EVALUATION OF ANTINUCLEAR ANTIBODIES IN GEORGIAN ALLERGIC PATIENTS POLYSENSITIZED WITH CROSS REACTIVE ALLERGENS

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Western countries have been challenged with an "allergy epidemic" during the last three to four decades, resulting in a very high burden of allergic rhinitis, allergic conjunctivitis, atopic eczema and asthma [18]. Interestingly, there has been a parallel increase in the incidence rates of several autoimmune disorders, including multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus (SLE) [2].

The immune system is a tightly regulated network that is able to maintain a balance of immune homeostasis under normal physiological conditions. However, under particular circumstances, this balance is not maintained and immune responses either under or over react [21]. Allergy and autoimmunity are two potential outcomes of dysregulated immunity. They have many parallels and at the same time, they have many differences. They can be considered, in philosophic conception, to be the yin and yang of immunopathology [3]. However, the parallel appearance of allergic and autoimmune conditions in some patients may reveal that such aberrations of the immune system have a common pathophysiologic mechanism [22]. Several studies showed that patients with primary Sjögren's syndrome (SS) are associated with an increased risk of developing asthma [24] and vice versa: it exists a significant relationship between atopic diseases and the risk of SLE, especially for females [11]. The presence of atopic triad diseases is significantly associated with risks of systemic lupus erythematosus, rheumatoid arthritis, and Sjögren syndrome, and their coexistence exacerbates this risk [10].

Allergic and autoimmune diseases are the result of a combination of congenital, genetic causes and acquired, external triggering factors, which are common to a certain extent to both entities - infection, medications, chemicals, food, UV radiation, etc. Different immune mechanisms are thought to underlie allergy and autoimmune disorders; a predominant Th2 response has been detected in allergic patients, whereas Th1-driven response has been found in most patients with autoimmune disorders [25]. An autoimmune disease occurs when the antigens of an organism are attacked by the autoantibodies as a result of disturbed self-tolerance. Antinuclear antibodies (ANA) usually target specific antigens in the nuclear part of the cell, although they can sometimes show affinity against all types of subcellular structures and cell organelles, including the cytoplasm, nuclei, nucleoli, or cell surfaces.

Allergic reactions can be considered as abnormal IgE immune responses towards environmental antigens. Allergic sensitization is the outcome of a complex interplay between the allergen and the host in a given environmental context [26]. It is estimated that the polysensitization are the strongest risk factors for the development of multimorbidity in allergy patients [20]. Among another factors sensitization with cross reactive allergens, belonging to panalergen families, determines the formation of distinct allergic entities, syndromes and associations [19]. Although usually considered as minor allergens, sensitization to panallergens might be problematic as it bears the risk of developing multiple sensitizations. Clinical manifestations seem to be tightly connected with geographical and exposure factors [7].

The aim of our study was to evaluate antinuclear antibodies (ANA) in Georgian allergic patients polysensitized with cross reactive allergens, in order to establish connection between allergic and autoimmune diseases in our population.

Material and methods. Two groups of patients referred to the Center of Allergy and Immunology (Tbilisi, Georgia) were included in the study: patients with atopy (group 1, n=97) and without (group 2, n=42).Diagnostic workup was performed according to local and international guidelines. ImmunoCAP Phadiatop (Thermo Fisher Scientific, Uppsala, Sweden) serum test was used as first-line screening tools for allergic sensitization. The level >0.35 KU/L was defined as positive.

