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Role of Autoantibody in the Pathogenesis of Patients with Atopic Bronchial Asthma

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ABSTRACT

Bronchial Asthma is considered as the most spreading human chronic diseases. The diagnostic of the disease at its beginning is very difficult because the light forms of the disease can't be diagnosed as the symptoms are not very well developed at the outbreak of the disease. The objective of this study was to correlate the climatic and geographic factors and the environmental conditions in the occurrence of Atopic Bronchial Asthma and other autoimmune phenomena, for example the prevalence of abzymes in the pathogenesis of Atopic Bronchial Asthma. In the present work, enzyme linked to immune sorbent assay method and the methods of electrophoresis in agarose gel were used. The results of our study showed the discovery of an excessive auto-antibodies to DNA in the blood vessels of patients with atopic bronchial asthma and there was a direct correlation dependence ($r = 0.0005$) between the level of auto-antibodies to DNA and the severity of the Atopic Bronchial Asthma. The detected auto-antibodies possess catalytic activity of DNA, enzymatic specificity which is associated with the degree of severity of disease. The auto antibodies in patients suffering from severe forms of Bronchial Asthma are specific for monofilament DNA and antibodies in the blood serum of the patients with the light form of asthma is heterogenic: besides antibodies with monofilament substratum, some specific antibodies with bi-filament DNA circulate. Therefore, in the serum of the patients suffering from Atopic Bronchial Asthma antibodies with Catalytic activity DNA was observed-that is abzymes. It was suggested that these "abzymes" may be directly involved in the removal of debris produced by the metabolism of organism under physiological conditions. Considering all these facts, Abzymes can be regarded as serological markers of autoimmunity and needs to be tested while investigating autoimmunity especially in Atopic Bronchial Asthma and it may also serve as an additional criterion for the diagnosis of asthma even in the early stages and can also help in the evaluation of the effectiveness of the treatment.

Key words: Abzymes, auto-antibodies, atopic bronchial asthma

INTRODUCTION

The Asthma's index dynamic slowly occurs but steadily. In spite all the ongoing measures for early asthma's discovery, nowadays the disease has been diagnosed in its first stages with about 5-15% world's population suffering from asthma (Chuchalin, 2007). In Russia, specialists consider Bronchial Asthma as an important social disease (Perez, 2008). According to many data, the spread in the adults population varies from 2.2-7 even 15% (Masoli *et al*., 2004) but within children's population this index is about 10% (National Program, 2008). Nowadays, we must observe that despite the success in the study of the disease's mechanisms, the tendency in the rising of patients with severe form of bronchial asthma remains unchanged. The same trend is also observed with patients who died of this allergic form of the disease. Over a century, the attention has been focused on the allergy as the cause of the bronchial asthma. However, all medical attempts to cure the allergy in order to do away with asthma were unsuccessful. For this reason, during a conference, the International Committee of Experts has declared «... the outstanding question is to establish if the influence of allergens is really the beginning's factor of Bronchial Asthma ....(GINA, 1995)».

In 2003, the research of Rottem and Shoenfeld (2003) was published. These authors reported that allergy and autoimmunity can have the same mechanisms in the pathological ways. The two scientists described Asthma as a paradigm of autoimmune diseases by studying the characteristics of the correlation of the allergic diseases and autoimmunity. Autoimmune diseases, virtually unknown a few years ago, rank 3rd cause of death behind cardiovascular diseases and cancers. These diseases result from the disruption of the immune system attacking the body gradually as if it was a foreign body. The cells of the immune system permanently collide with components of the organism itself. The solutions that were offered in the field of medicine provide only approximate results to this phenomenon.

The analysis of auto-antibody profiles has shown to be important in the diagnosis of some auto-immune diseases, such as anti-dsDNA and anti-Sm auto-antibodies in the Systemic Lupus Erythematosus (SLE) (Von Muhlen and Tan, 1995; Brouwer *et al*., 2001) and anti-cyclic citrullinated protein in rheumatoid arthritis (Schellekens *et al*., 2000). But it is important to emphasize right away that these auto-antibodies may have a role in the pathogenesis of auto-immune diseases, they are most of the time markers associated with certain diseases. Therefore, the measurement of these antibodies is a diagnostic aid, for it allows either confirming an autoimmune disease where the clinic is suggestive, or excluding a diagnosis. An increase in the levels of these antibodies can precede the clinical manifestations of an outbreak (Cortes-Hernandez *et al*., 2004). According to the authors (Rigopoulou *et al*., 2007) of the above studies mentioned (Beland *et al*., 2004; Vitozzi *et al*., 2004), anti-LC1 (liver cytosol type 1 antibody) and anti-SLA (antibody against soluble liver antigen) auto-antibodies can be regarded as a serological markers of autoimmunity and need to be tested while investigating autoimmunity, especially in chronic HCV infection (Rigopoulou *et al*., 2007). Immunological disorders have been frequently described in the course of Hepatitis C Virus (HCV)-related chronic hepatitis and non-organ-specific auto-antibodies (NOSAs) in particular are common examples of auto-reactivity associated with HCV infection (Lenzi *et al*., 1999; Muratori *et al*., 2003, 2005). For example, Radioligand assays of higher sensitivity than the conventional assays have increasingly been reported in the auto-antibody diagnostic setting of a plethora of autoimmune diseases such as diabetes, multiple sclerosis, systemic lupus erythematosus and AIH (Dalekos *et al*., 1999; Ma *et al*., 2002; Wiik *et al*., 2004). Paul (1998) found in the blood serum of the patients with Bronchial Asthma and the natural antibodies that belong to Immunoglobulin G with proteolysis activity. This

brought new perspectives in the study of this problem. Szczeklik *et al.* (1995) while studying the autoimmune status of patients suffering from Bronchial Asthma, found in the blood some antinuclear antibodies, but in any case, none of the patients got some antibodies with $_{N}$DNA, however the quantity of autoimmunity of the patients suffering from bronchial pulmonary system reveal the advantage of the synthesis of specific organ AT, for example AAT $_{N}$DNA and/or $_{DN}$DNA (Markin *et al.*, 2001). The auto-antibodies-abzyme's appearance is the general tendency of the answer to the immune system in the severe contaminations. We can say that catalytic activity can find its reflection in the natural potential defence of immune system for different kinds of autoimmune disturbances.

