Trakia Journal of Sciences, Vol. 10, Suppl. 2, 63-70, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

RESPIRATORY FUNCTION AND MUCOSAL IMMUNITY IN ADOLESCENTS RELATED TO ATMOSPHERIC POLLUTION

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ABSTRACT

Adolescents are a sensitive group to adverse synergetic effects of atmospheric pollutants in urban areas. PURPOSE of this study is to estimate the functional status of respiratory system and mucosal immunity in adolescents from three towns in the region of Stara Zagora and atmospheric air pollution with particulate matter (PM_{10} and $PM_{2.5}$), SO_2 and NO_2 in the same towns. METHODS: Spirometric tests were performed under the ATS/ERS Protocol among 281 eighteen years old students: 129 from Stara Zagora, 115 from Kazanlak, and 37 from Chirpan. IgA containing immune complexes (IgAIC), as a marker for mucosal immunity, as well as IL-10, were determined in plasma by ELISA. Concentrations of PM_{10}, $PM_{2.5}$, SO_2 and NO_2, were measured in the towns observed in the year of investigation. RESULTS: The significantly lowest average values for FEV_1/FVC, PEF% and FEV_{25-75}% were found in the adolescents from Stara Zagora in comparison with these from the other two towns, regardless of the smoking status. FEV_1/FVC under 70% was estimated in 3.1% of all students from Stara Zagora, in 2.7% of students from Chirpan and in no one from Kazanlak. The significantly higher mean values of plasma IgAIC were observed in student from Stara Zagora compared to Kazanlak and Chirpan. The same tendency was observed for quantity of plasma IL-10. The annual average concentrations of PM_{10} and $PM_{2.5}$ were highest in Stara Zagora. The percentages of daily average $PM_{2.5}$ concentrations exceeding the limit value were 57% for Stara Zagora, 29% for Chirpan and 14% for Kazanlak. All measured 1-hour average concentrations of SO_2 and NO_2 were several times lower than the norms for protection of human health, but the maximum daily average values were highest in Stara Zagora also. CONCLUSION: Atmospheric pollution, especially $PM_{2.5}$ influences harmfully the spirometric parameters and modulates mucosal immunity in adolescents from the region investigated.

Kay words: spirometry, immunity, air pollution

INTRODUCTION

Major pollutants in the ambient air in urban areas are dust, sulfur dioxide and nitrogen oxides. Their synergistic harmful effect on human health is well known. Entering the body occurs primarily in the airways, which may cause local irritant, ie of acute respiratory symptoms, and exacerbate already existing lung disease. Particularly vulnerable populations are children, the elderly and people with chronic lung and cardiovascular diseases (1).

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Epidemiological methods are particularly suitable for determining the groups with increased or decreased sensitivity to adverse effects of atmospheric pollution (2). Examination of Mexican children who received antioxidants indicates that they are protected from harmful oxidative effects of pollutants on pulmonary function (3). Another study found a higher incidence of myocardial infarction in humans exposed to high transport traffic related to increased air pollution than the general population (4).

Chronic cough and phlegm, and lower lung function are associated with long-term impact of the environment and the inhalation of particulate matter. The results of studies in the U.S. and Europe show that respiratory symptoms are more prevalent among those living near main streets, regardless of the concentrations of pollutants (5-8).

Many epidemiological studies demonstrated that ambient air pollution particles can adversely affect human health and exposure to different air pollutants form like solid particles, liquid droplets, or gases has been related to risk of cardiovascular and pulmonary disease and cancer (9). Although these finding determined the concentration (dose) response of pollutants that cause these effects, the underlying mechanisms cannot be readily clarified.

Barraza-Villarreal et al. 2008 emphasize that the immune mechanisms influencing on inflammatory reaction consequent the exposure to atmospheric pollutants are not completely understood (10).

The main objective of this work was to estimate the functional status of respiratory system and mucosal immunity in adolescents from three towns in the region of Stara Zagora and atmospheric air pollution with particulate matter (PM_{10} and $PM_{2.5}$), SO_2 and NO_2 in the same towns.

