The level of tlr-2 and tlr-9 genes expression at polysensitization.

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Abstract: The study of innate immunity at allergies is vital; however, there are not enough data on the role innate immunity in polysensitization or drug hypersensitivity. Five groups of patients with different nature of the hypersensitivity were studied: a group with a proved hypersensitivity to one drug (n =49); a group with multiple drug hypersensitivity (n=18); a group with a non-drug hypersensitivity (n=37); a group with multiple non-drug hypersensitivity (n=53) and the control group with no any signs of drug and no-drug hypersensitivity (n = 39). The level of TLR-2 and TLR -9 gene expressions were assessed in peripheral blood mononuclears by relative quantification PCR compared with Beta-actin gene as a reference. As a result, expression of the TLR-9 gene was down-regulated in the group with multiple drug hypersensitivity. There were no significant differences in TLR2 and TLR9 gene between other groups.
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1. Introduction

Much has been expected from the results of the investigation of innate immunity system in the development of allergic and autoimmune diseases. A number of researchers find correlation between TLR-receptors and the hygiene hypothesis allergy [1]. The link between TLR gene polymorphisms and the development of allergy is of interest for researchers [2-4], drugs, modeling TLR activity, are developed to treat allergy [5-8]. So far, 13 human genes encoding TLR receptors have been identified [9-10]. At first, TLR were thought to recognize only molecular pattern of microorganisms, but recent data have shown that they can respond to various allergic agents [11, 12].

The purpose of this investigation was to study TLR-2 and TLR-9 gene expression in the peripheral blood mononuclears in patients with multiple or mono drug and non-drug sensitization.

2. Material and Methods

2.1 Patients

Five groups have been formed including a control group of 39 subjects (29 women and 10 men) who did not have any complaints of allergy or hypersensitivity to any allergy agent. The criterion of subject selection to the control group was the general IgE contents of less than 100 mME/l. The average age of the subjects was 39.0±4.6 years.

The second group of patients with allergy, comprising 49 people of whom 35 were females and 14 males, was made of those who had classical allergic symptoms (such as Quincke's oedema, urticaria, maculopapular exanthems) to only one allergen. The patients of this group were with proved sensitivity (increased level of serum-specific IgE and skin positive tests) to the pollen allergen (in 50% cases), to domestic allergen (in 20% cases) and to food allergen (in 10% cases). The average age of the subjects was 41.2±6.7 years.

The third group of 53 subjects had typical clinical signs of allergy to 3 or more allergens. This group included the patients who revealed food sensitivity (76%), reactions to domestic allergens (60%), those to pollen (26%) and to animal allergens (16.6%). The polysensitivity was proved by positive skin tests and increased level of serum-specific IgE. The most common reactions were allergic rhinitis, conjunctivitis, urticaria and Quincke's oedema. The average age of the patients was 42.3±6.4 years.

Fourth group with drug allergy (hypersensitivity) comprised 34 females and 3 males. These patients reacted to 1 drug: 25% showed reactions to antibiotics, 28% reacted to nonsteroidal antiinflammatory drugs (NSAIDs), 8 % - to vitamins, 15% reacted to local anesthetics and 24%, to other drugs. The drug hypersensitivity was proved by skin tests and/or patient hystory. The patients did not complain of hypersensitivity or sensitivity to any allergen other than drugs. The average age of the patients was 47.4±6.8 years.

The last group with multiple drug hypersensitivity comprised 18 patients, all being women, who had records of multiple hypersensitivity reactions to drugs (the so called Multiple drug hypersensitivity); 76% of the subjects showed reactions to antibiotics, 29% - to NSAIDs, 17% - to vitamins and 17.4% - to anesthetics. The sensitivity was proved through skin tests, provocation tests

and/or patient hystory. The average age of the patients was 45.3±8.1.