Many studies confirmed the negative influence of the environmental pollution on the level of the sickness and on the spread of Bronchial Asthma. Kobets and Tanaga (2011) confirmed that climatic factors played a vital role in the Bronchial Asthma's development. Pearce *et al.* (2007) reported the rising index of the spreading symptoms in Africa and in Latin America, this shows that Asthma continues to mount at alarming rate. However, scientists do not agree upon the causes of Asthma; therefore, the objective of this study was to correlate the climatic and geographic factors and the environmental conditions in the occurrence of Asthma and other autoimmune phenomena in the population of the State of Tatarstan, for example the prevalence of abzymes in the pathogenesis of Atopic Bronchial Asthma (ABA).

MATERIALS AND METHODS

Study population: A total of 273 samples of peripheral blood serum collected from patients were used in this study. The first group consist of patients from 15-70 years old (80 samples) and healthy donors from 21-59 years old who did not get any clinical diagnostic (15 samples). Three sub-groups were made within the patients of the first group: Subgroup (1) Patients with ABA (36 patients), subgroup, (2) Patients without ABA but with allergic Rhino-conjunctivitis (polypus) (24 Patients) and subgroup and (3) Patients without ABA but with nose's polypus (20 patients) (Chuchalin, 2007). All the patients were monitored by the doctors of Allergic Diseases Polyclinic of Scientific Research of Epidemiology and Microbiology institut of Kazan and Municipal Clinical Hospital No. 7 of Kazan. The second group comprises 193 patients from the division of allergic disease of Republic Clinical Hospital with diagnostic of ABA. The 193 patients were 18-60 years old. For the test to be positive on antibodies with catalytic Activity of DNA, the blood serum of patients suffering from Systemic Lupus Erythematosus (SLE) and Auto-Immune Thyroiditis (AIT) was used. All the patients were informed and they gave their written consent. The severity of asthma in these patients was assessed according to the Global Initiation for Asthma guideline (GINA, 1995; NAEPP, 1997). The diagnosis of ABA was established on the basis of the data of allergic anamnesis and based on the results of cutaneous experiments of skin with allergens and dust (GINA, 1995; NAEPP, 1997). The study was performed in accordance with the rules of the Ethics Committee in the laboratory of Clinical Immunology and Allergy of RKB and with the regulations of the Ministry of Health of the Russian Federation in compliance with the Helsinki Declaration.

Methods: Enzyme-linked immunosorbent assay (ELISA) was used in order to find IgG auto-antibodies against DNA in the blood serum (Engvall and Perlman, 1971; Goldsby *et al.*, 2003). For the study of the catalytic activity of IgG auto-antibodies against DNA in the blood serum the reaction was carried out in vitro. Commercial preparation of Plasmids DNA $_{p}$BR322 extracted from *E. coli* XL-1Blue («Sib Enzyme, Russia) was used as substratum. The initial preparation of DNA

of plasmids and the samples blood serum were put in a sterile eppendorf: 25 mM tris-HCl (pH 7.5), 5 mM $MgCl_2 6H_2O$, NaCl. Hydrolysis of plasmid DNA was done at 37°C for 24 h. Each sample was run for 4 h. Catalytic activity of antibody against DNA was estimated by the transformation of the super-coiled plasmid DNA pBR322 in the circular and linear form by electrophoresis. Electrophoresis was performed in 0.7% of agarose gel, prepared on TBE containing 0.09 M Tris-OH, 0.09 M boric acid, 0.002 M EDTA and at pH 8.0-8.2 with a voltage of 3-5 V cm^{-1} for 1 h 30 min at 20°C. The gel stained for 15 min in the TBE containing 1 μg mL^{-1} ethidium bromide. The evaluation of the results of the plasmid DNA pBR322 was carried out on gel analyzer system of brand Chemi Doc TMX RS (Bio-Red Laboratories, USA).

Statistical analysis: The processing of the results was carried out with the help of calculation of mean values (M), the Coefficient of variation (Cv) and the dispersion of the average (Δm). The analysis of the obtained data with the use of structural medium-median (IU) and the coefficient of asymmetry (As) showed that the distribution of individual indicators at the level of the AAT to the DNA in some groups was different from the normal. Therefore, to assess the differences within and between samples non-parametric (rank) criteria were used (Mann and Whitney, 1947).

RESULTS

To evaluate the possible involvement of the autoimmunity in the pathogenesis of bronchial asthma in the population of Tatarstan State, the availability and the level of circulating IgG antibodies (auto-antibodies) were identified in the blood serum of patients' peripheral blood and relatively healthy donors. According to the results of ELISA test, some IgG antibodies to $_N$DNA were found in patients with atopic (ABA) and non atopic (N/ABA) bronchial asthma. As shown in Fig. 1, in patients with asthma regardless of the pathogenesis of the disease, it was revealed an increase in the level of IgG anti-$_N$DNA compared to the control group and in patients with infectious-dependent variant of which the proliferation reached the level of significance ($r<0.5$). In the patients with infectious dependent variant the level reached was significant ($r<0.5$).

