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Banana lectin (BanLec) induces non-specific activation of basophils and mast cells in atopic subjects

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Keywords
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Abbreviations
BanLec, banana lectin; BSA, bovine serum albumin; Con A, concanavalin A; HR, histamine release; OVA, ovalbumin; PBS, phosphate-buffered saline; PECs, peritoneal exudate cells; SPT, skin prick test; Tris-CAM buffer, 10 mM Tris-HCl buffer, pH 7.4 containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.03% BSA.

Summary
Dietary lectins play a major role in the activation of mast cells/basophils by bridging cell surface IgE glycans to release histamine and other mediators. In the present study, the effect of mannose/glucose-specific banana lectin (BanLec) on the activation of mast cells/basophils from non-atopic and atopic subjects has been investigated. BanLec was purified from banana pulp in a yield of 7 mg/kg. Leukocytes isolated from heparinized blood of non-atopic/atopic subjects were used for quantitation of the released histamine. Approximately 28.2% of the atopics (n = 117) was positive by skin prick test (SPT) to purified BanLec (100 µg/mL concentration), and all the non-atopics (n = 20) were negative. Maximal release of histamine was seen at 2 µg of BanLec. In percent histamine release, an increase of 35-40% is observed in case of atopics (n = 7) compared to non-atopics (n = 5), and the histamine release from atopic and non-atopic subjects correlates fairly well with the total serum IgE levels (R^2 = 0.817). BanLec also induces release of histamine (26.7%) from mast cells present in rat peritoneal exudate cells. BanLec can significantly activate and degranulate mast cells and basophils by cross-linking the trimannosidic core mannose of IgE glycans in atopic population as compared to non-atopic population; the activation is marginal in the case of non-atopics.

Introduction

Lectins are proteins or glycoproteins of plant or animal sources that bind specific carbohydrates and agglutinate cells of various types (1); they are a diverse group of multivalent sugar-binding proteins of non-immune origin and are ubiquitous in all forms of living matter, including bacteria and viruses (1,2). Their characteristic agglutination properties imply possible involvement of the membrane glycoproteins or glycolipids containing specific carbohydrate residues in the interaction with lectins (2,3).
Several lectins from plant sources have been well studied and characterized. Concanavalin A (Con A) from jack bean (*Ca-*

navania ensiformis) has been investigated extensively in terms of the protein structure, saccharide specificity and its interaction with a variety of cells (4); it has been shown to activate basophils and mast cells specifically by binding to the carbohydrates on the Fc portion of cell-bound IgE, resulting in the release of histamine and other biological mediators (5,6). Many dietary lectins have been investigated for their interactions with cells of the immune system (thymocytes, splenocytes, dendritic cells and macrophages) and allergic system (mast cells, basophils and eosinophils) (7).

Bridging of IgE molecules on the cell surface of mast cells/basophils by an allergen or bivalent antibody against IgE or artificially aggregated IgE is a necessary event for IgE-mediated basophil or mast cell degranulation (8,9). The interaction of lectins with basophils/mast cells (4,5,10) leads to release of histamine and other biological mediators, and thus resembles the response of the interaction of food allergens with mast cells/basophils from allergic subjects (11,12). Allergic reactions caused by the activation of mast cells/basophils by dietary lectins are commonly termed as non-allergic food hypersensitivity (formerly termed as "false food allergy"). Lectins can either interact with carbohydrates on cell-bound IgE or directly with the carbohydrates of cell surface glycoproteins and glycolipids on basophils and mast cells (5,10); however, the end result is similar to the food allergen-specific IgE interaction seen in food allergy (13). A detailed study showed that potato lectin (*Solanum tuberosum* agglutinin) induces non-specific activation of mast cells and basophils of atopic subjects, and an excellent correlation was seen between histamine release from the buffy coat and serum total IgE level (14). Therefore, it appeared important to address the role of other common dietary plant lectins in mediating false-food allergic reactions.

Banana is widely consumed all over the globe as a wholesome nutritional fruit containing 75% moisture, 23% carbohydrates, 1.1% protein and 0.33% fat (wet weight basis) (15). Among the proteins, BanLec is a minor protein present in relatively low amounts: 4 mg lectin/100 g fruit, edible portion (16,17). Banana lectin (BanLec) is a homodimeric plant lectin (subunit molecular weight: 15 kDa; isoelectric point: 7.2-7.5) belonging to the jacalin-related lectin family (16,17). BanLec belongs to a subgroup of this family that binds to glucose/mannose, but is unique in recognizing internal α-1,3 linkages as well as β-1,3 linkages at the reducing termini (18,19). The structure of BanLec has a β-prism I fold, similar to other family members, but differs from them in its mode of sugar binding; the reducing unit of the sugar is inserted into the binding site causing the second saccharide unit to be placed in the opposite orientation compared with the other ligand-bound structures of family members (20,21). BanLec does not agglutinate untreated human or sheep erythrocytes, but agglutinates rabbit erythrocytes, and is known to stimulate T-cell proliferation (22).

The present study is focused on studying the effect of purified BanLec on basophils and mast cells from different atopic subjects. Since lectins are often present in significant amounts in many plant foods (1,2,7,11,16), it appeared interesting to study the interactions of BanLec with mast cells and basophils of atopic and non-atopic subjects, to understand their physiological significance and role in non-allergic food hypersensitivity reactions.

Materials and methods

Reagents, allergenic extracts and animals

Sephadex G-75, compound 48/80, *o*-pthalaldehyde (OPT), pectinase, concanavalin (Con A), Favin from *Vicia faba* (broad bean, fava bean or field bean) and murine anti-human IgE (monoclonal)-alkaline phosphatase (AP) conjugate were products of Sigma-Aldrich Co., St. Louis, MO, USA. Lysozyme, ovalbumin (OVA) and bovine serum albumin (BSA) were purchased from Bangalore Genei, Bengaluru, India. Flat-bottom 96-well microtiter plates (MICROLON) were bought from Greiner Bio-One GmbH, Frickenhausen, Germany. All other chemicals/reagents used in this study were of analytical grade.

Southern grass pollen mix (no. 1651, Bayer Corp., Spokane, WA, USA) contained pollens from Bermuda, Johnson, Kentucky blue, Orchard, Redtop, sweet Vernal, and timothy grasses; this is referred to as grass pollen mix 1. Grass pollen mix (no. P28, Greer Laboratories, Lenoir, NC, USA) contained pollens from Bermuda, Johnson, Kentucky blue, Orchard, Redtop, Timothy, sweet Vernal meadow, fescue, and perennial rye grasses; this is referred to as grass pollen mix 2. House dust mite extract (*D. farinae*, 10,000 AU/mL) and weed pollen mix were also products of Greer Laboratories, Lenoir, NC, USA. Skin prick tests (SPT) were performed with grass pollen mix 1, grass pollen mix 2, weed mix and house dust mite extract to classify whether the patient was atopic or non-atopic.

Experiments involving animals have been conducted in accordance with the "International Guiding Principles for Biomedical Research Involving Animals" guidelines recommended by the World Health Organization (WHO) for the use of laboratory animals, after obtaining approval from the Institutional Animal Ethics Committee (IAEC). Adult male Wistar rats (4-week-old) housed in the animal house facility of our institute were used for the preparation of peritoneal exudate cells (PEC) as per standard operating procedures described later.

Identification of atopic and non-atopic subjects

All procedures involving human subjects were approved by the Institutional Research Ethics Committee (approval number: IHEC-07-04), and were conducted in accordance with the ethi-

cal standards established in the Declaration of Helsinki of 1946 and its later amendments or comparable ethical standards. Written informed consent was obtained from all atopic (allergic) and non-atopic (normal) subjects before enrollment in this study.
The atopic (n = 117 of which 57 were males and 60 were females) and non-atopic (n = 20 of which 11 were males and 9 were females) subjects were identified based on case history (allergic subjects are chosen at random who had symptoms of at least one allergic condition such as allergic rhinitis, atopic dermatitis, asthma, food allergy, and allergic conjunctivitis) of the subjects and the results of skin prick test (SPT) of certain common allergenic extracts (grass pollen, weed, house dust mite) and prepared banana extracts. The age of the subjects was in the range of 18 to 60 years with a median of 41 years. Banana extract (50% w/v) prepared from the commonly consumed variety (*Musa acuminata*) was used for this study.

Eosinophil count, serum IgE and serum/plasma histamine levels

Eosinophil count was determined using whole blood, and expressed as numbers per μL of blood (23). Murine monoclonal anti-human IgE antibody (murine IgG2a, κ; hybridoma cell line ATCC HB-121, designation E5BB3IIA2) was purified by hybridoma cell culture supernatant on protein A-agarose; this cell line was obtained from National Centre for Cell Science, Ganeshkhind, Pune, India. Serum total IgE (expressed as IU/mL, and hereafter referred to as serum IgE) was quantitated by ELISA (24) using this antibody. Following TCA precipitation of serum, histamine was extracted, determined by fluorometry (25), and expressed as ng/mL serum.

Preparation of banana extract from banana pulp

Banana was purchased from the local grocery market; after peeling the skin, the pulp portion (500 g) was chopped into pieces to prepare 50% (w/v) extract in salt solution (250 mM NaCl/5 mM $MgCl_2$/5 mM $MnCl_2$/5 mM $CaCl_2$) containing 0.5% pectinase. The extract was stirred for 2 h and left at 25 °C overnight; later, the extract was filtered through Whatman no. 1 filter paper. The filtrate was concentrated using Amicon ultrafiltration system using a membrane having 3 kDa molecular weight cut-off, and the retentate obtained was extensively dialyzed against 50 mM sodium acetate buffer, pH 4.

Purification of banana lectin (BanLec)

This was essentially performed as per the method described by Koshte et al. (16); this is an affinity-based purification wherein banana lectin having specificity to glucose/mannose will bind to the dextran matrix of Sephadex G-75, whereas all the other proteins will not bind to the column and elute based on their molecular masses. Banana lectin was isolated from 50% (w/v) banana extract on Sephadex G-75. The concentrated, dialyzed sample (28 mL) was loaded onto Sephadex G-75 column (2 × 110 cm), which was pre-equilibrated with 50 mM sodium acetate buffer, pH 4.0. After loading the sample, the column was washed with the equilibration buffer until the absorbance has fallen to 0.005. The bound proteins were then eluted with 50 mM sodium acetate buffer, pH 4, containing 0.15 M NaCl and 0.5 M D-glucose. The eluted fractions were pooled and analyzed by SDS-PAGE and hemagglutination assay.

SDS-PAGE (reducing) and hemagglutination assay

SDS-PAGE (12%, reducing) was carried out following the standard procedure (26) to assess the purity and relative molecular mass of the purified banana lectin. Hemagglutination activity of the purified lectin was carried out using trypsinized rabbit erythrocyte suspension as described by Burger (27). Briefly, a 2% suspension of trypsinized rabbit erythrocytes (0.2 mL) was added to a serially diluted lectin solution, gently mixed and incubated at 37 °C for 1 h, and the agglutination was visualized. The amount of protein present at the highest dilution represents the minimum quantity of protein necessary for agglutination and is taken as the titer.

Preparation of samples (BanLec and banana extract) for SPT

Based on protein estimation by Bradford method (28), banana extract and banana lectin were prepared at 2 mg/mL and 200 μg/mL in phosphate-buffered saline, respectively. The samples were then diluted 1:1 using glycerol (analytical grade) to obtain banana extract at 1 mg/mL and BanLec at 100 mg/μL concentration; these samples were used for performing SPT on non-atopic and atopic subjects.
Banana extract (1 mg/mL) and BanLec (0.1 mg/mL) was used for SPT; these concentrations were chosen since in many allergological studies, purified allergens (natural or recombinant) have been used in the concentration range of 20 μg/mL to 1 mg/mL, and most whole food extracts have been used for SPT at a maximum concentration of 10 mg/mL. Glycerinated-PBS and histamine base (at 1 mg/mL) were used as negative and positive reference standards. SPT was carried out as per the described protocol (29). After 20 min, the wheal and flare diameters were measured; a wheal diameter of 3 mm greater than that of the negative control was considered as positive.

ELISA for detection of BanLec-specific IgE

BanLec-specific IgE was detected by indirect ELISA (24). Briefly, microtiter wells were coated with 10 μg of BanLec at pH 9.6 at 4°C overnight. After the blocking step, the wells were incubated with

subjects' sera at 1:3 dilution in PBS containing 1% BSA/0.05% Tween-20 at 4 °C overnight. Next, incubation was done with murine monoclonal anti-human IgE-AP conjugate (1:1500 dilution) at 37 °C for 2 h, followed by color development.

Isolation of leukocytes containing basophils

The buffy coat (leukocyte layer containing basophils) was isolated from 10 mL of venous blood drawn from non-atopic (n = 10) or atopic (n = 20) subjects as described (30) using 6% dextran T-700 gradient. The buffy coat was washed 4-5 times with isotonic PBS and resuspended in Tris-CAM buffer (10 mM Tris-HCl buffer, pH 7.4 containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.03% BSA). The isolated leukocytes were counted using crystal violet stain. Percentage viability of leukocytes in the buffy coat was determined by Trypan blue dye exclusion.

Isolation of rat peritoneal exudate cells (PEC)

PECs were isolated from male Wistar rats (adult; 4-weeks-old weighing ~250-300 g) following the standard procedure (31) using Tyrode buffer, pH 7.4 containing 0.1% BSA. After injecting the peritoneal cavity, the fluid containing PECs was collected and the cells were pelleted, washed with physiological salt solution, and finally resuspended in Tris-CAM buffer. PECs were stained for mast cells using toluidine blue, and their viability was assessed by Trypan blue dye exclusion. The PEC preparation was found to contain 15-20% mast cells.

Histamine release (HR) assay

Cells and reagents (BanLec or other proteins) in Tris-CAM buffer were added to polystyrene tubes at a final volume of 1 mL in an ice bath. Each tube containing ~2 × 10^6 cells/mL was incubated at 37 °C for 45 min (30). In each experiment, perchloric acid (final concentration: 3%) was added to one set of samples (alternatively, one set of samples was boiled at 100 °C for 10 min), to obtain the total histamine content of cells (Pc). Blank tubes containing only cells and buffer were used as controls for non-specific or spontaneous release (Ps), which was generally < 10%. After 45ß min, the tubes were transferred to an ice bath to stop the reaction and centrifuged at 1600 rpm at 4 °C for 20 min; the supernatants were assayed for histamine content (Pt).

The released histamine was quantitated by a fluorometric assay (32). Briefly, the histamine in the supernatant was extracted initially into *n*-butanol, and then HCl; the histamine extracted into HCl was neutralized, derivatized using OPT, and the reaction arrested using phosphoric acid. The fluorescence intensity of the derivatized histamine was measured using a spectrofluorometer (λ_{ex} = 360 nm; λ_{em} = 450 nm). The formula for the calculation of percent HR (A%) is = (Pt - Ps) / (Pc - Ps) × 100, where Pt refers to test release, Ps refers to spontaneous release, and Pc refers to complete release.

Statistical analysis

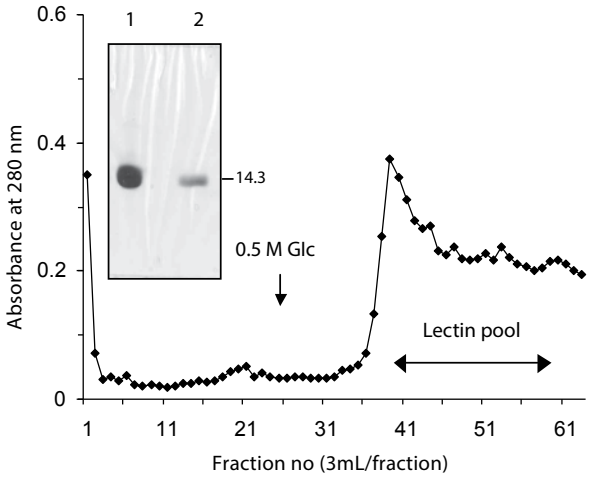
Each datum represents the arithmetic mean and standard deviation (s.d.) of the different experiments under identical conditions. Student's t-test was used to make a statistical comparison between the paired and unpaired groups. The correlation between histamine release and serum IgE was analyzed to find the correlation coefficient. A p-value of < 0.05 was considered statistically significant. All the statistical analyses were performed using software SPSS, version 10 (SPSS Inc., Chicago, IL, U.S.).