For the evaluation of polysensitization the component resolved diagnosis was used. The detection of specific IgE to multiple allergen components was performed using the 112 component ImmunoCAP ISAC allergen microarray immunoassay (Termo Fisher Scientifc, Uppsala, Sweden). ISAC is a test for semi-quantitative determination of IgE in serum samples. The solid phase in this test is provided by the surface of a plate on which 112 components (43 native and 69 recombinant) have been adsorbed and arranged in triplets. Antibody levels were expressed in standardized units, ISU-E (ISAC Standardized Unit for specific IgE). The measured values ranged from 0.3 to 100 ISU-E, and values ≥ 0.30 ISU-E were considered to be positive results. Based on ISAC results atopy patients were divided into two subgroups: 1) allergic patient sensitized to at least one of the cross reactive allergen family members and 2) allergic patientswith mono- or poly-sensitized to species –specific allergens without cross reactive allergen sensitization.

ANA Measurement. Allergic and non allergic individuals were screened for ANAs by IIFA (Indirect Immunofluorescence assay) on HEp-2 cells (Bio-Rad Laboratories, Hercules, CA).Twofold serial dilution in 0.01 M phosphate buffered saline (PBS) was used for autoantibody titration. A positive and a negative reference controls were tested in each slide for quality control. Prediluted sera were overlaid on fixed HEp-2 cells for 20 min at room temperature. Slides were washed for 10 min with PBS, overlaid with fluorescein conjugated (FITC) antiserum and incubated for an additional 20 min. After a slide was washed a cover slip was placed over the slide with mounting medium. The slides were evaluated with a fluorescence microscope (ata X 600-fold magnification). A titer of 1:40 or higher was considered toindicate ANA positivity[9].

Statistical characteristics of quantitative variables were presented as arithmetic means (x), standard deviations (SD), minimum (min.) and maximum (max.) values. Frequencies of qualitative variables were expressed as percentages. The percentage of ANA (+) individuals in different groups was compared by Fisher's exact probability test. The threshold of statistical significance for all tests was set at $\alpha = 0.05$. Statistical analysis of the results was carried out with SPSS (SPSS, Inc., Chicago, IL, USA).

Results and discussion. One hundred and thirty-nine individual were studied. For non-atopic group the male/female proportion and mean age (with standard deviation) were 11/31 (26.3%/73.8%) and 40.17± 11.97 years respectively. Slightly different characteristics were obtained for atopic individuals: male/female proportion was 55/42 (56.7%/43.3%) and mean age (with standard deviation) 35.72±14.34years (Table 1).

Table 1. Baseline characteristics of study patients

	Non-atopic individuals n=42	**Atopic individuals n=97**	**Atopic individuals sensitized with cross reactive allergen n=67**	**Atopic individuals without cross reactive allergen sensitization n=30**
Gender (n/%)				
Male	11(26.2)	55(56.7)	41(62.2)	14(46.7)
Female	31(73.8)	42(43.3)	26 (38.8)	16(53.8)
Age (± Std. Deviation)	40.17± 11.97	35.72± 14.34	34.76 ± 14.39	37.87 ± 14.21
Clinical phenotypes (ICD10 codes) (n/%)				
Acute atopic conjunctivitis (H10.1)	NA	26(26.8)	23(34.3)	3(10.0)
Allergic rhinitis (J30)	NA	56(57.7)	42(62.7)	14(46.7)
Asthma (J45)	NA	15(15.5)	12 (17.9)	3 (10.0)
Atopic dermatitis (L20)	NA	18(18.6)	12(17.9)	6(20.0)

Table 2. ANA positivity among different groups

	Non-atopic individuals n=42	**Atopic individuals n=97**	**Atopic individuals sensitized with Cross reactive allergen n=67**	**Atopic individuals without Cross reactive allergen sensitization n=30**
ANA positive (n%)	1(2.4)	26(26.8)	18(26.9)	8(26.7)
ANA negative (n%)	41(97.6)	71(73.2)	49(73.1)	22(73.3)

In general 67(69.1%) allergic patients were sensitized to at least one of the cross reactive allergen. Among them 26 (38.8%) showed the sensitization to PR-10 family members; 19(28.4%) to CCDs; 18(26.9%) to profilins; 11(16.4%) to nsLTP; 10 (14.9%) to tropomyosin; 9 (13.4%) to TLPs, 7(10.4) to serum albumins and 1(1.5%) patient to polcalcins (Table 1).