In patients with bronchial asthma exacerbation often occurs in the spring-summer period, the distribution of the level of anti-$_N$DNA IgG was investigated in summer and winter periods and seasonal changes of circulating auto-antibodies in the blood serum were identified. Figure 2 shows that the level of anti-$_N$DNA IgG in healthy donors was lower in the summer period compared to the winter period (0.6 and 0.976, respectively). Seasonal change of anti-DNA IgG level was reported

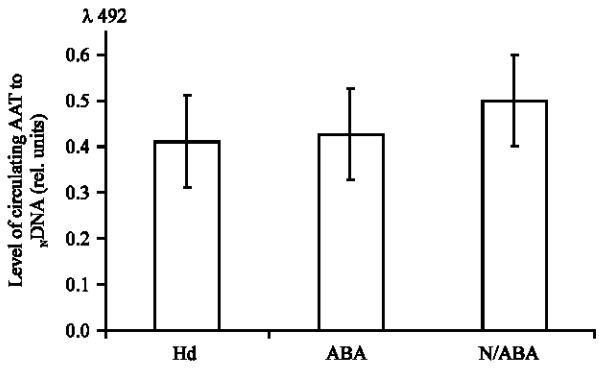

Fig. 1: Dependence level of antibodies to $_N$DNA bronchial asthma variant ABA: Atopic bronchial asthma, N/ABA: Non atopic bronchial asthma, Hd: Healthy donor

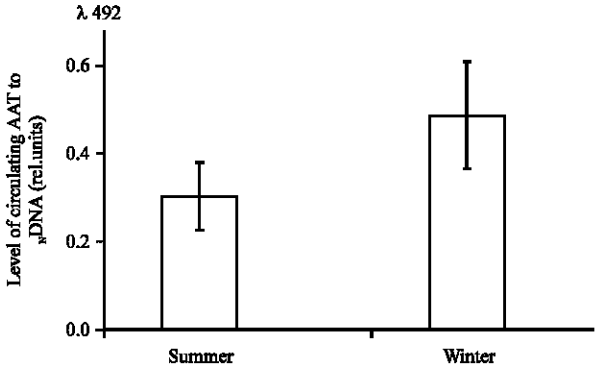

Fig. 2: Level of IgG AAT to $_{N}$DNA in the blood serum of healthy donors in summer and winter periods

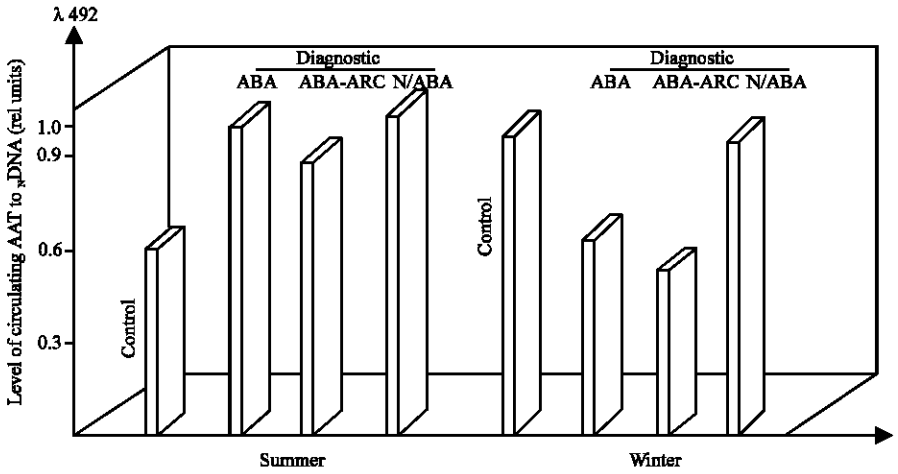

Fig. 3: Change in the level of IgG AAT in patients with asthma in the summer and winter period: ABA: Atopic bronchial asthma, ABA-ARC: Atopic bronchial asthma, combined with allergic rhino conjunctivitis and N/ABA: Infectious-dependent bronchial asthma with polyposis

in some patients (Fig. 3). In summer the number of circulating IgG auto-antibodies in patients with ABA increased up to 66% (1.00±0.2) and in patients with ABA on the background of allergic rhino-conjunctivitis (ABA-ar/To) reached 50% (0.89±0.2) and in patients with infectious-dependent bronchial asthma (with polyposis) was 75% (1.05±0.15) compared with the control group in the spring-summer period (0.60±of 0.08) (Fig. 3). In winter a decrease was observed with the level of anti-DNA IgG in these groups compared to the control group in winter (0.976±of 0.07) apart from 34.4% (0.64±0.06), 45.6% (0.53±0.08) and 3.6% (0.86±0.1), respectively (Fig. 4). It should be noted that in patients with ABA in combination with other allergic disease (rhino conjunctivitis), there was a decrease in the content of auto-antibodies to DNA in the winter period. In patients with infectious-dependent variant in combination with the polyposis of nose, an increased level of auto antibodies to $_{N}$DNA was found in the serum during the summer period but the level of auto-antibodies in the winter period was at the control level in winter and did not differ from the level of antibodies in the blood in the spring-summer period. Thus, we have found the seasonal frequency detection of circulating IgG auto antibodies to DNA and the dependence of the

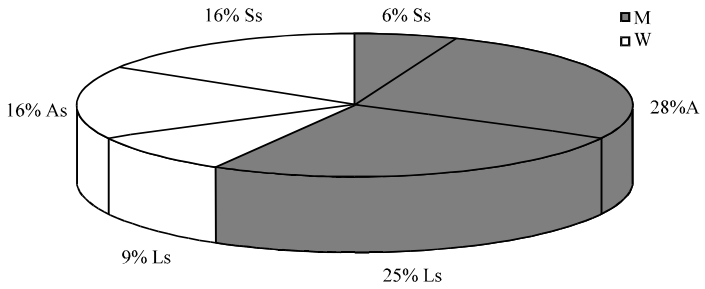

Fig. 4: Frequency of asthma among men and women based on the severity of the disease, Ls: light severity, As: Average severity, Ss: Serious severity

concentration of the seasons of the year. Non atopic asthma is characterized by the high content of IgG auto-antibodies to DNA throughout the year, i.e., it is characterized by the lack of seasonal effect in the number of auto-antibodies in the blood of patients (Fig. 4).