MATERIALS AND METHODS

For the study, cities of Stara Zagora region with different air pollution were chosen. Selected cities were Stara Zagora, a highly industrialized city with intense traffic, Kazanlak with relatively underdeveloped industry and Chirpan little rural town.

We investigated groups of 18-year old healthy adolescents, grew up in those cities. The selection of adolescents of the same age was in order to create relation between long time exposure to the local environmental pollutions and time-dependent accumulation of harmful health effects leading to aberrant immune response. The informing consent was obtained from each participant.

Pulmonary function tests were carried out with 281 eighteen years old students, allocated as follows: 129 from Stara Zagora (population 136 363), 115 from Kazanlak (population 54 357), and 37 from Chirpan (population 16 822). Spirometry was performed under the ATS/ERS Protocol with Spirolab III, Italy. Respiratory parameters were measured as percent from the predicted normal values for each subject's age, height and weight (%pred). FEV1/FVC ratio (Tiffeneau index) was also calculated.

40 volunteers living in Stara Zagora, 27 from Kazanlak and 27 living in Chirpan were recruited for immunological blood test. The peripheral venous blood (3 ml) was taken by venepuncture and collected in sterile tubes with ethylenediamine tetraacetic acid (EDTA). The plasma samples were separated at room temperature within 4 h of collection and stored at $-70^{\circ}C$ until use.

The assessment of circulating IgA immune complexes (IgAIC) in human plasma was performed by CIF-ELISA according to Stanilova and Slavov, 2003 (11). The data are presented as OD 492 units. Nunc's maxisorp immunomodules was used for the performance of IgAIC CIF-ELISA. CIF were isolated in our laboratory from total water extract of the seeds of parasitic plant *Cuscuta europea*, as described previously by Zhelev et al., 1994 (12). For the detection of human IgA, goat anti-human IgA conjugated with peroxidase (Sigma) was used.

The quantity determination of IL-10 were performed by ELISA in plasma samples according to the manufacturer's protocol (Invitrogen). Color reaction developed was measured as OD units at 450 nm. The concentration of IL-10 was determined by using standard curve constructed with kit's standards and was expressed in pg/ml. The minimum detectable concentration of the IL-10 ELISA kit was less than 1 pg/ml.

Daily mean concentrations of PM_{10}, $PM_{2.5}$ and hour mean concentrations of SO_2 and NO_2, were measured in the towns observed during 2010 according standard methods for air pollution monitoring.

Student's t-test was used to determine the statistical differences between mean values. Differences were considered significant when the *p* value was less than 0.05.

RESULTS

Mean values of respiratory parameters FEV1/FVC, PEF and FEV25-75% (% pred) in the groups of students from the towns observed are shown at **Figure 1.** The ratio FEV1/FVC was significantly lowest among the students from Ztara Zagora (89.7±8.5%) compared with these from Kazanlak (95.6±4.7%) - $p<0.0001$ and Chirpan (90.5±6.6%). The mean group level of PEF was significantly lowest for adolescents from Stara Zagora also (78.1±15.6%) compared with Kazanlak (89.9±13.1%) – $p<0.0001$ and Chirpan (92.9±13.7%) - $p<0.0006$. For respiratory parameter $FEV_{25-75\%}$ the results were similar- the mean value for students from Stara Zagora was the lowest (98.1±23.1%) compared with the other two towns, significantly with Kazanlak only- $p<0.009$. Mean respiratory volumes FVC, FEV_1 (% pred) were lowest for the students from Kazanlak followed with these from Stara Zagora.