In allergic patients sensitized to cross-reactive allergens allergic rhinitis (42/62.7%) was the most prevalent symptom followed by acute allergic conjunctivitis (23/34.3%), asthma (12/17.9%) and atopic dermatitis (12/17.9%). In allergic patients without cross reactive allergen sensitization allergic rhinitis was also the most prevalent symptom followed by atopic dermatitis (6/20.0%), acute allergic conjunctivitis (3/10.0%) and asthma (3/10.0%) (Table 1).

All individuals involved in the study were tested for antinuclear auto-antibodies (ANA). Among allergic patients 26 (26.8%) tested positive for the presence of auto-antibodies; for non-allergic patient this value was only 1(2.4%).The difference was statistically significant [(OR 13.47, 95% CI: 1.76-103.3) p=0.001)].

The ANA expression was separately evaluated for subgroups of polysensitized patients divided according their sensitization profile: 18(26.9%) patients sensitized to cross reactive allergens showed positive results for ANA testing and 8(26.7%) allergic patient without cross allergen sensitization were positive for ANA. The difference between these two subgroups was not statistically significant [(OR 0.86, 95% CI: 0.32-2.31) (p=0.8)] (Table 2).

The morphological characteristics observed in the indirect immunofluorescence assay on Hep-2 cells were interpreted according the International Consensus on ANA staining Patterns (ICAP). The positive sera showed the following patterns of fluorescence: AC-2 (nuclear dense fine speckled), AC-4 (nuclear fine speckled), AC-8 (homogenous nucleolar) and AC-16 (Cytoplasmic fibrillar filamentous). The AC-2 was only one detected pattern among non-atopic individuals (1/2.4%) and the most frequent pattern in allergic patients (19/73%). The different ANA pattern expression among the study groups is showed in Table 3.

The highest titer at which the ANA positivity was observed was 1:160.The most frequent dilution was 1:80 (20/74.07%), followed by 1: 160 (4/14.8%) and 1:40 (3/11.1%).

In the ANA positive group of allergic patients the atopic dermatitis (13/50%) and asthma (6/23.1%) were most frequently diagnosed, in comparison to the ANA negative group where allergic rhinitis (43 /60.6%) and atopic conjunctivitis (20/28.2%) were most common (Table 4).

Table 3. The main types of ANA patterns observed during the study

ANA pattern	**Non Atopic individuals**	**Allergenic patients sensitized with Cross allergens**	**Allergenic patients without Cross reactive allergen sensitization**
AC-2 (n/%)	1(2.4%)	12 (17.9%)	7(23.3%)
AC-4 (n/%)	NA	NA	1(3.3%)
AC-8 (n/%)	NA	4(6%)	NA
AC-16 (n/%)	NA	2(3%)	NA

Table 4. Allergic diseases among ANA negative and ANA positive atopic patients

	Positive ANA (n/%)	Negative ANA(n/%)
Acute atopic conjunctivitis	6 (23.1%)	20 (28.2%)
Allergic Rhinitis	13 (50.0%)	43 (60.6%)
Asthma	6 (23.1%)	9 (12.7%)
Atopic dermatitis	13(50.0%)	5 (7.0%)

Allergy and autoimmunity are characterized by localized inflammation that leads to the injury and/or destruction of target tissues. Until recently, it was generally accepted that the mechanisms that govern these disease processes are quite disparate; however, new discoveries suggest possible pathogenesis linkage. In both cases, an increased production of IgE antibodies and presence of ANA in selected disease entities is observed [23].

We found that the positivity of ANA antibodies was significantly higher in allergic patients than in the control group of non-atopic individuals (p= 0.001.). In our study it was showed that positive antinuclear antibodies were more common in the patients with atopic dermatitis and asthma. In comparison of this the percentage of allergic rhinitis, and atopic conjunctivitis were higher in ANA negative allergic patients.