The impact of climatic and geographic factors on àuto-serotherapy in healthy people, during the winter and summer period, is known for quite a long time. The authors showed that based on the nature and type of auto-antibody in winter the frequency of their detection and the average content in the serum was 3-5 times higher than in summer. For example, for antibodies to DNA percentage of positive reactions in winter was 23.31% and in summer was 5.52% ($r<0.02$-0.001). The content of auto-antibodies in winter was 92.23±3.48 U mL^{-1} and in summer-17.99±of 2.93 U mL^{-1} (Dobrodeeva *et al*., 2006). Such information about the higher level auto-serotherapy based on the climatic conditions of the North exists in regard to the pulmonary and cardiac antigens. To highlight the cause of autoimmune reaction, authors consider the development of micro destructive processes, catabolic effect of increased activity of glucocorticoids and others (Mirrakhimov *et al*., 1985). In terms of continuation of the study of auto antibodies to DNA in patients with asthma, the level of anti-DNA IgG in men and women and the changes in their level based on the degree of severity were examined. Among the examined patients 34.4% had light severity and 43.3% had average severity and 22.4% had grave severity of disease. In spite of the fact that the selection of the patients was accidental, they were examined in the hospital by doctors. Our results for the dissemination of the severity of the disease among the patients with bronchial asthma were close to the medium. In Russia, 3-7% even 15% of the population suffers from asthma, of which about 30% of the light form, 50% of the average severity and 20% of the grave severity of the disease (Badoev *et al*., 2011).

The frequency of the disease severity among the male and female population: Women were particularly prone to autoimmune disease due probably to a difference between the hormonal regulation and the immune systems. According to Brouwer *et al*. (2001), a person's sex seems to have some role in the development of autoimmunity, classifying most of autoimmune diseases as sex-related diseases. Roughly 75% (Brouwer *et al*., 2001) of more than 23.5 million Americans who suffer from autoimmune disease are women, although it is less-frequently acknowledged that millions of men also suffer from these diseases. The reasons for the sex role in autoimmunity are unclear. In spite of the fact that there was no significant difference in the content of IgG antibodies to DNA in male and female patients, nevertheless the results showed (Fig. 4) that men often suffer from the light forms (25 against 9% of women) and women suffer from a severe form (16 against 6% for men). This could be the raison shorter life expectancy in women with asthma. According to

GINA (Chuchalin, 2007), the incidence ratio of boys and girls was 2/1, in adult men and women (aged 30 and above) the disease is common, approximately with the same frequency. At the same time, bronchial asthma is among the ten major non-communicable chronic diseases which is the main cause of death in middle and old age and shortens the average life expectancy of men to 6.6 years and that of women to13.5 years.

Given the particularities of the occurrence of circulating auto antibodies to DNA in patients with atopic dermatitis and infectious-dependent variant of bronchial asthma, we have increased samples of patients with atopic bronchial asthma and investigated on the character of distribution of the levels of anti-DNA IgG in blood with the progression in ABA, i.e., in the light, average and high severity of the disease. Figure 5 shows that there was a significant tendency ($r<0.01$) of growth in AAT to $_N$DNA to the extent of the deterioration of the disease. Correlation analysis showed a direct correlation between the level of $_N$DNA and the severity of bronchial asthma (Fig. 6). Thus, the main contribution to the emergence of auto antibodies to $_N$DNA in patients with ABA makes the severity of the disease.

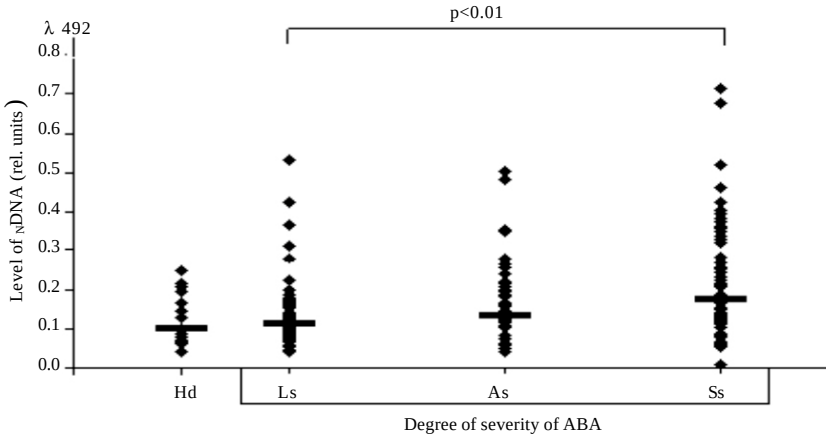

Fig. 5: Distribution of the levels of circulating IgG auto antibodies to DNA in the surveyed contingent based on the seriousness of the ABA-Me