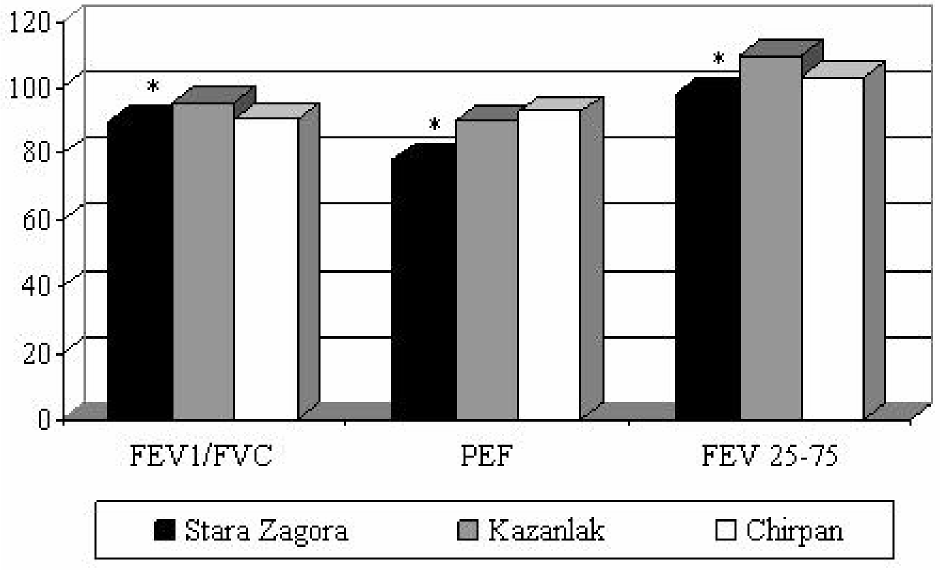

Figure 1. Mean values of respiratory parameters FEV_1/FVC, PEF and $FEV_{25-75\%}$ (% pred).

Figure 2 shows the percentage of students with Tiffeneau index (FEV_1/FVC) reduced fewer than 70% in the groups from the towns observed. The rate of students with impaired pulmonary function was 3.1 % for Stara Zagora, 2.7 % for Chirpan and no one from Kazanlak.

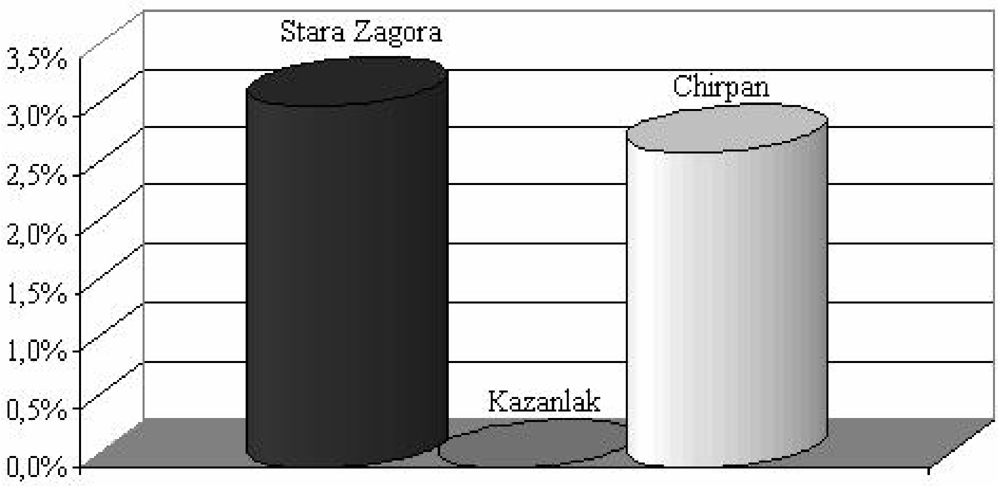

Figure 2. Rate of adolescents with Tiffeneau index (FEV_1/FVC) reduced fewer than 70%.

The results obtained and differences between groups were the same when the smoking habit of adolescents was taken into account.

The study has recruited to examine the effect of ambient air pollution on the human immune system in healthy adolescent. Figure 3 shows an overview of evaluated IgA immune complexes in students from selected cities. Results clearly demonstrated that students from Stara Zagora have the highest level of

IgA containing immune complexes. Statistically significant increase of IgA IC was observed for group from Stara Zagora (0,147±0,024) in comparison with youth living in areas of Kazanlak (0,125±0,025; p=0.000001) and Chirpan (0,125±0,025; p=0.000001).

