PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ESTUDO COMPARATIVO DA PREVALÊNCIA DA REATIVIDADE DE IGE A ALÉRGENOS RECOMBINANTES

COMPARATIVE STUDY OF THE PREVALENCE OF IGE REACTIVITY TO RECOMBINANT ALLERGENS ON THE BACKGROUND OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ РАСПРОСТРАНЕННОСТИ IGE РЕАКТИВНОСТИ К РЕКОМБИНАНТНЫМ АЛЛЕРГЕНАМ

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Received 12 April 2020; received in revised form 10 June 2020; accepted 14 June 2020

RESUMO

As doenças alérgicas são um dos problemas mais prementes da medicina prática. Segundo estatísticas da Organização Mundial da Saúde, cerca de 40% da população mundial sofre de alergias. Atualmente, o diagnóstico de alergia molecular (AM) é o método mais útil para selecionar os pacientes para prescrever imunoterapia com o objetivo de tratar alergias (imunoterapia com alérgenos), determinar a reatividade cruzada e a gravidade da reação associada a vários alérgenos. O diagnóstico de alergia molecular tem vantagens no diagnóstico de pacientes com sintomas alérgicos – asma, febre do feno, eczema, urticária, sintomas gastrointestinais, síndrome alérgica oral ou anafilaxia. Baseia-se na escolha pelo médico de alérgenos individuais ou no uso de microchips e oferece uma grande quantidade de informações relacionadas ao perfil de IgE de pacientes sensíveis a alérgenos. O método mais eficaz de tratamento de doenças alérgicas é a imunoterapia com alérgenos, que afeta todas as partes patogenéticas do processo alérgico e tem um longo efeito preventivo após a conclusão dos cursos de tratamento. O objetivo do artigo é estudar a prevalência de reatividade de IgE a importantes alérgenos recombinantes no contexto de tipos de imunoterapia com alérgenos. Durante o trabalho, os pacientes foram examinados pelo método de diagnóstico molecular alérgico. O artigo apresenta os resultados do estudo comparativo da presença de IgE específica no soro de pacientes com sensibilidade confirmada aos principais alérgenos recombinantes: absinto Art vl, Art v3, Phleum Phl p1, Phl p5 e bétula Bet v1 por um período de três anos. Os dados obtidos confirmam a alta eficácia da imunoterapia com alérgenos e seu efeito positivo na diminuição da sensibilidade em pacientes com febre do feno em comparação com os resultados do grupo de imunoterapia parenteral.

Palavras-chave: *febre do feno, diagnóstico de alergia molecular, principais alérgenos recombinantes, imunoterapia com alérgenos.*

ABSTRACT

Allergic diseases are one of the most urgent problems of practical medicine. According to statistics of the World Health Organization, about 40% of the world's population suffers from allergies. Molecular allergy diagnostics (MA) is currently the most useful patient selection method for prescribing allergen-specific immunotherapy (ASIT), determining cross-reactivity, and the severity of the reaction associated with various allergens. Molecular allergic diagnosis has advantages for the diagnosis of patients with allergic symptoms – asthma, pollinosis, eczema, hives, gastrointestinal symptoms, oral allergic syndrome, or anaphylaxis. It is based on the choice of individual allergens by a doctor or the use of microarrays and offers a large amount of information related to the IgE profile of sensitized patients. The most effective method of treatment for allergic diseases is allergen-specific immunotherapy, which affects all pathogenetic links of the allergic process and has a long-term preventive effect after the completion of treatment courses. The aim of the article is to study the prevalence of IgE

DOI: 10.52571/PTQ.v17.n35.2020.52_SALTABAYEVA_pgs_609_620.pdf

reactivity to major recombinant allergens on the background of the types of allergen-specific immunotherapy. In the course of the work, patients were examined by allergic molecular diagnostics. The article shows the results of a comparative study of the presence of specific IgE in the serum of patients with confirmed sensitization to major recombinant allergens: wormwood Art vl, Art v3, timothy Phl p1, Phl p5 and birch Bet v1 for a three-year period. The data obtained confirm the high efficiency of sublingual immunotherapy (SLIT) and its positive effect on the reduction of sensitization in patients with pollinosis compared with the results of the parenteral immunotherapy group (sublingual immunotherapy).

Keywords: *pollinosis, molecular allergodiagnostics, major recombinant allergens, allergen-specific immunotherapy.*