Results

Purified banana lectin exhibits hemagglutination activity

Banana lectin was isolated from banana pulp by affinity chromatography on Sephadex G-75. The column-bound proteins were eluted with elution buffer containing 0.5 M D-glucose. The elution profile is shown in **figure 1**. Fraction numbers 35-58 were pooled, and analyzed by SDS-PAGE (12%) and hemag-

Figure 1 *- Affinity chromatography of BanLec from pectinase-treated banana pulp on Sephadex G-75 (2 × 110 cm). Equilibration and loading buffer: 50 mM sodium acetate buffer, pH 4 containing 0.15 M NaCl. Elution buffer: 50 mM sodium acetate buffer, pH 4 containing 0.15 M NaCl and 0.5 M D-glucose. Fraction volume: 3 mL. Inset: 12% SDS-PAGE (reducing); lane 1, purified banana lectin (10 µg); lane 2, molecular weight marker, 6 µg (lysozyme, 14.3 kDa).*



glutination assay. The pooled fraction showed a single band by SDS-PAGE having a molecular mass of 15 kDa (**figure 1**, inset) and exhibited hemagglutination activity towards 2% rabbit erythrocytes; the activity was mannose-specific as analyzed by glycoprotein-binding ELISA assay (data not shown). The hemagglutination activity of the pooled fraction was ~28.6 units/mg protein, wherein one unit of hemagglutination activity is referred to as the minimum amount of protein required for causing hemagglutination activity. The yield of banana lectin from 500 g of banana pulp was found to be 3.5 mg.

SPT of BanLec on atopic subjects indicates a high positivity

Atopic and non-atopic subjects were selected based on detailed case history and clinical symptoms. The subjects' status of atopic or non-atopic was confirmed in all the subjects under evaluation (n = 117 for atopics, and n = 20 for non-atopics) based on SPT. Subjects were considered atopic if they had generalized symptoms characteristic of at least one allergic condition and a positive SPT (> 3 mm over the negative control) to one of the allergens tested. Subjects were considered non-atopic if they did not have any clinical symptoms suggestive of allergies and had a negative SPT. Consecutive 117 atopic subjects consenting to participate in the study were included; non-atopic subjects were selected from the general population. The results are summarized in **table I,** which shows the results of SPT with BanLec tested on 117 atopic subjects and on 20 non-atopic subjects. BanLec at 100 µg/mL showed a positive SPT in 33 out of 117 atopic subjects (28.2%). It is interesting to note that SPT using banana extract shows that 29 out of 117 atopic subjects (24.8%) were positive. SPT reactions as assessed by means of wheal/flare diameter were barely positive (designated as +; 3-3.5/5 mm) or moderately positive (designated as 2+; 4-4.5/10-15 mm) compared to the positive control, histamine base (6/25 mm). None of the non-atopic subjects gave a positive SPT (wheal/flare diameter of 0-1/0 mm) for both BanLec and banana extract (**table I**).

Table I *- Skin prick test[a] of banana lectin (BanLec) on human atopic and non-atopic subjects.*

Sample used for SPT	Subjects tested	Number of subjects tested	Number of subjects positive	Percent positive	Wheal/flare diameter (mm)
BanLec (100 µg/mL)	atopic[b]	117 (m, 57; f, 60)[c]	33 (m, 15; f, 18)	28.2	3.0 - 4.5/8 - 15
Banana extract (50% w/v)	atopic	117	29 (m, 11; f, 18)	24.8	3.0 - 5.0/8 - 16
BanLec/banana extract	non-atopic[d]	20 (m, 11; f, 09)	0	0.0	0 - 1/0

[a]The positive control used for SPT is glycerinated histamine base (1 mg/mL), and the negative control is glycerinated PBS.

[b]Subjects displaying characteristic symptoms from any one of the following: asthma, allergic rhinitis, urticaria or food allergy (age range: 18-60 y).

[c]m, male; f, female.

[d]Healthy subjects without any clinical symptoms of allergy (age range: 18-60 y).

Table II *- Eosinophil counts, serum IgE, and serum/plasma histamine levels in a subset of non-atopic/atopic subjects.*

Subjects	Eosinophil	Serum IgE		Histamine level	
	mean ± s.e.m. (counts/µL)[a]	mean ± s.e.m. (A_{492})	IgE (IU/mL)[b] ± s.e.m.	serum mean ± s.e.m. (ng/mL)[c]	plasma mean ± s.e.m. (ng/mL)[d]
non-atopic (n = 10)	302 ± 11	0.267 ± 0.010	39.3 ± 4.6	28.2 ± 3.6	1.5 ± 1.2
atopic (n = 20)	776 ± 18	1.205 ± 0.120	253.7 ± 76.4	184.2 ± 10.1	11.6 ± 1.0

[a]Reference normal value for eosinophil counts = 40 - 400 cells/µL (23); $p < 0.001$ ($t = 38.2$);

[b]Reference normal value for serum total IgE = < 120 IU/mL (24); $p < 0.001$ ($t = 13.40$);

[c]Value for non-atopic subjects is 5 - 27 ng/mL (25); $p < 0.001$ ($t = 15.74$);

[d]Value for non-atopic subjects is 0.5 - 2 ng/mL (25); $p < 0.001$ ($t = 10.64$).

In a subset of 20 atopic and 10 non-atopic subjects, eosinophil counts, serum IgE and serum/plasma histamine levels were measured. The results are shown in **table II**. The serum total IgE was found to be significantly higher in atopic subjects, and represents approximately a 5 to 7-fold increase over the value for non-atopic subjects. In atopic subjects, the eosinophil counts were increased by ~2.6-fold over the mean value of non-atopic subjects (302 cells/µL). The serum and plasma histamine levels were found to be significantly higher in atopic subjects (~6 to 8-fold) as compared to the mean value for non-atopics.

Based on the clinical symptoms, the atopic subjects were subgrouped as representing allergic rhinitis, asthma, or both; this is summarized in **table III**. Again, in the sub-groups the sensitivity for BanLec was maximal in the sub-group who had clinical symptoms of both allergic rhinitis and asthma, compared to the sub-groups with only allergic rhinitis or asthma. The number of subjects who avoid eating banana was found to be 60 out of 117 (51.3%); these subjects reported that they avoided eating banana as they had experienced an increase in their allergic symptoms upon consumption of banana on most occasions. However, some of them have no SPT reactivity to either banana extract or to BanLec. On the other hand, none of the non-atopic subjects reported the avoidance of banana consumption. About half of the atopic subjects tested in each sub-group were having case history positive to banana, and they were found to avoid banana consumption.

Table III *- Results of SPT to BanLec in atopic subjects[a] characterized into three subgroups as allergic rhinitis, asthma, and allergic rhinitis with asthma.*

Subjects' status	Subjects positive to BanLec[b]	Percent positive	Subjects positive to banana extract[c]	Percent positive	Avoidance to banana[d] (n)	Avoidance to banana (%)
allergic rhinitis (n = 33)	07 (m 3, f 4)[e]	21.2	11 (m 4, f 7)	33.3	15 (m 6, f 9)	45.5
asthma (n = 39)	10 (m 5, f 5)	25.6	09 (m 4, f 5)	23.1	20 (m 9, f 11)	51.3
allergic rhinitis with asthma (n = 45)	16 (m 7, f 9)	35.5	09 (m 3, f 6)	20.0	25 (m 14, f 11)	55.6

[a]Atopic subjects were selected for SPT based on their case history and SPT results to allergenic extracts including house dust mite, and classified into subgroups based on their clinical symptoms;

[b]Atopic subjects who showed skin prick test positive (> 3 mm wheal) for BanLec (100 µg/mL);

[c]Atopic subjects who tested positive for banana extract (50% w/v) by SPT were tested here at 1 mg/mL banana extract;

[d]Number of atopic subjects who avoid eating banana and have a positive case history for banana consumption;

[e]m, male; f, female.

Table IV *- Summary of in vivo and in vitro diagnostic tests in the subgroup of atopic subjects positive to BanLec by SPT.*

Subjects' status[a]	Subjects positive to BanLec	Wheal/flare diameter (mm)[b]	Specific IgE ELISA units[c] (A_{405}) mean ± s.e.m.	Serum IgE ELISA units[c] (A_{492}) mean ± s.e.m.	Histamine release[d] (%) (range)
non-atopic	00	0 - 1/0	0.036 ± 0.016	0.280 ± 0.044	23.4 - 28.9
atopic (mildly sensitive to BanLec)	00	1 - 3/0 - 5	0.049 ± 0.011	0.466 ± 0.100	37.8 - 42.2
atopic (moderately sensitive to BanLec)	23	3 - 4/5 - 10	0.063 ± 0.023	0.737 ± 0.155	44.9 - 56.1
atopic (highly sensitive to BanLec)	10	4 - 5/> 10	0.084 ± 0.018	1.311 ± 0.347	58.5 - 68.3

[a]n = 10 in each group;

[b]Positive control, 1 mg/mL histamine base (5 - 6/20 - 25 mm);

[c]Value for non-lectin control (BSA) (n = 6), 0.018 (non-atopic); 0.020 (atopic); value for lectin control (Con A) (n = 6), 0.056 (non-atopic), 0.099 (atopic); mean of 3 values;

[d]measured at 2 µg/mL BanLec concentration; mean of 3 values.

Atopic subjects show a higher level of serum IgE compared to non-atopic subjects

Serum samples of non-atopic (n = 10) and atopic (n = 10) subjects were tested for the presence of BanLec-specific IgE. Con A (Man/Glc-specific lectin) and BSA (non-lectin protein) were used as negative controls. BanLec-specific IgE values for moderately BanLec-sensitive atopic subjects were very similar to those seen for mildly BanLec-sensitive atopic and non-atopic subjects (**table IV**). However, the serum IgE level of moderately BanLec-sensitive subjects is ~1.5 to 2-fold higher than that of mildly BanLec-sensitive subjects, and ~2 to 3.5-fold higher than that of non-atopic subjects who are not sensitive to BanLec.

BanLec induces a higher magnitude of HR release from atopics as compared to non-atopics

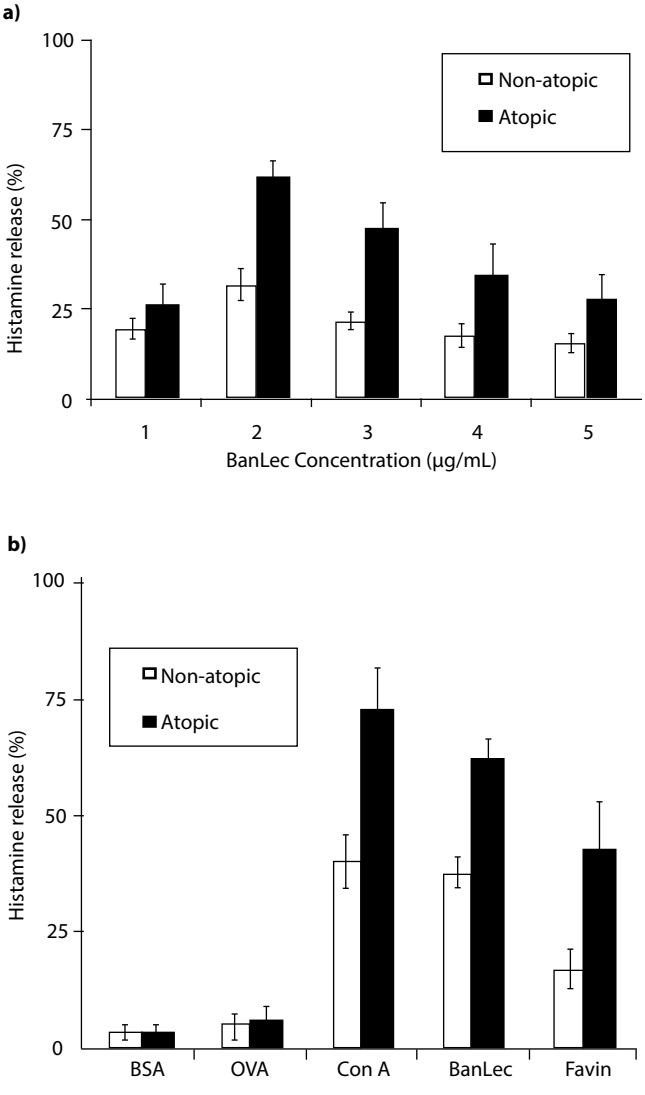
The results of percent HR from non-atopic (n = 5) and atopic subjects (n = 7) by BanLec in a concentration range of 1 to 5 µg/mL are presented in **figure 2** (panel **a**). Maximal release of histamine occurs at a concentration of 2 µg/mL; an increase of about 1.5-fold in HR was observed in the case of atopic subjects (62.0 ± 4.7%) compared to non-atopic subjects (37.8 ± 3.7%). The HR was found to be significant ($p < 0.005$) at 2 µg/mL concentration.

Con A (positive lectin control) was found to induce HR from both non-atopic and atopic subjects; here again, maximal release was found at 2 µg/mL concentration. The percent HR was ~40% in non-atopics (healthy individuals without any symptoms of allergy) and ~73% in atopic subjects (**figure 2**, panel **b**). Both the non-lectin proteins (BSA and ovalbumin) do not release histamine in either non-atopic or atopic subjects; HR using these non-lectin proteins was found to be only 4% in non-atopics and 6% in atopics. Generally, the HR is considered negative if the value is < 10%, and the percent HR as a function of BanLec was comparable with the positive lectin control Con A. Another mannose-specific lectin Favin (from *Vicia fava*) shows a similar trend as seen for Con A and BanLec, although of a lower magnitude (**figure 2**, panel **b**).

The magnitude of HR by BanLec correlates with the serum IgE level

Ten subjects in the non-atopic group and 20 subjects in the atopic group were analyzed for HR and total IgE levels. The mean value of percent HR as well as the serum total IgE values for both non-atopic and atopic (including its sub-groups) groups are shown in **table IV**. Since all non-atopic subjects showed serum IgE ELISA unit of < 0.33, 0.35 value was arbitrarily taken as the cut-off point of serum IgE (ELISA unit) for demarcating non-atopics and atopics. On the basis of the serum total IgE level and HR, the atopics can be sub-grouped roughly into three types: (i) mild sensitivity to BanLec with marginal HR, (ii) moderate sensitivity to BanLec with moderate HR, and (iii) high sensitivity to BanLec with high HR. It is seen that all atopic subjects showed a HR of > 35%. Further, the results show that the percent HR was found to have a fairly good correlation with the serum total IgE levels ($R^2 = 0.817$, n = 30).

Figure 2 *- Panel* **a**, *comparison of percent histamine release from the leukocytes of non-atopic (n = 5) and atopic (n = 7) subjects as a function of BanLec concentration (1 to 5 µg/mL). Panel* **b**, *Comparison of histamine release from the leukocytes of atopic (n = 7) and non-atopic (n = 5) subjects as a function of BanLec with the lectin control (Con A) and non-lectin controls (non-lectin proteins: BSA and OVA). Protein amount used: 2 µg in all cases.*



BanLec also induces HR from rat peritoneal exudates cells (PECs)

Con A (Man/Glc-specific lectin), which is taken as a reference lectin showed ~58% HR and can be regarded as positive control for HR assay using rat peritoneal exudates cells (**figure 3**). BSA (data not shown) and ovalbumin (non-lectin proteins) show minimal HR and can, therefore, be considered as negative controls. Favin and BanLec were found to induce the release of histamine (about 4-fold and 6-fold, respectively) in comparison to the HR value of the negative control, OVA.

Discussion

BanLec shows specificity for molecules containing D-glucopyranosyl, D-mannopyranosyl and other related carbohydrate structures (33-35); it shares many properties with Glc/Man-recognizing legume lectins (Con A). The yield of BanLec was found to be 7 mg/kg banana pulp; its purity was confirmed by SDS-PAGE (single band of 15 kDa) and hemagglutination activity indicated glucose/mannose specificity.

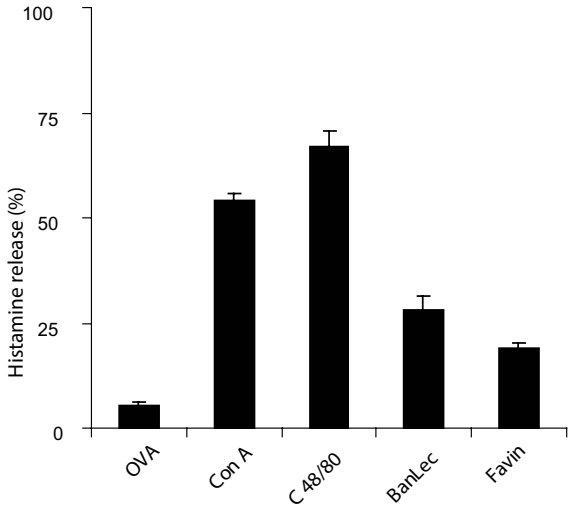
SPT of BanLec revealed that roughly one fourth of the atopic subjects were positive, whereas non-atopic subjects were negative. A positive SPT of 28.2% to a purified protein (BanLec) from banana pulp appears to be unusual for food allergy, since the incidence of food allergy in adults is generally 2-4% (8,9). This may be due to the non-specific interaction of BanLec with the carbohydrate structures of mast cells *in vivo* followed by activation. Similar results have been observed from our earlier studies on the effects of potato lectin in SPT of atopic subjects (14). In the case of atopic subjects who showed a positive SPT for BanLec, the serum IgE level was, in general, 3-8-fold higher than in non-atopic subjects. The banana reactors (24.8% among atopic patients as assessed by positive SPT) also had BanLec reactivity (28.2% among atopic patients as seen by positive SPT); however, the reactors did not show any specific IgE to banana protein(s). It is likely that the sensitivity is due to the effect of BanLec both in pure form or as crude form (in banana extract) which causes wheal/flare reaction through non-allergic food hypersensitivity by interacting with non-specific IgE on mast cells or basophils.