Data regarding the association of asthma with autoimmune disorders are controversial.According the study carried out by Tamai and al. asthma tends to involve autoimmunity associated with antinuclear antibody more frequently than COPD because asthma is the more robust factor for antinuclear antibody positivity. Another study showed that the presence of ANA is an independent risk factor in asthma for evolution with death, severe exacerbations, high inhaled corticosteroid intake and FEV1 decline >100 ml [1].

The relationship between ANA and atopic dermatitis (AD), which is a chronic, genetically predisposed skin disease of type I immediate mechanism related to IgE antibodies, has been confirmed to date. Positive antinuclear antibodies (ANAs) wererereportedin twenty to thirty percent of patients with atopic dermatitis (AD) [9].

Antinuclear antibodies (ANA) are primarily significant in the diagnosis of systemic connective tissue diseases. The assay for antinuclear antibodies (ANA) is commonly used in the screening of autoantibodies in systemic autoimmune diseases, and the indirect immunofluorescence assay (IIFA) utilizing HEp-2 cell substrates remains the recommended methodology[4]. ANA detection by IFA has the advantage of obtaining information on the IIF staining pattern, which is considered of added clinical value [5]. In the original ICAP classification algorithm, 28 distinct immunofluorescence patterns recognized by HEp-2 IIFA were defined and summarized into three main categories, comprising 14 nuclear, nine cytoplasmic and five mitotic patterns, respectively[8].

In our study the dominating pattern in allergic and in non-allergic patient was dense fine speckled pattern (AC-2). This nuclear pattern of staining is strongly correlated to the presence of autoantibody to DFS70 and, importantly, is seen in very low frequency in SjS, SSc, and SLE [13,14]. These autoantibodies have also been detected at varied frequencies in patients with diverse non-SARD inflammatory and malignant conditions such as atopic diseases, asthma, eye diseases, and prostate cancer. These observations have recently stimulated vigorous research on their clinical and biological significance.Both in apparently healthy individuals as well as patients who do not have a systemic autoimmune rheumatic diseases (SARD)the AC-2 pattern may be caused by autoantibodies to other antigens than DFS70 [17].

It is important fact that mono-sensitized groups and poly-sensitized groups are immunologically different, and allergic indices are more severe in the poly-sensitized group [12].Considering this aspect, we examined the ANA expression in patients poly-sensitized with cross reactive allergens. In the case of allergens and IgE, cross-reaction is based on the binding of an IgE antibody to homologous allergen structuresshared linear orin most casesconformational epitopes (i.e., structural similarities). Such structures may be conserved among proteins with similar functions [6].The pathogenesis-related (PR) protein family 10, the non-specific lipid transfer proteins (nsLTP) and profilins are well-known panallergens in pollen and plant foods. Tropomyosin is the hallmark of IgE cross-reactivity among invertebrates such as shellfish, molluscs and arthropods. In vertebrates, the only known panallergens are parvalbumins, the major fish allergens, and serum albumins, minor allergens of mammals [16]. Molecular based allergy diagnostic tests have been recently introduced in the clinical practice, allowing defining and characterizing exactly the sensitization profile [15]. The ImmunoCAP ISAC test is a novel molecular assay used in the diagnostics of allergic diseases.According the ISAC testing PR-10, profilins, nLTP, TLP and tropomyosins are the cross-reactive allergens, which are significantly associated with the different allergic phenotypes in Georgian population. In the present study we investigated ANA expression in patients poly-sensitized with cross-reactive allergens and patients without, but did not find any relationship between IgE cross reactivity and ANA positivity (P=0.8).

