Evaluation of Circulating Immune Complexes in Healthy Smokers

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Abstract

Background: The phagocytic activity of macrophages is important to clear naturally occurring circulating immune complex (CIC) from the body. Smoking leads to a decrease in their phagocytic ability.
Objectives: The aim of the present study was to investigate CIC in healthy smokers.
Methods: The study included 33 subjects, divided into 2 groups. Group 1 consisted of 18 healthy smokers [female/male: 12/6, mean age: 34 (23-57), mean duration of smoking: 12.5 years (6-30 years)]. Group 2 included 15 healthy nonsmokers [female/male: 9/6, mean age: 30 (25-48)]. The symptoms associated with circulating immune complex diseases were investigated. General physical examination and examination directed to autoimmune and collagen-vascular diseases were performed. Serum and urine samples were obtained for analysis in all subjects.
Results: Protein electrophoresis was in normal ranges. Rheumatoid factor, anti-DNA, Coomb's test, and cryoglobulin examination were negative in all subjects. Protein was not found in the urine samples. Mean serum Ig G and IgA levels was lower in group 1 than in group 2 ($p < 0.05$). There was no significant difference between groups with respect to mean serum IgE and IgM levels. Serum C3 levels were higher in the smoker subjects ($p=0.002$). Mean CIC level was 0.263±0.183 mgEq/ml in group 1 and was 0.216±0.104 mgEq/ml in group 2 ($p>0.05$).
Conclusions: Our results suggest that smoking does not lead to immune complex diseases. We conclude that while this result is being evaluated, several factors such as the role of differences in immune complexes, research methods, selection of the subjects, and low number of the subjects should be taken into account.

Turkish Respiratory Journal, 2001;2 (3):32-35

Key words: *circulating immune complexes, evaluation, healthy smokers*

Abbreviations: CIC: *Circulating immune complexes*, C3: *Complement 3*, CI: *Confidence interval*

Introduction

Cigarette smoking is a major public health problem. The number of deaths attributed to cigarette smoking in the United States is estimated to be more than 400.000 annually (1). It is known that cigarette smoking is the major risk factor associated with the development of chronic obstructive pulmonary disease and lung cancer (2). Also cigarette smoking affects many of the organs or systems from heart to gastrointestinal system (1). The number of studies investigating the effects of smoking on the immune and inflammatory responses has been increasing (3-5). Smoking leads to an increase in the number of alveolar macrophages while decreasing their phagocytic ability (4). The phagocytic activity of macrophages is important to clear natu-

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rally occurring circulating immune complexes from the body (4,6). In cases in which phagocytosis is insufficient, they may be deposited in endothelial and vascular structures, giving rise to damaged tissues and increased inflammatory response, all of which have been associated with several diseases (7). These results raise the question as to whether smoking cause accumulation of immune complexes in various structures, resulting in a multiplicity of diseases. We aimed to investigate the circulating immune complexes in the healthy smokers and the parameters demonstrating the damage produced by circulating immune complexes.

Methods

Subjects

The present study included 33 subjects, divided into 2 groups. Group 1 consisted of 18 smoking subjects [female: 12, male: 6, mean age: 34 (range 23 to 57), mean duration of smoking: 12.5 years (range: 6-30)]. Thirteen subjects were heavy smokers (<20/day) and 5 were light smokers (<20/day) (4). Group 2 included 15 nonsmoker subjects [female: 9, male: 6, mean age: 30 (range 25 to 48)]. Two groups were comparable with respect to age and sex ($p>0.05$). All of the subjects had normal pulmonary function test and normal chest x-ray. Informed consent was obtained from all subjects.

Study design

The smoking history of the subjects was determined. The symptoms associated with circulating immune complex diseases were investigated. General physical examination and examination directed to autoimmune and collagen-vascular diseases were performed. Blood and urine samples were obtained. Serum samples had been stored at -20°C until they were examined.

Laboratory studies

Standard techniques were used. Circulating immune complexes were measured by using C1q-enzyme immunoassay method. Circulating immune complexes from patient sera are incubated with human C1 adsorbed to wells of microtiter plates. After washing, an alkaline phosphatase labelled tracer is added, attaching to the Fc-region of human immunoglobulins. After another washing, the enzyme substrate is pipetted followed by a stopping reaction. The absorption is read at 405 nm. The upper limit of normal value for circulating immune complexes is 0.96 mgEq/ml. Serum IgE level was quantified by ELISA method. The upper limit of total IgE level is 180 IU/ml. Serum IgG, IgM, IgA and C3 levels were measured by radial immunodiffusion method. Normal serum value is between 800 and 1800 mg/dl for IgG, 70 and 280 mg/dl for IgM, 90 and 450 mg/dl for IgA, and 55 and 120 mg/dl for C3. Rheumatoid Factor and Anti-n-DNA were measured by using latex agglutination method. Cellulose acetate method was used for protein electrophoresis. Coomb's test was performed by reactivas cromatest method.

Statistical analysis

The characteristics of the groups were compared by using student-t and Fisher Exact tests. The results of the groups were compared using the Mann-Whitney U test.