Mannose-binding lectins (Con A, *Lens culinaris* agglutinin, and pea agglutinin) bind strongly to human IgE (5); the binding is mainly attributable to the complex-type glycopeptide of IgE (36). BanLec has specificity for Glc/Man similar to Con A (18,19). Since cell-bound IgE is a glycoprotein rich in oligosaccharides (~12%) in its Fc portion (both oligomannose and complex bi-antennary types), we examined the composition and structures of the N-linked glycans on the heavy chain of IgE (37). Among the glycans of human IgE, ~86% of glycans terminate in galactose or sialic acid, which represent complex bi-antennary type glycans (36). Though the serum IgE level was 2 to 8-fold higher in atopic subjects (who are positive to BanLec by SPT) as compared to non-atopics, BanLec-specific IgE was found to be very similar in the serum of both atopic and non-atopic subjects confirming that none of the atopic subjects were truly allergic to BanLec. Although 6 allergens (Mus a 1 to Mus a 6) have been identified as allergens in banana so far, BanLec has not been reported as an allergen in the WHO/IUIS Allergen Nomenclature home page (www.allergen.org). Lectins have been ranked ninth in their assignment as plant food allergen families in Pfam database (38), and are generally regarded as minor allergens.

Koshte et al. (22) observed that IgG4 antibodies to banana were found to occur far more frequently than expected, and the most important antigen involved proved to be BanLec; the authors firmly established the antigen-antibody nature of the BanLec-IgG4 interactions. Their results support the earlier suggestion that some lectins are particularly prone to induce an immune response upon oral feeding (7). Several studies in the past decade have shown that BanLec is a mucosal immunostimulator, and that oral administration of BanLec modulates cytokine profile and abundance of T-cell populations in mice (39-41).

HR from the leukocytes of non-atopic and atopic subjects by BanLec was found to be dependent on serum IgE levels; the release shows a fairly good correlation to serum IgE levels (R^2 = 0.8166); such a correlation clearly indicates that the effect of BanLec depends on the basophil IgE density in causing non-spe-

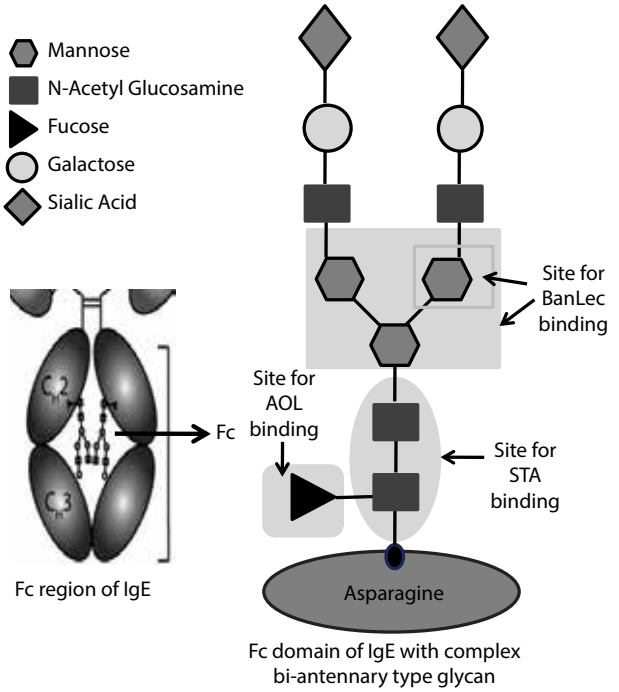
Figure 3 *- Histamine release from rat PECs containing mast cells at 2 µg protein. Compound 48/80 was used as a positive control for histamine release from mast cells. Con A was used as a lectin control, and BSA and OVA were used as non-lectin controls.*



cific activation. This is strikingly similar to the effect of Con A, wherein the HR is higher than spontaneous release (seen for non-lectin proteins) in non-atopics, and comparatively more so in atopics (4,6,13).

It is interesting to note that a clear correlation between serum IgE and expression of FceRI on basophils has been established earlier in several allergic conditions (42). Con A-induced HR has been shown to be dependent on the IgE density on basophils (4,6,8); Con A binds to terminal/internal mannose on IgE glycans, and cross-links cell-bound non-specific IgE (4,13,42) leading to degranulation. Dam et al. (43) showed that a significant correlation exists between the histamine-releasing properties of Diocleinae lectins (Glc/Man specificity) and their relative affinity constants for biantennary complex carbohydrates.

Figure 4 *- Structure of complex bi-antennary type N-glycan of IgE (3,36) showing the binding site for BanLec (specificity for mannose shown as Man (shown in a small box), and for trimannoside core (shown in a large box)); the specificity for Man and trimannoside core is similar to that of concanavalin A (Con A). Solanum tuberosum agglutinin (potato lectin or STA) has specificity for GlcNAc oligomers (14), and the core chitobiose unit is indicated by an arrow. Aspergillus oryzae lectin (AOL) has specificity for α1,6-fucosyl residue (44) which is generally referred to as the "core fucose" in complex N-glycans, and this is also indicated by an arrow. One of the two branches of the IgE glycans may or may not contain sialic acid.*



Non-specific degranulation of mast cells/basophils has also been demonstrated by the interaction of potato lectin (specific for core chitobiose) and *Aspergillus oryzae* lectin (specific for α1,6-fucosyl residue or "core fucose") of non-specific IgE (14,44). The binding sites of potato lectin, *Aspergillus oryzae* lectin and BanLec to the respective sugars on the complex bi-antennary type *N*-glycans of IgE are represented summarily in **figure 4**.

In addition to binding to the trimannoside core of IgE glycans, one may speculate that BanLec can also bind to the mannose residues of the *N*-glycans of α-chain of human FcεRI, the high-affinity IgE receptor on mast cells/basophils (8-10); the extracellular domain of α-chain is heavily glycosylated (38-42% *N*-linked, and 4% *O*-linked glycans of α-chain molecular mass) (45). The expression of FcεRI is dependent on serum IgE; since the receptor number is certainly higher in the case of atopics (42), BanLec can potentially cross-link free cell-surface FcεRI through the α-chain, and cause activation.

Conclusions

SPT using 100 μg/mL BanLec was positive in 28.2% of atopic subjects although there was no evidence of type I allergy to BanLec as shown by the absence of BanLec-specific serum IgE. HR from the leukocytes of non-atopic and atopic subjects by BanLec was found to show a moderately good correlation to serum IgE levels (R^2 = 0.817). Based on the results of HR from rat PECs, leukocytes of atopics, and positive SPT to BanLec in a majority of atopic population, it can be concluded that the binding of BanLec to basophils and mast cells is primarily through its interaction with the trimannoside core of *N*-glycans of cell-bound non-specific IgE; on the contrary, non-atopic subjects show only marginal activation and degranulation of mast cells/basophils. This may explain why several atopic subjects (suffering from allergic rhinitis, asthma, or both) experience adverse reactions upon consumption of banana fruit and therefore avoid eating banana, although they are not truly allergic to banana fruit.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Hyperlipidemia in association with pro-inflammatory cytokines among chronic spontaneous urticaria: case-control study

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Key words
chronic spontaneous urticaria; hyperlipidemia; IL6; lipid profile; TNFα

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Summary
Chronic spontaneous urticaria (CSU) is a disorder characterized by recurrent transient itchy wheels of 6 weeks duration or longer. The cause cannot be pinpointed in about 40% of patients. To elucidate the possible association between CSU and hyperlipidemia, 40 CSU patients and 40 group matched healthy individuals were assessed for hyperlipidemia. Data on history, urticaria activity score (UAS-7), physical examination and routine laboratory investigations including lipid profile (serum IL6 and TNF α) was recorded. Statistically significant in-crease of serum cholesterol, triglycerides (TG), low density lipoprotein (LDL), IL6, TNFα and decrease of high density lipoprotein (HDL) was found in CSU in comparison to control group. Regarding the different disease variables, both TG and cholesterol were positively correlated with duration of illness, urticaria activity score and serum TNF α. Serum LDL detected significant positive correlation with duration of illness, urticaria score, CRP and TNF α while serum HDL detected significant negative correlation with TNF α. IL6 and TNFα associated systemic inflammation could be a common pathogenic mechanism of CSU and hyperlipidemia. Patients with CSU should be evaluated for hyperlipidemia.

Introduction

Urticaria is described as wheals (swelling/erythema) and/or angioedema lasting for 1-24 hours. The European Academy of Allergy and Clinical immunology (EAACI), the Global Allergy and Asthma European Network (GA2LEN), the European Dermatology Forum (EDF) and the World Allergy Organization (WAO) guidelines sorted urticaria according to the duration and cause. Urticaria lasting more than 6 weeks is divided into two categories; inducible or spontaneous urticaria (1,2).

Chronic spontaneous urticaria (CSU) is stated as an immuno-inflammatory disorder characterized by the recurrent occurrence of wheals and/or angioedema lasting for more than 6 weeks (1). Causes of CSU may be known (e.g. autoantibodies) or unknown. Autoimmune mechanisms have been considered in 30-50% of cases (3). The inducible urticaria has variable triggers (cold, delayed pressure, solar, heat, vibratory, cholinergic, contact, and aquagenic) urticaria. More than one type of urticaria can coexist in the same patient (4). The natural course of CSU is self-limiting, with spontaneous remissions and exacerbations, however, impairment of the quality of life is usually severe (5).

Though the pathogenesis of CSU remains unknown, some studies have revealed the existence of intrinsic abnormalities in basophils or mast cells (6,7). Nonetheless, CSU inflammatory processes are not restricted to the consequences of mast/basophil degranulation (7). CSU is characterized by cutaneous mast cell degranulation, in addition to infiltration of the skin by eosinophils, neutrophils and T lymphocytes (8).

Foregoing studies suggested that immunological malfunction is the primary background in CSU. This is confirmed by the dysfunctional innate immune response which was found in CSU patients in the form of increased levels of C-reactive protein (CRP), proinflammatory cytokines such as interleukin (IL-6), tumor necrosis alpha (TNF-α), and matrix metalloproteinase-9 with an altered pattern of regulatory cytokine secretion (9). These inflammatory markers were noticed to harmonize well with activity score of urticaria and its severity (7,9,10).

Also, a systemic pro-inflammatory state marked by the elevation of serum level of inflammatory mediators as IL-6, TNF, and CRP are detected in patients with metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, and plasma glucose level). Dyslipidemia was incriminated to be involved in the inflammatory mechanisms of atherosclerosis associated with metabolic syndrome (11).

Consequently, both CSU and metabolic syndrome have been characterized by systemic inflammation which was pinpointed in various studies (12,13). Furthermore, a greater frequency of metabolic syndrome was detected in patients with CSU compared to healthy controls (13). However, few studies have yet specifically determined a link between hyperlipidemia and CSU. Thus, the aim of the current study was to clarify the possible association between hyperlipidemia and proinflammatory cytokines among chronic urticaria patients.

Methodology

Study participants

Forty patients with established diagnosis of chronic spontaneous urticaria according to standard European Academy of Allergology and Clinical Immunology/the Global Allergy and Asthma European Network (EAACI/GA2LEN) guidelines (2) were enrolled in the study. They were recruited from the allergy clinic of Ain Shams University hospital during the period from February 2015 to March 2017. The diagnosis of CSU was considered if the wheals last for 6 weeks or longer at least 2 times a week and its underlying cause remained unidentified notwithstanding the appropriate investigations with pseudoallergen-free diet for 3 weeks. No one of the participants was smoker, body mass index more than 25, had food/medication induced allergy including NSAIDS hypersensitivity or autoimmune diseases including thyroid autoimmune diseases, or urticarial vasculitis. Other exclusion criteria were inducible urticarial lesions by chronic infections, H pylori, environmental agents, stress or physical agents as cholinergic stimulation, dermatographism or pressure. Another forty group-matched healthy individuals were enrolled as a control group. The study was approved by the allergy and clinical immunology review board and research ethics committee of Ain Shams University. All participants did sign an informed consent.

Study design

All participants were subjected to detailed history including past and family history as well as urticaria activity score (UAS-7) and complete clinical examination excluding the aforesaid etiologies. The onset, duration, characteristics and distribution of lesions, history of any associated medical illness or allergies, drug history were recorded. Standard investigations were executed as skin test for allergy including physical tests, autologous serum skin test, urine analysis, stool analysis, Helicobacter pylori antigen, liver enzymes, kidney function tests, complete blood picture, thyroid antibodies, ESR, CRP, hepatitis C virus antibody, hepatitis B virus surface antigen and human immunodeficiency virus antibodies.

Assessment of urticaria activity score

We assessed CSU disease activity using the weekly urticaria activity score (UAS-7). It is evaluated according to the number of wheals and pruritus intensity assumed on the EAACI/GA2LEN/EDF guidelines. We asked the patients to record scores of 24 hours self-evaluation for 7 days and documented it as: no wheals = 0; < 20 wheals/24 hour = 1; 20-50 wheals/24 hour = 2; and > 50 wheals/24 hour = 3; and pruritus intensity: no = 0; mild = 1; moderate = 2; and severe = 3. The weekly UAS-7 (equal to the sum of the scores on 7 consecutive days) ranges from (0-42) (2).

Skin Prick Test

We performed the skin prick test using the most conventional aeroallergen extracts. The result was interpreted after 15-20 minutes. It was considered justifiable when the difference in mean wheal diameters was at least 1 mm between the histamine (positive) and saline (negative) controls. Patient is considered sensitized to that peculiar allergen when a wheal diameter is ≥ 3 mm more than the negative control (14).

Autologous serum skin test

Participants stopped all medications containing antihistamines and corticosteroids for at least 5 days before performance of the test. We performed the test according to the EAACI/GA2LEN task force consensus guidelines. The test is positive when a serum-induced wheal is at least 1.5 mm more than that induced by saline after 30 minutes (15).

Anti-nuclear antibody, anti-thyroid antibodies and inflammatory markers

Anti-nuclear antibody, anti-thyroglobulin, and thyroid microsomal antibodies were measured using indirect fluorescent antibody technique using (INOVA Diagnostics, San Diego, CA). Serum levels of C-reactive protein were assayed by immunoturbidimetry using kit supplied by Behring Diagnostics (GmbH, Marburg, Germany). Commercially available enzyme-linked immunosorbent Assay (ELISA) kits were utilized for assessment of TNF- α (Sigma-Saint Louis, Missouri, Germany) (Catalog number: CKH-200A) with kit sensitivity 4.4 pg/ml, and IL-6 ELISA kits (Biocompare; antibodies-online; Schloss-Rahe-Str. 15, Aachen, Germany) (Catalog number: ABIN414297) with assay sensitivity 6.4 pg/mL.

Lipid profile

Lipid profile was done including fasting cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides measured on Synchron CX9 auto-analyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634-3100, USA).

Statistical Analysis

Data were analyzed using SPSS version 22 (IBM). Shapiro-Wilk test was used to examine normality of numerical data distribution. Mean and SD represented the normally distributed numerical variables and the two independent sample (unpaired) Student t-test was used to compare intergroup differences between the two groups. Levene's test was used to examine equality of variances between groups. Mean and SD represented the non-normally distributed numerical data and Mann-Whitney U test was used to compare intergroup differences between the two groups. Pearson's correlation was used to check normally distributed variables for linear correlation. However, Spearman's Rank correlation was used for Non-Normally distributed variables. Categorical variables were presented as number or proportion and percentage, and intergroup differences were compared using Fischer's exact test (for nominal data) or Chi-Squared Test for Trend (for ordinal data). The diagnostic value of serum continuous variables was examined by Receiver-operating characteristic (ROC) curve analysis. The area under the ROC Curve (AUC) was compared with that of random prediction. The relationship between normally distributed variables was investigated by linear regression and curve estimation was used to plot these relations. A two-sided p-value < 0.05 was considered statistically significant. A two-sided p-value < 0.01 was considered highly statistically significant.

