the highest consideration. Furthermore, the treatment choices should be carefully evaluated

Table 3 - Level of asthma control reported in published studies performed in community pharmacy setting.

First Author (Ref)	N. patients	Country	Questionnaire used	Controlled asthma %	Uncontrolled asthma %
Nishiyama (13)	306	UK	JMA	50	50
Laforest (14)	1.559	France	ACT	28	72
Mehuys (15)	166	Belgium	ACT	51	49
Laforest (16)	1.048	France	ACT	30	70
Armour (17)	396	Australia	JMI	21	79
Mehuys (18)	201	Belgium	ACT	52	48
Mendes (19)	5.551	Portugal	ACT	39	61
Giraud (20)	727	France	ACQ6	49	51
Saini (21)	570	Australia	JMI	23	77
Garcia-Cardenas (22)	336	Spain	ACQ	34	66
Armour (23)	570	Australia	JMI	23	77
Le May (24)	354	Australia	PACS	23	77
Laurenco (25)	224	Portugal	CARAT	13	87

in the light of the complex comorbidity frame, which often characterizes the older patients.

The duration of this study is shorter than one year, and therefore it does not cover the whole pollen seasons. However, the time-frame from January to July includes the pollination of cypress, olive tree, birch and grass, which are the most relevant seasonal allergens in our region. We did not include a PEF measurement in the study design. However, PEF assessment is very useful in the monitoring of the disease over the time, but it is less relevant as an isolated measurement (30).

One potential flaw of our study is that the Verona County is not representative for the whole country. Nevertheless, this is the first Italian report assessing the level of asthma control in the community pharmacy setting.

In other studies, the preliminary cross-sectional evaluation of asthma control was followed by longitudinal interventions with different outcomes, such as PEF monitoring, control of asthma, quality of life, asthma education and assessment of the inhalation technique. In most of these studies an increase of the knowledge of asthma (17,18,21), an improvement of asthma control (18,20) and quality of life (17,21), a better technique in the use of the devices (18,21) was observed, whereas conflicting results were reported about the lung function improvement (18,21,31). In our study, the very low number of patients with an asthma action plan to be used in case of asthma exacerbations, can be considered a relevant drawback in the current management of asthma, as well as the not negligible proportion of subjects reporting SABA as the only reference treatment. The absence of a regular anti-inflammatory treatment has been described in literature as the result of both poor adherence and, in some cases, inappropriate prescription (32). However, the overuse of SABA has been identified as a risk factor not only for severe exacerbations, but also for fatal asthma attacks (33,34). The UK National Review of Asthma Deaths (32) and a recently published Italian fatal asthma case series (35) described the use of SABA alone as a key recurrent finding. A strong educational action is thus mandatory, which involves all the health care providers, including pharmacies.

However, though the positive results of pharmacy interventions suggest their feasibility in real life, the possible drawbacks are the costs of the training courses as well as the waste of time for pharmacists (36). Differently from other countries such as Australia, in Italy there is an easy access to the medical facilities. For these reasons, on practical ground the community pharmacies interventional role can be focused on less time consuming outcomes, such as the assessment of asthma control or the inhaler technique verification. Furthermore, an active role in asthma management can be feasible for a limited number of pharmacies, maybe through the creation of dedicated networks (37).

A regular inclusion of spirometry testing was recently promoted in the pharmacy-based assessment of asthma, on the basis of positive results registered in several studies (38). However, the interpretation of this test needs specific background and expertise, and therefore the inclusion of spirometry in the pharmacy facilities must be carefully evaluated.

In conclusion, to our knowledge this is the first Italian pharmacy-based study on asthma control. The community pharmacies can play a relevant role in the preliminary assessment of asthma control, by using easy and not time consuming tools, such as ACT. A higher asthma control level has been observed in the present study in comparison with other trials, nevertheless it has to be noticed that one out of two patients are still insufficiently controlled. Thus, there is room for an active role of pharmacists in the management of asthma. In particular, the implementation of the inhalation technique and the educational support in order to improve the knowledge of the disease, could represent areas of intervention. However, a careful cost / effective evaluation should be performed for every interventional plan involving other health care providers, and the central role of the GP and of the specialist in the management of asthma should not be neglected.

References

1. Cazzoletti L1, Marcon A, Janson C, Corsico A, Jarvis D, Pin I, et al. Asthma control in Europe. A real world evaluation based on an international population-based study. *J Allergy Clin Immunol.* 2007;120:1360-7.
2. Slejco GS, Ghushchyan VM, Sucher B, Globe DR, Lin SL, Globe G, et al. Asthma control in the United States, 2008-2010: indicators of poor control. *J Allergy Clin Immunol.* 2014;133:1579-87.
3. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, et al. Can guideline-defined control be achieved? The Gaining Optimal Asthma Control study. *Am Resp Crit Care Med.* 2004;170:836-44.
4. Howell G. Non adherence to medical therapy in asthma : risk factors, barriers and strategy for improvement. *J Asthma.* 2008;45:723-9.
5. Chen W, Fitzgerald JM, RusseauR, LyndLD, Tan WC, Sadatsafavi M. Complementary and alternative asthma treatments and their association with asthma control. *BMJ Open.* 2013;3:e003360.
6. Canonica GW, Baena-Cagnani C, Blaiss MS, Dahl R, Kaliner MA, Valovirta EJ, GAPPsurvey Working Group. Unmet needs in asthma: Global Asthma Physician and Patient (GAPP) survey: global adult finding. *Allergy.* 2007;62:668-74.
7. Caminati M, Magnoni MS, Rizzi A, Braido F, Foresi A, Bettoncelli G et al. Asthma management among different specialists: results from a national Italian survey. *Eur Ann Allergy Immunol.* 2014;46:74-82.
8. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test : a survey for assessing asthma control. *J Allergy Clin Immunol.* 2004;117:59-65.
9. Reddel HK, Bosnic-Anticevich SZ, Armour CL, Bashedi I. Pharmacy intervention in asthma. *Eur Resp J.* 2008;32:812.

10. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2015. Available from: <http://www.ginasthma.org/>. (accessed 31 Nov 2015).
11. Corrado A, Renda T, Polese G, Rossi A. Assessment of asthma control : the SERENA study. *Resp Med*. 2013;107:1659-66.
12. Caminati M, Bettoncelli G, Magnoni MS, Rizzi A, Testi R, Passalacqua G et al. The level of control in mild asthma in general practice: an observational community-based study. *J Asthma*. 2014;51:91-6.
13. Nishiyama T, Chrystyn H. The Jones Morbidity Index as an aid for community pharmacists to identify poor asthma control during the dispensing process. *Int J Pharm Pract*. 2003;11:41-6.
14. Laforest L, Van Ganse E, Devouassoux G, Chretien S, Bauguil G, Pacheco Y, et al. Quality of asthma care: results from a community pharmacy based survey. *Allergy*. 2005;60:1505-10.
15. Mehuys E, Van Bortel L, Annemans L, Remon JP, Van Tongelen I, Van Ganse E, et al. Medication use and disease control of asthmatic patients in Flanders: a cross-sectional community pharmacy study. *Respir Med*. 2006;100:1407-14.
16. Laforest L, Van Ganse E, Devouassoux G, Osman LM, Brice K, Massol J, et al. Asthmatic patients' poor awareness of inadequate disease control: a pharmacy-based survey. *Ann Allergy Asthma Immunol*. 2007;98:146-52.
17. Armour CL, Lemay K, Saini B, Reddel HK, Bosnic-Anticevich SZ, Smith LD, et al. Using the Community pharmacy to identify patients at risk of poor asthma control and factors which contribute to this poor control. *J Asthma*. 2011;48:914-22.
18. Mehuys E, Van Bortel L, De Bolle L, Van Tongelen I, Annemans L, Remon JP, et al. Effectiveness of pharmacist intervention for asthma control improvement. *Eur Respir J*. 2008;31:790-9.
19. Mendes Z, Madeira A, Suzete C, Sonia I, Marianela V, Artur TA, et al. Asthma Control Assessment using Asthma Control Test in Portuguese Pharmacies. *Rev Port Imunoalergologia*. 2010;18:313-30.
20. Giraud V, Allaert FA, Roche N. Inhaler technique and asthma: feasibility and acceptability of training by pharmacists. *Respir Med*. 2011;105:1815-22.
21. Saini B, LeMay K, Emmerton L, Krass I, Smith L, Bosnic-Anticevich S, et al. Asthma disease management - Australian pharmacists' interventions improve patients' asthma knowledge and this is sustained. *Patient Educ Couns*. 2011;83:295-02.
22. Garcia-Cardenas V, Sabater-Hernandez D, Kenny P, Martinez-Martinez F, Faus MJ, Benrimoj SI. Effect of pharmacy intervention for asthma control. A cluster randomised trial. *Resp Med*. 2013;107:1346-55.
23. Armour CL, Reddel HK, LeMay KS, Saini B, Smith LD, Bosnic-Anticevich SZ, et al. Feasibility and effectiveness of an evidence-based asthma service in Australian community pharmacies: A pragmatic cluster randomized trial. *J Asthma*. 2013;50:302-09.
24. Le May KS, Armour CL, Reddel HL. Performance of a brief asthma control screening tool in community pharmacy: a cross-sectional prospective longitudinal analysis. *Prime Care Resp J*. 2014;23:79-84.
25. Lourenco O, Calado S, Sa'-Souza A, Fonseca J. Evaluation of allergic rhinitis and asthma control in a Portuguese community pharmacy setting. *J Manag Care Pharm*. 2014;20:513-22.
26. Boulay ME, Boulet LP. Discordance between asthma control clinical, physiological and inflammatory parameters in mild asthma. *Respir Med*. 2013;107:511-8.
27. Caminati M, Caimmi C, Dama A, Schiappoli M, Passalacqua G, Senna G. What lies beyond Asthma Control Test: suggestions for clinical practice. *J Asthma*. 2016;53(6):559-62.
28. Scichilone N, Ventura MT, Bonini M, Braido F, Bucca C, Caminati M, et al. Choosing wisely: practical considerations on treatment efficacy and safety of asthma in the elderly. *Clin Mol Allergy*. 2015;13(1):7.
29. Lombardi C, Raffetti E, Caminati M, Liccardi G, Passalacqua G, Reccardini F, et al. ; ELSA Study Group. Phenotyping asthma in the elderly: allergic sensitization profile and upper airways comorbidity in patients older than 65 years. *Ann Allergy Asthma Immunol*. 2016;116(3):206-11.
30. Brusasco V. Usefulness of peak expiratory flow measurements : is it just a matter of instrument accuracy. *Thorax*. 2003;58:375-6.
31. Mangiapane S, Schulz M, Muhlig S, Ihle P, Schubert I, Waldmann HC. Community pharmacy-based pharmaceutical care for asthma patients. *Ann Pharmacother*. 2005;39:1817-22.
32. Royal College of Physicians. Why asthma still kills: the National Review of Asthma Deaths (NRAD) Confidential Enquiry report. London: RCP; 2014.
33. Al-Dorzi HM, Al-Shammary HA, Al-Shareef SY, Tamim HM, Shammout K, Al Dawood A, et al. Risk factors, management and outcomes of patients admitted with near fatal asthma to a tertiary care hospital in Riyadh. *Ann Thorac Med*. 2014;9:33-8.
34. Gonzalez-Barcala FJ, Calvo-Alvarez U, Garcia-Sanz MT, Bourdin A, Pose-Reino A, Carreira JM, et al. Characteristics and prognosis of near fatal asthma exacerbations. *Am J Med Sci*. 2015;350:98-102.
35. Vianello A, Caminati M, Crivellaro M, El Mazloum R, Snenghi R, Schiappoli M, et al. Fatal asthma. Still an epidemic? *World Allergy Organ J*. 2016; in press.
36. Van Mil JW, Tromp TF. European barriers to the implementation of pharmaceutical care. *Int J Pharm*. 2001;9:163-8.
37. Berry TM, Prosser TR, Wilson K, Castro M. Asthma friendly pharmacies : a model to improve the communication among pharmacists, patients and health care providers. *J Urban Health*. 2011;88(suppl 1):113-25.
38. Cawley MG, Warning WJ. Pharmacists performing quality spirometry testing: an evidence based review. *Int J Clin Pharm*. 2015;37:726-33.