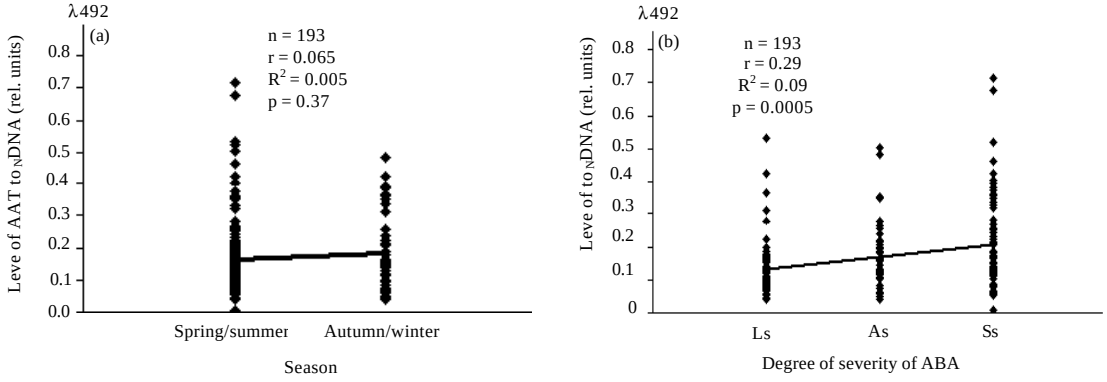

Fig. 6(a-b): Results of the correlation between the level of circulating AAT to $_N$DNA in relation to (a) Season and (b) Degree of severity of disease, Ls: Light severity, As: Average severity, Ss: High severity

Considering the nature of the distribution of severe ABA among the population and the direct correlation dependence between the level of circulating auto-antibodies and the severity of the disease, the level of autoimmune response (the presence of circulating IgG-abzymes in the peripheral blood) in patients with ABA was examined. The serum of patients with Systemic Lupus Erythematosus (SLE) was used as a positive control. The example of the serum of SLE showed the link of autoimmunity (i.e., the presence of catalytic DNA of IgG auto- antibodies) with pathology of apoptosis. The serum of patients with ABA for the presence of catalytic activity of DNA was investigated. It should be particularly noted that the antibodies, enzymes (enzyme-antibody) as a rule, were absent in the body of healthy people (this could be due to the fact that their number was extremely small), so until recently, the question about the possibility of the existence of abzymes in the absence of autoimmune pathologies remained open. But in recent years, accurate and reliable data on the immune nature of the malfunction in atopic diseases were reported not only on the trigger allergies but also on atopic symptoms that was connected with the malfunction of the adrenergic functioning receptors in these diseases and in particular in asthma. The existence of antibodies to b-receptors is about to be classified in atopic asthma, putting the disease into the category of autoimmune pathology. Therefore, auto antibodies found in the peripheral blood of patients with asthma were studied for the presence of catalytic activity. To check the function of the enzyme system, the serum of Systemic Lupus Erythematosus (SLE) and Auto-Immune Thyroiditis (AIT) were used (Fig. 7) and which were characterized by the presence of antibodies-abzymes with catalytic activity of DNA. DNA activities of antibodies serum of patients with asthma were evaluated based on their ability to turn substrate-super-spiral DNA pBR322 into the ring and linear form.

Figure 7 illustrates that the serum of patients with ABA possesses catalytic activity of DNA along the serum of patients with autoimmune thyroiditis. Then, the DNA catalytic activity level of antibodies' serum of patients with asthma depending on the severity of the asthma (within 24 h) was studied. Figure 8 shows that the dynamics in the growth of the number of hydrolysis products were identified based on the time. The level of catalytic activity of DNA depends on the severity of asthma: auto-antibodies serum of patients with severe asthma were more active and show the affinity to monofilament areas of super spiral DNA (form 1), as proved by the rise of the circular DNA (form 2) (Fig. 8, well-4). After 12 h, the reaction of the whole super spiral DNA entered the ring shape, thus, the influence of protein serum on the substrate specificity of catalytic activity of

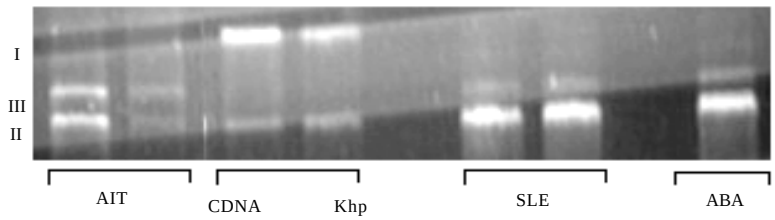

Fig. 7: Manifestation of DNA activity of circulating AAT to $_{N}$DNA of serum of patients with systemic lupus erythematosus (SLE) and a light form of Auto-immune thyroiditis (AIT) and with atopic bronchial asthma. Systemic lupus erythematosus (SLEs), a light form of auto-immune thyroiditis (AIT) on the example of the hydrolysis of plasmid DNA pBR 322. Reaction is after 24 h (I) Super spiral, (II) Ring and (III) Linear-structural forms of pBR322; CDNA-DNA without reactionary environment, K_{hp}-DNA+serum of healthy people

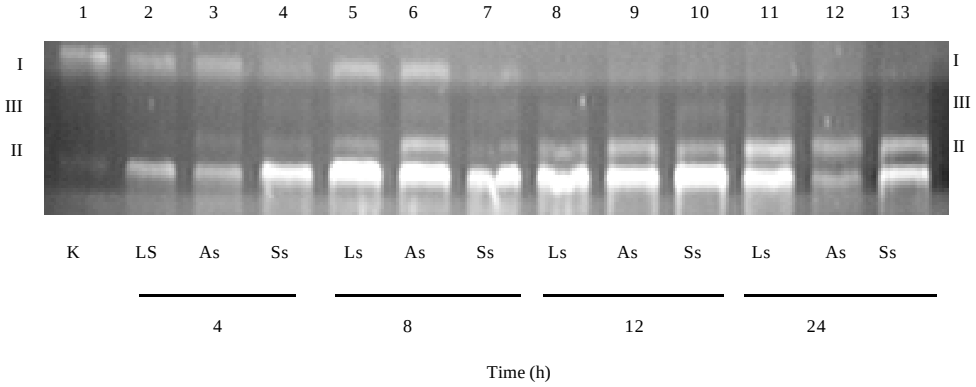

Fig. 8: Alteration in the activity at the $_{N}$DNA serum of patients with asthma based on incubation time (37°C, 24 h) and the severity of the disease (I) Super spiral, (II) Ring and (III) Linear structural forms of pBR322; CDNA-DNA without reactionary environment; LMS light, average and severe forms of asthma, respectively