Results

Clinical and laboratory characteristics of both cases and control groups are shown in **table I**. Cases included 22 males and 18 females. Their mean age was 37.13 ± 10.371. Mean age of controls was 37.03 ± 10.58 years. 19 were males and 21 were

Table I *- Demographic data and laboratory investigations of both studied groups.*

		Group				test of significance	
		cases		control			
		mean/n	SD/%	mean/n	SD/%	p-Value	sig.
age (years)		37.13	10.37	37.03	10.58	0.966[T]	ns
gender	male	22	55%	19	47.50%	0.655[F]	ns
	female	18	45%	21	52.50%		
duration of illness (month)		8.25	4.37				
urticarial severity score		18.9	12.3				
serum IL6 (pg/ml)		8.73	17.79	28.87	31.64	< 0.001[M]	s
serum TNFα (pg/ml)		11.25	16.6	38.71	34.89	< 0.001[M]	s
serum CRP (mg/dl)		11.58	5.65	19.89	18.37	0.266[M]	ns
serum TG (mg/dl)		143.8	23.36	179.23	31.26	< 0.001[T]	s
serum cholesterol (mg/dl)		150.68	49.41	248.45	58.43	< 0.001[M]	s
LDL (mg/dl)		175.2	61.9	77.9	50.3	< 0.001[M]	s
HDL (mg/dl)		40.43	6.87	44.38	5.02	0.004[T]	s

[M]Mann-Whitney test; [T]t-test; [F]Fisher's Exact test.

females. Serum level of IL_6, TNFα, Cholesterol, TG, LDL and HDL were highly elevated with statistical significance in group of patients compared to control group with p-value (< 0.001, < 0.001, < 0.001, < 0.001, < 0.001, 0.004) respectively. However, non-significant difference regarding gender, age or CRP was found.

Table II exhibits the correlation of serum IL6 and TNF α level with different disease variables, in which serum IL6 show significant positive correlation with urticaria activity score (p = 0.031). Serum TNF α was positively correlated with duration of illness (p-value < 0.001) and urticaria activity score (p-value < 0.001) with statistical significance. However, no statistically significant difference of both in relation to age or gender.

The descriptive and statistical difference of serum TG and cholesterol levels as regard the different disease variables is shown in **table III**, in which both TG and cholesterol were positively correlated with duration of illness (p < 0.001, < 0.001), urticaria activity score (p = 0.003, < 0.001) and serum TNF α (p = 0.024, < 0.001), respectively.

Regarding the different disease variables, serum LDL detected significant positive correlation with duration of illness (p = < 0.001), urticaria activity score (p = < 0.001), serum CRP (p = < 0.001) and TNF α (p = < 0.001) while serum HDL was negatively correlated with serum TNF α (p = 0.033) as shown with statistical significance in **table IV**.

Diagnostic performance of (cholesterol/TG) as a cause of CSU is shown in **figure 1**. At the cut-off value of Cholesterol 163 mg/dl; the sensitivity = 92%, specificity = 85%, PPV = 86.0% and NPV = 91.9%. While at the cut-off value of TG 154 mg/dl; the sensitivity = 80%, specificity = 67.5%, PPV = 68.1% and NPV = 75.8%. At the cut-off value of LDL > 88 mg/dl; the sensitivity = 92.500 %, specificity = 80%, PPV = 82.2 % and NPV = 91.4%. While at the cut-off value of HDL < 40 mg/dl; the sensitivity = 57.5 %, specificity = 80%, PPV = 74.2% and NPV= 65.3%.

Table II *- Correlation between serum levels of both IL6, TNF*α *with different disease variables.*

Correlation between cases group		age	urticaria severity score	duration of illness
serum IL6 (pg/ml)	Pearson Correlation	- 0.129[P]	0.342[P]	0.15[S]
	p-Value	0.254	0.031	0.354
	sig.	NS	S	NS
serum TNFα (pg/ml)	Pearson Correlation	0.105[S]	0.869[S]	- 0.819[S]
	sig. (2-tailed)	0.355	< 0.001	< 0.001
	sig.	NS	S	S

[S]Spearman's correlation; [P]Pearson's correlation.

Table III *- Correlation between serum levels of both cholesterol and TG with different disease variables.*

Correlation between cases group		age	urticaria score	duration of illness	IL6	TNFα	CRP
serum cholesterol (mg/dl)	Pearson correlation	- 0.072[P]	0.819[S]	0.869[S]	0.291	0.637	0.0585
	p-Value	0.528	< 0.001	< 0.001	0.069	< 0.001	< 0.001
	sig.	ns	s	s	ns	s	s
serum TG (mg/dl)	Pearson correlation	- 0.08[S]	0.46[S]	0.504[S]	0.292	0.356	0.217
	sig. (2-tailed)	0.478	0.003	<0.001	0.068	0.024	0.179
	sig.	ns	s	s	ns	s	ns

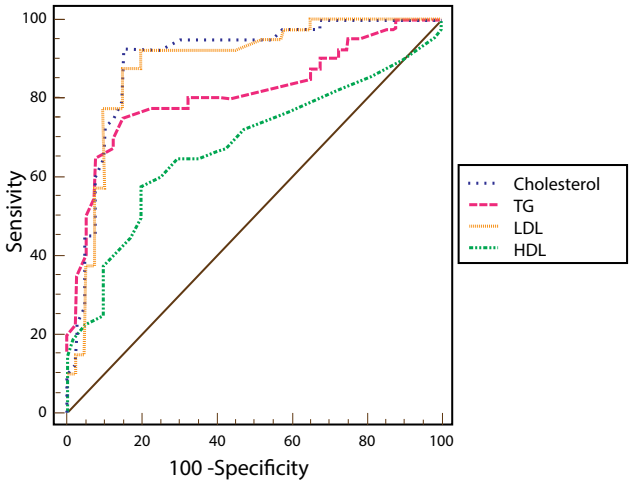
[S]Spearman's correlation;

[P]Pearson's correlation.

Table IV *- Correlation between serum level of both LDL and HDL with different disease variables.*

Correlation between cases group		age	urticaria score	duration of illness	IL6	TNFα	CRP
LDL (mg/d)	Pearson correlation	- 0.198	0.843	0.708	0.272	0.739	0.566
	p-Value	0.222	< 0.001	< 0.001	0.089	< 0.001	< 0.001
	sig.	ns	s	s	ns	s	s
HDL (mg/d)	Pearson correlation	0.119	- 0.182	- 0.149	- 0.086	- 0.341	- 0.169
	sig. (2-tailed)	0.464	0.260	0.360	0.599	0.033	0.296
	sig.	ns	ns	ns	ns	s	ns

Figure 1 *- ROC for significance of lipid profile as cause of urticaria.*



Discussion

The pathogenesis of chronic spontaneous urticaria (CSU) remains unknown, but autoimmune abnormalities, disorders of histamine-releasing factors, and cellular defects have all been involved (16). Patients with CSU have an increased risk of antecedent hyperlipidemia. Little studies had formerly reported a link between hyperlipidemia and CSU, however, an increased level of inflammatory markers as IL-1, Il-6, and TNF-α in both patients with CSU and those with metabolic syndrome has been signified (9,10,11). Hence, the rationale of the current study was to investigate the possible association between chronic urticaria and hyperlipidemia.

In our study, there was highly statistically significant increase in group 1 compared to group 2 in serum Cholesterol, TG, LDL, and statistically significant decrease in HDL. However, non-significant difference regarding gender, age or CRP was found. TG and cholesterol were positively correlated with duration of illness, urticaria activity score and serum TNF α. Serum LDL was positively correlated with duration of illness, urticaria activity score, serum CRP, TNF α, and serum HDL detected significant negative correlation with serum TNF α.

This is in correspondence with Chung and colleagues, who found a 1.65-fold increased risk of antecedent hyperlipidemia in 36.1% of CU patients in comparison to controls after adjusting for possible confounding factors (17). Another cross-sectional study was conducted on 131 patients in Korea and concluded that metabolic syndrome was an independent predictor of uncontrolled CSU (13). This is in agreement with our study, but ours detected only the hyperlipidemia among patients with CSU.

In addition, Kobayashi detected in patients with in comparison to with controls elevation of serum lipids, cholesterol, B-lipoprotein and phospholipids (18). Also, Kobayashi and coworkers investigated serum lipids further (19) and found a correlation between serum lipids, fatty acids and chronic urticaria (20). These laboratory results suggested that serum lipids and fatty acids are chemical mediators and play a role in inducing urticaria.

These outcomes can be clarified by several probabilities. Various studies found an association between hyperlipidemia and inflammatory markers which were also observed in CSU (9,10,21). Besides, CSU is considered a skin disease, induced by mast cells activation (22), which were also linked with hyperlipidemia or atherosclerotic disease. Mast cells, as a part of both innate and adaptive immune system, could have a role in endothelial inflammation (23) which was found to be corresponding to the atherosclerotic disease immensity (24).

Vascular mast cells, present within the adventitia and atherosclerotic plaques, (25) can modify lipid metabolism by hindering the ApoE- and ApoA-II-dependent cholesterol efflux (26). Besides, increased collagen content was observed in mast cell-deficient mice in addition to fibrous cap development, and reduced local inflammation leading to decreased atherosclerosis (27).

Mast cells which accumulate in the atherosclerotic lesions (28) are then activated by the pro-inflammatory stimuli, as C3a and C5a, and oxidized low-density lipoprotein (LDL) (29). Activated mast cells, in turn, release a broad array of proinflammatory cytokines affecting the development of CSU (22). Thus, the potential link between hyperlipidemia and CSU may be due to the presence of systemic inflammation which influences them both. Whether systemic inflammation or mast cells activation are involved in the pathogenesis of CSU following hyperlipidemia need further research to clarify.

The current study detected a highly statistically significant increase in group 1 in contrast to group 2 as regards serum IL6 and TNFα. Serum IL6 show significant positive correlation with urticaria activity score while serum TNF α shows significant positive correlation with duration of illness (p-0.003) and urticaria activity score.

This is in agreement with Kasperska-Zajac and coworkers, who observed significant elevation of serum IL-6 and CRP in CSU patients compared with the healthy controls. IL-6 concentration was positively correlated with weekly UAS-7 and the different degrees of urticarial activity. Increased serum IL-6 in association with CRP changes reflect the link between CSU and systemic inflammation (10).

Our results are also in accordance with Young-Min and colleagues who found patients with metabolic syndrome to be older, and had a higher mean UAS and serum levels of TNF-α compared with controls (13). However, our study studied only patients with hyperlipidemia who had higher level of TNF α and positively correlated with urticaria activity score but had non-significant correlation with age.

Mast cells and basophils are the predominant cells in the development of chronic urticaria (30), in which proinflammatory cytokines as TNF-α and IL-6 are incremented in the initiation and progression of their degranulation process (31,32). Levels of TNF-α was found to be significantly elevated in patients with CU with metabolic syndrome, and were directly harmonized with higher UAS. This is in agreement with foregoing studies, which showed that plasma levels of TNF-α, IL-6 and CRP were significantly correlated with CSU clinical activity (10). Consequently, TNF-α inhibitors may beneficial in refractory CSU (33) as elevated serum levels of IL- 6 and TNF-α induce basophil activation, histamine release and leukotriene production in CSU (34).

Moreover, a link between hypertriglyceridemia and increased TNF-α serum level is detected (36). TNF-α can affect serum level of TG by acting on both adipose tissue and liver. It increases the production of free fatty acids (37), decrease level of TG-rich lipoproteins (VLDLs) in the circulation by diminishing its clearance (36) and stimulates lipolysis in human adipose tissue (38). TNF-α can also increase plasma TG concentrations by the inhibition of lipoprotein lipase activity (39).

Besides, TNF-α may interfere with cholesterol metabolic pathways. TNF-α can inhibit the expression and activity of the rate-limiting enzyme of cholesterol elimination leading to decrease hepatic cholesterol catabolism and excretion (40). Moreover, it can down-regulate the activities of rate-limiting enzymes in the alternative pathway of bile acid synthesis. Hence, TNF-α decreases cholesterol elimination from the body and its availability during the acute phase response for other hepatic processes (41). IL-6 concentration is increased in CSU (42) and coordinated with its activity (10). IL-6, in addition, can lead to lipid abnormalities (43) through inhibition of adipocyte lipoprotein lipase activity (44), increased induction of hepatic triglyceride secretion (45) and increased plasma free fatty acids (FFAs) (46). Hence cytokines operate both as a cascade and as a network regulating the production of each other, increased IL-6 may reflect the actions of other cytokines as TNF-α.

Different immunomodulatory therapies were assessed in CSU including statins (47). Statins, apart from their lipid-lowering activity, have anti-inflammatory effects on basophils and the major proinflammatory effector cells by multiple mechanisms (48). Statins can inhibit the growth and activation of human basophils, as detected by Majlesi and colleagues (49). Administration of statins for 3 months, decreased the patients' symptoms assessed by urticarial score compared to before treatment. Hence, statins could be effective not only in hyperlipidemia but also in the treatment of chronic urticaria and it could alleviate the patients' symptoms in severe and resistant forms of urticaria (47).

Conclusion

There is a link between CSU and hyperlipidemia. IL6 and TNF-α associated systemic inflammation could be a common pathogenic mechanism of CSU and hyperlipidemia. More studies are needed on wider scales to clarify the possible pathophysiological mechanisms for prevention and early identification. Patients with severe and uncontrolled CSU should be evaluated for hyperlipidemia to improve CSU outcomes. We can reduce atherosclerosis and its complications with prompt detection and suitable management. Thus, both of CSU consequences and patients' quality of life might be better. Early detection of hyperlipidemia among CSU patients will pave a way for better interventions for this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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Impact of allergy on children with Attention Deficit Hyperactivity Disorder

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Summary
Attention deficit hyperactivity disorder (ADHD) has gained importance lately, because it has become common and has caused serious implication to those affected. DSM-IV-TR defined ADHD by symptoms of inattention, hyperactivity and impulsivity (1).
Studies estimated that 4% to 8% of children worldwide have ADHD, which is more prevalent in boys than girls by three folds (2). In Egypt, the prevalence raised to 9.4% (3).
There are many speculations about the possible relationship between ADHD and allergy, owing to the fact that ADHD children had allergic disorders. It is putative that ADHD might be a complication of allergy, as it was found that allergic reactions led to a sequence of imbalanced cholinergic/adrenergic activity in the central nervous system (4). On the other hand, ADHD can occur secondary to side effects of antiallergic drugs (5).
The pathogenesis of allergy and ADHD both rely on gene-environment interaction, which is complex in nature (6).
Surprisingly, ADHD and allergy share the hypersensitivity phenomenon. When exposed to certain stimuli which are tolerated by normal subjects, a sequence of symptoms occur. As inhalants like mite or ingestants like milk can trigger an allergic reaction, certain foods and pollens can activate ADHD symptoms (7).
Due to this hypersensitivity concept in pathogenesis of both allergic disease and ADHD, integrated evaluation, proper diagnosis, prevention and management should be revised and put in consideration to improve quality of care of these patients (5).
Therefore, the aim of this work was to investigate the percentage of allergic conditions among clinically diagnosed children with ADHD, and to study the effect of allergy on symptom patterns, severity and its association with demographic variables in ADHD children.
Secondary outcomes were to detect the most common allergens in ADHD children with concurrent allergic disorders.

Subjects and methods

This case-control study included 127 ADHD children and 60 healthy children. The patients were recruited from the Outpatient Clinic of the Child Psychiatry, Institute of Psychiatry, Faculty of Medicine in Ain Shams University, Cairo, Egypt, over a six month period from September 2014 to March 2015. The patients were then referred to the Allergy and Clinical Immunology Clinic for further assessment for allergic diseases. The clinics are located in eastern Cairo and serve as catchment area for about a third of greater Cairo. The Clinic serves both rural and urban areas, including areas around greater Cairo as well. The age of patients ranged between six and twelve years. They were diagnosed according to DSM-IV criteria (8). Patients with

co-morbid neuropsychiatric disorders, below average IQ, and chronic illnesses were ineligible for the study. An informed consent was taken from all guardians of the participants prior to enrollment in this study. The study was approved by the Ain Shams Medical Research Ethics Committee.

