Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2006, Article ID 85019, Pages 1–4
DOI 10.1155/MI/2006/85019

Research Communication

Comparison of Effects of Smoking and Smokeless Tobacco "Maras Powder" Use on Humoral Immune System Parameters

Murat Aral,[1] Hasan Cetin Ekerbicer,[2] Mustafa Celik,[3] Pınar Ciragil,[1] and Mustafa Gul[1]

[1] *Department of Microbiology, KSU Medical School, 46050 Kahramanmaras, Turkey*
[2] *Department of Public Health, KSU Medical School, 46050 Kahramanmaras, Turkey*
[3] *Department of Family Medicine, KSU Medical School, 46050 Kahramanmaras, Turkey*

Received 26 November 2005; Revised 8 February 2006; Accepted 14 February 2006

Background. The aim of this study is to assess the impacts of "*Maras powder*" and cigarette smoking on the parameters of the humoral immune system. *Material and Methods.* One hundred seventy seven subjects were included in the study. The IgA, IgG, IgM, C3 and C4 levels were detected via nephelometric method. *Results*. In 1.4% of the control group IgM levels were below normal where it was 10.8% and 18.6% in Maras powder group and in cigarette smoking group respectively. The IgM levels of both groups were significantly lower compared to the control group ($P < .05$). Nonetheless, the IgE levels of Maras powder group and smoking group were found to be remarkably higher compared to the control group ($P < .01$). *Conclusion.* Effects of *Maras powder* on humoral immune response were found to be similar to that of smoking.

BACKGROUND

In Turkey a kind of smokeless tobacco called *Maras powder* has a lot of addicts in the city of Kahramanmaraş and its surroundings [1–3]. The leaves of Nicotina rustica L. are powdered, mixed, crushed with the ash obtained from the oak, walnut tree, or vine stick in the proportion of 1/2 or 1/3, and humidified a little before it is used. It is known that the ash blended during the preparation stage of *Maras powder* eases the absorption of nicotine from the mouth mucous membrane by making the medium alkaline. *Maras powder* is a kind of smokeless tobacco that is used by the addicts through buccal mucosa instead of cigarette or in order to give up smoking. On the other hand, it is more addictive than smoking. Its negative impacts on human health could not yet be understood fully. A similar kind of smokeless tobacco used in Sudan is known as *Toombak*. It is reported that *Toombak* use may play an important role in the etiology of oral squamosus cell carcinoma of the oral cavity and also may be associated with salivary gland cancers [4, 5]. Due to the fact that it is taken orally, it is reported that the cronic stimulation of the lenfoid tissues in oral mucous membrane may be related to the increased gingivitis, leukoplacis, and oral cancer incidence. Similarly, it is stated that it has a stronger potential of leading to addiction compared to cigarette smoking because of its higher nicotine concentration and prolonged mean usage time [6].

Most of the diseases related to smoking have been known in detail so far. More than 400 000 people die in the USA because of smoking and the direct expenditures for medical purposes regarding smoking-related morbidity exceed 50 million US dollars. The effect of smoking on the immune system and its parameters is not understood fully and the data about this is limited and somewhat contradictory. The studies up till now put forth that the immunotoxic and genotoxic impacts of cigarette arise from the particle phase more than the smoke-phase. The particle phase is composed of thousands of substances, but mainly nicotine. There are a lot of findings about the fact that nicotine is the major immunosuppressive in cigarette and/or smokeless tobacco. Nicotine causes the secretion of chatecolamines that have suppressive effects on immune system by inducing ACTH secretion [7]. It is crucial to clarify the relation between tobacco smoking and immune system in order to understand this biological process fully.

The aim of this study was to determine the effects of *Maras powder* use and cigarette smoking on the parameters of humoral immune response.

MATERIALS AND METHODS

The study was conducted between January 2004 and June 2004 in Kahramanmaras Sutcu Imam University, Faculty of Medicine. All subjects included in the study population were healthy volunteers recruited from visitors. Informed consent was obtained from all the subjects participating in the study. The control group was composed of 33 women (41.1%) and 37 men (52.9%) with no history of smoking or *Maras powder* usage; the smokers group was composed of 31 women (42.2%) and 39 men (55.8%); and *Maras powder* group was composed of 17 women (45.9%) and 20 men (54.1%). The mean ages were 37.8 ± 12.5 SD (standard deviation) (min. 19–max. 73), 37.6 ± 12.0 SD (min. 20–max. 71), and 41.9 ± 10.2 SD in the control, smoking, and *Maras powder* groups, respectively. Mean ages and the sex distribution of the groups were similar ($P > .05$). Selection criteria of the individuals were as follows: cigarette smokers have been smoking a pack of cigarettes (twenty in number), *Maras powder* addicts have been using at least 2 packs of *Maras powder* (a pack is of 16 ± 3 g SD) for at least 5 years and nonpassive smoking for control and *Maras powder* users.