antibody against DNA depending on the severity of the disease was studied (Fig. 9). The results showed that the substrate specificity of antibodies was correlated with the severity of the disease. According to Fig. 9, the nature of the accumulation of the products of hydrolysis of plasmid DNA pBR322 of circulating antibodies, patients with a severe form of ABA possessed endonucleases activity and have a preference to a chain of DNA-after 12 h in the reaction mixture among the products of hydrolysis and approximately 78% of circular DNA were found. Increasing 10 times the concentration of protein changed slightly the number of circular DNA (up to 69.5%). The fact is that in solutions with a small ionic force (0.15 M NaCl) and the neutral or light basic solution, plasmid DNA pBR322 had "super-twisted" structure as a result formed over spiral (form 1). The formation of super-twisted forms of DNA was followed by the denaturing of a certain area of molecules which disappeared while changing the DNA from form 1-2, i.e., as a result of a single gap which was the instant recovery of hydrogen bonds in denatured division of the molecule. These properties of DNA pBR322 make them easy targets for the study of endo-nucleases activity of enzymes and antibodies-abzymes (thanks to the attribute of DNA pBR322, it was really easy to target them for the study of endo-nucleases activity of enzymes and antibodies-abzymes).

Therefore, based on the stability of the bi-filament circular DNA pBR322 to the action of the studied circulating antibodies of patients with severe form of ABA revealed that the addition of excessive amounts of antibodies of patients with severe asthma or the increase in the reaction time did not lead to a complete transfer of circular DNA (form 2) into the linear (form 3). Circulating auto-antibodies in blood serum of patients with light asthma were heterogeneous. The analysis of results of Fig. 9 shows that in the serum of patients with the light form of ABA along with antibodies to monofilament substrate, the circulating antibodies were specific to bi-filament DNA, because there was a dynamics of hydrolysis of circular DNA and which increases the number of linear DNA (form 3) (to 68.5) at the rising level of protein serum in the reactionary environment. These data could serve as control and the lower catalytic activity of DNA of antibodies, abzymes

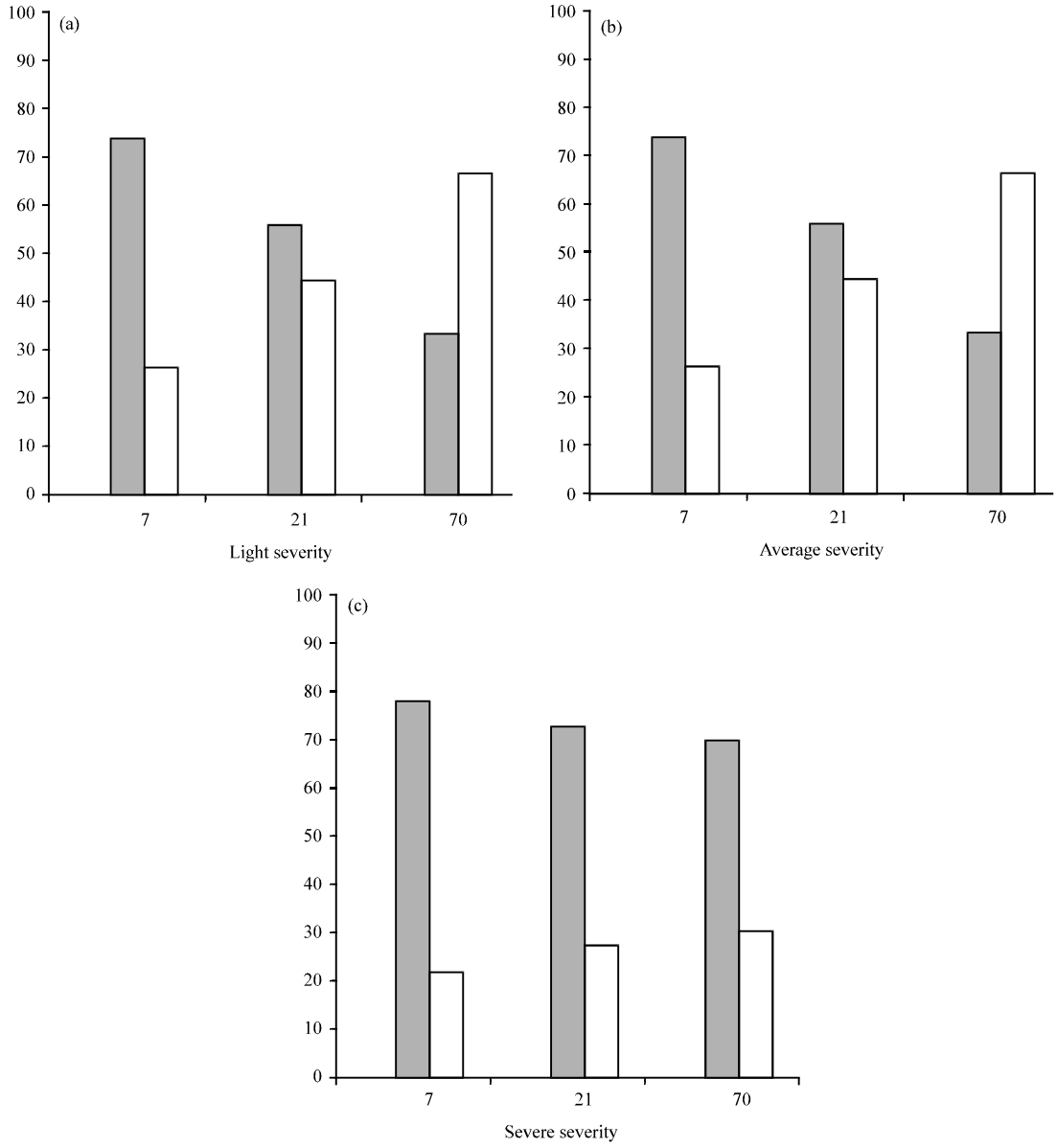

Fig. 9(a-c): Substrate specificity of circulating antibodies serum of patients with atopic bronchial asthma based on the severity of the disease. Quantity of introduced protein per sample: 7. 21. 70 μg μL^{-1}. Time of incubation (37°, 12 h). Hydrolysis product pBR322 (form DNA) in percentage. Grey color: II (circular), White color: III (linear)