In conclusion, we can underline that the occurrence of antinuclear antibodies is more frequentin atopic patients and associate mostly withasthma and atopic dermatitis phenotypes of allergic diseases. The most frequent coexisting ANA pattern is dense fine speckled pattern (AC-2), but the small amount of data does not allow for a clear definition of the relationship between autoimmunization with DFS70 and allergic diseases. Despite the fact that IgE cross reactivity is of interest for various reasons in present study didn't showed an influence on the autoimmunization. The occurrence of ANA antibody inatopic patients and its role in allergy remains the subject for future research.

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SUMMARY

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Western countries have been challenged with an "allergy epidemic" during the last three to four decades. Interestingly, there has been a parallel increase in the incidence rates of several autoimmune disorders.The aim of our study was to evaluate antinuclear antibodies (ANA) in Georgian allergic patientspolysensitized with cross reactive allergens, in order to establish connection between allergic and autoimmune diseases in our population.

Two groups of patients were included in the study: patients with atopy (group 1, n=97) and without (group 2, n=42).ImmunoCAPPhadiatop and ISAC assay platforms were used for atopy screening and polysensitization patterns evaluation. Screening for ANAs was performed by IIFA (Indirect Immunofluorescence assay) on HEp-2 cells.

In general 67(69.1%) allergic patients were sensitized to at least one of the cross reactive allergen. Among allergic patients 26 (26.8%) tested positive for the presence of auto-antibodies; for non-allergic patient this value was only 1(2.4%). 18(26.9%) patients sensitized to cross reactive allergens showed positive results for ANA testing and 8(26.7%) allergic patient without cross allergen sensitization were positive for ANA. The AC-2 was only one detected pattern among non-atopic individuals (1/2.4%) and the most frequent pattern in allergic patients (19/73%). In the ANA positive group of allergic patients the atopic dermatitis (13/50%) and asthma (6/23.1%) were most frequently diagnosed.

The occurrence of antinuclear antibodies is more frequent in atopic patients and associate mostly with asthma and atopic dermatitis phenotypes of allergic diseases. The most frequent coexisting ANA pattern is dense fine speckled pattern (AC-2).

The occurrence of ANA antibody in atopic patients and its role in allergy remains the subject for future research

Keywords: allergy, cross reactivity, autoimmunity, ANA antibody.

РЕЗЮМЕ

ОЦЕНКА АНТИНУКЛЕАРНЫХ АНТИТЕЛ У ПАЦИЕНТОВ С АЛЛЕРГИЕЙ, ПОЛИСЕНСИБИЛИЗИРОВАННЫХПЕРЕКРЕСТНО-РЕАКТИВНЫМИАЛЛЕРГЕНАМИ, В ГРУЗИИ

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Западные страны столкнулись с проблемой «эпидемии аллергии» в течение последних трех-четырех десятилетий. Интересно, что параллельно увеличилась частота возникновения нескольких аутоиммунных заболеваний.

Целью исследования явилась оценка антинуклеарных антител у пациентов с аллергией, полисенсибилизированных перекрестно-реактивными аллергенами, для установления связи между аллергическими и аутоиммунными заболеваниями в грузинской популяции.

В исследование включены две группы пациентов: пациенты с атопией (группа 1, n=97) и без (группа 2, n=42). ImmunoCAP Phadiatop и ISAC использованы для скрининга атопии и оценки паттернов полисенсибилизации. Скрининг антинуклеарных антител (ANA) проводили с помощью непрямого иммунофлуоресцентного анализа на клетках HEp-2.

67 (69,1%) пациентов с аллергией были сенсибилизированы, по крайней мере, к одному из перекрестно-реактивных аллергенов. Среди пациентов с и без аллергии положительный результат на наличие аутоантител выявлен у 26 (26,8%) и 1 (2,4%) пациента, соответственно. Одинаковым было количество положительных результатов теста на ANA для пациентов с и без сенсибилизации к перекрестно-реактивным аллергенам. AC-2 был единственным выявленным паттерном среди лиц, не страдающих атопией (1/2,4%) и наиболее частым паттерном у пациентов с аллергией (19/73%). В группе пациентов с аллергией, положительной на ANA, чаще диагностировались атопический дерматит (13/50%) и астма (6/23,1%).