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Acute cardiac disease in a patient with hyper-IgE syndrome

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KEY WORDS

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Summary

We describe the case of a 24-year-old male with hyper-IgE syndrome (HIES) which was diagnosed at 4 years of age and died from a very rare cardiac complication. He had typical clinical and laboratory manifestations of HIES, including total serum IgE as high as > 100,000 IU/mL. Stem cell transplantation was not available. During the 20-year follow-up, he suffered numerous various infections of the skin and deep organs, partial lung resection, as well as multiple bone fractures. At age 24, he developed acute decompensated heart failure associated with elevated serum troponin I and brain natriuretic protein. Two-dimensional echocardiogram revealed global hypokinesis of the left ventricle with estimated ejection fraction 20-25%, and catheterization revealed ectasia of multiple coronary arteries. Endomyocardial biopsy showed lymphocytic myocarditis, focal necrosis, mild fibrosis, and myxoid degeneration, but cultures were negative. The patient improved on corticosteroid therapy and was discharged on heart failure therapy and external defibrillator. Six weeks later, he developed supraventricular tachycardia and persistent global hypokinesis and was treated with amiodarone. A trial of intravenous immunoglobulin was initiated and was repeated as outpatient every four weeks for four times. However, his cardiac function did not improve and he developed severe hypotension and pulseless electrical activity arrest. Resuscitation was unsuccessful. To the best of our knowledge, this is the first reported case of HIES complicated with lymphocytic myocarditis. Both immunologists and cardiologists need to be aware of such a complication and practice caution in using immunosuppressants when the patient's immune status is markedly compromised.

Introduction

Immunodeficiency diseases are often complicated with a variety of comorbid conditions, mostly infections and less common hematologic disorders, autoimmune diseases, and malignancy, but rarely heart disease.

Case report

We describe the case of a 24-year-old male with hyper-IgE syndrome (HIES) diagnosed at 4 years of age, with documented

mutation in signal transducer and activator of transcription 3 (STAT3). He had been closely followed for management of various infections and other complications (**table 1**). He had almost all typical manifestations of the disease (1) including total serum IgE as high as > 100,000 IU/mL, severe eczema, retention of primary teeth, recurrent *Staphylococcal aureus* and fungal skin infections, as well as the typical facial features including prominent forehead, deep set eyes, broad nasal bridge, wide nasal tip, and mild prognathism. Over the years, he suffered major complications including severe scoliosis, osteoporosis, multiple bone

fractures, and chronic skin infection on the chest caused by *Fusarium falciforme* resistant to multiple antifungals and requiring resection. He also had staphylococcal lung abscess that required partial resection of the left upper lobe.

At age 24, he developed fatigue, dyspnea on exertion, orthopnea, and mild right sided pleuritic chest pain. Upon

admission to the hospital, his symptoms were compatible with acute decompensated heart failure. He had no recent viral illness or previous heart disease. Physical examination was notable for tachycardia (120-130 beats/min), tachypnea (18-27 breaths/min), elevated jugular venous pressure to the jaw angle, pulmonary crackles bilaterally, and S3 gallop.

Table 1 - Recent and past clinical course of our patient with hyper-IgE syndrome.

Age (yr)	Clinical complication and/or infection(s)	Treatment
24	Recurrence of chronic <i>Staphylococcus aureus</i> (MSSA) ¹ and <i>Fusarium falciforme</i> infections of the anterior chest wall <i>Fusarium</i> septic elbow joint Lymphocytic myocarditis Multiple abscesses of right axillae, <i>Staphylococcus aureus</i> (MSSA)	Culture-directed antibiotics, prophylactic voriconazole, trimethoprim-sulfamethoxazole Irrigation, debridement As in text Incision/drainage, culture-directed antibiotics
23	Recurrence of chronic fungal anterior chest wall wound Abscess of right biceps, <i>Staphylococcus aureus</i> (MSSA)	Debridement/excision of anterior chest wall wound with graft complicated by <i>S. aureus</i> (MSSA) post-surgical wound infection Incision/drainage with culture-directed antibiotics
20	Multiple bilateral axillary abscesses Left upper lobe lung abscess with empyema Left flank abscess Right supraclavicular lymphadenitis Giardia	Vancomycin, lymphadenectomy Metronidazole
19	Vertebral osteomyelitis in T11 <i>Staphylococcus aureus</i> (MSSA) pneumonia	Vancomycin Nafcillin
17	Pulmonary abscess	Left lung partial resection
16	Groin abscess Neck abscess	Incision and drainage Incision and drainage
15	Chronic and recurrent <i>Fusarium</i> fungal infection of anterior chest wall subcutaneous tissue Developed osteoporosis	Surgical excision and placement of fasciocutaneous flap after proven resistance to multiple antifungals including fluconazole, voriconazole, itraconazole, terbinafine, amphotericin B, and posaconazole Bisphosphonates - later discontinued after development of esophagitis Vitamin D and calcium supplementation
12	Sepsis and osteomyelitis of left neck of femur due to <i>Staphylococcus aureus</i> (MRSA) ²	Culture-directed antibiotics
8-11	Recurrent fractures of radius and ribs	
7	Recurrent sinusitis Recurrent otitis media	Sinus surgery Multiple tympanostomy tube placements
6	Pneumonia	Antibiotic therapy
4	Pneumonia	Antibiotic therapy