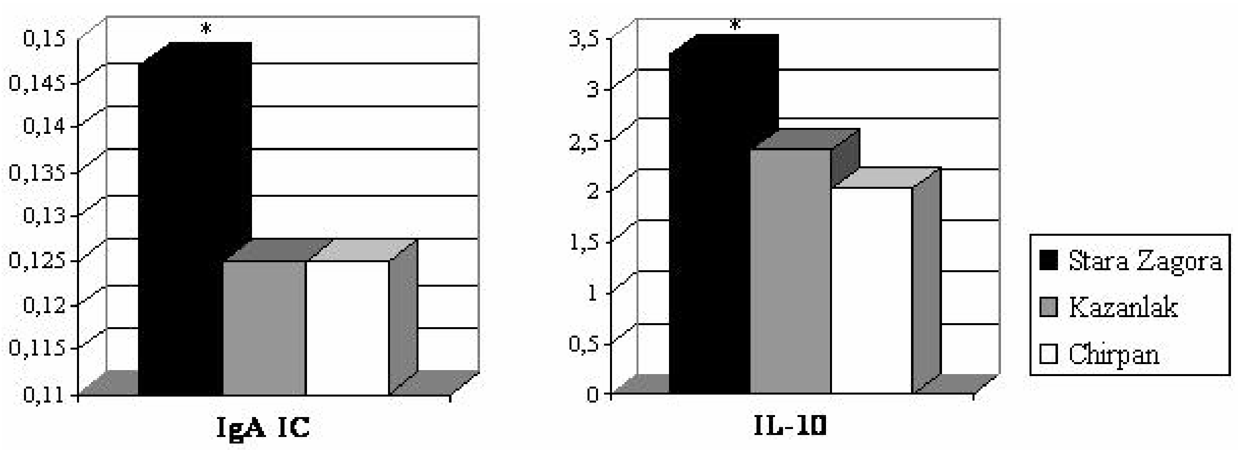

Figure 3. Average group levels of IgA IC and IL-10 in adolescents and statistically significant difference for group levels from Stara Zagora against these from Kazanlak and Chirpan.

Immune complex formation and its clearance is an immune process which completes humoral immune response. Generally, after pathogen invasion and clonal expansion of antigen-specific B cells, differentiated plasma cells produce antibodies which recognized soluble antigens and formed multicomponent aggregates, immune complexes. Depending on the isotype of predominant antibody they can be IgG, IgM or IgA immune complexes and can contains subfragments of the activated complement system, mainly C3b. Immune complexes are effectively removed by the system of mononuclear phagocytes. Uptake of the immune complexes by cells preferentially occurs through complement receptors and Fc receptors (FcR). Immune complex formation and clearance constantly occurs in healthy organisms and this counterpoise is the basic characteristic of healthy immune system.

Results from examination of plasma quantity of IL-10 are presented in Fig. 3 also. Reported are averages of cytokine ranging from 2 to 3.4 pg/ml in different cities. According to the literature data normal plasma concentrations of IL-10 in healthy subjects does not exceed 2 pg/ml. Therefore, we considered near-normal levels of this cytokine in plasma of young people living in Chirpan and significantly higher concentrations of IL-10 in plasma of young people from Stara Zagora (3.344±1.973). They were significantly higher than those of students in Kazanlak (2.405±1.81; p=0.0034) and Chirpan (2.026±1.755; p=0.0033). In the same vein are studies of Calderon-Garciduenas et al, 2009 (13), which establish the dysregulation of immune parameters and significantly increase the amount of IL-10 in blood of children from Mexico City, linking these changes with prolonged exposure to air pollution mainly fine particulate matter ($PM_{2,5}$). Elevated levels of IL-10 is associated with reduced expression of HLA-DR on monocytes (14). This process leads to monocytes deactivation and may be cause for weaker protective cell-mediated immune response in these individuals.

Monitoring of particulate matter air pollution in 2010 showed that the maximum daily average concentrations of PM_{10} (94.6 μg/m^3) and $PM_{2,5}$ (98.3 μg/m^3) were measured in Stara Zagora. Annual average values of PM_{10} were over the Guideline value (40 μg/m^3) in the three towns monitored, but in Stara Zagora was the highest. The highest annual average value exceeding Guideline value for $PM_{2,5}$ (25 μg/m^3) was found in Stara Zagora also– **Figure 4**.