АННОТАЦИЯ

Аллергические заболевания на сегодняшний день являются одной из наиболее актуальных проблем практической медицины. Согласно статистическим данным Всемирной организации здравоохранения, аллергией страдают около 40% населения нашей планеты. На сегодняшний день молекулярная аллергодиагностика (МА) является наиболее полезным методом отбора пациентов для назначения аллерген-специфической иммунотерапии (АСИТ), определения перекрестной реактивности и тяжести реакции, ассоциированной с различными аллергенами. Полисенсибилизированные пациенты с неясными симптомами, типом сенсибилизации, или у кого нет ответа на лечение, могут быть диагностированы в стандартной лаборатории при доступности в ней МА. Молекулярная аллергодиагностика обладает преимуществами для диагностики пациентов с аллергическими симптомами – астмой, поллинозом, экземой, крапивницей, желудочно-кишечными симптомами, оральным аллергическим синдромом или анафилаксией. Определение истинной сенсибилизации так же важно, как и выявление вторичной сенсибилизации под действием перекрестно реагирующих аллергенов. Молекулярная аллергодиагностика, основанная на выборе врачом отдельных аллергенов или использовании микроматриц, предлагает большой объем информации, относящейся к IgE-профилю сенсибилизированных пациентов. Наиболее эффективным методом лечения при аллергических заболеваниях является аллерген-специфическая иммунотерапия, воздействующая на все патогенетические звенья аллергического процесса и обладающая длительным профилактическим эффектом после завершения лечебных курсов. Целью исследования явилось изучение наличие специфических IgE в сыворотке крови пациентов с подтвержденной сенсибилизацией к рекомбинантным мажорным аллергенам на фоне аллерген-специфической иммунотерапии. В статье изображены результаты сравнительного изучения наличия специфических IgE в сыворотке крови пациентов с подтвержденной сенсибилизацией к рекомбинантным мажорным аллергенам: полыни Art vl, Art v3, тимофеевки Phl p1, Phl p5 и березы Bet v1 в течение трехлетнего периода. Полученные данные подтверждают высокую эффективность сублингвальной аллерген-специфической иммунотерапии (СЛИТ) и ее положительное влияние на снижение сенсибилизации у больных поллинозом в сравнении с результатами группы парентеральной аллерген-специфической иммунотерапии (ПИТ).

Ключевые слова: *поллиноз, молекулярная аллергодиагностика, рекомбинантные мажорные аллергены, аллерген-специфическая иммунотерапия.*

1. INTRODUCTION

Allergic diseases are one of the most urgent problems of practical medicine. This is due to the high prevalence and the continuous increase in the number of diseases, as well as the increase in severe clinical manifestations, which often cause a deterioration in the quality of life and disability of patients (Canonica *et al*., 2013a; Perkin and Genuneit, 2017; Brozek and Cuello-Garcia, 2016; Namazova-Baranova, 2011; Morenko, 2010). According to statistics of the World Health Organization, about 40% of the world's population suffers from allergies. The first place are respiratory allergies, about 12-45% of them, specifically pollinosis (Ilyina and Pavlova, 2016; Bousquet *et al*., 2016; FitzGerald *et al.*, 2014; Papadopoulos *et al*., 2012).

A feature of allergic diseases in the Republic of Kazakhstan is that the main allergenic plants are wormwood and other weeds, the intensity of sensitization is about one million times greater than that observed in Central Europe and the European part of the Russian Federation (Kurbacheva *et al*., 2013; Maslova, 2012; Calderon *et al*., 2011; Gorbas *et al*., 2015; Zhumambayeva *et al*., 2014). The main reason of the increase in the incidence of pollen and the prevalence of its medium and heavy forms is the leadership of symptomatic pharmacotherapy over pathogenetic allergen-specific immunotherapy

(ASIT) The most effective method of treatment for allergic diseases is allergen-specific immunotherapy, which affects all pathogenetic links of the allergic process and has a long-term preventive effect after the completion of treatment courses (Saltabaeva and Morenko, 2015; Durham, 2006; Canonica *et al*., 2014; Zhuravleva *et al*., 2019; Valovirta, and Berstad, 2011). If parenteral methods developed in 1911 have already received general acceptance worldwide, then oral methods for decades have been discredited by using untreated vaccines. Such vaccines have not been depolymerized and depigmented (Calderon *et al*., 2012; Abdrakhmanova *et al*., 2019).

Only in the last two decades, these methods began to be widely used first in European countries, later in North and Central America, and in the last years methods are already used in Kazakhstan. A comparative study of the efficacy of oral and parenteral allergen-specific immunotherapy (ASIT) has not been adequately studied, mainly in European countries (Cheryl *et al*., 2013, Douladiris, 2013; Kudabayeva *et al*., 2014; Kudabayeva *et al*., 2017; Valenta *et al*., 2014). In our republic, similar results of the study were not previously published. The region of Northern Kazakhstan, where the capital Nur-Sultan is located, is characterized by heterogeneous climatic conditions due to the long extent, heterogeneous surface structure, and characteristic landscapes. The specificity of pollination in Nur-Sultan is special in relation to a relatively short summer, where there are shifts in time of flowering of trees, weeds, and meadow grasses, which complicates the establishment of true sensitization to certain allergens. Nur-Sultan is located in a spacious steppe and is exposed to a wind rose, with a strong pollen direction from the southern regions and the influence of several different pollen sources of allergens at the same time, which lead to cross-allergy (The climate of Astana, 2019). To determine the true sensitization in cross-reactions in polysensitized patients when traditional diagnostic tests and case history data are insufficient to determine significant allergens for the correct selection of therapy, prognosis, and effectiveness, molecular allergy diagnostics with recombinant allergens is used (Becker *et al*., 2016; Canonica *et al*., 2013b; Ramilyeva *et al*., 2019; Burkitbaiev *et al*., 2017; Fooke-Achterrath *et al*., 2012; Spergel, 2010; Valenta *et al*., 2011).