¹Methicillin sensitive *S. aureus*

²Methicillin resistant *S. aureus*

Electrocardiogram showed sinus tachycardia with diffuse ST segment elevations. Laboratory findings included elevated serum troponin I at 6.3 ng/mL (normal < 0.05 ng/mL) and brain natriuretic protein at 6353 pg/mL (normal 5-125 pg/mL). He had mild anemia with Hgb level 12.1 g/dL (normal 12.5-16.3 g/dL), and elevated absolute eosinophil count of 1.43 K/ μ L (normal 0-0.5K/ μ L). His previous eosinophil levels during the past two years ranged from 0.29 to 1.53 K/ μ L and mostly were less than 1.0 K/ μ L.

Two-dimensional echocardiogram revealed global hypokinesis of left ventricle with estimated ejection fraction 20-25%. On day two of the hospital admission, he developed episodes of sinus node pauses and left heart catheterization revealed ectasia of multiple coronary arteries. To rule out giant cell myocarditis, endomyocardial biopsy of the right ventricle was performed. It showed lymphocytic myocarditis, eosinophils in some blood vessel walls, focal necrosis, mild fibrosis, and myxoid degeneration (**figure 1**). The biopsy cultures were negative. Laboratory evaluation did not reveal infectious etiology, including HSV, parvovirus, CMV, EBV, Borrelia, HIV, or hepatitis. Methylprednisolone (1 mg/kg daily) was administered for two days followed by a 2-week prednisone taper. The patient improved and was given an external defibrillator (life vest) on discharge with plan to repeat echocardiogram after three months of heart failure therapy.

Six weeks after discharge, the patient was re-admitted due to lightheadedness and palpitations. He admitted poor compliance with wearing the cardiac monitor. Electrocardiogram was consistent with supraventricular tachycardia. Echocar-

diogram showed persistent global hypokinesis without significant change from prior echocardiogram. He was treated with amiodarone. Because of a non-healing chest skin infection, placement of implantable cardioverter defibrillator was deferred. Further immunosuppressive agents were not considered due to his underlying immunocompromised state. A trial of intravenous immunoglobulin (IVIG) was initiated in a modest dose (400 mg/kg) because of the cardiac condition and was repeated as outpatient every four weeks for four times. However, his cardiac function did not improve and he had frequent hospital admissions for heart failure. Within 24 hours of his last admission, the patient became acutely dyspneic, cyanotic, and subsequently collapsed. He developed severe hypotension and pulseless electrical activity arrest. Bedside echocardiogram was negative for pericardial effusion. Resuscitation was attempted but all efforts were unsuccessful. The family did not consent for autopsy.

Discussion

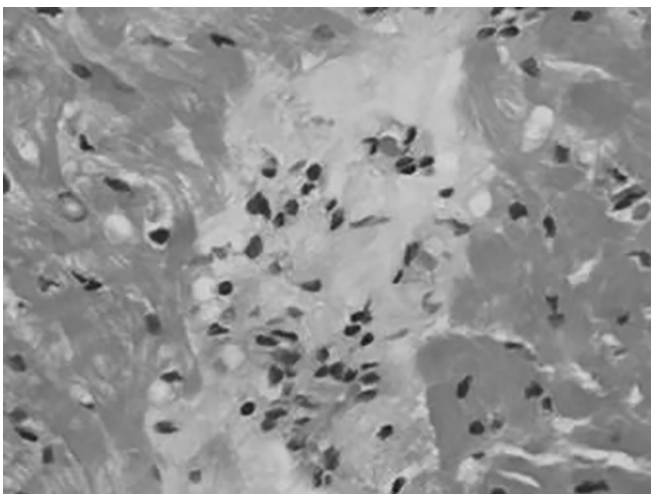
Hyperimmunoglobulin E syndrome (HIES) is extremely rare with an estimated prevalence of 1 in 100,000 individuals (2). The condition was initially called "Job's syndrome," based upon a description of the biblical story on Job in the Old Testament "so went Satan forth from the presence of the Lord, and smote Job with sore boils from the sole of his foot unto his crown" (Job 2:7).

HIES can be autosomal dominant or autosomal recessive. The majority of patients have the autosomal dominant (AD-HIES) form with an underlying STAT3 gene defect (3,4). In addition to eczema, AD-HIES is associated with high susceptibility to staphylococcal and fungal infections and defective dentition, skeletal, and connective tissues. The autosomal recessive form is not associated with skeletal or dental involvement but can have severe viral infections, marked eosinophilia, and severe neurologic complications (5).

In some patients, the connective tissue defect can lead to cardiac disease such as coronary arteries tortuosity, vascular ectasia, focal aneurysms, and pseudoaneurysms (6,7). In animal experiments, cardiac myocyte-specific STAT3 deficiency in mice leads to cardiomyopathy with early cardiac failure (8). However, to the best of our knowledge, neither cardiomyopathy nor myocarditis has been reported in patients with HIES, except for a presumptive diagnosis of myocarditis in a 2-year-old child that was not specified by a biopsy (9). In our patient, lymphocytic myocarditis was biopsy-diagnosed and did not seem to be secondary to infection. The biopsy did not show significant eosinophils and the patient's circulating eosinophil count was not different from those during the previous years.