The blood samples are collected from each subject by venipuncture of the cubital veins before labour and frozen at −20°C after aliquoting their sera until they are studied. Blood samples were analyzed for concentrations of the humoral immune system parameters (IgE, IgM, IgG, IgA, C3, and C4) using nephelometry (Dade Behring, Marburg GmbH, Germany). The normal values of humoral immune system parameters were accepted as 0.70–4.00 g/L for IgA, 7.0–16.0 g/L for IgG, 0.4–2.3 g/L for IgM, 0–100 IU/mL for IgE, 0.90–1.80 g/L for C_3, and 0.10–0.40 g/L for C_4. The values under these ranges were defined as "low" (there is no "low" value for IgE since its range starts from 0unIU/mL), and over these ranges as "high."

Data were expressed as mean values ± SD, median and range, or as number of subjects and percentages. Either nonparametric or parametric (if data was normally distributed) tests were used for statistical analyses. ANOVA and Kruskal-Wallis variance analysis (followed by post-hoc Mann-Whitney U test where needed) were used for comparison of numerical data. Chi-square tests were performed on categorical data. P values < .05 were considered statistically significant. Analyses were performed by using SPSS software, version 9.05 for Windows (SPSS Inc., Chicago, Ill).

RESULTS

Comparison of humoral immune system parameters in study groups were shown in Table 1. No statistically significant difference was detected among the parameters except for IgE ($P > .05$). IgE values of *Maras powder* and smoking groups were significantly higher than that of the control group ($P < .05$). Furthermore, the distribution of subjects according to ranges of humoral immune system parameters was compared (Table 2). For this purpose, data was classified as low, normal, and high for each parameter. The distribution of normal values was similar for each parameter except IgM and IgE. In control group 1.4% of the subjects had IgM values below normal where it was 18.6% and 10.8% in smoking and *Maras powder* groups, respectively ($P < .05$). After chi-square analysis, it was seen that IgE values above the normal had been similar in the smoking and *Maras powder* groups and significantly higher compared to the control group ($P < 0.01$).

DISCUSSION

It was stated that using tobacco affects both the cellular and humoral immunity negatively in various ways. Nevertheless, the level of the negative effects could not yet be explained clearly. In a number of studies carried out for finding out the possible effects of cigarette smoke on lymphocytes, a leukocytosis accompanying the increase in all lymphocyte populations is mentioned. The relation between cigarette smoking and effects of cigarette smoke on in vitro lymphocyte functions is disputatious. Some authors reported that there had been significant reductions in the proliferative response of lymphocytes to T cell mitogens. On the other hand, some others reported that there had been no significant difference in terms of this response between smokers and nonsmokers. Besides, it was stated that these differences might have been influenced by age, sex, dosage, duration of exposure, and ethnic origin [8–15]. Goud et al [6] reported increases in lymphocyte proliferation and polyclonal IgM response caused by smokeless tobacco. However, Lindemann and Park [16] reported that water-soluble smokeless tobacco extract had anticytolytic and antiproliferative effects on peripheral lymphocytes. Possible causes for this disagreement could not be determined. In white subjects, it was shown that cigarette smoking was closely related to a series of immunological deteriorations accompanied by decreases in immunoglobulin levels, the number and the functions of NK (natural killer) cells, and the number of T cell subgroups [9, 11, 12, 14]. Moszczynski et al [17] had observed a decrease of CD4/CD8 ratio due to the decrease in the serum concentration of lysozyme and immunoglobulins and a decrease in the number of ($CD\ 16^+$) NK cells particularly in the addicts who had smoked for more than 10 years and an increase in the number of ($CD\ 8^+$) cytotoxic T lymphocytes. It was shown that smoking decreases serum levels of almost all types of immunoglobulins except IgE (IgE increases) [18–20]. There are also some articles that defend the idea that it has no particular effect on immunoglobulin levels [7].

In our study, rates of subjects with a serum IgM level below normal were significantly higher in smoking and *Maras powder* groups than in control group ($P < .05$). However, the differences of serum IgM levels between the 3 groups were not statistically significant. This may be interpreted as contradictory. When the data was studied carefully, it was seen that the IgM values below normal were very close to the lower limit. As for IgE levels, rates of subjects with values above normal were significantly higher in smoking and *Maras powder* groups than in control group ($P < 0.01$). Nearly all of the individuals (86.5%–95.7%) have levels of IgA, IgG, C3, and C4 within the normal limits.

Table 1: Comparison of humoral immune system parameters in study groups.