Participants were subjected to the following:

1. detailed history taking and full general examination for exclusion of any medical condition that might interfere with the process of the study.
2. psychiatric assessment using (M.I.N.I. Kid) for diagnosis of ADHD and exclusion of other psychiatric co-morbidities in the patient and control group. There were two screening questions. If the patient/informant responded positively to one or both of the screening questions, more detailed symptom questions were asked (9). The version used in this study was the Arabic version and it was translated into Arabic language. In addition, the reliability and validity tests were done (10).
3. IQ assessment using Wechsler Intelligence Scale for Children (WISC) to exclude children with below average IQ. The version used in this study was the Arabic version. It was translated into Arabic language and the reliability and validity tests were done (11).
4. assessment of ADHD severity using Conners' parent rating scale-revised, long version. Its main use was assessment of the severity of ADHD, response to treatment, follow up studies, and DSM-IV diagnostic correspondence (12).
5. assessment for allergy. Both the patients' group and the control group were referred to the Allergy and Clinical Immunology Clinic of Ain Shams University, and were subjected to:
   a) history for allergy and clinical assessment, which included previous diagnosis of allergy, atopic manifestations of the disease, history of food allergy or food induced attacks, past history of illnesses, immunizations, history of child being breastfed or on artificial milk, and family history of allergy.
   b) Skin prick test, puncturing the skin with a calibrated lancet (1 mm) held vertically, or a hypodermic needle or blood lancet at an angle of 45°, and introducing a drop of diluted purified allergen. All patients were also introduced with prick of a drop of histamine as positive control, and a drop of normal saline as negative control. An itchy wheal should develop at the histamine puncture site within 10 minutes. Test solutions were standardized to give a mean wheal diameter of 6 mm. The maximum or mean diameter of the wheals to various allergen extracts, including mites, moulds, and mixed pollens extracts, were read at 15 minutes. A wheal of 3 mm or more in diameter was considered to represent a positive response (indicating sensitization to the allergen) (13).
   c) open food elimination challenge to common food allergens including milk, egg, fish, nuts, wheat, maize, chocolate and banana. After elimination of the food tested for two weeks, reintroduction of the food was done in very small amounts with gradual increase in amount until allergic symptoms appear.
   d) serum total IgE concentration (IU/mL) was evaluated using the total IgE enzyme immunoassay (ELISA) kit (DRG International Inc., USA), according to the instructions of the manufacturer. The minimum detectable concentration was 5 IU/mL. The normal limit of total IgE in children was 50 IU/ml.

Statistical analysis

Data was collected tabled and statistically analyzed using SPSS version 16. The sample size was determined by the ethic committee, based on the following assumptions: alpha error = 0.0500 (two-sided), power of study = 0.8000, and percentage of positive prick test in ADHD = 0.6700. Therefore, the estimated required sample size was = 100. Parametric data were expressed as means ± SDs and non-parametric data were expressed as number and percentage. Comparison of data was done using Student's t-test for parametric data and chi-square test for non-parametric data. Analysis of variance (ANOVA) was used to analyze the differences between groups. Two tailed p value of > 0.05 was insignificant, $p \leq 0.05$ was significant, $p \leq 0.01$ was highly significant and $p < 0.001$ was very highly significant.

Results

One hundred and twenty-seven children were enrolled. Twenty-seven patients dropped out of the study due either presence of co-morbidities or refusal to continue in the study, with a total of 100 male and female patients, of whom 83 of the children were males (83%) and 17 were females (17%) with male to female ratio 4.8:1 in the ADHD group. The control group consisted of 60 healthy children of whom 47 male (78.3%) and 13 female (21.7%) with male to female ratio 3.6:1 without significant statistical difference between the ADHD and the control ($x^2 = 0.536$, $p = 0.46$). The mean age was 8.54 (± 1.9) years in ADHD patients and 8.77 (± 1.82) in healthy control ($p = 0.62$) without significant statistical difference. Among the ADHD patients, most of the children (86%) lived in urban areas, while only 14 children (14%) lived in rural areas, and the rest of the children (86%) lived in urban areas. 35 children (35%) were clinically diagnosed to have allergy. The mean age of onset of ADHD was 6.0 (± 1.5) years, while the mean age of onset of allergy was 4.0 (± 2.6) years. 36 (36%) of the total sample had positive family history of allergy. Positive skin prick test was found in 16 (16%) of patients and 3 (5%) of the controls (p value 0.037), 45 (45%) of the patients and 11 (18%) of controls showed high total IgE (p value 0.0006).

Patients were divided into two groups according to allergy workup into allergic and non-allergic groups. There were no statistically significant differences when analyzing the socio-demographic data between the two groups, except for a statistically significant difference in past history of tonsillectomy and sinus problems, in the allergic group (as shown in **table I**).

According to the allergy-immunology questionnaire; 16% (16 patients) of the ADHD group and 5% (3 individuals) in the healthy control group had SPT +ve results to one or more of the allergens examined ($x^2 = 0.15$, $p = 0.7$). The most common allergic diseases in ADHD group were mixed allergic diseases 25% (the most common was allergic rhinitis and bronchial asthma, followed by bronchial asthma and urticaria, then allergic rhinitis and urticaria), followed by urticaria 8%, then asthma 2.0%, while there were no ADHD cases with pure atopic eczema or allergic rhinitis. Sensitization to mixed allergens comprised the vast majority of cases, followed by aeroallergens including mites and pollens and lastly food allergens (wheat, nuts and banana).

There was strong positive correlation between onset of allergy symptoms and onset of ADHD symptoms (r value = 0.947, where the correlation is significant at r value = 0.01) as shown in **figure 1**.

The co-existence of allergy in ADHD children revealed a statistically significant difference with type of ADHD (combined, DSM- IV hyperactive-impulsive, DSM-IV inattentive), severity of ADHD symptoms (oppositional, cognitive problem, DSM IV inattentive, lability, hyperactivity, DSM IV hyperactive-impulsive, psychosomatic, Conner's global index: total, and DSM-IV total score) and the presence of co-morbid psy-

Figure 1 *- Correlation between onset of allergy and onset of ADHD symptom.*

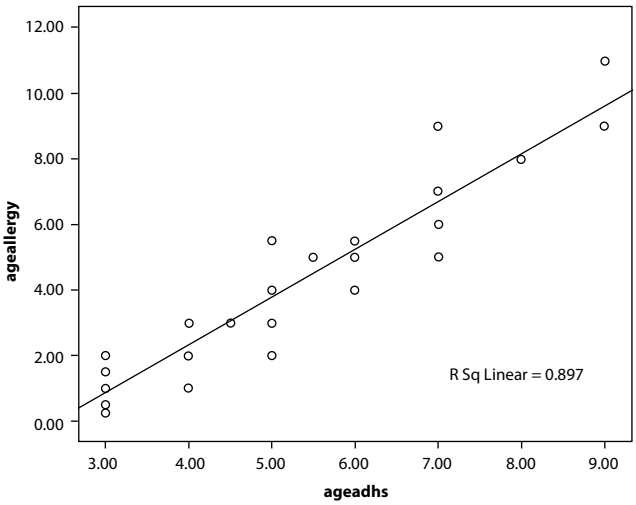
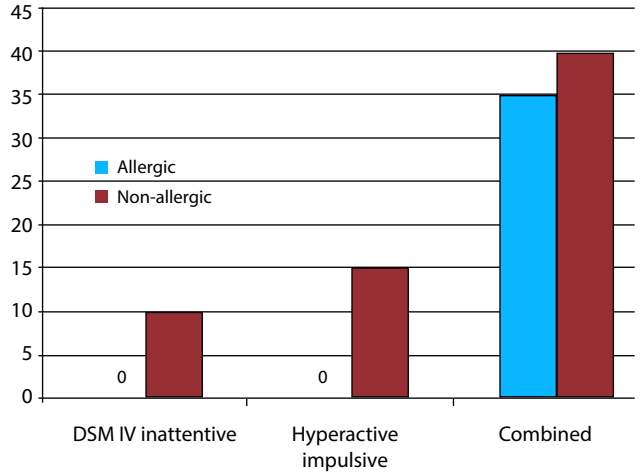


Table I *- Sociodemographic data of both groups.*

Socio-demographic data			ADHD children		Total	p-value (significance)
			allergic	non allergic		
gender	male	count	31	52	83	p = 0.2 non significant $x^2 = 1.1$
		%	88.6%	80.0%	83.0%	
	female	count	4	13	17	
		%	11.4%	20.0%	17.0%	
age (years)	mean		8.15	8.22		p = 0.867 non significant $x^2 = 0.17$
	stand. deviation		± 1.89	± 2.1		
residence	urban	count	29	57	86	p = 0.5 non significant $x^2 = 0.4$
		%	82.9%	87.7%	86.0%	
	rural	count	6	8	14	
		%	17.1%	12.3%	14.0%	
past medical history	none	count	26	60	86	p = 0.03 significant $x^2 = 6.7$
		%	74.3%	92.3%	86.0%	
	previous surgeries	count	8	5	13	
		%	22.9%	7.7%	13.0%	
	sinus problem	count	1	0	1	
		%	2.9%	.0%	1.0%	

Figure 2 *- Impact of allergy on ADHD type.*



chiatric symptoms (anxiety, oppositional deficient) (as shown in **figure 2** and **table II**).

The odds of those who had positive skin test to those who had more severe Conner's global total score was 34.25 (95% CI, 3.7 to 315) times to those who had negative skin prick test, which was statistically highly significant (Wald x2 (1) = 9.7, p = 0.002). While those who had positive skin test had a more severe Conner's DSM IV total score which was 18.3 (95% CI, 2 to 167.7) times to those who had negative skin prick test, which was also statistically highly significant (Wald x2 (1) = 6.6, p = 0.01).

Discussion

ADHD and allergic disorders are both hereditary diseases involving gene-environment interactions that may share a common biological background. Although children with ADHD have increased prevalence of allergic diseases, studying the association between allergic disorders and ADHD has received less attention (14).

Schmitt et al. (2010), identified and systematically reviewed 20 epidemiological studies that investigated the relationship between atopic disease and ADHD/ADHD symptoms.

Many studies suggested a positive association between eczema and ADHD (15,16). Furthermore, studies by Romanos and colleagues found an association between asthma and ADHD (17). Other published studies found no relation between ADHD and allergic rhinitis (18). This was in agreement with Schmitt et al., who found weak to moderate strong association between asthma and ADHD; however, they found no relationship between ADHD and allergic rhinitis (19).

In contrast to the previous studies, recently two cross sectional studies found that allergic rhinitis has a strong association with ADHD (20). Suwan et al. (2011), revealed that the most common allergic disease was allergic rhinitis (21). Also, Brawley et al. (2004) found that most patients with ADHD had atopic manifestations and skin prick test findings to common aeroallergens, which were consistent with a diagnosis of allergic rhinitis (22).

Camfferman et al. found a significant association between allergic eczema, allergic rhinitis and allergic conjunctivitis, and to la lesser extent with asthma in association with ADHD (23).

Table II *- The impact of clinically diagnosed allergy on ADHD severity of symptoms of ADHD.*

			mild	moderate	severe	Total	significance
Conners ADHD index	ADHD	allergic	0%	18%	17%	35%	p = 0.018
	children	non allergic	13%	27%	25%	65%	x^2 = 8.040
Conners global index:	ADHD	allergic	0%	14%	21%	35%	p = 0.069
restlessness-impulsive	children	non allergic	5%	34%	26%	65%	x^2 = 5.346
Conners lability	ADHD	allergic	0%	0%	35%	35%	p = 0.001
	children	non allergic	9%	50%	6%	65%	x^2 = 77.486
Conners global index	ADHD	allergic	0%	16%	19%	35%	p = 0.03
	children	non allergic	10%	31%	24%	65%	x^2 = 6.998
Conners DSM-IV inattentive	ADHD	allergic	0%	0%	35%	35%	p = 0.001
	children	non allergic	13%	46%	6%	65%	x^2 = 77.486
Conners DSM-IV	ADHD	allergic	0%	0%	35%	35%	p = 0.001
hyperactive-impulsive	children	non allergic	2%	45%	18%	65%	x^2 = 47.750
DSM-IV total score	ADHD	allergic	0%	12%	23%	35%	p = 0.001
	children	non allergic	18%	24%	23%	65%	x^2 = 14.286

Conversely, only one cross-sectional study did not observe the association (24).

In our study we found a statistically significant difference between ADHD patients and the healthy control group, regard both the SPT reactivity and the serum total IgE levels, and that the most common allergic diseases were mixed allergic diseases 25%, followed by urticaria 8%, then asthma 2.0%, while there were no ADHD cases with pure atopic dermatitis, allergic rhinitis or allergic conjunctivitis.

The present study revealed that sensitization to mixed allergens comprised the vast majority of cases, followed by aeroallergen then food allergen; these results matched the results of Suwan et al. (2011), that sensitization to aeroallergens was higher than for food allergens in both ADHD and healthy control groups. In agreement with previous studies, food allergen sensitization was most prevalent during the first years of life, diminishing in prevalence in later childhood. Conversely, the risk of sensitization to aeroallergens increased with age (25).

As regards the most common allergens causing sensitization, we found that mites comprised the commonest allergen, followed by pollens. These results are also consistent with those reported in an earlier study conducted by Suwan et al. (2011), who reported that the commonest allergen in both ADHD and healthy control groups was house dust mites. This result supports the finding from previous studies that house dust mites are the most important allergens in Thailand.

The mean age of the ADHD group in the current study was 8.54 years. The male to female ratio was about 4.8:1. Tsai et al. (2013) found a strong association between atopic disease below 7 years and ADHD (26). Also, these results matched the findings of Genuneit et al. (2014), who analyzed data of a population-based, prospective birth cohort study among 770 children included at baseline in 2000/2001 with follow-up up to age 11, and found that Atopic eczema (AE) was associated with an increased risk of subsequent ADHD only within the first few years after AE diagnosis, and possibly to a greater extent in early compared to later childhood (27). Despite the fact that most of our patients selected were above 7 years of age, according to the history of start of allergic disease and ADHD, we found that the earlier the age of onset of allergy in these patients, the earlier the symptoms of ADHD happened to those prone. We also found that presence of family history of allergy affected significantly the onset of ADHD. Suwan et al. (2011) also found that positive atopic family history was significant in ADHD children.

There are several explanations for the observed co-morbidity between early age of onset of ADHD and atopic disease. Some experimental evidence supported a theory of a link of both diseases, in a way, through neuro-immune pathways. The brain is still immature in early life, allowing allergic induced cytokines to pass and affect the ADHD-relevant brain circuits (28).

It is noteworthy that allergic ADHD patients in this study had history of tonsillectomy. Several studies demonstrated an association between allergy and tonsillar hypertrophy. Moreover, family history of allergy and clinical allergic disorders are related to early onset of tonsillar hypertrophy (29). This raises the question should children with history of tonsillar hypertrophy be evaluated for allergy and in turn screened for ADHD, and furthermore what is the response of allergy and ADHD symptoms after tonsillectomy.

The results of the current study found that the co-existence of allergy in ADHD children was significantly associated with severity of symptoms (oppositional, cognitive problem, inattentive, lability, hyperactivity, hyperactive-impulsive), type of ADHD (combined, hyperactive-impulsive, inattentive), and the presence of co-morbid sub-threshold symptoms (anxiety, oppositional defiant). The present results agreed with those reported by Suwan et al. (2011), who found a strong association between the presence of allergy and the severity of ADHD symptoms and decreased response to psycho-stimulants. Shyu et al. (2012), reported that allergic children (including those with AR, bronchial asthma, atopic dermatitis) had a more severe symptom pattern and a higher prevalence percentage in ADHD than the general population, and the impulsivity took the upper hand more than inattention in the AR children.

The strengths in this study are that the study looked at the percentage of allergic conditions among clinically diagnosed children with ADHD and studied the effect of allergy on symptom patterns and severity. Also demonstrating the effect of age of onset of allergy and presence of positive family history is crucial in determining the progress of ADHD. Limitations is that the study did not cover the whole range of ADHD patient, and therefore a large scale cohort study is recommended. Therefore, assessment and understanding the relationship between ADHD and allergy is important for early and proper diagnosis of these patients. Performance of workup necessary as regards allergy and ADHD will provide correct management, decrease the use of psychostimulants and prevent worsening and non-responsiveness of the condition. Determination and avoidance of the culprit allergen, either airborne or food, may be a turn point in improvement of ADHD patient with allergic disease along with proper care and treatment of allergy symptoms. In addition, screening of siblings of patient may help in prevention and decrease incidence of the disease.

Conclusion

Children with ADHD had an increased prevalence of allergic diseases. Allergic ADHD children had more severe ADHD symptoms (oppositional, cognitive problem, inattentive, lability, hyperactivity, hyperactive-impulsive) and more co-morbid sub-threshold disorders (anxiety, oppositional defiant). A bet-

ter relationship between atopic diseases and development of ADHD, especially at the biological, molecular and genetic level, is of significant public health relevance as it may lead to targeted treatments and improved preventive measures for those children with atopic disease who are at increased risk to develop ADHD.