The aim of the article is to study the prevalence of IgE reactivity to major recombinant allergens on the background of the types of allergen-specific immunotherapy.

2. MATERIALS AND METHODS

Molecular allergy diagnostics (MA) is currently the most useful patient selection method for prescribing allergen-specific immunotherapy (ASIT), determining cross-reactivity, and the severity of the reaction associated with various allergens. Polysensitized patients with unclear symptoms, type of sensitization, or who have no response to treatment can be diagnosed in a standard laboratory with the availability of MA in it. Molecular allergic diagnosis has advantages for the diagnosis of patients with allergic symptoms – asthma, pollinosis, eczema, hives, gastrointestinal symptoms, oral allergic syndrome, or anaphylaxis. Determining true sensitization is just as important as identifying secondary sensitization by cross-reacting allergens. Molecular allergy diagnosis, based on the choice of individual allergens by a doctor or the use of microarrays, offers a large amount of information related to the IgE profile of sensitized patients (Valenta *et al*., 1991; Smiyan *et al*., 2015; Cromwell *et al*., 2011; Jutel *et al*., 2005; Saltabaeva and Morenko, 2017).

The study focuses on the presence of specific IgE in the serum of patients with confirmed sensitization (by Molecular allergy diagnostics) to pollen from wormwood to recombinant major recombinant allergens: wormwood Art vl, Art v3, timothy Phl p1, Phl p5 and birch Bet v1 for a three-year period (Tables 1, 2, and 3). Surveys were carried out in the medical and health center “Umit” (Nur-Sultan, Republic of Kazakhstan). The study involved 228 patients with varying degrees of severity of hay fever, among whom were children from 5 to 18 years old and an adult population (113 patients were males, 115 were females). The average age was 23.5 ± 0.9 years, and the minimum age was 5 five years, t, the maximum was 60 years. The studied respondents were randomized into two groups: group 1 included 126 (55.3%) patients who took sublingual immunotherapy (SLIT) (Durham, 2006), group 2 included 102 (44.7%) patients who received parenteral immunotherapy (PIT) (Kurbacheva *et al*., 2013).

In the course of the work, 153 (67.1%) patients from the 1st group (SLIT) 95 (75.4%), from the 2nd group (PIT) 58 (56.9%) patients with pollinosis were examined by allergic molecular diagnostics and having positive skin scratch tests for native allergens with polysensitivity to mixes of weeds, meadow grasses, and trees (Douladiris, 2013).

Non-parametric methods of research,

Wilcoxon criterion, Mann-Whitney criterion, Friedman criterion, mean value, standard deviation, median, 25th, and 75th percentile were used to evaluate the parameters (Saltabayeva *et al*., 2016a; Saltabayeva *et al*., 2016b).

3. RESULTS AND DISCUSSION:

The enzyme immunosorbent assay using major recombinant allergens Art vl, Art v3, Phl p1, Phl p5, and Bet v1 was diagnosed with sensitization to these allergens as follows: Art v 1 – in 26 (17.0%), Art v3 – in 9 (5.9%), Art v1-Art v3 – in 73 (47.7%), Art v1.3-Bet v1 – in 20 (13.1%), Art v1.3-Phl p1.5 – in 16 (10.5%), Art v1,3-Bet v1-Phl p1.5 – in 9 (5.9%) (Figure 1).

Among inhaled pollen allergens, the highest degree of sensitization was determined to Art v1-Art v3. After a course of specific immunotherapy, the total number of patients with significant sIgE decreased 1.4 times. The results obtained correlate with known scientific data. In a number of works (Kulis, 2011; Radauer *et al*., 2010) a high level of sensitization to recombinant wormwood allergens is noted in various populations of the European part of Russia, Kyrgyzstan, including wormwood, which coincides with our data and confirms the presence of a large number of IgE epitopes in Art v1-Art v3 to weed pollen allergens.

At the same time, antibodies to the main allergen of the monopole Art v1-Art v3 are found in 73 (47.7%) patients with seasonal allergic rhinitis. The identification of high levels of Art V1-Art v3 major antibodies in patients was important for developing criteria for the selection of therapy and evaluating the effectiveness of ASIT. Most scientific sources confirm this data, which describes nine different allergens isolated from wormwood pollen (Artemisia vulgaris), including two major allergens (Art vl and Art v3), which are often responsible for cross-allergy (Eigenmann, 1998). The complete structure of all known wormwood allergens has recently been deciphered (Guideline on the clinical development…, 2008). The main native wormwood allergen Art vl is a highly glycosylated glycoprotein with a molecular weight of 24 to 28 kDa, has an unusual tertiary structure and the end portion of the molecule, the so-called “head and tail”, which explains its extraordinary biochemical properties (Incorvaia and Fuiano, 2013).

