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# Diagnostics of specific IgE-antibodies to oranges in individuals of various ages

**Key words:** *specific IgE, allergy, orange.*

**Background and objectives.** The prevalence of allergic diseases, including of alimentary genesis is rapidly growing worldwide. Thus above 20 % of the European population suffers from various types of allergies [6]. According to the latest studies, alimentary allergy is recorded in millions of people (about 1 − 2 % of the world population), and has become a pandemic of the XXI century. It is of some interest to observe that the frequency of food sensitization is significantly higher among children during the first years of life, and is about 6 % under the age of 3 years and gradually decreasing during the first decade of life [1].

At present, one of the recognized effective methods of alimentary allergy management is a diet with the elimination of products which cause its development. However, taking into account the exceptional significance of fresh fruit for maintaining all-round good health and preventing the development of various diseases, it becomes increasingly evident that their forced exclusion from the diet can have quite a negative impact on the quality of patients' life, suffering from allergies. [5]

Despite the fact that orange is widely recognized as one of the most allergenic products, the Russian and Ukrainian scientific publications practically lack the studies related to the detailed description of its sensitization. National test systems for diagnosing IgE-antibodies to the full orange allergen extracts and for detecting sensitization to its specific antigens have not been developed so far.

**The purpose** of this research is to investigate frequency and peculiarities of age sensibilization to the amount of orange antigen by identifying IgE of specific antibodies among patients with allergic background.

## Materials and Methods

We selected and examined serum of 235 patients aged from 7 months to 78 years with an allergic background who complained of frequent development of various allergic reactions, mainly atopic dermatitis, urticaria, etc.

All the patients were divided into three age groups: patients younger than 5 years old, patients from 5 to 10 years of age, patients up to 10 years old and over (the group under the age of 2 has not been studied separately because of the small number of patients in this category). According to the preliminary analysis, the identifying of 90 % of sensitization to orange allergens with the group of patients under 5 years of age and 50 % of sensitization in the group of 10 year-old patients and over was expected. Therefore considering the presence of the 3 test groups the level of significance of 1% and the power of 90 % were set. Consequently, we calculated a sufficient sample size, which amounted to 32 persons in each group. Accordingly, the number of individuals under the examination in the age groups made up: patients under 5 years of age − 38 persons, patients from 5 to 10 years old − 37 persons, 10 year-old patients and over −160 persons.

Each group of patients was singled out by levels of total IgE and specific IgE levels to orange. Total IgE levels were investigated in 161 patients using enzyme immunoassay system "TOTAL IgE» produced by LLC "Don Ukrmed" Donetsk. The specific IgE levels detection to complex of allergens to orange in serum of patients were performed using the first national test-system of the fourth-generation "ORANGE - IgE" of LLC "Ukrmed Don", Donetsk. The detailed characteristic of enzyme immunoassay system is presented in Table 1.

**Table 1**

**The characteristic of enzyme immunoassay system for the content detection of specific IgE to orange allergens produced by LLC «Don Ukrmed» Donetsk**

| № | Options | Characteristic |
|---|---------|----------------|
| 1. | Method principle | ELISA |
| 2. | The total time of the analysis | 3 h 30 min |
| 3. | Measurement range IU/ml | 0 – 100 |
| 4. | Optical density range | 0,04 – 3,50 |
| 5. | Sensitivity, IU/ml | 0,1 |
| 6. | Coefficient of variation | not > 5 % |
| 7. | Serum volume for research in doubles | 100 µl |

Statistical analysis was performed using the licensed program MedStat. The frequencies of the age-specific sensitization distributions to complex of orange allergens were investigated. Multiple comparisons of age distribution frequencies were performed using the criterion χ-square and Maraskulio - Lyah - Guryanov procedure. Moreover, median, median error, right and left borders of the 95 % confidence interval of the values of specific IgE to orange were calculated in each age group and for the total sample using the numerous amounts of comparisons data for those parameters. Using the Kruskal-Wallis one-way analysis of variance by ranks test and the Dunn's test multiple comparisons of the data indexes were performed. Additionally, the correlation coefficient between total IgE levels and levels of specific IgE-antibodies to orange both for the whole sample and in certain age groups was calculated by the Kendall's method.

**Results and discussion**

Regular consumption of fresh fruit and vegetables is generally accepted as the basis of a daily diet and promotes both an improvement of a general state of the human health and the prevention of developing of certain diseases. There are numerous studies that have found a correlation between the consumption of fresh fruit and the decrease in the frequency of cardiovascular diseases, asthma, diabetes, and cancer pathology [4]. This is the case not only due to good nutritional quality of fruit associated with the presence of vitamins, minerals and dietary fibers but also because of the "non nutritional" component which has an increased content of secondary metabolites (phenols, flavonoids, carotenoids) performing an important role of an antioxidant [4].