Conflict of Interest

The authors declare that they have no conflict of interest.

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The role of mobile apps in allergic respiratory diseases: an Italian multicentre survey report

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Keywords

mobile apps; allergic rhinitis; asthma; e-Health; m-Health

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Summary

We performed a multicentre survey about the role of mobile apps in allergic respiratory diseases. The main objective was to investigate the current use and usefulness of mobile phone apps in the management of allergic respiratory disease. An original questionnaire including 12 multiple-choice questions was administered to 360 participants (153 male and 207 female subjects). Of notice, 290 subjects owned a smartphone, 120 a personal computer and 68 a tablet (multiple answers were possible). 123 subjects reported to be regular mobile-apps users, 209 were occasional users and 76 has never downloaded an app. Indeed, 259 subjects have never dealt with a medical or healthcare app, with only 8 subjects answering to often take advantage of such supportive tools. Data were even more discouraging when asking whether subjects had ever downloaded an app directly related to their own medical condition (allergy, asthma and rhinitis). 87.2% provided a negative answer. Among the few individuals who reported a previous experience with allergy/asthma apps only 2 subjects reported to regularly use the apps they had downloaded, even after months. The majority of subjects believed the apps would provide a relevant support, but only 25/360 participants found that the apps are "truly helpful", while 44 considered them of "help but not essential". Our data seem to show that the apps in the medical field, especially for allergic respiratory diseases, are welcome by patients, but their continued use and utility wane with the passage of time from the date of the download. In the future it will therefore be important to focus on the quality of the apps themselves and on the careful selection of the most suitable patients to use them. Finally, it will be important to make use of the fundamental contribution of healthcare professionals for the development of the apps.

Introduction

The prevalence of allergic respiratory diseases and asthma is increasing worldwide and the complexity and severity of such diseases continue to grow, especially in young subjects, who are bearing the greatest burden. In Europe, approximately 23% of the population is affected by allergic rhinitis (1). Asthma also represents a major health and socio-economic concern considering that more than 300 million people worldwide are currently affected by this condition. Moreover, allergic rhinitis is frequently associated to allergic asthma, increasing the overall burden on patients (2). Despite extensive availability of effective therapies, a considerable number of patients do not manage to achieve satisfactory disease control (3).

Hence, the management of asthma and other allergic respiratory conditions needs to be prompt, accurate and personalized in order to be truly effective. In this complex and multi-parametric approach, the education and active involvement of patients has been shown to be central, highlighting the importance of self-management. Electronic health (e-Health) is defined as "an emerging field at the intersection of medical informatics, public health and business, referring to health services and information delivered or enhanced through the Internet and related technologies" (4) and represents an innovative tool for improving the management of chronic pathologic conditions, as systemic hypertension, chronic heart failure, and diabetes. With a potential role in enhancing the quality of care, improving patient's compliance to therapy and enabling early diagnosis of disease worsening and exacerbation, e-Health might revolutionize the concept of healthcare. Additionally, with the ongoing advances in information technology, an increasing number of patients claim for electronic tools and solutions to better manage their illness. E-Health mainly covers three areas of intervention: the delivery of information, for health professionals and consumers, through Internet and telecommunications; the use of information technology to improve public health services; the use of e-commerce and e-business practices in health systems management (5). E-Health includes several areas of interest such as mobile health, telemedicine, virtual healthcare teams, electronic health records (EHRs), medication trackers and clinical decision support systems. In particular, mobile health has been defined as a new social healthcare model, achievable through the use of mobile devices such as smartphones, apps, patient trackers, and personal digital assistants (PDAs). Mobile Health can be considered a part of Health Internet of Things (IoT), a compound of devices designed to detect bio-signals and bio-images resulting from connection to medical devices or other types of sensors. This allows to gather data and information placing the patient in a proactive position in the management of his own health status and ensuring at the same time a better interaction with healthcare professionals. Therefore, the application of mobile health allows to bring down spatial and temporal barriers, making the patient's management and monitoring more effective and profitable.

In the last decade, the mobile revolution has given a unique opportunity to offer medical support when and where people needed it. A large number and variety of medical and health-related apps is available on the market today; from basic app-based text message reminders, to sophisticated apps that play a multitude of functions.

A proper management of allergic respiratory diseases, like allergic rhinitis and/or asthma, includes decision-making processes based on patient's symptoms, environmental exposures and medication usage. Helping patients to understand how these variables impact their health and, when necessary, instructing them on how to take adequate actions and properly seek care, empowers them to develop relevant self-management skills (7-9).

We published a review to evaluate the web resources nowadays available and to analyze the studies about the web-based instruments used to improve asthma knowledge, control asthma outcomes (10). Reviews assessing the web-resources used, and analyzing the studies about the web-based instruments to improve asthma knowledge, control, and outcomes are now available (11). In general, studies revealed that the technology is well accepted, but the number of tools and apps available continues to increase, and agencies such as the FDA, become involved in their regulation, thus the m-Health landscape will continue to evolve. Although asthma tools and apps have great potential to improve care for asthma, the proof of data reproducibility, the demonstration of effectiveness, and the privacy issues still represent the major technical problems.

Patients, materials and methods

Recently, we performed a multicentre survey in Italy about the role of mobile apps in allergic respiratory diseases whose main objective was to investigate the current use and the possible usefulness of m-Health interventions – in the form of mobile phone apps – in the management of allergic respiratory disease. 13 centers participated in the study, with a territorial prevalence in northern Italy.

An original questionnaire including 12 multiple-choice questions with variable number of possible answers was administered to 360 participants. The first 5 questions referred to the anthropological, attitudinal and social characteristics of subjects (i.e. gender, age, type of job, educational level, preferred device used for internet connection), while the following 7 items investigated the individual's use of mobile phone apps and opinion on their usefulness in the management of allergic respiratory diseases. The study was carried out in accordance

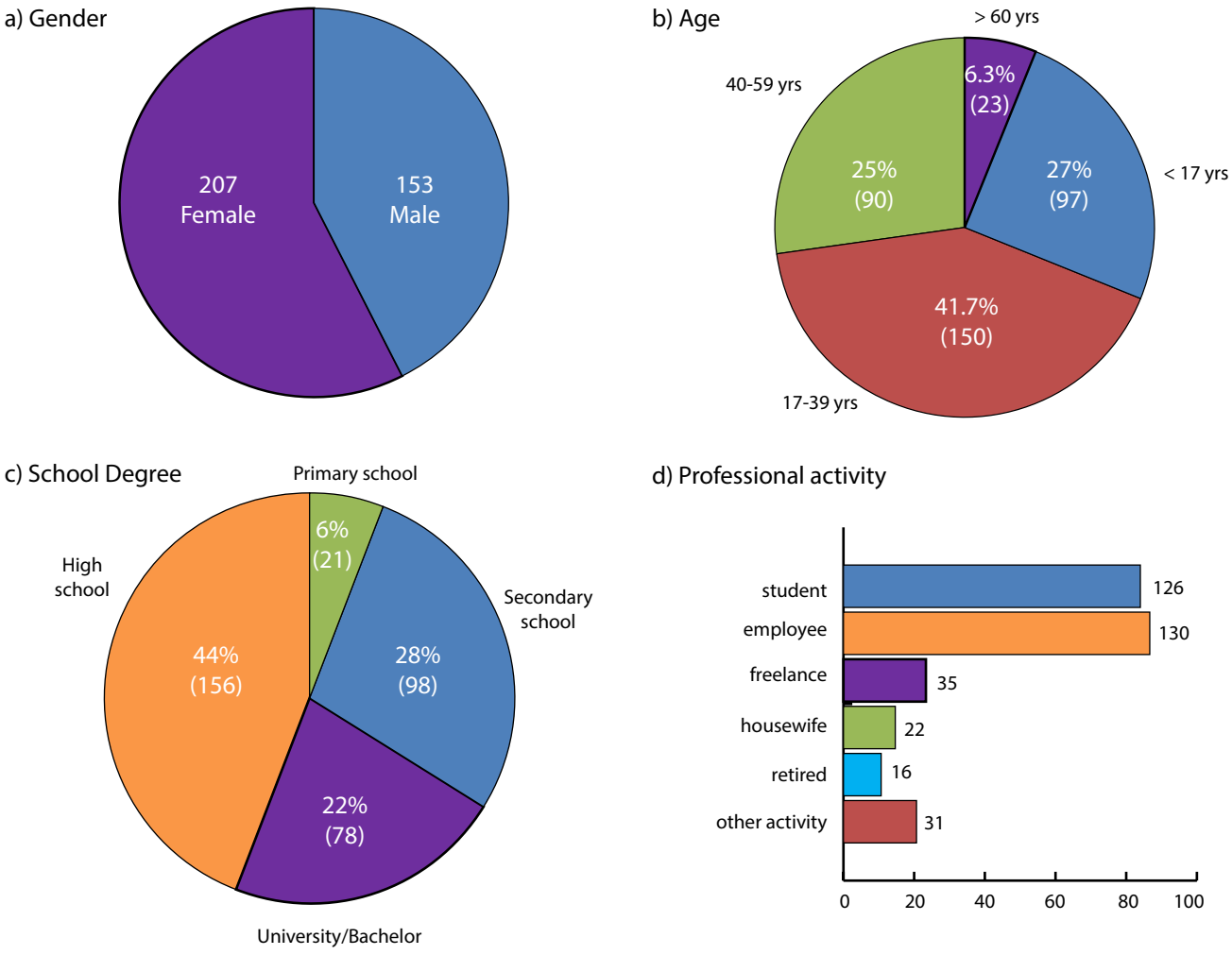
with the ethical standards established in the Declaration of Helsinki, and that informed consent was obtained from all participants before enrolment in the study.

The questionnaire was completed by 153 male and 207 female subjects (**figure 1a**). Both children/adolescents and adults took part in the study. Twenty-seven per cent (97/360) of subjects were 17 years old or younger, and only 23 individuals (6.3%) were older than 60 years (**figure 1b**). Within the sample assessed 21 subjects had a primary school degree, 98 had a secondary school degree, 156 had a high school degree, and 78 a bachelor degree (**figure 1c**). **Figure 1d** reports the professional activity distribution of participants.

Results

Of remark, 290 subjects owned a smartphone, 120 a personal computer (either a desktop or a laptop) and 68 a tablet (multiple answers were possible). A consistent number of people (123) reported to be regular mobile-apps users, 209 interviewees referred to be occasional beneficiaries, while 76 has never downloaded an app. Unsatisfactorily, 259 subjects have never dealt with a medical or healthcare app, with only 8 subjects answering to often take advantage of such supportive tools. Data were even more discouraging when it was asked whether subjects had ever downloaded an app directly related to their own

Figure 1 – **a**, *distribution by gender with a slight prevalence of the female sex;* **b**, *age distribution with a prevalence of the age group from 17 to 39 years;* **c**, *school degree distribution with a prevalence of high school degree subjects;* **d**, *about the work activity, the most represented were the students and the employees.*



medical condition (i.e. allergy, asthma and rhinitis). In fact, 314 out of 360 (87.2%) provided a negative answer. Among the few individuals who reported a previous experience with allergy/asthma apps only 2 resulted to be frequent users. The apps that these two patients used were respectively: Asma (Momento Medico s.r.l.) and Allergy Diary by MACVIA ARIA (Peercode B.V.).

The last three domains of the questionnaires addressed the individuals' perspectives on the role and usefulness of medical apps in improving disease monitoring and self-management. The majority of subjects believed the apps could mainly provide support to increase the knowledge of the disease, with a considerable share of subjects highlighting a role in symptom monitoring and treatment reminding (**figure 2**). However, only 25 participants found the apps to be extremely helpful, while 44 considered them of support but potentially improvable (**figure 3**). Despite the personal experience, the vast majority of subjects 289 stated they likely would advise patients affected by allergic and respiratory diseases to use mobile apps and other m-health interventions.

Conclusions

Our data seem to show that the apps in the medical field, especially for allergopathies, are welcome by patients, but their continued use and utility wane with the passage of time from the date of the download. As in the approach to adherence to drug therapy, definable as the extent to which a patient acts in accordance with the prescribed interval and dose of a dosing regimen, it is important to take into account the concept of "medication persistence", which must be understood as the duration of time from initiation to discontinuation of therapy (12) , as in the use of medical apps we must consider not only the aspects concerning the initial use, but above all the "persistence" over time to the use and correct use of the app itself. In the future it will therefore be important to focus on the quality of the apps themselves and on the careful selection of the most suitable patients to use them. There is great heterogeneity between the currently available mobile phone apps. It would be more useful to assess which specific types of apps (e.g. interactive vs data-recording vs information providing types) would be more widely accepted by patients and have highest compliance rates. It would also be useful to assess the willingness of subjects to use apps which are interactive and provider-linked (for example, if providers were able to download and view patient-recorded symptoms over time to tailor treatment accordingly). As such, providers and the industry might then focus on developing such apps to increase the quality of holistic patient care. Finally, it will be important to make use of the fundamental contribution of healthcare professionals for the development of the apps. In conclusion, we believe that also in the field of "medical apps" the following rule of advice should apply: "right app, to the right patient, and given by the right doctor".

Conflict of interest

The authors declare that they have no conflict of interest.

Figure 2.

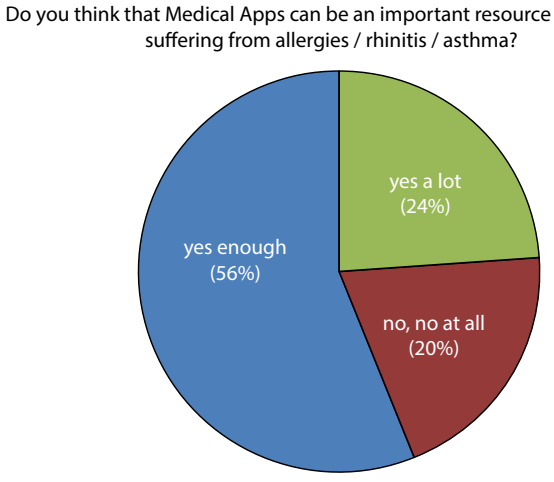
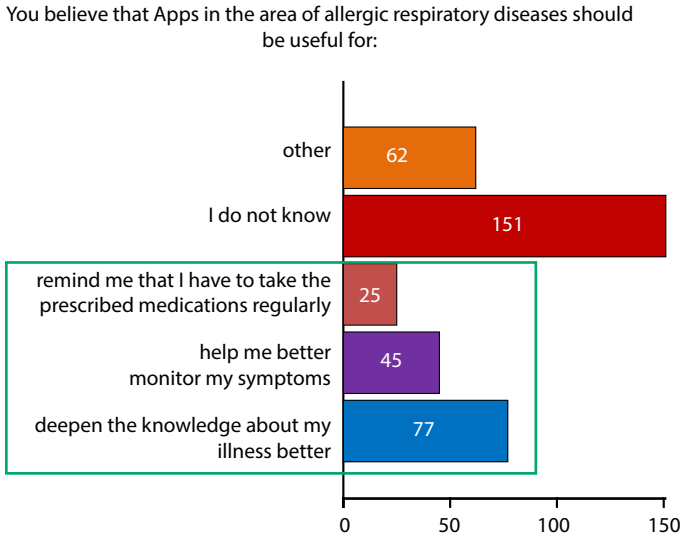


Figure 3.



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Paediatric case series of drug reaction with eosinophilia and systemic symptoms (DRESS): 12-year experience at a single referral centre in Hong Kong and the first reported use of infliximab

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Keywords

paediatric; drug reaction with eosinophilia and systemic symptoms; DRESS; drug-induced hypersensitivity syndrome; Chinese; Hong Kong

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Summary

DRESS (drug reaction with eosinophilia and systemic symptoms) is a rare but potentially life-threatening disorder characterized by fever, skin eruption, haematological abnormalities and multi-organ dysfunction after drug exposure. The pathophysiology is thought to be related to interactions between culprit drugs, viral reactivation and T-lymphocytes activation. We report 4 paediatric patients with DRESS who were treated at our centre over the past 12 years. Most cases improved after corticosteroids. Other immunosuppressive medications were attempted in refractory cases with varied outcomes. Patient 3 was the first reported case that involved the use of infliximab, a TNF-α inhibitor, for DRESS. Although clinical efficacy was not observed for this one patient, a previous study demonstrated that patients with DRESS, disease progression and HHV-6 reactivation had elevated pre-treatment TNF- α and IL-6 levels. Further research is needed to explore the role of these cytokines in DRESS..