and specific to double-stranded DNA. Thus, in the blood serum of patients with ABA we have detected antibodies to catalytic activity of DNA-àbzymes, i.e., signs of autoimmune disease development.

DISCUSSION

According to the Federal statistical observations it was established in Russia only in 2007 «diagnoses of bronchial asthma, asthma status» and the number of asthma patients was 1 266 282

people. It is especially important to note that bronchial asthma was the cause of around 1963720 days of temporary incapacity to work among the working age population and 3856744 days of hospitalization. The exacerbation of bronchial asthma often reduces not only the level of living of the patients but also of that of their families (Perez, 2008).

Studies on the incidence of Bronchial Asthma (BA) were conducted in the State of Tatarstan from 2005-2010. The results of these studies showed that the incidence of ASTHMA was 96 cases in 2005 and 60 cases $100{,}000^{-1}$ inhabitants in 2010. The prevalence of ASTHMA recorded was 744 cases in 2005 and 880 cases $100{,}000^{-1}$ inhabitants in 2010. Death rate caused by asthma in the State was relatively low (11.7 in 1996; 9.6 in 2000; 7.4 in 2005-2010 and it was characterized by a certain dynamics. Such a significant tendency in the reduction of asthma could be due to the efficiency of the free treatment program which consists in providing patients with medicines of basic therapy which resulted in the reduction in the frequency of asthma exacerbations and the number of deaths (Pronin, 2011). On the other hand, our results of the survey conducted showed that 34.4% had a light case and 22.4% had severe case of disease. This is above the average in Russia-30% among asthma patients suffering from a light form of asthma and 20% of a severe form of the disease against 5% in the United States. It was in 2007 that the cities of Kazan and Nizhnekamsk were excluded from the Priority list of cities with the highest level of pollution of atmospheric air, but the air pollution in these cities was characterized as high (Martinez, 2007). It is necessary to note that the study of asthma incidence showed net relationship between the patterns of the severity of Asthma and the morbidity. Although, the preferential program of providing patients with medicines (Pronin, 2011) is of the basic therapy which result in reducing the prevalence and severity of Asthma. The situation remains complicated, as at this time the real cause of the disease has not been identified (Emelyanov *et al*., 2007). Therefore, in the first place, the task of primary prevention of Asthma is the identification of persons with signs of a threat of BA. In the framework of developing the predictive medicine, genetic studies to identify the responsible genes for the disease of BA were performed (Ober and Hoffjan, 2006; Martinez, 2007; Asanov *et al*., 2008).

The algorithm of diagnostics and the treatment of Asthma are regulated by the modern international and Russian documents on this pathology. Nevertheless, even in patients with a characteristic history, the diagnosis of Asthma is difficult in the absence of objective evidence of bronchial patent and normal indicators of External Respiration Functions (ERF). A similar picture is often observed in intermittent or persistent light form of BA (Tsibulkina, 2005). Along with this, there is evidence of secondary autoimmune (immune-pathological) of clinico-pathological variant of bronchial asthma-the form of the disease with predominance in the pathogenesis of autoimmune processes. This variant is characterized by severe, continuous recurrent forms, a reduction in the overall immunological reactivity, resistance to therapy, including gluco-corticosteroid drugs. The observations of dual autoimmunity, in particular, in some patients who concurrently develop organ-specific and systemic disease have been reported (Penas *et al*., 1997; Malik and Ahmed, 2007). For a long time, scientists thought that the appearance of antibodies was a sign of serious problems in the body. Various diseases are due to the changes of auto-antibodies. On the basis of the determination of the content of auto-antibodies, the method allows to assess and predict the state of the health of any person. Rottem and Shoenfeld (2003) reported that the Allergy and autoimmunity can have common mechanisms in the pathogenic pathways. Among other things according to some authors, the evolution of the correlation between asthma and allergy is not always symmetrical. This correlation was demonstrated in Britain, but neither in Germany nor in