Different groups studied, sensitization to allergens from Art vl wormwood, Art v3, Timothy Phl p1, Phl p5, and Bet v1 birch had a similar distribution of the trait in most patients; no statistically significant difference was found between these groups (p = 0.501). The greatest sensitization was also detected in both groups to the major recombinant allergens of the wormwood Art vl-Art v3.

Also, when evaluating the results obtained by serological examination of patients' blood, we identified a statistically significant difference between the relative concentrations of major recombinant allergens of wormwood, ambrosia, timothy, and birch p <0.001 before and after sublingual immunotherapy. The distribution of signs was different from normal.

In the studied patients of group 1 (SLIT), the frequency of positive sensitization results to various recombinant major allergens Art vl, Art v3, Phl p1, Phl p5 and Bet v1 changed as follows: Art v 1 – from 17 (17.9%) to 11 (11.2%), Art v3 – from 6 (6.3%) to 4 (3.9%), Art v1-Art v 3 – from 43 (45.3%) to 27 (28.3%), Art v1,3-Bet v1 – from 13 (13.7%) to 8 (8.6%), Art v 1,3-Phl p1.5 – from 11 (11.6%) to 7 (7.2 %), Art v1,3-Bet v1-Phl p1.5 – from 5 (5.3%) to 3 (3.3%) (Figure 2).

For a comparative study of the immunological efficacy of SLIT and PIT, we studied the concentrations of major recombinant allergens before each course of ASIT. In patients of group 1, the level of specific IgE antibodies to the allergens of Art v1 wormwood to SLIT (Me, 25–75%) was 27.1 (11.2–35.8) Ku/l and corresponded to (Me, 25–75%) 3, 4 (3.1-3.9) class, after the 1st course SLIT (Me, 25-75%) was 23.6 (9.8–31.1) Ku/l and corresponded (Me, 25- 75%) 3.4 (3.1-3.9) class, after the 2nd course SLIT (Me, 25-75%) was 18.1 (7.5-23.9) Ku/l and corresponded to (Me, 25-75%) 3.4 (3.1-3.9) class, after the 3rd course SLIT (Me, 25-75%) was 17.4 (7.2-22.9) Ku/l, corresponding to (Me, 25-75%) 3.4 (3.1-3.9) class. When evaluating the effectiveness of SLIT in patients with pollinosis for three courses, the concentration of Art v1 in serum was statistically significantly reduced 1.6 times (Friedman test: x2 = 72.2; p <0.001) (Figure 3).

The level of specific IgE antibodies to wormwood Art v3 allergens to SLIT (Me, 25-75%) was 8.0 (0.4-14.0) ku/l and corresponded to (Me, 25-75%) 1.3 (1, 1-3.9) class, after the 1st course SLIT (Me, 25-75%) was 7.3 (0.4-12.7) ku/l, corresponding to (Me, 25-75%) 1.3 (1.1-3.9) class, after the 2nd course SLIT (Me, 25-75%) was 5.6 (0.3-9.8) ku/l, corresponding to (Me, 25-75%) 1.3 (1.1-3.9) class, after the 3rd course SLIT (Me, 25-75%) was 5.4 (0.3-9.3) ku/l and corresponded (Me, 25-75%) 1.3 (1.1-3.9) class. Thus, the concentration of Art v3 in serum was statistically

significantly reduced by 1.5 times (Friedman test: x2 = 64.1; p <0.001).

The level of specific IgE antibodies to meadow grass allergens Phl p1 to SLIT (Me, 25-75%) was 1.0 (0.0-1.5) ku/l, corresponding to (Me, 25-75%) 0, 2 (0, 1-2.9) class, after the 1st course SLIT (Me, 25-75%) was 0, 9 (0.0-1.2) ku/l and corresponded to (Me, 25-75%) 0 , 2 (0.1-2.9) class, after the 2nd course SLIT (Me, 25-75%) was 0.7 (0.0-1.0) ku/l, corresponding to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 3rd course SLIT (Me, 25-75%) was 0, 6 (0.0-0.9) ku/l and corresponded (Me, 25-75%) 0, 2 (0.1-2.9) class. Thus, the concentration of Phl p1 in serum was statistically significantly reduced 1.6 times (Friedman test: x2 = 42.4; p <0.001) (Figure 4).

The level of specific IgE antibodies to meadow grass allergens Phl p5 to SLIT (Me, 25-75%) was 0, 2 (0.0-1.1) ku/l and corresponded to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 1st course SLIT (Me, 25-75%) was 0.15 (0.0-0.8) ku/l, corresponding to (Me, 25-75%) 0, 2 (0.1-2.9) class, after the 2nd course SLIT (Me, 25-75%) was 0.11 (0.0-0.7) ku/l, corresponding to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 3rd course SLIT (Me, 25-75%) was 0.10 (0.0-0.6) ku/l and corresponded to (Me, 25-75%) 0, 1 (0.1-1.9) class. That is, the concentration of Phl p 5 in serum was statistically significantly reduced by 1.5 times (Friedman test: x2 = 36.0; p <0.001).