Initially, the cardiac dysfunction seemed to improve on corticosteroids in addition to appropriate treatment of heart

Figure 1 - Histology specimen of biopsy from the endomyocardium of right ventricle demonstrating lymphocytic infiltrate within the myocyte fibers, focal necrosis, and myxoid degeneration.



failure including beta blockers, spironolactone, furosemide, ACE-inhibitors, and fluid restriction. Immunosuppressants were not considered because of their high risk in such patients. IVIG has been used, as an immunomodulatory therapy in a few scattered cases of acute myocarditis, presumably viral-induced, with good outcomes (10,11,12). However, a recent Cochrane review concluded that more studies are needed before IVIG is routinely recommended for myocarditis (13). When our patient was readmitted, IVIG was administered in a modest dose (400 mg/kg) to avoid circulation overload on top of his heart failure. He showed some improvement that permitted his discharge from the hospital. It was repeated in the clinic every four weeks and he received a total of four doses. Unfortunately, his cardiac condition deteriorated and he died despite standard treatment of heart failure. Normally, transcription factor STAT1 has a proapoptotic effect and STAT3 has a protective effect on cardiac myocytes by antagonizing STAT1, and thus protecting against ischemia / reperfusion (14). Therefore, STAT3 mutation, as in our patient, could be a risk factor for myocarditis.

Conclusions

We report a biopsy-proven lymphocytic myocarditis as a complication of HIES in a young adult who was maintained in a relatively satisfactory clinical condition from early childhood in spite of multiple complications. Both immunologists and cardiologists should be aware of such a complication. Also, caution is required in considering the use of immunosuppressants when the immune status is markedly compromised. More data is needed on the dosage and potential benefit of using IVIG in myocarditis. To the best of our knowledge, this is the first reported case of HIES complicated with lymphocytic myocarditis.

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References

1. Buckley RH. The Hyper-IgE Syndrome. *Clin Rev Allergy Immunol.* 2001;20(1):139-54.
2. Mogensen TH. STAT3 and the Hyper-IgE syndrome: Clinical presentation, genetic origin, pathogenesis, novel findings and remaining uncertainties. *JAK-STAT.* 2013;2(2):e23435.
3. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature.* 2007;448:1058-62.
4. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med.* 2007;357:1608-19.
5. Renner ED, Puck JM, Holland SM, Schmitt M, Weiss M, et al. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. *J Pediatr.* 2004;144:93-9.
6. Yavuz H, Chee R. A review on the vascular features of the hyperimmunoglobulin E syndrome. *Clin Exp Immunol.* 2010;159(3):238-44.
7. Gharib AM, Pettigrew RI, Elagha A, Hsu A, Welch P, et al. Coronary abnormalities in hyper-IgE recurrent infection syndrome: depiction at coronary MDCT angiography. *AJR Am J Roentgenol.* 2009;193(6):W478-W481.
8. Hilfiker-Kleiner D, Hilfiker A, Fuchs M, Kaminski K, Schaefer A, et al. Signal transducer and activator of transcription 3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury. *Circ Res.* 2004;95:187-95.
9. el Noor IB, Venugopalan P, Johnston WJ, and Froude JR. Ventricular aneurysm and myocarditis in a child with the hyperimmunoglobulin E syndrome. *Eur Heart J.* 1995;16(5):714-15.
10. Ozyigit T, Unal Z, Ozben B. Successful intravenous immunoglobulin therapy in a case of acute fulminant myocarditis. *Arch Turk Soc Cardiol.* 2011;39(1):59-63.
11. Fairley SL, Herron B, Wilson CM, Roberts MJD. Acute fulminant necrotising lymphocytic myocarditis in a patient with mixed connective tissue disease: a rapid clinical response to immunosuppression. *Ulster Med J.* 2014;83(2):119-20.
12. Kato S, Morimoto S, Hiramitsu S, Uemura A, Ohtsuki M, et al. Successful high-dose intravenous immunoglobulin therapy for a patient with fulminant myocarditis. *Heart Vessels.* 2007;22:48-51.
13. Robinson J, Hartling L, Vandermeer B, Klassen TP. Intravenous immunoglobulin for presumed viral myocarditis in children and adults. *Cochrane Database of Systematic Reviews* 2015, Issue 5. Art. No.: CD004370. DOI: 10.1002/14651858.CD004370.pub3.
14. Scarabelli TM, Townsend PA, Chen Scarabelli C, Yuan Z, McCauley RB, et al. Amino acid supplementation differentially modulates STAT1 and STAT3 activation in the myocardium exposed to ischemia/reperfusion injury. *Am J Cardiol.* 2008;101(11):S63-S68.

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New therapeutic approach by sirolimus for enteropathy treatment in patients with LRBA deficiency

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KEY WORDS

Sirolimus; enteropathy; LRBA deficiency; common variable immune deficiency; autoimmunity

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Summary

Purpose. To report the successful use of sirolimus for management of enteropathy in four patients with LPS-responsive beige-like anchor protein (LRBA) deficiency. **Methods.** Case series. **Results.** sirolimus therapy led to a complete improvement of symptoms including decrease in frequency and severity of diarrhea, as well as patients' weight gain. No signs of abdominal cramps and anorexia were also detected during the follow up period after treatment. **Conclusions.** sirolimus with its potential efficacy and immunomodulatory properties may be recommended for the treatment of severe enteropathy refractory to conventional therapy in patients with LRBA deficiency.

Doi

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Introduction

LPS-responsive beige-like anchor protein (LRBA) deficiency is a rare genetic disorder caused by biallelic loss-of-function mutations in the *LRBA* gene. This disorder is characterized by early-onset hypogammaglobulinemia, chronic diarrhea and autoimmune manifestations (1-4). Similar to common variable immune deficiency (CVID) patients, affected individuals show a reduced levels of immunoglobulin (Ig) isotypes and suffer from recurrent infections, hepatosplenomegaly, chronic pulmonary disorders as well as auto-inflammatory conditions including idiopathic thrombocytopenic

purpura (ITP), autoimmune hemolytic anemia (AIHA) and enteropathy (1,5-9).