Oranges as one of the most widely consumed fruits are included in the diet year round both worldwide and in our country. This information apparently led to the fact that orange is registered as one of the most allergenic products of plant origin. [4] It should be pointed out that this fruit may be the cause of both nutritional allergies and the development of atopic reactions like hay fever. [4]

Of 235 investigated patients in 151 patients diagnostically significant levels of specific IgE- antibodies to orange were detected in the serum, the allergy occurrence to this product was laboratory confirmed in (64.26 ± 3,13) % of patients.

Multiple comparisons of sensitization frequency to orange allergens for 3 groups showed a statistically significant difference at a significance level p < 0,001 and was conducted by using the criterion χ-square. While conducting the Maraskulio-Lyah-Guryanov procedure to assess age-specific differences in the distribution of the frequency detection of diagnostically significant levels of specific antibodies of class E (Table 2), a significantly higher sensitization frequency in the group of patients under 5 years of age comparatively to the group of patients of 10 years of age and over (p < 0,001), and in the group of 5 to 10 year old patients in comparison with the group of 10 year-old patients and over (p < 0,002) was revealed. This result confirms the research data obtained in previous studies according to which the food sensitization frequency is significantly higher in childhood and decreases during the first decade of life [4].

The Kruskal–Wallis one-way analysis of variance by ranks test showed the occurrence of significant difference in the 3 treatment groups (p <0,001). In addition, by the Dunn's test criterion, statistically significant differences in the levels of specific IgE to the antigens to orange were found in the groups with patients under 5 years of age and from 5 to 10 years old (p <0,05), from 5 to 10 year-old patients and patients of 10 years of age and over (p <0,05), from 5 to 10 year-old patients and patients of 10 years of age and over (p <0,01) (Table 3).

On the other hand, the significant (p <0.01) direct correlation between the values of total IgE levels and specific IgE-antibodies to orange (tau = 0,305) for the full sample has been

**Table 2**

**Frequency detection of specific IgE antibodies to allergens of orange in individuals of various age groups**

| № | Age groups | Specific weight of children with various levels of IgE antibodies | | | | | |
|---|-----------|---------------------|---|-------------|---|-------|---|
| | | Sensitization absence | | Sensitization | | TOTAL | |
| | | № | % | № | % | № | % |
| 1. | under 5 years | 3 | 7,89+4,37 | 35 | 92,11+4,37 * | 38 | 16,17+2,40 |
| 2. | 5-10 years | 6 | 16,22+6,06 | 31 | 83,78+6,06 ** | 37 | 15,74+2,38 |
| 3. | Over the age of 10 years old | 75 | 46,88+3,95 | 85 | 53,12+3,95 | 160 | 68,09+3,04 |
| 4. | TOTAL | 84 | 35,74+3,13 | 151 | 64,26+3,13 | 235 | 100,00+0,00 |

Note: * – significant differences with group over the age of 10 years old (p < 0,001); ** – significant differences with group over the age of 10 years old (p < 0,002).

| | | | | | |
|---|---|---|---|---|---|
| | | | | | *Table 3* |
| The levels of specific IgE to the antigens of orange in individuals of various age groups | | | | | |
| № | Age groups | number № | median | median error | 95% CI |
| 1. | under 5 years old | 38 | 0,26*,** | 0,04 | 0,24; 0,31 |
| 2. | 5-10 years old | 37 | 0,21*** | 0,08 | 0,18; 0,25 |
| 3. | Over the age of 10 years old | 160 | 0,37 | 0,05 | 0,33; 0,39 |
| 4. | TOTAL | 235 | 0,32 | 0,04 | 0,30; 0,34 |

Note: CI – confidence interval; * – significant differences with group over the age of 10 years old (p<0,05); ** – significant differences with group over the age of 10 years old (p<0,05); *** – significant differences with group over the age of 10 years old (p<0,01).

found. In the groups of patients of from 5 to 10 years old and from 10 years old and over that correlation turned out to be observed (tau = 0,260 with p = 0.04 and tau = 0,347 with p < 0.01 respectively) while in the group of patients under 5 years of age it was not revealed.