The level of specific IgE antibodies to Bet v1 birch allergens to SLIT (Me, 25–75%) was 1.4 (0.2–2.1) ku/l and corresponded to (Me, 25–7%) 0, 2 (0, 1-2.9) class, after the 1st course SLIT (Me, 25-75%) was 1, 2 (0.0-1.9) ku/l, corresponding to (Me, 25-75%) 0, 2 (0.1-2.9) class, after the 2nd course SLIT (Me, 25-75%) was 0, 9 (0.0-1.2) ku/l and corresponded to (Me, 25-75%) 0, 2 (0.1-2.9) class, after the 3rd course SLIT (Me, 25-75%) was 0.8 (0.0-0.9) ku/l, corresponding to (Me, 25-75%) 0.2 (0.1-2.9) class, the concentration of Bet v1 in serum significantly decreased 1.5 times (Friedman test: x2 = 56.1; p <0.001).

After SLIT, in patients of group 1, the content of serum specific IgE antibodies to wormwood allergens Art v1 significantly decreased (Wilcoxon matched pairs test: z = 8.3; p <0.001), to Art v3 (Wilcoxon matched pairs test: z = 7.5; p <0.001), to meadow grass allergens Phl p1 (Wilcoxon matched pairs test: z = 4.1; p <0.001), to Phl p5 (Wilcoxon matched pairs test: z = 3.4; p <0.05), to Bet v1 birch allergens (Wilcoxon matched pairs test: z = 4.5; p <0.001). In the study, the content of recombinant major allergens in the serum of patients with polysensitization showed their statistically significant decrease after SLIT (p <0.001).

As in group 1 and group 2, when evaluating the results obtained by a serological method for examining patients' blood, a statistically significant difference was found between the relative concentrations of recombinant allergens of wormwood, timothy and birch (p <0.001) before and after the ICU, but which was less SLIT. The distribution of signs was also distinguishable from normal. Therefore, non-parametric research methods were used to evaluate the parameters.

In patients of group 2 (PIT), the frequency of positive sensitization results to various recombinant major allergens Art vl, Art v3, Amb a1, Phl p1, Phl p5 and Bet v1 changed as follows: Art v1 – from 9 (15.5%) to 7 (11.9%), Art v3 – from 3 (5.2%) to 2 (4.0%), Art v1-Art v3 – from 30 (51.7%) to 23 (39.8%), Art v1,3-Bet v1 – from 7 (12.1%) to 5 (9.3%), Art v1,3-Phl p1.5 – from 5 (8.6%) to 4 (6.6%), Art v1,3-Bet v1-Phl p1,5 – from 4 (6.9%) to 3 (5.3%) (Figure 5).

In the group of patients who received parenteral immunotherapy, the level of specific IgE antibodies to wormwood Art v1 allergens to PIT (Me, 25-75%) was 32.5 (13.4-42.9) Ku/l and corresponded to (Me, 25-75%) 3.4 (3.1-3.9) class, after the 1st course PIT (Me, 25-75%) was 29.5 (12.2-39.0) Ku/l, corresponding to (Me, 25-75%) 3.4 (3.1-3.9) class, after the 2nd course PIT (Me, 25-75%) was 24.6 (10.2-32.5) Ku/l, corresponding to (Me, 25-75%) 3.4 (3.1-3.9) class, after the 3rd course of PIT (Me, 25-75%) was 23.6 (9.8-31, 2) Ku/l and corresponded to (Me, 25-75%) 3.4 (3.1-3.9) class. When evaluating the effectiveness of PIT in patients with pollinosis during three courses, the concentration of Art v1 in serum significantly decreased 1.4 times (Friedman test: x2 = 64.2; p <0.001).

The level of specific IgE antibodies to wormwood Art v3 allergens before PIT (Me, 25–75%) was 8.8 (0.3–15.4) Ku/l and corresponded to (Me, 25–75%) 3.4 (3 , 1-3.9) class, after the 1st course PIT (Me, 25-75%) was 8.7 (0.3-15.3) Ku/l, corresponding to (Me, 25-75%) 1, 3 (1.1-3.9) class, after the 2nd course of PIT (Me, 25-75%) was 7.2 (0.2-12.7) Ku/l and corresponded (Me, 25-75%) 0.3 (0.1-3.9) class, after the 3rd course of PIT (Me, 25-75%) was 6.8 (0.2-11.9) Ku/l, corresponding to (Me, 25-75%) 0.3 (0.1-3.9) class, the concentration of Art v3 in serum was statistically significantly reduced 1.3 times (Friedman test: x2 = 48.6; p <0.001).