The enteropathy phenotype includes autoimmune enteropathy, inflammatory bowel disease (IBD)/IBD-like disease and non-infectious recurrent diarrhea. LRBA deficiency has been reported to be common among patients with CVID-like phenotype underwent genetic diagnosis (2,10,11). CVID patients and patients with LRBA deficiency resemble symptoms of enteropathy presenting in immunocompetent individuals, but the pathology is usually documented to be not similar and the symptoms often do not respond to the conventional therapies. In LRBA deficient

patients, chronic diarrhea is characterized by duodenal villous atrophy and large bowel lymphocytic infiltration (5). Recent studies have reported that the chronic and severe diarrhea in patients with LRBA deficiency may not improve despite intravenous Ig (IVIg) treatment (6,12). Medical therapy typically with corticosteroids (budesonide and prednisone), empiric antibiotic therapy and gluten free diets have been used commonly (13). In patients refractory to corticosteroids, treatment with immunosuppressive drugs such as azathioprine, 6-mercaptopurine, tacrolimus, mycophenolate mofetil, infliximab, and rituximab have been reported. Side effects are commonly documented in administration of this group of medications, and maintaining remission has been reported to be unsuccessful in previous studies (5,14-16). sirolimus, also known as rapamycin, is a macrocyclic lactone antibiotic which also has a profound immunosuppressive property on the cellular immune response, particularly on T cells. sirolimus binds to the same intracellular receptor as tacrolimus and cyclosporine, however does not inhibit calcineurin. sirolimus blocks the "mammalian target of rapamycin" (mTOR) which subsequently interrupts signaling pathways for several cytokines and growth factors including interleukin 2 (IL2). Recent studies have suggested the effectiveness of sirolimus to reduce chronic diarrhea in patients with enteropathy. Here, we report for the first time the successful use of sirolimus for management of enteropathy in four patients with LRBA deficiency.

Case presentation

Case 1. A 14 years old female patient with LRBA deficiency was diagnosed at the age of five years old with hypogammaglobulinemia. She is a child of related (first cousin) parents. Her first manifestation was diarrhea which started at six months of age. The patient underwent antibiotic therapy for the diarrhea but there was no improvement in her symptoms. Other manifestations included splenomegaly, hepatomegaly and juvenile rheumatoid arthritis at the age of four. She underwent treatment for immunodeficiency at five years of age with IVIg, accordingly her diarrhea was controlled. Diarrhea became more severe since a year ago, up to 20 times during the day and 8 times during the night, and consequently six kilograms-weight loss was detected. Infliximab was administrated for five months, but no improvement was observed in diarrhea and weight loss symptoms. The pathological report of the colonoscopy showed edema and excess infiltration of lamina propria with lymphocytes and eosinophils. Focal micro-abscess formation and cryptitis were also detected. Her microscopic reports were conclusive of mild chronic gastritis, esophagitis and active colitis but no parasite or *Helicobacter pylori* infection was reported.

Case 2. A 21-year-old LRBA deficient female patient with refractory diarrhea is a child from the first cousin related parents. The first presentation of her disease was diarrhea which started

at the age of 13. She was diagnosed with celiac based on the endoscopic evaluation, thus she underwent gluten free diet, but there was no improvement in her symptoms. The patient has a history of other autoimmune complications including autoimmune thyroiditis, ITP and AIHA. IVIg therapy had been started two years ago which was not effective for her autoimmune symptoms. Splenectomy was performed one year ago for the treatment of the cytopenia. She was also on prednisolone treatment in the last year, but no improvement in her diarrhea and weight loss symptoms were seen. The patient experienced a nine kilograms weight loss and cachexia during the last year. The results of the endoscopic and colonoscopy samples were suggestive of celiac like enteropathy, chronic active gastritis and atrophic duodenal mucosa with villus atrophy.

Case 3. This case is a six years old female patient with LRBA deficiency and a history of recurrent diarrhea has been documented for this patient. She is a child from a first cousin related parents, with no family history of immunodeficiency. Until the age of four, the patient had no symptoms or signs of diarrhea. She was hospitalized at the ages of four and six years old, both due to chronic diarrhea and consequent severe dehydration. Frequency of diarrhea was more than 10 times a day (with an increased volume and wateriness). The pathologic report of the colonoscopy revealed cryptitis and crypt abscess associated with lymphocytic infiltration and neutrophils in the lamina propria. Results were suggestive of active colitis and especially of IBD.

Case 4. The fourth case was a 27 years old male, born to consanguineous parents. His symptoms started at the age of two with respiratory tract infection (RTI) and identified with tentative diagnosis of CVID at the age of 10. He was under observation since age two, and several episodes of pneumonia, sinusitis and diarrhea were detected. Other complications included arthritis, bronchiectasis, failure to thrive and clubbing. Patient's diarrhea did not respond well to treatment with corticosteroids. Histopathologic evaluation showed infiltrations of lymphocytes, plasma cells and eosinophil cells in the lamina propria of the intestinal epithelium; suggestive of acute ileitis. Furthermore, infiltration of lymphocytes, plasma cells and polymorphonuclear leukocytes (PMNs) were reported in the colonic mucosa with preserved cryptic architecture which was suggestive of non-crypt destructive colitis.

Immunologic characteristics of four patients with LRBA deficiency are illustrated in **table 1**. For all patients, colonoscopy was performed and the diagnosis of enteropathy and acute colitis were confirmed. As prior therapy including infliximab, cotrimoxazole, clarithromycin and gluten free diet had failed to control the disease process, sirolimus therapy with the dosage of 1 mg/day for three months was started for all patients. This treatment led to a complete improvement of their symptoms including decrease in frequency and severity of diarrhea and im-

provement in the patients' weight. Evidence of a response trend is further documented by normal serum level of albumin, calcium and potassium.