Considerable problem for the town is air pollution with fine particulate matter- 57.1% from all daily average concentrations of $PM_{2,5}$ measured in Stara Zagora were over the Guideline value (30 μg/m^3) – **Figure 5**.

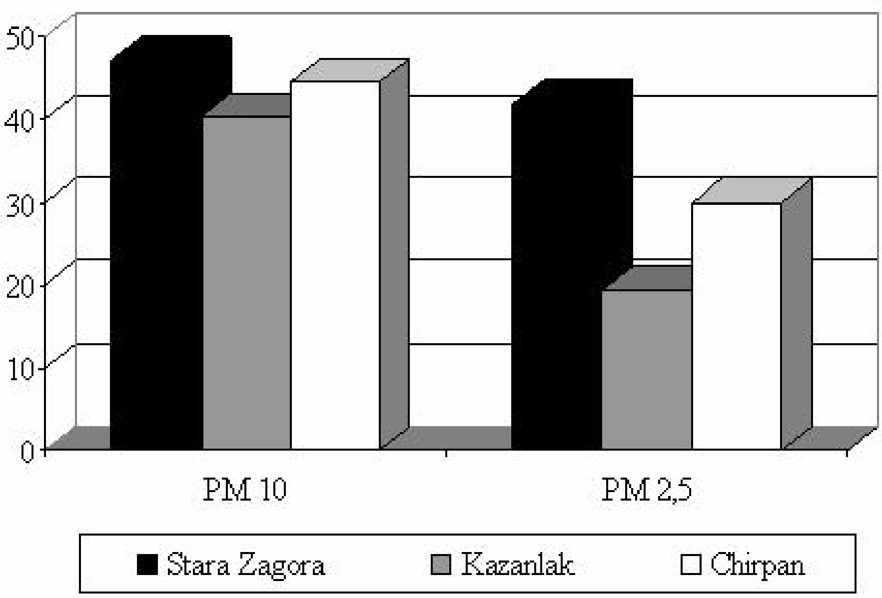

Figure 4. Annual average values of PM_{10} (Guideline value- 40 µg/m^3) and $PM_{2,5}$ (Guideline value – 25 µg/m^3).

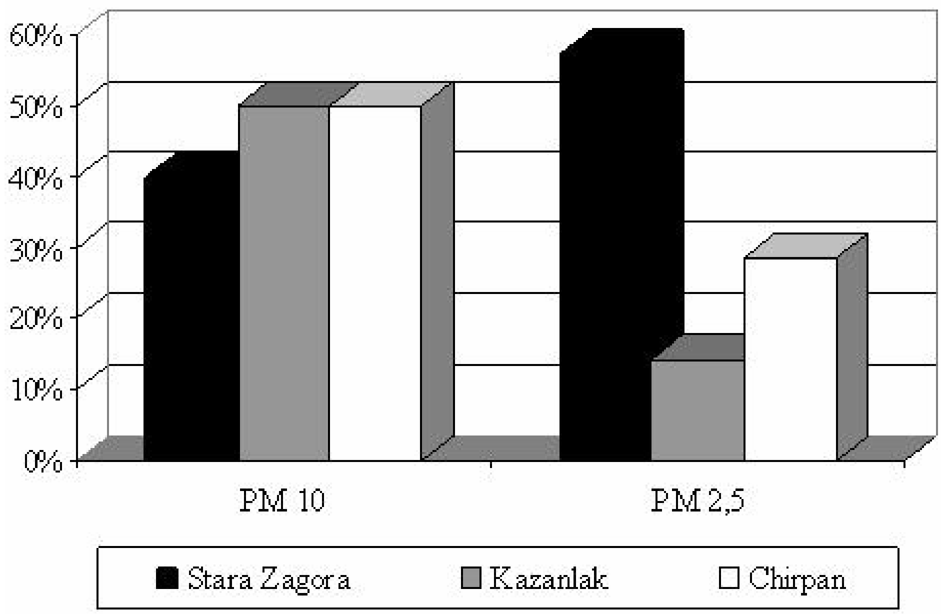

Figure 5. Rate of daily average concentrations exceeding Guideline Values for PM_{10} (GV- 50 µg/m^3) and $PM_{2,5}$ (GV- 30 µg/m^3).