It is noteworthy that in the present study the orange antigen - Cit s 1 was isolated by the affine chromatography method. It is common to differentiate between three major orange allergens currently. They include Cit s 1 – herminoid protein, Cit s 2 – profilin [3], and Cit s 3 – pan allergens member of the family of protein - carrier lipids [4]. Cit s 1 is a glycoprotein with a molecular weight of 24 kDa.

It should be noted that the allergen can be represented by different isoforms with molecular weight of 20 to 120 kDa [6]. According to quite a few clinical studies data sensitization to Cit s 1 was detected in 78 % of patients with confirmed allergy to orange [1]. The biological activity of the allergen in the fruit has not been sufficiently investigated yet, but it is confirmed that Cit s 1 is one of the major allergens of orange. It shows high reactivity *in vitro* by glycans forming the main IgE-epitopes [4]. Thereby, the discovery of this allergen offers new prospects for the development of modern national test systems for the diagnostics of specific sensitization to this molecular allergen.

### Conclusions

1. Thus we have found that the frequency of specific allergy to orange among individuals with allergic background is quite high and amounts to (64.26 ± 3.13) %.

2. Analysis of the age distribution of specific IgE-antibodies levels to orange revealed the presence of significantly higher sensitization frequency in the group of patients under 5 years of age as compared to the group of 10 year-old patients and over (p < 0,001), as well as in the group of patients from 5 to 10 years of age as compared to the group of 10 year-old patients and over (p < 0,002). A statistically significant difference of specific levels of IgE-antibodies was found in each of the three age groups.

3. It was found that there is a direct significant correlation between the values of total IgE levels and specific IgE - antibodies to orange (tau = 0,305, with p < 0.01) that was revealed in the groups of patients from 5 to 10 years of age (tau = 0,260 with p = 0.04) and in the group of 10-year-old patients and over (tau = 0,347 with p < 0.01), but in the age group under 5 years old it was not available.

4. The data obtained from the study confirm the purpose and the potential use of specific sensitization to orange allergens of the first national test of the fourth generation systems for diagnostics both in public health and in further research.

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### ДІАГНОСТИКА СПЕЦИФІЧНИХ IgE-АНТИТІЛ ДО АПЕЛЬСИНУ У ОСІБ РІЗНОГО ВІКУ

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**Резюме**

**Вступ.** *Доведено, що близько 20 % жителів Європи страждають на різноманітні алергічні захворювання. Апельсини є одними з найбільш вживаних фруктів та входять до раціону харчування в усьому світі та нашій державі впродовж цілого року. Апельсини вважаються одними з найбільш алергенних продуктів харчування рослинного походження. Одним з головних алергенів апельсину є Cit s 1.*

**Метою** *цього дослідження є опис частоти сенсибілізації до алергенів апельсину у різних вікових групах з визначенням рівнів IgE-антитіл до вищеназваних алергенів за допомогою перших вітчизняних імуноферментних тест-системи четвертого покоління.*

**Матеріали та методи.** *Авторами досліджено сироватки 235 пацієнтів у віці від 7 місяців до 78 років, що мали обтяжений алергічний анамнез. Визначення рівнів загального та специфічного IgE до алергенів апельсину проводилося за допомогою перших вітчизняних імуноферментних тест-системи четвертого покоління («Укрмед-Дон», Донецьк, Україна). Статистична обробка даних була виконана з використанням ліцензійної програми «MedStat» (Донецьк, Україна). Згідно з попереднім аналізом було визначено, що розмір вибірки n = 32 для кожної з груп є достатнім для вияву різниці у частоті випадків*

з 90 % потужністю на 1 % рівні значущості. Для повної вибірки та кожної з трьох досліджуваних груп (1 — 38 осіб у віці від 7 місяців до 5 років; 2 — 37 осіб у віці від 5 років до 10 років; 3 — 160 осіб у віці від 10 років до 78 років) було розраховано медіану, помилку медіани, правий та лівий ліміти 95 % довірчого інтервалу. Множинні порівняння у цих трьох групах проводили з використанням рангового однофакторного аналізу Крускала–Уолліса та критерію Данна. Також проводилися множинні порівняння частоти сенсибілізації у різних вікових групах. Крім того, було проведено кореляційний аналіз, спрямований на виявлення можливого зв'язку між загальним IgE та специфічним IgE у різних вікових групах з використанням коефіцієнту Кендалла (тау, $p \le 0,05$).