Italy, where the incidence of allergies did increase but not that of asthma (Eder *et al.*, 2006). This tends to prove that asthma could be intrinsic causes as in autoimmune reaction or a genetic predisposition, although there is no study, till date that strictly proved it. This is consistent with the results of Goodnow (2007) who suggested that the combination of genetic susceptibility and environmental factors of an outbreak is needed to cause an autoimmune disease. These findings may therefore help to clearly perceive that the autoimmune diseases, resulting from the combination of genetic and environmental factors affect a variety of tissues and produce a range of diverse diseases and which may be unknown to the scientific world (Goodnow, 2007). The genetic risk factor accounts for almost half of the susceptibility to autoimmune diseases. The most likely among them are the common ways of regulation of the immune response, in which antibodies are frequent, in particular auto antibodies to DNA. In order to assess the idea of the possible involvement of autoimmune response against the mucous membrane of the bronchi in the pathogenesis of non atopic asthma, a group of researchers used indirect immune-fluorescent staining of fresh frozen mucous membrane of the bronchial tissues of human serums of patients with atopic and non-atopic asthma; the serum was from healthy donors and patients suffering from systemic lupus erythematous. The immune application of circulating IgG antibodies against the mucous membrane of the bronchi were determined in 2 (9.1%) of the 22 patients with non-atopic asthma and in neither case of the 22 patients with atopic asthma and nor in 22 samples of IgG antibodies control from two patients with non atopic asthma whom the cytoplasmic membrane of the basal cells in the bronchial epithelium were mainly stained. Serum from 10 patients of SLE with the nucleus of the epithelial cells throughout a layer of epithelium of the bronchi was stained. This work showed the presence of circulating IgG-antibodies against epithelial bronchial cells in small numbers of patients with non atopic asthma. But the authors continued this work in order to evaluate the possible involvement of autoimmune mechanism in the pathogenesis of non atopic asthma. Nahm *et al.* (2001) measured the presence of circulating auto antibodies in the culture of the epithelial cells of human being with the aid of ELISA. In this work, they used the serum of healthy donors (control) and 26 patients with atopic asthma, 16 patients with non atopic asthma and 12 patients with SLE. Therefore, the authors showed that the level of IgG antibodies to epithelial cells of the bronchi was significantly higher in patients with non atopic asthma (Mean value±SD absorption; 0.135±0.03) and in patients with SLE (0.293±0.181), than in persons who served as control samples (0.12±0.016) and patients with atopic asthma (0.116±0.031) ($r<0.05$). The work of these authors showed the absence of circulating auto-IgG to the epithelial cells of the bronchi in atopic asthma that is, the absence of autoimmune phenomenon. Later, Tedeschi and Asero (2008) confirmed that the detection of antinuclear antibodies and antibodies against antigens of the epithelial bronchi cells in patients with non-allergic asthma involves autoimmune basis of the disease and they further reported the involvement of auto-regency mechanism for allergic (atopic) asthma. Thus, the search for new biomarkers related to the severity of asthma could be very promising. It should be noted that the question of antinuclear in the blood of patients with asthma were studied long time ago, but the results were contradictory. Even in the work of Howard and Ellsworth (1965), it was shown that the method of anti-nuclear in the lungs epithelium cells was not available. According to Lidor *et al.* (1980) individual with anti-nuclear antibodies was 21% among patients with ABA against 55% with non atopic BA, 25% among patients with bronchitis and 16% of relatively healthy persons. Very similar results were obtained which shows the following dynamics: later, anti-nuclear was detected in 53% of patients with non atopic BA, 30% among patients with lung cancer and 20% with ABA against 4.7% in relatively healthy persons.

The presence of antinuclear antibodies was studied by Menon *et al.* (1989) by using indirect method of fluorescent antibodies with asthma based on age, sex, atopic status, dose and resistance to anti asthmatic medications, immunotherapy, the disease severity and the presence or absence of myalgia. In this article the occurrence of fluorescing complex antinuclear and anti-cytoplasmatic Antibodies was higher in the serum of patients with atopy asthma ($r = 0.03$) than among people with asthma and myalgia ($r \leq 0.05$). The results of our studies showed that the presence of circulating AAT to $_{N}$DNA in the blood serum of adults with ABA and demonstrated through mathematical analysis methods that the direct correlation was most likely established between the level of the DNA and the severity of Asthma ($r = 0.0005$), as it is also known that the IgG has the ability to be recorded in the tissues, it could be assumed that this is one of the damage mechanisms of the tissue-target during a progressive course of the disease and the development of autoimmune form of ABA. Circulating auto-antibodies in blood serum possess catalytic activity of DNA. In blood serum of patients with the light form of ABA along with antibodies specific to monofilament substrate, there are circulating antibodies specific for hydrolyzing bi-filament DNA. In the serum of patients with severe asthma were mostly antibodies to monofilament DNA. Thus, in the blood serum of patients with ABA was found antibodies with different substrate specificity and a different level of catalytic activity of DNA - Abzymes. It was suggested that these "abzymes" may be directly involved in the removal of debris produced by the metabolism of organism under physiological conditions (Friboulet *et al.*, 1999; Lacroix-Desmazes *et al.*, 2003). The presence in normal human milk of catalytic antibodies with a protein kinase activity and antibody with DNase activity suggests a protective role of "abzymes" under physiological conditions (Kanyshkova *et al.*, 1997; Lacroix-Desmazes *et al.*, 2002). However, most studies on human being showed that the prevalence of "abzymes" increases in pathological conditions, especially during autoimmune diseases (Lacroix-Desmaze *et al.*, 2003). The first "abzymes" described in humans were isolated in patients with asthma and could cleave Vaso-active Intestinal Peptide (VIP) (Ponomarenko *et al.*, 2006). Some researchers isolated from the serum of patients with various systemic autoimmune diseases: Disseminated lupus erythematosus, scleroderma, rheumatoid poly-arthritis or sclerosis in plate (Baranovskii *et al.*, 2001) and among patients suffering from diseases of the light chains (Bence-Jones disease) (Shuster *et al.*, 1992) of antibodies hydrolyzing DNA and RNA. Our results are consistence with those of their works which described asthma by characterizing the relationship of allergic diseases and autoimmunity as a paradigm of autoimmune diseases (Rottem and Shoenfeld, 2003; Tirosh *et al.*, 2006).

CONCLUSION

Considering all these facts, We can say that the degree of bronchial asthma severity reflects the transition of the disease to autoimmune variant of bronchial asthma which is considered to develop in the further progression and the intensification of the course of atopic and non atopic bronchial asthma. And the detection of antibodies in the serum of peripheral blood in asthma and analysis of the relative DNA activity of auto antibodies may serve as an additional criterion for the diagnosis of asthma even in the early stages and evaluation of the effectiveness of the treatment.

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