All 1-hour average concentrations of SO_2 and NO_2 measured in the three towns were under the Guideline values (350 µg/m^3 for SO_2 and 200 µg/m^3 for NO_2). Maximum Daily average value for SO_2 was under the reglamented Guideline value- 125 µg/m^3. Annual average value for NO_2 was under the reglameted Guideline value- 40 µg/m^3. Nevertheless, maximum daily average concentrations for SO_2 (55.8 µg/m^3) and NO_2 (19.0 µg/m^3) were found in Stara Zagora **(Figures 6).**

In conclusion, the atmospheric air pollution with the main pollutants monitored (PM_{10}, $PM_{2,5}$, SO_2, NO_2) is the most adversely in Stara Zagora that correspond with results from spirometry tests and immunologic investigations among adolescents.

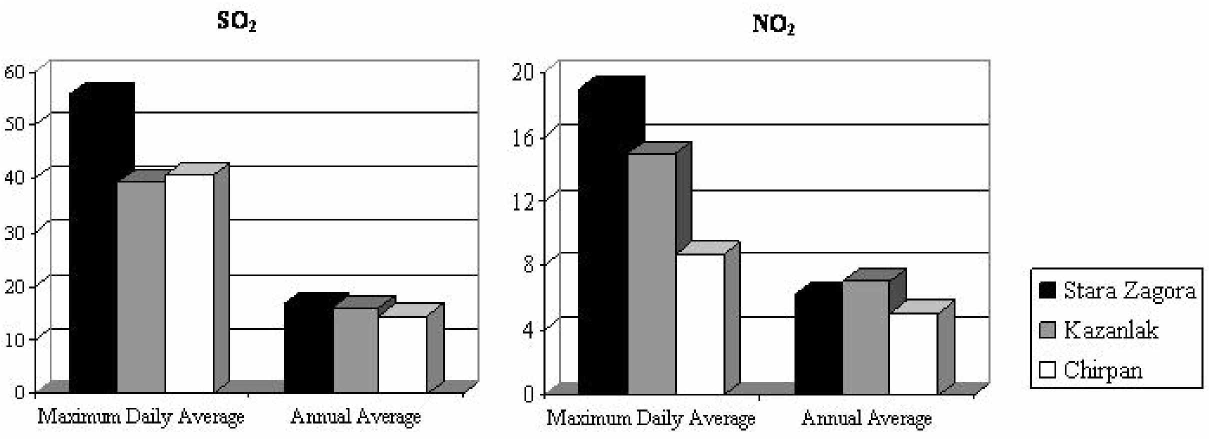

Figure 6. Maximum Daily average and Annual average value of SO2 and NO2.

DISCUSSION

Spirometry parameters FEV_1/FVC, PEF and $FEV_{25-75\%}$ were the lowest in Stara Zagora regardless of the smoking habit of students, which suggest a possible connection with the highest air pollution in the town. Calderón-Garcidueñas at al., 2003 have concluded that exposure to air pollutants in the towns bring about respiratory damages in children and increases the risk of chronic lung diseases and other system adverse effects (15). According to research of Avol E.L et al. PM_{10} has adverse influence on spirometry volumes FEV_1, PEF and FEV_{25-75} in children 10 to 15 years (16). In the towns with higher levels of air pollutants the rate of students with decreased respiratory volumes was raised. This adverse effect is most pronounced for PEF and $FEV_{25-75\%}$, which is relating to unfavourable changes in small lung airways. These respiratory volumes were lowest in adolescent from Stara Zagora where the $PM_{2,5}$ concentrations were the highest and exceeded the Guideline value. The rate of students with Tiffeneau index $FEV_1/FVC<70\%$ was highest (3.1%) in Stara Zagora also.

Our results correspond with these of Lagorio. S et al., 2006 which found reduced FVC and/or FEV_1 associated with increased concentrations of $PM_{2,5}$, NO_2 and some heavy metals (17). Other researches report about reduced lung volumes in children living in environmentally polluted regions. Exposure to $PM_{2,5}$ cause acute inflammation of upper respiratory tract and decline lung function in children with asthma as well as in children without diseases (10). J. Maloney and S. Sicherer, 2004 found five time increased rate of 18 years adolescents with lowered FEV_1 when $PM_{2,5}$ concentrations were higher (18).