Discussion

LRBA deficiency is characterized by combined immunodeficiency, enteropathy, and autoimmune complications. According to previously published studies, clinical features in patients with LRBA deficiency are heterogeneous, and first presentations of the disease often occur during childhood (2). The cohort study of Alkhairy et al. (2) divided the disease phenotypes into categories of RTI, autoimmunity, organomegaly, and enteropathy. They described the enteropathy phenotype as an overlapped group of autoimmune enteropathy, IBD/IBD-like disease and non-infectious diarrhea (6). In our study, all patients were early onset, and a broad range of complications including RTI and autoimmune manifestations were reported. Moreover, gastrointestinal complications including chronic diarrhea were seen in all patients.

Treatment of the autoimmunity, chronic diarrhea and associated colitis is challenging in patients with antibody deficiency (17,18). Uzzan et al. (19) reported that Ig supplementation does not significantly effect on the course of non-infectious gastrointestinal disease in CVID patients. Currently available treatments including steroids and cyclosporine have resulted in remission only in a subset of patients, then large doses of steroids are often necessary to control active disease. Patients with autoimmune enteropathy commonly do not respond to conventional treatment or other non-specific

immune suppression therapies, however autoimmune enteropathy has been reported with partial response to immunosuppressive drugs such as cyclosporine, azathioprine and 6-mercaptopurine (20). Tacrolimus has been used as a treatment option for enteropathies, especially in autoimmune patients. Its mechanism of action is similar to cyclosporine. Both drugs block the gene activation for cytokine production by inhibiting the antigenic response of helper T lymphocytes (21). Bousvaros et al. (16) in 1996 used tacrolimus for the first time as an alternative therapy for autoimmune enteropathy, and concluded that it can be efficacious when other immunosuppressive regimens fail (16). Mycophenolate mofetil has been also proposed as an alternative therapeutic option after the successful induction of remission in an infant with autoimmune enteropathy (22).

In the present report, we used sirolimus for clinical management of enteropathy in patients with LRBA deficiency. We showed that disease symptoms such as chronic diarrhea and weight loss were successfully controlled after administration of sirolimus. Massey et al. (23) reported effectiveness of sirolimus in treatment of refractory Crohn's disease in an adult patient. Mutalib et al. (24) showed that sirolimus, by inducing both clinical remission and mucosal healing, is effective in children with severe IBD refractory to conventional therapies. In another study, Araki et al. (25) found that treatment of a severe refractory colonic and perianal Chron's disease with sirolimus may result in a marked improvement in symptoms of enteropathy. Yong et al. (26) also reported the impact of sirolimus in children with IPEX and IPEX-like enteropathy. Although satisfying results of ad-

Table 1 - Immunologic characteristics of patients with LRBA deficiency underwent sirolimus therapy.

Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Normal Range
IgG (mg/dL)	0	765	111	360	656-1351
IgM (mg/dL)	0	69	28	44	34-255
IgA (mg/dL)	4	5	24	0	86-320
IgE (IU/ml)	0	0.3	3	-	Up to 46
White blood cell count (cell/ μ L)	8210	8730	14700	14400	4000-11000
Lymphocytes (cell/ μ L)	2320	2095	7497	2016	1000-2800
CD3 ⁺ (cell/ μ L)	1717	1739	4273	1492	700-2100
CD4 ⁺ (cell/ μ L)	116	649	1724	363	300-1400
CD8 ⁺ (cell/ μ L)	1554	670	2624	1109	200-900
CD4 ⁺ /CD8 ⁺ ratio	0.07	0.968	0.66	0.32	1-3
CD16-56 ⁺ (cell/ μ L)	819	98	-	-	90-600
CD19 ⁺ (cell/ μ L)	93	147	675	121	100-500

ministration of sirolimus have been documented in patients with autoimmune enteropathy prior to our study (26,27), there was no evidence of using sirolimus in treatment of enteropathy in LRBA deficient patients. In the current study, for the first time, four LRBA deficient patients unresponsive to non-specific immune-suppressive agents underwent sirolimus therapy. Following administration of sirolimus, the frequency of diarrhea decreased and the patients' weight gradually normalized. Therefore, sirolimus with its potential efficacy and immunomodulatory properties may be recommended for the treatment of severe enteropathy in LRBA deficiency. Further studies should be designed to provide evidence for the effectiveness of sirolimus administration in management of diarrhea in immunodeficient patients by providing detailed pathological and microbiological evidences after treatment.