Introduction

Drug reaction with eosinophilia and systemic symptoms (DRESS), also known as drug-induced hypersensitivity syndrome, is a rare but potentially life-threatening disorder characterized by fever, skin eruption, haematological abnormalities and multi-organ involvement. There are three proposed diagnostic criteria (**table I**). The estimated incidence ranges from 1 in 1,000-10,000 drug exposures, with a mortality rate of 10%. The typical clinical course involves a latency period of 3 to 76 days after culprit drug exposure (1,2). Here we present 4 Chinese paediatric patients with DRESS managed at our centre over the past 12 years who experienced highly variable clinical courses. Most of these cases of DRESS improved after the administration of high-dose corticosteroids, while in refractory cases other immunosuppressive medications were attempted with variable outcomes.

Case Summaries

Patient 1

A 13-year-old boy with chronic urticaria and asthma first presented in May 2001, with autoimmune hepatitis suspected to be an idiosyncratic drug reaction related to doxepin and famotidine. This diagnosis was made based on neutrophilic and eosinophilic liver infiltration evident in a liver biopsy, and disease resolution after pulse methylprednisolone

and high dose prednisolone were given. Two months later, he presented again with fever, cough and urticarial exacerbation while prednisolone was weaned. Amoxicillin-clavulanate was prescribed empirically for 10 days, but his neutrophilia and eosinophilia persisted. The prednisolone dose was increased for suspected flare of autoimmune hepatitis.

Nevertheless, he developed progressive respiratory distress requiring ventilatory support. Meropenem and clarithromycin were empirically administered. Sepsis workup including blood culture and bronchoalveolar lavage was negative. High resolution computed tomography (HRCT) and lung biopsy confirmed autoimmune pneumonitis and bronchiolitis obliterans with organizing pneumonia. He also developed multiple skin ulcers, and a skin biopsy confirmed a drug eruption. The overall features were compatible with DRESS triggered by amoxicillin-clavulanate. Subsequently, four doses of weekly pulse methylprednisolone and an eight-week course of cyclophosphamide were given, followed by oral prednisolone for 2 years and azathioprine for 3 years. His skin condition, lung function and repeat HRCT showed gradual disease resolution. He remained in clinical remission with no further exacerbations for more than 15 years of follow up.

Patient 2

A 5-year-old girl with a complex congenital heart disease status-post surgical repair complicated by subsequent left-sided stroke and focal seizures was started on carbamazepine after confirming negative HLA-B1502 status. Two months later, in February 2014, she developed generalized and blistering erythroderma, conjunctivitis, oral ulcers and fever. Blood testing demonstrated eosinophilia (1.3 x 10^9/L), 21% atypical lymphocytes and deranged liver function. Serum cytomegalovirus pp65 antigen and oral ulcer swab for herpes simplex virus (HSV) were positive, and therefore ganciclovir was given. Human herpesvirus type-6 (HHV-6) and HHV-7 DNA PCR were negative. Stevens-Johnson syndrome was initially suspected, so carbamazepine was discontinued while intravenous immunoglobulin (IVIG) and prednisolone were administered. Her rash improved and her skin biopsy confirmed a drug eruption.

Despite these treatments, she suffered from progressive liver dysfunction, and a liver biopsy revealed vanishing bile duct syndrome. She also began to have renal impairment, acute pancreatitis and pneumonitis. The overall presentation was compatible with DRESS.

Table I *- Diagnostic criteria for drug reaction with eosinophilia and systemic symptoms (DRESS).*

Bocquet, Bagot and Roujeau Criteria	**SCAR-J**	**RegiSCAR**
Drug rash Hematological abnormalities: - eosinophilia > 1.500/mm^3 - presence of atypical lymphocytes. Systemic involvement: - adenopathy > 2cm in diameter - hepatitis (increase in transaminases at least twice of normal values) - interstitial nephritis - pneumonitis - carditis.	Paculopapular rash developing more than 3 weeks after starting therapy with a limited number of drugs. Persistent clinical findings after drug withdrawal. Fever > 38 °C. Hepatic abnormalities (alt > 100u/l). Leucocyte abnormalities with the presence of at least one of the following: - leucocytosis > 11.000/mm^3 - atypical lymphocytosis > 5% - eosinophilia > 1.500/mm^3. HHV-6 reactivation.	Hospitalization. Reaction suspected to be drug related. Acute skin rash. Fever above 38 °C. Enlarged lymph nodes at ≥ 2 sites. Involvement of at least one internal organ. Blood count abnormalities: - lymphocytes above or below the normal range - eosinophils above the normal range (in percentage or absolute count) - platelets below the normal range.
All 3 criteria required, with at least 1 hematologic and 1 systemic feature included.	Typical DRESS syndrome: presence of 7 findings; Atypical DRESS syndrome when the first 5 findings are present.	Patients with the first 3 findings and 3 out of 4 systemic features will enter a scoring system ranging from -4 to 9 points to decide whether the case is definite, probable or possible for DRESS.

Her clinical course remained stormy, complicated by corticosteroid-induced duodenal ulcer resulting in massive bleeding requiring endoscopic haemostasis. Despite aggressive antimicrobial therapies and supportive measures, she died of disseminated infections.

Patient 3

A previously healthy 14-year-old girl presented with Salmonella paratyphi A septicaemia in June 2007. She was treated with a week of ceftriaxone and co-trimoxazole upon discharge. One week later, she developed fever, a generalized maculopapular and blistering rash, bilateral conjunctivitis and hepatosplenomegaly (**figure 1**). Blood testing demonstrated eosinophilia (1.18 x 10^9/L) and 26% atypical lymphocytes, cholestatic liver derangement and coagulopathy. Ultrasound of the liver was suggestive of cholangitis, and her skin biopsy confirmed a drug eruption.
The patient was diagnosed with DRESS due to co-trimoxazole and she was started on prednisolone. Her liver dysfunction and coagulopathy improved, but her skin condition did not. A course of pulse methylprednisolone was given, followed by prednisolone, azathioprine, cyclosporine A and mycophenolate mofetil (MMF). Monthly IVIG, infliximab, topical corticosteroids, acitretin and phototherapy with narrow band UVB and PUVA were attempted but these therapies did not result in any improvement. Her skin condition was further complicated by photosensitivity after ultraviolet therapy, which led to erythroderma and skin exfoliation. Therefore, phototherapy was withheld. Her skin disease eventually evolved into psoriatiform lesions and waxy papulosis with significant palmoplantar keratoderma. Weekly methotrexate was given for four years which was able to stabilize her skin condition.
Five years after her DRESS diagnosis, she developed Graves' disease requiring carbimazole and later radioactive iodine ablation. Her latest dermatological assessment showed she had generalized vitiligo and alopecia totalis.

Figure 1 *- Patient 3, who presented with erythroderma and a blistering skin eruption.*

Patient 4

This is a 17-year-old female with juvenile idiopathic arthritis and IgG_2 deficiency, who initially presented in August 2007 with tonsillitis treated with co-trimoxazole. Nine days later, she developed an erythematous, maculopapular rash over her face and body, massive lymphadenopathy, fever, acute renal failure and respiratory failure. Microbiological investigations were unrevealing. Her respiratory and skin condition deteriorated, and she required extracorporeal membrane oxygenation support (ECMO). Skin biopsy was suggestive of a drug reaction and the overall picture was compatible with DRESS triggered by co-trimoxazole.
Her condition was also complicated by pseudomembranous colitis and acute cholangitis. Immunological investigations showed persistent hypogammaglobulinemia and B-cell lymphopenia. The patient was managed with corticosteroids and her skin condition gradually improved. She was weaned off from ECMO and continued to receive prednisolone and monthly IVIG for her chronic hypogammaglobulinemia.

Discussion

The four Chinese paediatric DRESS patients described have variable disease courses and outcomes. While using the proposed diagnostic criteria was helpful in making the diagnosis, identification of the culprit drug remained challenging. Drug patch test may be used to identify the culprit drug, which is commonly utilized to diagnose non-IgE mediated cutaneous adverse drug reactions or delayed drug hypersensitivity. It is recommended to be performed at least six months after the disappearance of adverse drug reactions. Positive predictive value of the test varies between different drugs. The sensitivity and specificity appears to be higher for certain anticonvulsants, such as carbamazepine, and antimicrobials, such as beta-lactams, but lower for medications such as allopurinol and salazopyrin (3). However, skin patch tests were unable to be performed in our cases since testing supplies were not available for patients 1 and 4 at the time they presented, while patient 2 was critically ill and the skin condition for patient 3 was not suitable for the test all along. Therefore, identification of the culprit drugs in these

cases mainly relied on their clinical history and the temporal sequences of the events.

The mainstay management approach to DRESS includes avoidance of unnecessary empirical use of medications during the acute phase of disease to minimize potential immune cross-reactivity, early recognition and withdrawal of the culprit drug, and aggressive immunosuppressive therapies and supportive measures (4). The first-line treatment remains to be high-dose corticosteroids, which are generally effective during the acute phase. For long-term treatment, or in steroid-unresponsive cases, steroid-sparing agents may be used. Cyclosporine (5-7), IVIG (8-11), and cyclophosphamide (12) have been reported to be effective in treating steroid-refractory DRESS. However, there has been no randomized trial so far comparing the efficacies between these agents.

As demonstrated in our cases, patients with DRESS have highly variable clinical courses and responses to immunosuppressive agents. To our knowledge, patient 3 was the first reported case of DRESS that involved the use of infliximab, a TNF-α inhibitor. Although clinical efficacy was not observed for this patient, large-scale studies using infliximab and other immunomodulating therapies are required to fully determine the optimal treatment for patients with DRESS refractory to corticosteroids. Moreover, Uno et al. demonstrated that elevated pre-treatment levels of TNF-α and IL-6 in patients with DRESS and HHV-6 reactivation were correlated with disease progression, and therefore TNF-α and IL-6 levels may potentially serve as a biomarker for this syndrome (13). Further research is needed to explore the role of TNF-α and IL-6 in DRESS.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Hypersensitivity to antiretroviral drugs

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Keywords
hypersensitivity; antiretroviral drugs; patch tests; emtricitabine; tenofovir; nevirapine

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Abbreviations
NW, North-West; NE, North-East; C, Centre; S, South; Is, Islands subset.

Summary
Background. *Antiretroviral treatment improved the prognosis of patients with Human Immunodeficiency Virus (HIV) infection. Antiretroviral drugs may be responsible for hypersensitivity reactions varying in severity, clinical manifestations and frequency. Individuals infected with HIV show an increased frequency of drug eruptions when compared with general population. Reports of delayed allergic reactions to antiretroviral drugs in patients with HIV have been described, but diagnostic methods are scarce.* ***Case report.*** *We report the case of a 47-year-old woman, with diagnosis of HIV infection since 2009, who developed a delayed mucocutaneous reaction after treatment with antiretroviral drugs. Hypersensitivity reaction (HR) to emtricitabine and tenofovir was considered probable based on positive patch tests (PT), which were negative in 7 controls. Delayed HR to nevirapine was confirmed by drug provocation test.* ***Discussion.*** *The diagnosis of HR to antiretroviral drugs in patients with HIV infection remains a diagnostic challenge, partly due to unknown mechanism and to the absence of validated diagnostic tools. Patch testing may represent a useful method for confirming hypersensitivity to antiretroviral drugs, however the use of PT is not widespread, so the predictive value of testing has not been ascertained. Further investigation in this area is required to elucidate the mechanisms in HIV-infected patients, so that successful management strategies can be offered, preventing loss of potent and viable antiretroviral agents.*

Introduction

The availability of potent antiretroviral drugs - highly active antiretroviral therapy (HAART) - has led to a dramatic decline in the morbidity and mortality associated with HIV infection (1). Since the introduction of HAART in the late 1990s, hypersensitivity to antiretroviral agents has increased (2). The increased frequency of drug reactions in patients with active viral illness has been attributed to dysregulation of the immune system and particular vulnerability to oxidative stress (3). Evidence that these reactions are immune mediated is largely based on typical symptomatology, and few studies have been done to determine the pathogenic mechanisms (4). The pathophysiology of drug hypersensitivity in HIV is most likely multifactorial and related to a number of metabolic, immunologic, host and viral factors (5). Concurrent illnesses such as immune reconstitution syndromes and viral illnesses may themselves present with fever, rash and multisystem disease, and hence may confound the diagnosis of drug hypersensitivity reactions (HR) (5).

There are currently six groups of antiretrovirals agents, comprising nucleoside reverse transcriptase inhibitors (NRTIs), non nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, fusion inhibitors, CCR5 inhibitors and integrase inhibitors (6).

The diagnosis and management of drug hypersensitivity in HIV-infected patients is particularly difficult because of the multiple medication regimens that are used to treat patients (2). Skin reactions are the most common manifestation (6)

and drug-related rashes have been estimated to be 100 times more common in HIV-positive patients than in general population (7,8) and up to 1000 times greater when entities like Stevens-Johnson syndrome or toxic epidermal necrosis (SJS/TEN) are assessed (9). The diagnosis is based on clinical criteria (10) and diagnostic work-up includes a detailed clinical history, physical examination, symptom resolution on withdrawal, and reintroduction of the suspected drugs and exclusion of other causes.

Usually the onset of an allergic reaction is delayed, between 1-6 weeks after commencing the drug (6).

The authors present a case of HR to nevirapine confirmed by drug provocation test (DPT) and probable HR to emtricitabine and tenofovir based on positive patch tests (PT).

Case report

We report the case of a 47-year-old Caucasian woman with diagnosis of HIV infection since 2009, that started HAART with tenofovir, emtricitabine and nevirapine in 2011. The genetic screening for HLA B5701 was negative, pretreatment $CD4^+$ T-cell count was 345 cells/mm^3 and HIV-1 RNA level was 463 copies/mL.

On the second day of treatment, she developed pruritic exanthema and palpebral edema that improved two weeks after the infectiologist discontinued HAART and prescribed anti-histamines (AH) and oral corticosteroids (CS). Laboratory tests (complete blood count and biochemistry with liver and renal function tests) performed at the time of reaction were normal.

One month later, treatment with tenofovir and emtricitabine was restarted in association with darunavir/ritonavir. The patient reported a reproducible reaction on the second day of treatment which improved after discontinuation of therapy and treatment with AH and CS, with symptoms resolution in 8 days.

Two months later, the patient restarted darunavir/ritonavir in association with abacavir and lamivudine. On the second day she developed palpebral edema and discontinued the treatment once again. She was then referred to our Drug Allergy Clinic for suspected HR to antiretroviral drugs.

PT were performed with ritonavir 1% and 10% in petrolatum and with 1%, 10% and 30% in petrolatum with the other suspected drugs. Results were recorded using a standardized scoring system (11).

As summarized in **table I**, PT were considered strong positive for emtricitabine (1%, 10% and 30%) and tenofovir (10% and 30%), doubtful (erythema only) for lamivudine (10 and 30%) and negative to the other suspected drugs.

PT with emtricitabine, tenofovir and lamivudine (1%, 10% and 30%) were negative in 7 controls (**table II**).

DPT with darunavir (800 mg), ritonavir (100 mg) and abacavir (600 mg) were followed by home treatment with no adverse reactions. DPT with nevirapine up to 400 mg was positive, with development of pruritic exanthema in the upper limbs one hour after the conclusion of the oral challenge and palpebral edema 24 hours after.

DPT with raltegravir (800 mg) as alternative drug was negative.

She currently maintains treatment with darunavir/ritonavir, raltegravir and abacavir uneventfully.

Table I *- Results of patch testing.*

Patient							
patch tests	**ABC**	**FTC**	**TDF**	**3TC**	**NVP**	**TV**	**TMC114**
48 h							
1%	-	++	-	-	-	-	-
10%	-	++	++	?	-	-	-
30%	-	++	++	?	-	np	-
negative control	-	-	-	-	-	-	-

Standard patch test scores (adapted from Brockow K., et al. Allergy 2002; 57:45-51).	
?	doubtful (faint erythema only)
+	weak positive (erythema, infiltration, possibly papules)
++	strong positive (erythema, infiltration, papules, vesicles)
+++	extreme positive (bullous, ulcerative)
-	negative

ABC, abacavir; FTC, emtricitabine; TDF, tenofovir; 3TC, lamivudine; NVP, nevirapine; TV, ritonavir; TMC114, darunavir; np, not performed.

Discussion

Although nearly all antiretroviral drugs have been reported to cause HR, the most commonly associated with such syndromes include abacavir, nevirapine, efavirenz, etravirina, rilpivirina, fosamprenavir and enfuvirtide (5).

The diagnosis of drug hypersensitivity in HIV-infected patients is a challenging task. It is only based on clinical criteria, and complicated by the fact that many patients take multiple drugs and develop diseases such as opportunistic infections and immune restoration disease that can make determination of causality difficult (6).