Katsouyanni et al., 2011 on the basis of meta-analysis for short-term effects of fine particulate matter and toxic gasses in the atmospheric air, concluded that the most of authors reported about adverse influence of air pollutants, which appears as an increase of overall and hospital mortality from respiratory and heart diseases as well as exacerbation of existing respiratory diseases. The impact assessment shows that the diminution of daily average concentrations of PM_{10} to 20 mg/m^3 all over the Europe will lead to reduction of premature death by 15 cases per 100 000 annually. The authors consider the need for further research projects clarifying the effects of air pollution on human health (19).

The effects of airborne pollutants on the immune system have been most widely studied in the respiratory tract (20, 21). Some understanding of the immune mechanisms underlying enhanced disease is required. IgA play a significant role in mucosal immunity including respiratory immune response. In this study IgA containing immune complexes (IgAIC), as a marker for mucosal immunity, were determined in plasma of selected groups of students by ELISA. Our results demonstrated significantly higher level of IgAIC in group of Stara Zagora then groups of Kazanlak and Chirpan. Enhanced immune complex is associated with increased immune activation, particularly with B-cell mediated immune response. Prevalence of IgA containing IC in blood combined with secretory and mucosal activities of IgA suppose involvement of these complexes in airway hyperresponsivess. Elevated levels of immune complexes, detected in plasma of students from Stara Zagora showed more activation than the suppression of the immune system and this refers mainly to antibody production. Our data are in the same direction, as data of Hadnagy et al., 1996 for activating the immune system in residents of two industrial cities of Germany (22). These changes in the immune system can lead to increased risk of developing chronic lung disease.

In the same direction are results from determination of IL-10 quantity in plasma. It was detected the highest concentration of IL-10 in plasma of students from Stara Zagora. Подобни резултати съобщават Calderón-Garcidueñas at al., 2003 reported similar results about increased level of IL-10 and IL-6 in 5 to 17 years children living in environmentally polluted areas. (15). Barraza-Villarreal et al. 2008 found increased IL-8 levels in nasal secretion from children suffering from asthma (1,07 pg/ml), as well as in children without asthma (1,16 pg/ml), associated with exposure to higher concentrations of $PM_{2,5}$ (10).

IL-10 is a main cytokine responsible for IgA class switching leading to IgA production and IgA immune complex formation. As well known Interleukin-10 (IL-10) is the most important regulatory cytokine that controls the progress of the immune response. IL-10 has immunosuppressive effects on the inflammatory and cell-mediated immune response because of its inhibiting functions on the proinflammatory cytokine production from activated macrophages and the Th1 cytokine synthesis. In the same time, IL-10 has been shown to have stimulatory effects on B cell growth and differentiation and to facilitate the induction of Th2 cell types, favored IgA class switching and IgA antibody production.

Data from measurement of air pollution shown the highest concentrations of PM_{10} and $PM_{2,5}$, SO_2 and NO_2 in Stara Zagora. $PM_{2,5}$ concentrations exceeding the limit value were found in 57.1% of all samples from Stara Zagora, in 28.6% of samples from Chirpan and in 14.3% of samples from Kazanlak.

Taken together these data suppose that atmospheric air pollutants, may alter mucosal immunity to enhance IgA production by initiating a Th2 cytokine environment.

Modulation of respiratory immune response as a result of exposure to air pollutants could bring to enhanced susceptibility to respiratory infection and increased susceptibility to allergic lung disease.

In conclusion, the unfavorable changes in respiratory function and activation of mucosal immunity, which were most expressed in the adolescents from Stara Zagora could be related with the highest air pollution in the town, especially with fine particulate matter.

AKNOLEDJEMENTS

This research was in the frame of Project “Assessment, reduction and prevention of air, water and soil pollution in Stara Zagora region”, Norwegian cooperation programmes with Bulgaria, 2009–2011. The authors would like to thank for support and opportunity to participate in the Project.

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