The level of specific IgE antibodies to meadow grass allergens Phl p1 to PIT (Me, 25-

75%) was 1.5 (0.0-2.2) Ku/l and corresponded to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 1st course PIT (Me, 25-75%) was 1.2 (0.0-1.8) Ku/l, corresponding to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 2nd course of PIT (Me, 25-75%) was 0.04 (0.0-0.6) Ku/l and corresponded (Me, 25-75%) 3, 0, 1 (0.1-1.9) class, after the 3rd course SLIT (Me, 25-75%) was 1.1 (0.0-1.6) Ku/l Corresponding to (Me, 25–75%) 0, 2 (0.1–2.9) class, the concentration of Phl p1 in serum significantly decreased 1.3 times (Friedman test: x2 = 29.6; p < 0.001).

The level of specific IgE antibodies to meadow grass allergens Phl p5 to PIT (Me, 25–75%) was 0.17 (0.0– 1.0) Ku/l and corresponded to (Me, 25–75%) 0.2 (0.1-2.9) class, after the 1st course of PIT (Me, 25-75%) was 0.15 (0.0-0.9) Ku/l, corresponding to (Me, 25-75%) 0.2 (0.1-2.9) class, after the 2nd course SLIT (Me, 25-75%) was 0.14 (0.0-0.6) Ku/l, corresponding to (Me, 25 - 75%) 0.1 (0.08-1.9) class, after the 3rd course PIT (Me, 25-75%) was 0.14 (0.0-0.5) Ku/l and corresponded to (Me, 25-75%) 0, 1 (0.1-1.9) class. Thus, the concentration of Phl p5 in serum was statistically significantly reduced by 1.2 times (Friedman test: x2 = 54.0; p <0.001).

The level of specific IgE antibodies to Bet v1 birch allergens before PIT (Me, 25–75%) was 2.3 (0.0–2.9) Ku/l and corresponded to (Me, 25–75%) 0.2 (0, 1-2.9) class, after the 1st course of PIT (Me, 25-75%) was 2.1 (0.0-2.7) Ku/l, corresponding to (Me, 25-75%) 0, 2 (0.1-2.9) class, after the 2nd course PIT (Me, 25-75%) was 1.8 (0.0-2.1) Ku/l, corresponding to (Me, 25- 75%) 0.2 (0.1-2.9) class, after the 3rd course of PIT (Me, 25-75%) was 1.7 (0.0-1.9) Ku/l and corresponded to (Me, 25-75%) 0.2 (0.1-2.9) class. The serum Bet v1 concentration was statistically significantly reduced 1.5 times (Friedman test: x2 = 56.1; p <0.001). After PIT, the content of serum specific IgE antibodies to Art v1 wormwood allergens (Wilcoxon matched-pairs test: z = 8.4; p <0.001) and Art v3 (Wilcoxon matched-pairs test: z = 7.6; p <0.001), to meadow grass allergens Phl p1 (Wilcoxon matched-pairs test: z = 5.1; p <0.01), to Phl p5 (Wilcoxon matched-pairs test: z = 3.8; p <0.05), to birch allergens Bet v1 (Wilcoxon matched-pairs test: z = 5.4; p <0.001).

As it is known, a significant clinical task in allergology for polysensitization and cross-allergy is to make an accurate diagnosis. For diagnostic accuracy and optimization of therapy, in particular immunotherapy, the development of recombinant allergens was an important step in medicine (Nelson, 2016; Pauli *et al*., 2008; Ferreira, 1996; Valenta *et al*., 2010).

Scientists of the Research Institute of Molecular Biology V.A. Engelhard, O. Smoldovskaya, G. Feyzkhanova, and their co-authors (2016) were studied in a multicenter comparative study of patients with hypersensitivity to birch pollen and cat dander using biological microchips, as well as skin scarification samples. As a result of the work, it was established that the diagnostic accuracy with the use of recombinant allergens was higher compared to skin test samples (Smoldovskaya *et al*., 2016). An important conclusion and our study show that among all inhaled allergens, the highest rate relates to wormwood, which significantly decreased after the courses ASIT. Similar data were obtained in domestic surveys in epidemiological studies, where the main cause of seasonal allergy in Kazakhstan was pollen of weeds (Saltabayeva *et al*., 2017).

Therefore, further study of allergic anamnesis and skin scarification tests with other allergens that have common allergenic properties, including an assessment of pollen, timothy and birch pollen reactivity, is needed to study cross-section properties with recombinant allergens, which is necessary to determine a more accurate profile of specific IgE reactivity to recombinant allergens in patients living in the north of Kazakhstan. Continuing research to determine whether the recombinant allergens match the spectrum of allergen-specific antibodies detected in this population will facilitate the use of recombinant allergens for diagnosing, monitoring, and selecting the right treatment with specific immunotherapy for patients with pollinosis in our region with multiple pollen influences.

4. CONCLUSIONS:

Upon completion of the three courses of PIT, a statistically significant (p <0.001) decrease in the concentration of major recombinant allergens in the blood serum was detected, but which was relatively inferior to the SLIT group. The data obtained confirm the high efficiency of SLIT and its positive effect on the reduction of sensitization in patients with pollinosis compared with the results of the parenteral immunotherapy group.

Thus, the presence of a positive response from patients to major allergens of pollen from Art vl wormwood, Art v3, Timothy Phl p1, Phl p5 and Bet v1 birch with cross allergy decreased after three courses of immunotherapy in group 1 (SLIT) 1.5 times and in group 2 (PIT) 1.3 times.