HLA-B*5701 has great utility as a screening test, with 100% negative predictive value generalizable across different ethnicities to identify patients at risk to develop abacavir hypersensitivity (12,13). The complexity of HLA associations across different phenotypes and ethnicities with other drugs like nevirapine is such that currently HLA testing has limited utility as a screening strategy to prevent nevirapine hypersensitivity syndromes before nevirapine prescription (14). MHC HLA class II allele HLA-DRB1*0101 has been associated with nevirapine hypersensitivity (15).

Drug-related rashes occur at a much higher frequency in HIV-positive patients than in the general population (2). Cutaneous eruptions are the most common manifestation, but significant systemic findings, including fever and internal organ involvement can occur (10). The most common cutaneous drug reaction in HIV-infected patients are maculopapular exanthemas, often accompanied by pruritus without fever (15). These eruptions usually appear between 2 and 10 weeks after primary exposure to antiretroviral therapy and within 1 to 2 days of rechallenge (14).

Cutaneous problems and hepatoxicity are the main side effects induced by NNRTIs (14). All NNRTIs have been associated with rash and less commonly with HR marked by combinations of fever, rash and internal organ involvement or severe skin involvement such as Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) (5).

The most common drug-related adverse event secondary to nevirapine is nonurticarial eruption (2). Female sex, ethnicity (Hispanic, Chinese and African), individuals with higher $CD4^+$ T-cell count (> 250 cells/mm^3 for women and > 400 cells/mm^3 for men), and uncontrolled HIV viremia in early studies seemed to be at higher risk for nevirapine-related rash (2,16).

Hypersensitivity to tenofovir has been rarely reported, as described by Borras-Blasco, et al. (17); another case of hypersensitivity to emtricitabine/tenofovir was reported with similar clinical symptoms to abacavir HR (18).

All HR in our patient were mild cutaneous reactions on the second day of HAART: first with combination of emtricitabine, tenofovir and nevirapine; then with the combination of emtricitabine, tenofovir, darunavir/ritonavir; and last with the combination of darunavir/ritonavir, lamivudine and abacavir.

Our patient had at least three risk factors that may facilitate nevirapine-related rash: higher pretreatment $CD4^+$ T-cell count, lower HIV-1 RNA level, and female sex.

PT is the most studied cutaneous testing approach in delayed drug HR (11). In the case of HR to antiretroviral drugs the true diagnostic sensitivity is unknown, except for abacavir. Guidelines have recommended concentrations of 1-10% of the pure drug and 30% of the commercialized form (19). Based on this, PT were prepared 1% and 10% in petrolatum of ritonavir, and with 1%, 10% and 30% in petrolatum with the other suspected drugs (emtricitabine, tenofovir, nevirapine, darunavir, lamivudine and abacavir).

Table II *- Results of patch testing in controls.*

	Controls					Patch tests									
	sex	**age (years)**	**atopy**	**HIV**	**exposure to ART**	**1%**	**FTC 10%**	**30%**	**1%**	**TDF 10%**	**30%**	**1%**	**3TC 10%**	**30%**	**NC**
1	f	45	NP	+	3TC	-	-	-	-	-	-	-	-	-	-
2	m	49	NP	+	FTC, TDF	-	-	-	-	-	-	-	-	-	-
3	m	52	NP	+	FTC, TDF	-	-	-	-	-	-	-	-	-	-
4	f	46	A	+	3TC	-	-	-	-	-	-	-	-	-	-
5	f	63	NA	-	-	-	-	-	-	-	-	-	-	-	-
6	m	34	A	-	-	-	-	-	-	-	-	-	-	-	-
7	f	61	A	-	-	-	-	-	-	-	-	-	-	-	-

f, female; m, male; HIV, human immunodeficiency virus; ART, antiretroviral treatment; FTC, emtricitabine; TDF, tenofovir; 3TC, lamivudine; NC, negative control; NP, not performed; NA, non atopic; A, atopic.

HR to emtricitabine and tenofovir was considered probable based on positive PT in the patient and negative PT in 7 controls (**table II**).

Delayed HR to nevirapine was confirmed by DPT. It was possible to exclude HR to darunavir, ritonavir and abacavir based on DPT.

Although anti-retroviral drugs may share a structure, such as a shared sulfa antimicrobial group in the case of darunavir and fosamprenavir, or shared mechanism of action and propensity to develop skin rash, as for the HIV NNRTIs, clinical and immunologic cross-reactivity between antiviral drugs is uncommon in clinical practice (14).

Since lamivudine is chemically similar to emtricitabine (20), the doubtful result of PT with lamivudine could be explained by a possible cross-reactivity between both drugs, although new sensitization to lamivudine can not be excluded.

This is an interesting case, because HR to antiretroviral drugs in HIV-infected patients are increasing, and management of these patients represents a diagnostic and therapeutic challenge. In addition, HAART affects the prognosis of patients with HIV infection.

PT may represent a useful method for confirming hypersensitivity to antiretroviral drugs. PT performed with emtricitabine and tenofovir in 7 controls (**table II**) were negative, suggesting that the concentrations used were not irritative. However, the use of PT is not widespread, so the predictive value of testing has not been ascertained.

Further investigation in this area is required to elucidate the mechanisms in HIV-infected patients, so that successful management strategies can be offered, preventing loss of potent and viable antiretroviral agents.

Informed consent

The authors have obtained the informed consent of the patients mentioned in the article.

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Spontaneous disappearance of severe latex allergy in an adult

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Keywords
latex allergy; desensitization; skin testing; IgE ; anaphylaxis; contact allergy

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To the Editor

A 56 year-old woman was recently seen at this allergy center. The patient had been known since 1995 for severe natural rubber latex (NRL) allergy. At that time, she had experienced an episode of immediate anaphylaxis (dyspnea, generalized pruritus, hypotension, and tachycardia) after wearing latex gloves. That episode was treated by systemic corticosteroids, antihistamines, and beta-agonists in an Emergency Room. The subsequent allergy workup demonstrated a clear-cut reactivity on skin prick testing with both latex glove (by the prick-prick technique) and commercial NRL extract (ALK/Abellò, Lainate, Italy; 500 µg protein/ml) with wheals whose mean diameters exceeded 10 mm. The SPT with the common seasonal and perennial airborne allergens showed moderate hypersensitivity to ragweed pollen. Severe NRL allergy was diagnosed, and the woman was recommended to avoid any contact with latex.

During the following years the woman experienced severe rhino-conjunctivitis whenever she entered a hospital department to visit an inpatient or when she had to undergo medical visits. Some surgical interventions were carried out successfully throughout the years strictly following latex-safe procedures.
About 3 years ago the patient entered in a hospital to visit the mother as an inpatient without any consequence. Further, about 6 months ago, while undergoing dental treatments the woman noticed that she had forgotten to remind the dentist about her latex allergy and that he was wearing latex gloves but, to her great surprise, she did not experience any adverse reaction. In view of these facts she asked for a control visit at this department. The SPT with the same commercial NRL extract scored negative while the mean wheal diameter induced by the positive control, histamine 10 mg/ml, was 7 mm; similarly, the measurement of latex-specific IgE by ImmunoCAP (Thermo Fisher Scientific) scored negative (< 0,10 kU/L). A latex glove-wearing test

did not cause any reaction. Based on these findings and on the absence of any reaction following the recent exposures to NRL, it was concluded that the patient was no longer sensitized to natural rubber latex proteins. Notably, ragweed hypersensitivity was still clearly detectable at the control visit, as shown by clear-cut positive SPT and a specific IgE level of 4,55 kU/L.

Discussion

The disappearance of both hypersensitivity and clinical allergy to an allergen is a well-known phenomenon in children allergic to cow's milk and/or hen's egg who, in most cases, outgrow their allergic status (1). In contrast, hypersensitivities that develop subsequently both in children and in adults are considered to be life-long. To the best of our knowledge this is the first reported case of spontaneous disappearance of natural rubber latex hypersensitivity in an adult patient. It is generally accepted that the long-term absence of contact with an allergen may result in a gradual lowering of specific IgE levels to the point that both in-vitro and in-vivo diagnostic tests score negative at the first re-exposure. IgE-mediated beta-lactam allergy is a typical example in this sense. However, it is also well-known that the re-exposure during the in-vivo diagnostic procedures elicits a secondary immunologic reaction by memory B cells that leads to a clinically measurable re-sensitization status after a few weeks (2). The patient described here was possibly re-exposed to NRL during a hospital visit 3 years ago and certainly and massively re-exposed during the more recent dental therapeutic treatments. Nonetheless, she seems presently no-longer sensitized to NRL allergens.
It is not possible to say how frequently spontaneous desensitization occurs in adults, nor whether this is a specific feature of NRL allergy or may happen also in patients with other food or respiratory allergies. Similarly, the mechanisms by which the patient got desensitized are unclear, although it could be hypothesized that in this case a population of specific regulatory T cells proliferated and got activated, leading to the disappearance of the IgE-mediated response to NRL. As a matter of fact, the specificity of this process for NRL was demonstrated by the significant persistent ragweed hypersensitivity.
In conclusion, this case report shows that in some patients NRL allergy may disappear. Patients with this type of allergy might undergo allergological re-evaluation if a long time has gone from the initial diagnosis.

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Dog allergy: can a prevalent or exclusive sensitization to Can f 5 be considered a lucky or negative event in "real life"?

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Summary
Recent studies have shown the increasing relevance of allergic sensitization to Can f 5 (a prostatic kallicrein), which is an androgen-regulated protein expressed in the prostate and detectable only in male dogs. Can f 5 can be a prevalent or exclusive sensitizing agent in a considerable percentage of dog-allergic patients. Its specific allergenic characteristics are able to induce possible "negative" as well as "positive" clinical effects in individuals sensitized to dogs. In the present article we pointed out the possible pros or cons of sensitization to this allergen in "real life".
Further studies should be carried out to correctly assess some peculiar characteristics of Can f 5, in order to support the most of "positive" aspects and remedy at best the "negative" effects.

Dog allergens are a common cause of allergic sensitization and triggering respiratory symptoms worldwide. The impact of dog allergens is particularly relevant in geographical areas characterized by a high level of pet ownership, such as US and Northern Europe (1).
Common described dog allergens belong to lipocalins (Can f 1, Can f 2, Can f 4 and Can f 6) or albumins (Can f 3) families of proteins (2). In 2009 Mattson et al. (3) identified a new dog allergen named Can f 5, a prostatic kallicrein which is an androgen-regulated protein expressed in the prostate and detectable only in male dogs (small amounts might also be present in dog epithelia). Recent studies have highlighted the increasing importance of allergic sensitization to Can f 5, which has been found as exclusive allergen in about a third (3) up to 37% of dog-sensitized individuals (4). However, further studies should confirm the real value of Can f 5 sensitization in "real life" (5).
Considering this background, we will describe in the present article some specific aspects of Can f 5 and will try to balance possible pros or cons of being prevalently of exclusively sensitized to this allergen in "real life".

Reasons for possible "negative" events

It is well known that literature data on dog allergen immunotherapy (AIT) demonstrated poor and conflicting results on clinical efficacy, correlated with the poor-quality extracts and the inherent complex allergenic profile of dog materials. It is also likely that the concomitant sensitization to lipocalins

and/or albumins of other furry animals, especially in those patients directly exposed, could be a further aspect which determines the efficacy of dog AIT in sensitized individuals (6). Molecular-based diagnosis (CRD) can be considered a prototype of so-called "Precision Medicine", because CRD provides the possibility of a better targeted prescription of AIT, discriminating against primary and cross-sensitization to allergens (7). Considering the presence of different allergenic materials in extract of mammalian origin, it is evident that a standard dog AIT is not likely to be effective in Can f 5 mono-sensitized individuals.

Since Can f 5 is a prostatic kallicrein, similar to human prostatic antigen, some studies have shown that it can be involved in cases of human seminal plasma allergy (8,9).

Possible necessity of re-location of male dogs owned by patients with established allergic sensitization exclusively to Can f 5 and uncontrolled respiratory symptoms should be considered (see also the possible "lucky events" below).

Reasons for possible "lucky" events

We have previously shown that pet (cat or dog) ownership, or their presence in indoor environments, cannot be considered the main criterion to assess the exposure to animals. The use of this criterion represents a potential bias of underestimation in clinical practice and in large epidemiological studies (10-12). In fact, exposure to dogs and cats can happen via several direct and indirect modalities, such as through various pet allergen-contaminated items. The indirect modality of exposure may explain the common findings that dog allergens (Can f 1 and Can f 2) can be present in indoor environments where dogs cannot be kept. In developed countries, the consequence of pet allergen ubiquity is a persistent stimulation of airways, similar to that induced by dust mite, that may consequently increase the risk of allergic sensitization.

To the best of our knowledge, no studies have previously demonstrated a passive transport of Can f 5 in dog-free indoor environments. Therefore, also considering that the source of Can f 5 is dog prostate, we believe that the main way of exposure to this allergen should be the direct dog exposure for ownership or direct contact elsewhere. In a previous study on a similar topic we demonstrated that sensitization to urine allergens was exclusive of patients with rabbit at home, whereas individuals exposed indirectly to rabbit-derived materials exhibited allergic sensitization only to epithelial allergens (13).

The possible lack of passive transport of Can f 5 could decrease the risk of allergic sensitization through indirect modalities linked to ubiquity of dog allergens, so reducing the risk due to domestic exposure. However, further studies should be planned to evaluate the presence of Can f 5 in dog-free indoor environments as consequence of a possible passive transport through various items (e.g. clothes of dog owners). This modality of exposure has been demonstrated with the main dog allergens Can f 1 and Can f 2. Moreover, further studies should be planned on the possible reduction of Can f 5 production after castration of male dog.

With the exclusion of the similarity with the human prostatic antigen, Can f 5 should not cross-react to other mammalian "pan-allergens" belonging to albumins or lipocalins family. Thus, further investigations should evaluate a possible "cross reactivity" with prostatic antigen of other pets or domestic mammals. In fact, we have previously shown, by using an *in vivo* (skin prick test) and *in vitro* model (the micro-array technique ImmunoCAP ISAC), that exposure and allergic sensitization to common pets (including dogs) may increase the risk of developing sensitization to other furry animals (14,15). These findings are likely the consequence of cross–reaction mechanisms involving lipocalins and albumins.

The peculiarity of Can f 5 of being produced only by male dogs may lead to pros or cons consequences on dog ownership. In fact, Schoos et al. (16) have recently suggested that dog-allergic patients mono-sensitized to Can f 5 seem to tolerate female dogs, since a 54-year-old female patient reported that respiratory symptoms occurred only after exposure to male dogs. Diagnostic procedures (*in vitro* and *in vivo* tests including ocular provocation test) confirmed the absence of reaction to allergenic materials extracted from a female dog but not to those from a male dog (16). If other studies will confirm this first case-report, it would open up the possibility of trying to own a female dog, with lower risks, to fulfill the wish to get a dog in the house (this is a crucial aspect in "dog-lover patients", especially children).

Conclusions

Recent studies have highlighted the role of Can f 5 as sensitizing agent of airways that can be prevalent in a considerable percentage of dog-sensitized patients. Its particular antigenic profile is likely to determine positive or negative situations in "real life" (**figure 1**).

For this reasons, further studies should be performed to evaluate some essential aspects of Can f 5 in order to make the most of "positive" characteristics and to remedy at best the "negative" effects.

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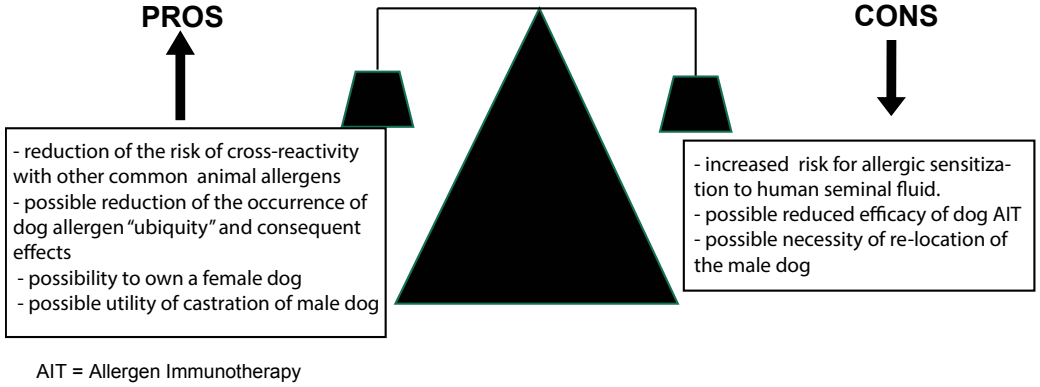
Figure 1 *- Possible Pros or Cons of being exclusively or prevalently sensitized to dog allergen Can f 5.*



Conflict of interest

The authors declare that they have no conflict of interest.

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