References

- Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *American journal of human genetics*. 2012;90(6):986-1001. PubMed PMID: 22608502. Pubmed Central PMCID: 3370280.
- Alkhaairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of Phenotypes Associated with Mutations in LRBA. *Journal of clinical immunology*. 2016;36(1):33-45. PubMed PMID: 26707784.
- Azizi G, Rezaei N, Kiaee F, Tavakolinia N, Yazdani R, Mirshafiey A, et al. T-Cell Abnormalities in Common Variable Immunodeficiency. *Journal of investigational allergology & clinical immunology*. 2016;26(4):233-43. PubMed PMID: 27374799.
- Yazdani R, Abolhassani H, Rezaei N, Azizi G, Hammarstrom L, Aghamohammadi A. Evaluation of Known Defective Signaling-Associated Molecules in Patients Who Primarily Diagnosed as Common Variable Immunodeficiency. *International reviews of immunology*. 2016;35(1):7-24. PubMed PMID: 26959802.
- Burns SO, Zenner HL, Plagnol V, Curtis J, Mok K, Eisenhut M, et al. LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia. *The Journal of allergy and clinical immunology*. 2012;130(6):1428-32. PubMed PMID: 22981790. Pubmed Central PMCID: 3930010.
- Alangari A, Alsultan A, Adly N, Massaad MJ, Kiani IS, Aljebreen A, et al. LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. *The Journal of allergy and clinical immunology*. 2012;130(2):481-8 e2. PubMed PMID: 22721650. Pubmed Central PMCID: 3582381.
- Azizi G, Abolhassani H, Asgardoorn MH, Alinia T, Yazdani R, Mohammadi J, et al. Autoimmunity in common variable immunodeficiency: epidemiology, pathophysiology and management. *Expert review of clinical immunology*. 2016Sep16:1-15. PubMed PMID: 27636680.
- Azizi G, Abolhassani H, Asgardoorn MH, Shaghghi S, Negahdari B, Mohammadi J, et al. Managing patients with side effects and adverse events to immunoglobulin therapy. *Expert Rev Clin Pharmacol*. 2016;9(1):91-102. PubMed PMID: 26496172.
- Marefat H, Abolhassani H, Ghareje Daghi M, Azizi G, Aghamohammadi A. Early onset steroid induced posterior subcapsular cataract in a patient with common variable immunodeficiency: case reports and review of literature. *European annals of allergy and clinical immunology*. 2016;48(5):197-201. PubMed PMID: 27608477.
- Serwas NK, Kansu A, Santos-Valente E, Kuloglu Z, Demir A, Yaman A, et al. Atypical manifestation of LRBA deficiency with predominant IBD-like phenotype. *Inflamm Bowel Dis*. 2015;21(1):40-7. PubMed PMID: 25479458.
- Azizi G, Ahmadi M, Abolhassani H, Yazdani R, Mohammadi H, Mirshafiey A, et al. Autoimmunity in Primary Antibody Deficiencies. *International archives of allergy and immunology*. 2016;171(3-4):180-93. PubMed PMID: 28013299.
- Azizi G, Abolhassani H, Rezaei N, Aghamohammadi A, Asgardoorn MH, Rahnavard J, et al. The Use of Immunoglobulin Therapy in Primary Immunodeficiency Diseases. *Endocrine, metabolic & immune disorders drug targets*. 2016Jul24. PubMed PMID: 27456825.
- Teahon K, Webster AD, Price AB, Weston J, Bjarnason I. Studies on the enteropathy associated with primary hypogammaglobulinaemia. *Gut*. 1994;35(9):1244-9. PubMed PMID: 7959231. Pubmed Central PMCID: PMC1375701.
- Cunningham-Rundles C. How I treat common variable immune deficiency. *Blood*. 2010;116(1):7-15. PubMed PMID: 20332369. Pubmed Central PMCID: 2904582. Epub 2010/03/25. eng.
- Charbonnier LM, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. *The Journal of allergy and clinical immunology*. 2015xw;135(1):217-27. PubMed PMID: 25468195. Pubmed Central PMCID: 4289093.
- Bousvaros A, Leichtner AM, Book L, Shigeoka A, Bilodeau J, Semeo E, et al. Treatment of pediatric autoimmune enteropathy with tacrolimus (FK506). *Gastroenterology*. 1996;111(1):237-43. PubMed PMID: 8698205. Epub 1996/07/01. eng.
- Azizi G, Ziaee V, Tavakol M, Alinia T, Yazdai R, Mohammadi H, et al. Approach to the Management of Autoimmunity in Primary Immunodeficiency. *Scandinavian journal of immunology*. 2017;85(1):13-29. PubMed PMID: 27862144.
- Azizi G, Mirshafiey A. Imatinib mesylate: an innovation in treatment of autoimmune diseases. *Recent patents on inflammation & allergy drug discovery*. 2013;7(3):259-67. PubMed PMID: 23947692.
- Uzzan M, Ko HM, Mehandru S, Cunningham-Rundles C. Gastrointestinal Disorders Associated with Common Variable Immune Deficiency (CVID) and Chronic Granulomatous Disease (CGD). *Curr Gastroenterol Rep*. 2016;18(4):17. PubMed PMID: 26951230. Pubmed Central PMCID: PMC4837890.
- Gentile NM, Murray JA, Pardi DS. Autoimmune enteropathy: a review and update of clinical management. *Curr Gastroenterol Rep*. 2012;14(5):380-5. PubMed PMID: 22810979. Pubmed Central PMCID: 3912565. Epub 2012/07/20. eng.
- Vanderhoof JA, Young RJ. Autoimmune enteropathy in a child: response to infliximab therapy. *J Pediatr Gastroenterol Nutr*. 2002;34(3):312-6. PubMed PMID: 11964960. Epub 2002/04/20. eng.
- Baud O, Goulet O, Canioni D, Le Deist F, Radford I, Rieu D, et al. Treatment of the immune dysregulation, poly-

- endocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. *N Engl J Med*. 2001;344(23):1758-62. PubMed PMID: 11396442. Epub 2001/06/09. eng.
23. Massey DC, Bredin F, Parkes M. Use of sirolimus (rapamycin) to treat refractory Crohn's disease. *Gut*. 2008;57(9):1294-6. PubMed PMID: 18719139.
24. Mutalib M, Borrelli O, Blackstock S, Kiparissi F, Elawad M, Shah N, et al. The use of sirolimus (rapamycin) in the management of refractory inflammatory bowel disease in children. *Journal of Crohn's and Colitis*. 2014;8(12):1730-4.
25. Araki K, Ellebedy AH, Ahmed R. TOR in the immune system. *Curr Opin Cell Biol*. 2011;23(6):707-15. PubMed PMID: 21925855. Pubmed Central PMCID: PMC3241972.
26. Yong PL, Russo P, Sullivan KE. Use of sirolimus in IPEX and IPEX-like children. *Journal of clinical immunology*. 2008;28(5):581-7. PubMed PMID: 18481161. Epub 2008/05/16. eng.
27. Bindl L, Torgerson T, Perroni L, Youssef N, Ochs HD, Goulet O, et al. Successful use of the new immune-suppressor sirolimus in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). *J Pediatr*. 2005;147(2):256-9. PubMed PMID: 16126062. Epub 2005/08/30. eng.