Analysis of the effects of ASIT in patients

with positive *in vitro* results on major recombinant allergens showed that after a three-year course of ASIT in two groups, a decrease in serum concentration of major recombinant allergens was observed, but more significant results were obtained in the group of patients who took the sublingual type of immunotherapy compared with the parenteral immunotherapy group ($p < 0.001$).

5. ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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Table 1. *Frequency of joint positive results in vitro testing of patients for major recombinant allergens*

Recombinant major allergens	Total patient	Number of patients with significant sIgE (> 0.35 kE/l), (p<0.01)		% of the total number of patients (p <0.01)	
		before	after	before	after
Art v 1	153	26	17	17.0	11.1
Art v 3	153	9	5	5.9	3.3
Art v 1 – Art v 3	153	73	47	47.7	30.7
Art v1 – Art v3 – Bet v1	153	20	13	13.1	8.5
Art v1 – Art v3 – Phl p1 – Phl p5	153	16	11	10.5	7.2
Art v1 – Art v3 – Bet v 1 – Phl p1 – Phl p5	153	9	7	5.9	4.6
Total	153	153	100	100.0	65.4

Table 2. *Frequency of joint positive results in vitro testing of patients of group 1 (SLIT) for recombinant allergens*

Recombinant major allergens	Total patient	Number of patients with significant sIgE (> 0.35 kE/l), (p<0.01)		% of the total number of patients (p <0.01)	
		before	after	before	after
Art v 1	95	17	10	17.9	10.5
Art v 3	95	6	3	6.3	3.2
Art v 1 – Art v 3	95	43	26	45.3	27.4
Art v1 – Art v3 – Bet v1	95	13	8	13.7	8.4
Art v1 – Art v3 – Phl p1 – Phl p5	95	11	8	11.6	8.4
Art v1 – Art v3 – Bet v 1 – Phl p1 – Phl p5	95	5	4	5.3	4.2
Total	95	95	59	100.0	62.1

Table 3. *Frequency of joint positive results in vitro testing of patients of group 2 (PIT) for recombinant allergens*

Recombinant major allergens	Total patient	Number of patients with significant sIgE (> 0.35 kE/l), (p<0.01)		% of the total number of patients (p <0.01)	
		before	after	before	after
Art v 1	58	9	7	15.5	12.1
Art v 3	58	3	2	5.2	3.4
Art v 1 – Art v 3	58	30	22	51.7	37.9
Art v1 – Art v3 – Bet v1	58	7	5	12.1	8.6
Art v1 – Art v3 – Phl p1 – Phl p5	58	5	3	8.6	5.2
Art v1 – Art v3 – Bet v 1 – Phl p1 – Phl p5	58	4	3	6.9	5.2
Total	58	58	42	100.0	72.4

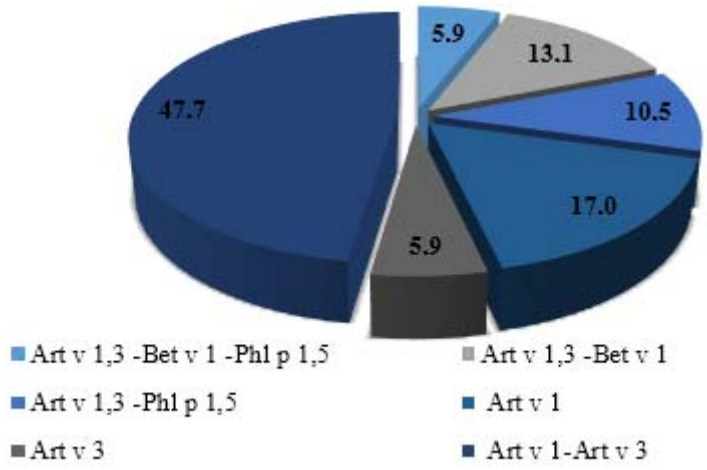

Figure 1. *The degree of sensitization to various recombinant allergens Art vl, Art v3, Amb a1, Phlp p1, Phl 5 and Bet v1*

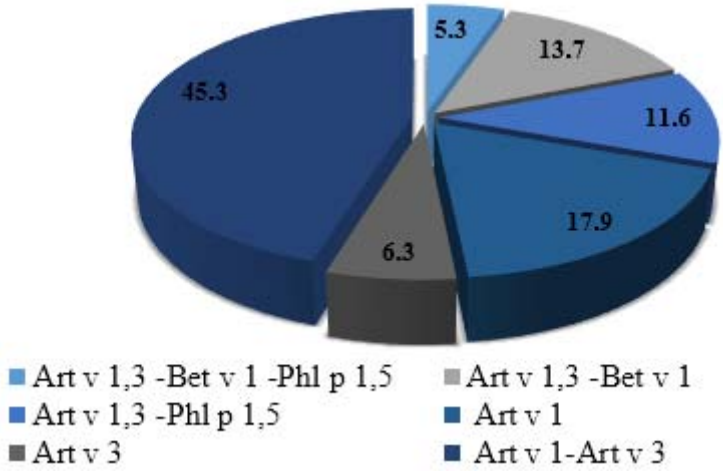

Figure 2. *The degree of sensitization of group 1 (SLIT) to various recombinant major allergens Art vl, Art v3, Phl p1, Phl 5 and Bet v1*