Присутсвие антинуклеарных антител чаще встречается у пациентов с атопией и связано, в основном, с фенотипами астмы и атопического дерматита. Наиболее частым сосуществующим паттерном ANA является плотный мелкий крапчатый паттерн (AC-2). Возникновение ANA у пациентов с атопией и их роль в развитии аллергии является предметом будущих исследований.

რეზიუმე

საქართველოში ანტინუკლეარული ანტისხეულების შესწავლა ჯვარედინად მორეაგირე ალერგენებით პოლისენსიბილიზირებულ პაციენტებში

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[1]ალერგიისა და იმუნოლოგიის ცენტრი, თბილისი; [2]დავით ტვილდიანის სამედიცინო უნივერსიტეტი, თბილისი, საქართველო

კვლევის მიზანს წარმოადგენდა საქართველოს პოპულაციის მაგალითზე ალერგიასა და აუტოიმუნურ დაავადებებს შორის კავშირის დადგენა, ანტინუკლეარული ანტისხეულების არსებობის შესწავლა ალერგიულ პაცინტებში, რომლებიც პოლისენსიბილიზირებულნი არიან ჯვარედინად მორეაგირე ალერგენებით.

კვლევაში ჩართული იყო პაციენტების ორი ჯგუფი: პაციენტები ატოპიით (ჯგუფი 1, n=97) და ატოპიის გარეშე (ჯგუფი 2, n=42). ImmunoCAP Phadiatop და ISAC ტესტირების პლატფორმები გამოყენებული იყო ატოპიის სკრინინგისა და პოლისენსიბილიზაციის მახასიათებლების შესასწავლად. ანტინუკლეარული ანტისხეულების (ANA) განსაზღვრა განხორციელდა HEp-2 უჯრედულ ხაზებზე არაპირდაპირი იმუნოფლუორესცენციის მეთოდით.

ალერგიული პაციენტებიდან 67 (69.1%)-ს აღენიშნებოდა სენსიბილიზაცია სულ მცირე ერთი ჯვარედინად მორეაგირე ალერგენული ოჯახის წარმომადგენელზე. ასევე, ალერგიული პაციენტებიდან 26 (26.8%) აღმოჩნდა პოზიტიური ANA-ს მიმართ; არაალერგიული პაციენტებისთვის კი ეს მაჩვენებელი შეადგენდა 1 (2.4%)-ს. ჯვარედინად მორეაგირე ალერგენებით სენსიბილიზირებულ და არასენსიბილიზებულ პაციენტებში ANA-ს დადებითობა იყო ერთგვაროვანი. AC-2 მორფოლოგიური სურათი წარმოადგენდა არაატოპიურ პაციენტებში ერთადერთ (1/2.4%) და ალერგიულ პაციენტებში ყველაზე ხშირ (19/73%) იმუნოფლუორესცენტულ ნათების ტიპს. დადებითი ANA-ს მქონე ალერგიულ პაციენტებში ატოპიური დერმატიტი (13/50%) და ასთმა (6/23.1%) წარმოადგენდა ყველზე ხშირ ალერგიულ ფენოტიპს.

ANA-ს დადებითობა უფრო მაღალი იყო ატოპიურ პაციენტებში და ის უმეტესწილად ასთმასთან და ატოპიურ დერმატიტთან ასოცირდებოდა. AC-2 (მკვრივი, წვრილი, ლაქოვანი) მორფოლოგიური სურათი წარმოადგენდა ყველაზე ხშირად გამოვლენილ ANA-ს ნათების ტიპს. ANA-ს თანაარსებობა ატოპიურ პაციენტებში და მისი როლის შესწავლა ალერგიის ჩამოყალიბებაში შემდგომი კვლევების საგანს წარმოადგენს.