	Control ($n = 70$)		Smoking ($n = 70$)		*Maras powder* ($n = 37$)		P
	Mean ± SD	Min.–Med.–Max.	Mean ± SD	Min.–Med.–Max.	Mean ± SD	Min.–Med.–Max.	
IgA (g/L)	2.2 ± 0.9	0.6–2.0–5.7	1.9 ± 0.8	0.5–1.8–4.8	2.3 ± 0.9	0.3–2.3–4.5	> .05
IgM (g/L)	1.3 ± 0.5	0.4–1.3–3.2	1.3 ± 0.7	0.3–1.2–3.3	1.2 ± 0.4	0.5–1.2–2.3	> .05
IgG (g/L)	11.7 ± 2.7	7.9–11.3–20.8	12.9 ± 2.3	8.2–12.9–17.8	12.0 ± 3.7	6.9–11.8–31.3	> .05
IgE (IU/mL)	64.6 ± 43.9	18.0–54.0–236.0	127.9 ± 76.2	18.6–113.5–290.0	160.5 ± 85.9	119.8–172.0–351.0	< .01*
C3 (g/L)	1.3 ± 0.3	0.7–1.3–1.9	1.4 ± 0.3	0.8–1.4–2.0	1.1 ± 0.3	0.4–0.9–1.8	> .05
C4 (g/L)	0.3 ± 0.0	0.1–0.2–0.6	0.2 ± 0.0	0.1–0.2–0.5	0.2 ± 0.1	0.1–0.2–0.8	> .05

*The difference arose from control group.

Table 2: Distribution of the humoral immune system parameters with respect to low, normal, and high ranges in study groups.

	Low n (%)	Normal n (%)	High n (%)	Total n (%)	P
IgA* (g/L)	< 0.70	0.70–4.00	> 4.00	—	—
Control	1 (1.4)	67 (95.7)	2 (2.9)	70 (100.0)	—
Smoking	7 (10.0)	62 (88.6)	1 (1.4)	70 (100.0)	—
Maras powder	3 (8.1)	34 (91.9)	—	37 (100.0)	—
IgM (g/L)	< 0.4	0.4–2.3	> 2.3	—	—
Control	1 (1.4)	68 (98.6)	—	70 (100.0)	< .05*
Smoking	13 (18.6)	57 (81.4)	—	70 (100.0)	—
Maras powder	4 (10.8)	33 (89.2)	—	37 (100.0)	—
IgG* (g/L)	< 7.0	7.0–16.0	> 16.0	—	—
Control	1 (1.4)	66 (94.3)	3 (4.3)	70 (100.0)	—
Smoking	—	66 (94.3)	4 (5.7)	70 (100.0)	—
Maras powder	1 (2.7)	35 (94.6)	1 (2.7)	37 (100.0)	—
IgE (IU/mL)	—	0–100.0	> 100.0	—	—
Control	—	53 (75.7)	17 (24.3)	70 (100.0)	< .01*
Smoking	—	25 (35.7)	45 (64.3)	70 (100.0)	—
Maras powder	—	10 (27.0)	27 (73.0)	37 (100.0)	—
C3* (g/L)	< 0.90	0.90–1.80	> 1.80	—-	—
Control	—	62 (88.6)	8 (11.4)	70 (100.0)	—
Smoking	—	65 (92.6)	5 (7.1)	70 (100.0)	—
Maras powder	1 (2.7)	32 (86.5)	4 (10.8)	37 (100.0)	—
C4* (g/L)	< 0.10	0.10–0.40	> 0.40	—	—
Control (none)	—	66 (94.3)	4 (5.7)	70 (100.0)	—
Smoking	—	67 (95.7)	3 (4.3)	70 (100.0)	—
Maras powder	2 (5.4)	33 (89.2)	2 (5.4)	37 (100.0)	—

*Statistical analysis could not be performed due to the limited number of cases.
**The difference arose from control group.

In some studies it was reported that alcaloid, nitrosamine, and nicotine contents distinguish between Nicotiana rustica L. and Nicotina tobacum L. alcaloid content of *Maras powder* (Nicotiana rustica L.) may be 6–10 times more than that of Nicotina tobacum L. [21]. It was reported that tobacco-specific nitrosamine content of *Toombak* was one hundred times more than that of other types of smokeless tobacco used in Sweden and USA, and *Toombak* use was described as the highest nonoccupational carcinogen exposure documented [4, 5]. Three times more urinary cotinine levels were found in *Maras powder* addicts than in smokers [22]. However, Lindemann et al reported that plasma nicotine levels were found to be similar in smokeless tobacco users and smokers [23]. Nevertheless, in our study, the effect of *Maras powder* on humoral immune response was found to be similar to that of smoking.

As noted earlier in our study, many of the researchers stated that smoking affects the immune system and its parameters negatively at various levels. Nonetheless, the level of this negative effect could not be determined yet effectively

and evidently. The information in literature does not show any coherence at all. We believe that further studies investigating the effects of different types of tobacco usage on humoral immune system parameters are needed.

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