2.2 StudyTLRs

Blood sampling was performed in the morning using the EDTA-tube. Then, mononuclear suspension was separated using density gradient. After the separation, the cellular mass was washed and its quantitative composition was determined, which was followed by making up cell concentration to 1000 cell per milliliter.

Total RNA was extracted using a Pure Link RNA Mini Kit (Ambion). The quality and quantity of obtained RNA was estimated using IMPLEN nanophotometer P330. The reverse transcription reaction was performed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The level of TLR-2 and TLR-9 gene expression was determined with reverse transcription polymerase chain reaction (RT-PCR) made by ACGT LAB (Astana, Kazakhstan). The beta-actin gene was chosen as a reference.

Statistical analysis was performed using software qBase+ (Biogazelle 2008-2013, Version: 2.5.1, Belgium). A value of $p \leq 0.05$ has been considered as a statistically significant.

3. Results

The total 196 blood samples of patients from five groups with different types of hypersensitivity were collected from 2012 to 2013. The mononuclear cells were separated from samples where the levels of TLR-2 and TLR-9 gene expression were studied. The results of TLR-2 and TLR-9 gene expression in peripheral blood mononuclears for monosensitivity and polysensitivity are presented in Table 1 and Table 2.

Table 1: TLR-2 gene expression in peripheral blood mononuclears for monosensitivity and polysensitivity groups.

	Control group	Mono-sensitivity	Poly-sensitivity
Average relative level of expression	0.744	0.656	1.913
CI ±95%	0.355±1.5	0.288±1.4	0.696±5.6
p-level	0.172		

The study did not show any significant difference between the groups.

Table 2: TLR-9 gene expression in peripheral blood mononuclears for monosensitivity and polysensitivity groups

	Control group	monosensitivity	polysensitivity
Average relative level of expression	0.778	0.690	.3
Confidence interval ±95%	0.420-1.87	0.442-2.8	0.397-4.98
p-level	0.106		

The study did not show any significant difference between the groups.

The results of TLR-2 and TLR-9 gene expression in peripheral blood mononuclears for drug monosensitivity and multiple drug hypersensitivity are presented in Table 3 and Table 4.

Table 3: TLR-2 gene expression in peripheral blood mononuclears for drug hypersensitivity groups

	Control group	Drug hypersensitivity	Multiple drug hypersensitivity
Average relative level of expression	0.740	1.316	0.109
Confidence interval ±95%	0.358-1.56	0.542-3.1	0.10-4.96
p-level	0.140		

The study did not show any significant difference between the groups.

Table 4: TLR-9 gene expression in peripheral blood mononuclears for drug hypersensitivity and multiple drug hypersensitivity groups.

	Control group	Drug hypersensitivity	Multiple drug hypersensitivity
Average relative level of expression	0.778	1.27	**0.042**
Confidence interval ±95%	0.302-2.0	0.558-2.91	0.01-1.82
p-level	0.029		

Interestingly, the analysis of gene expression in the case with drug polysensitivity has revealed the significant difference in the level of expression TLR9 gene ($p = 0,042$). This gene was down-regulated in the group with multi-drug in comparison with control and mono-drug allergy (figure 1).

At the same time, there was no significant difference ($p>0.05$) in the level of TLR-2 gene expression in mononuclear cells for groups with polysensitization to drugs.

4. Discussions

The changes in topography, altitude, It has been found in our research that of all the groups studied only one showed a significant difference in TLR expression, that of Multiple drug hypersensitivity, which presented a significant decrease of the level of TLR-9 gene expression . Though the importance of TLRs in the immune response has been proved by a number of experiments, the role of the innate immunity in drug hypersensitivity and multiple drug hypersensitivity has not been studied.

Basing on the fact that there is a link between cell TLR gene polymorphisms and the development of various phenotypes of asthma and dermatitis [13-15], the level of TLR gene expression, as a one form of epigenetic regulation, might play the important role in the cases with a Multiple drug hypersensitivity. This issue is required to investigate further.

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