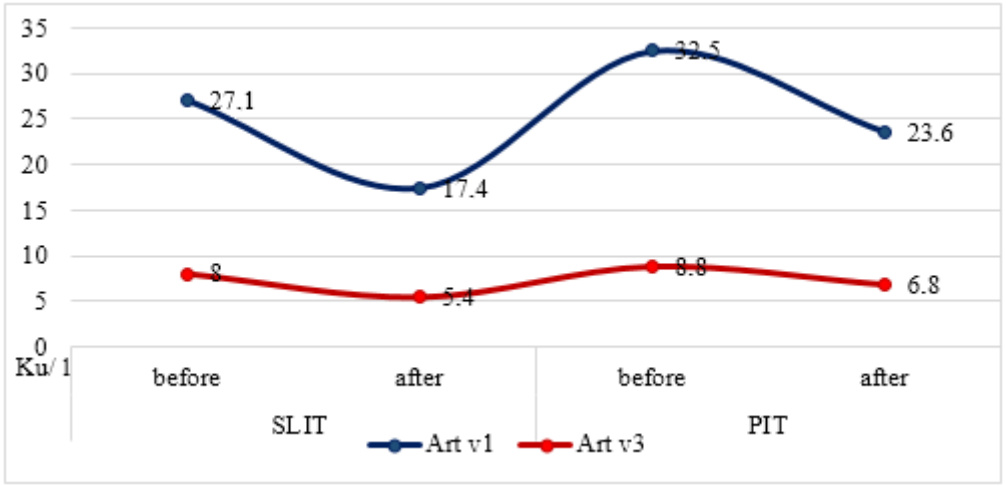

Figure 3. *Comparative evaluation of recombinant major allergens Art vl, Art v3 against the background of SLIT and PIT*

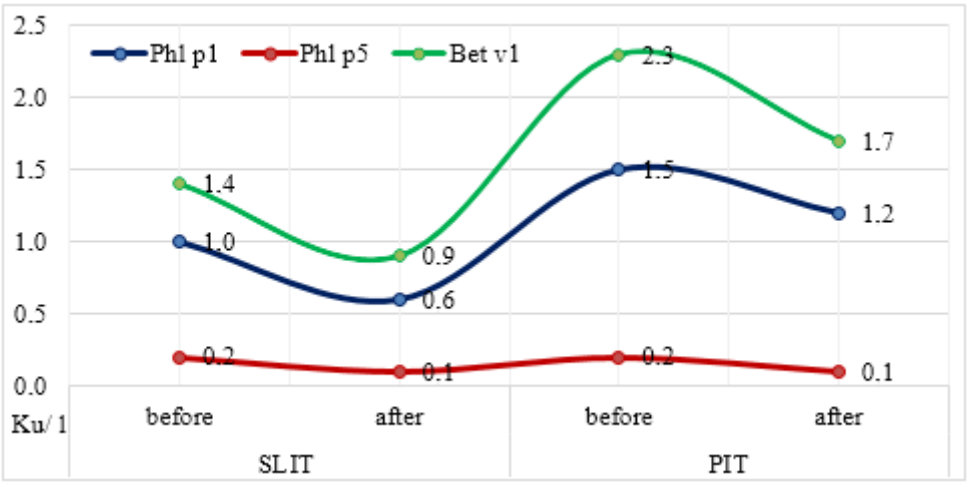

Figure 4. *Comparative evaluation of recombinant major allergens Phl p1, Phl 5, Bet v1 on the background of SLIT and PIT*

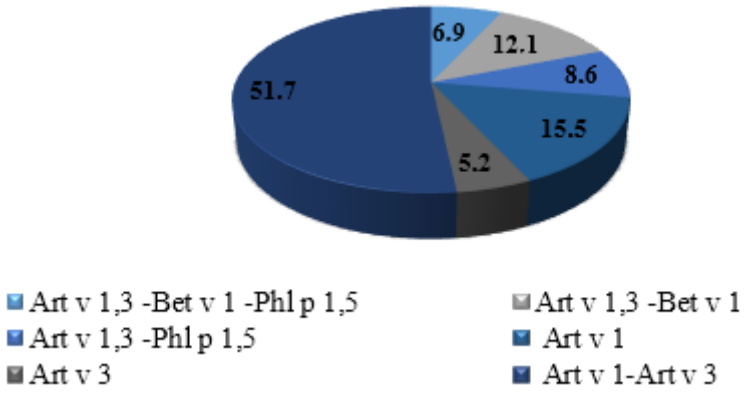

Figure 5. *The degree of sensitization of patients of group 2 (PIT) to various recombinant major allergens*