**Результати та обговорення.** Діагностично значущі рівні IgE до алергенів апельсину було визначено у $(64,26 \pm 3,13)$ % пацієнтів. Численні порівняння частоти виявили різницю між першою $(92,11 \pm 4,37)$ і третьою $(53,12 \pm 3,95)$ групами на рівні значущості $p < 0,001$ та між другою $(83,78 \pm 6,06)$ і третьою $(53,12 \pm 3,95)$ групами на рівні значущості $p < 0,002$. Ранговий однофакторний аналіз Крускала–Уолліса виявив наявність достовірної різниці у рівнях специфічного IgE ($p < 0,001$). Критерій Данна також показав достовірну різницю між першою та другою групами ($p < 0,05$), між першою та третьою групами ($p < 0,05$) та між другою та третьою групами ($p < 0,01$).

Достовірний прямий кореляційний зв'язок між загальним IgE та специфічним IgE до антигенів апельсину було знайдено у загальній вибірці (тау $= 0,305$, $p < 0,01$), другій (тау $= 0,260$, $p = 0,04$) та третій віковій групах (тау $= 0,347$, $p < 0,01$). На додаток у віковій групі від 7 місяців до 5 років такий зв'язок був відсутнім.

**Висновки.** Використання для діагностики специфічної сенсибілізації до алергенів апельсину перших вітчизняних тест-систем четвертого покоління є доцільним та перспективним. Отримані результати можуть бути використані як у медичній практиці, так і в науковій роботі з метою вдосконалення діагностики серед пацієнтів, сенсибілізованих до апельсину.

**Ключові слова:** специфічний IgE, алергія, апельсин.

## DIAGNOSTICS OF SPECIFIC IgE-ANTIBODIES TO ORANGES IN INDIVIDUALS OF VARIOUS AGES

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**Summary**

**Introduction.** *It is known that above 20 % of Europeans suffer from various allergies. Oranges as one of the most widely consumed fruit being included in the diet year round both worldwide and in our country. Orange is registered as one of the most allergenic products of plant origin. One of the major orange allergen is Cit s 1.*

**The purpose** *of this research is to describe the frequency of sensitization to orange allergens in the different age groups using the first national ELISA test systems of the fourth generation for the detection of IgE-antibodies to the above mentioned allergens.*

**Material and methods.** *We investigated the serum of 235 patients aged from 7 months to 78 years with allergic background. The level detection of total and specific IgE to the orange allergens was carried out using the first national ELISA test systems of the fourth generation («Ukrmed-Don», Donetsk, Ukraine). Statistical analysis was performed by the licensed program «MedStat» (Donetsk, Ukraine). According to the preliminary analysis the sample size being n = 32 in each group was sufficient to detect the difference of occurrence frequency at a power of 90 %, for significance level of 1 %. Both for the total sample of specific IgE and for the three different age groups (the first group — 38 persons 7 months to 5 years old; the second group — 37 persons 5 to 10 years old; the third group — 160 persons 10 to 78 years old), the median, the median error, left and right limits of confidence interval of 95 % were calculated. Numerous data comparisons for those three samples were performed due to the one-way analysis of the Kruskal-Wallis one-way analysis of variance by ranks and the Dunn's test. Multiple comparisons of occurrence frequency of sensitization were performed.*

*The correlation analysis aimed at revealing possible links between total IgE and specific IgE in the different age groups using the Kendall's rank correlation coefficient (tau, p < 0,05) was carried out.*

**Results and discussion.** *Diagnostic relevant levels of IgE to the orange allergens were detected in (64,26 ± 3,13) % of patients. Multiple comparisons of occurrence frequency revealed the differences between the first group (92,11 ± 4,37) and the third (53,12 ± 3,95) age group at significance level p < 0,001 and between the second (83,78 ± 6,06) and the third (53,12 ± 3,95) age group at significance level p < 0,002. The Kruskal-Wallis one-way analysis of variance by ranks test revealed the significance differences of specific IgE levels (p < 0,001). The Dunn's test also showed significance differences in the first and the second age groups (p < 0,05), in the first and the third age groups (p < 0,05) and in the second and third age groups (p < 0,01). The significant direct correlation link between total IgE-antibodies and specific IgE-antibodies to the orange antigens was found in the total sample (tau = 0,305, p < 0,01), in the second (tau = 0,260, p = 0,04) and the third age groups (tau = 0,347, p < 0,01). The significant correlation link in the first age group (7 months to 5 years old) was not found.*

**Conclusions.** *The diagnostic benefit of specific sensitization to orange allergens of the first national test-systems of the fourth generation is reasonable and promising. The obtained data may be used both in medical practice and in further research for diagnostic improvement among sensitized patients to orange allergens.*

**Key words:** *specific IgE, allergy, orange.*