Results

In either group, arthralgia, purpura, or other signs and symptoms suggestive of collagen-vascular diseases were not detected. There was no hematological pathology. Protein electrophoresis was in normal ranges. All cases were found to be negative in terms of rheumatoid factor and anti-DNA, Coomb's test and cryoglobulin examination. Protein was not found in the urine samples. Table 1 shows serum IgA, IgE, IgM, and IgG levels of the groups. Mean serum IgG and IgA levels was lower in Group 1 than in Group 2 ($p<0.05$). There was no significant difference between Group 1 and Group 2 with respect to IgE and IgM levels ($p>0.05$). The serum level of circulating immune complexes and C3 of the groups are shown in Table 2. Serum C3 levels were higher in the smoker subjects ($p<0.005$). Five nonsmoker subjects had higher serum C3 levels than the upper limit of normal range. Serum circulating immune complex levels were in the normal ranges in all subjects and there was no significant difference between Group 1 and Group 2 ($p>0.05$).

Discussion

Smoking leads to a decrease in the phagocytic activity of macrophages (4). Macrophages are the major cells respon-

Table 1. Serum IgA, IgE, IgG, and IgM levels of the groups

	Group 1	Group 2	p value
IgA			
Mean ± SD	212.2 ± 81.7†	311.7 ± 137.8†	†p = 0.019
Median	215.5	285.0	
95% CI	179.5-240.5	235.3-388.0	
IgE			
Mean ± SD	41.3 ± 34.4‡	54.8 ± 46.4‡	‡p>0.05
Median	35.5	39.0	
95% CI	13.2-101.1	29.2-80.6	
IgG			
Mean ± SD	1579 ± 397.5 §	1782 ± 278.4 §	§ p= 0.041
Median	1466	1760	
95% CI	1381.8-1777.2	1628.5-1936.8	
IgM			
Mean ± SD	274.1 ± 100.1*	303.8 ± 108.4*	*p> 0.05
Median	247.5	290.0	
95% CI	224.3-323.9	243.8-363.9	

Table 2. Serum C3 and circulating immune complex levels of the groups

	Group 1	Group 2	p value
C3			
Mean ± SD	158.5 ± 27.3†	121.4 ± 31.1†	†p = 0.002
Median	163.5	116.0	
95 % CI	144.9-172.1	104.2-138.6	
CIC			
Mean ± SD	0.263 ± 0.183*	0.216 ± 0.104*	*p > 0.05
Median	0.21	0.14	
95 % CI	0.16-0.36	0.13-0.31	

sible for phagocytosis of circulating immune complexes (4,6,8,9). Circulating immune complexes are a natural defense mechanism of the body which are produced by the interaction of antigens and antibodies. If phagocytosis is insufficient, they may be deposited in endothelial and vascular structures and may cause several diseases (7).

In our study, C1q enzyme immunoassays and cryoglobulin assays were used to identify possible circulating immune complexes. Rheumatoid factor activity was tested to detect antiglobulins which might have produced immune complexes. Serum-protein electrophoresis was performed to show plasma cell proliferation and paraproteinaemia. Moreover, assays for C3 and urine protein were carried out to detect immune complexes by their complement-activating properties, and immune complex-induced renal injury, respectively.

Our results suggest that smoking does not lead to immune complex diseases. There was no significant difference between Group 1 and Group 2 with respect to circulating immune complex levels. Also, smokers' circulating immune complex levels were in the normal ranges. Cryoglobulin was found as negative in all subjects. None of the subjects had positive rheumatoid factor activity. Protein electrophoresis was in normal ranges. None of the subjects had symptoms and findings of immune complex diseases. In our study, although serum concentrations of IgA, IgG, IgM, and IgE were lower in smokers than in nonsmokers, the p values for IgG and IgA reached statistical significance. It is known that concentrations of these immunoglobulins are reduced by 10-20% in the serum of smokers (4,10-12). Serum concentration of IgE shows biphasic changes. Serum IgE levels of heavy smokers (<20/day) are below those of nonsmokers (13). There were 13 heavy smokers and 5 light smokers among our smoking subjects. We found that serum C3 concentration was higher in smokers than in nonsmokers. *In vitro* studies show that smoking is capable of directly activating the complement pathway (14). Five nonsmoking subjects had high serum C3 concentration in our series. The reasons of high serum C3 levels in nonsmokers may be environmental tobacco smoke or air pollution (15,16). Serum complement levels are also associated with body mass index, serum glucose level and serum lipid levels of the subject (17).

Cosio et al. (3) reported that cigarette smoking decreased binding of immune complexes by macrophages. Palosuo et al. (18) explained that there was no difference between smokers and nonsmokers with respect to CIC. This finding is similar to our result. It has been known that the technique used to detect circulating immune complexes affects the results considerably. Elevated values obtained from the study of Cano et al. (19) have been attributed to the diverse methods employed. Stein et al. (20) report that the Raji cell technique and C1q binding assays lack sufficiency related to the size of complexes and to the immunoglobulin classes producing immune complexes. They have recommended that while seeking immune complexes, at least two methods, e.g., monoclonal rheumatoid factor and cryoglobulin assay, should be employed along with studies depending upon complement binding techniques (20).

In conclusion, our results suggest that smoking does not lead to immune complex diseases. We conclude that while this result is being evaluated, several factors such as the role of differences in immune complex research methods, selection of the subjects, and low number of the subjects should be taken into account.

Acknowledgement: The authors thank Dr Mualla Partal and Dr Okşan Güner for their helps in this study.

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