The investigation of the effect of Maraş powder (smokeless tobacco) on hematological parameters

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ABSTRACT

Nicotine is used in different forms including smokeless tobacco. A special kind of smokeless tobacco also known as Maraş powder (MP) is widely used in southeastern region, especially Kahramanmaraş, Gaziantep and other southeastern cities of Turkey. The aim of this study was to investigate the effect of nicotine on hematological parameters in MP users. Ninety-two MP users from Kahramanmaraş and its environs and sixtyeight healthy controls who did not use MP were included in the study. We measured haematological parameters in the blood samples of MP users and controls. Our results showed that while iron and WBC levels were higher in MP users than the controls ($p< 0.001$), monocyte and platelet counts were lower ($p< 0.05$ and $p< 0.001$, respectively). Increased leukocyte counts in MP users may be an indicator of the present inflammatory events in various tissues. So, we assume that MP, because of either high nicotine content or high tobacco-specific nitroso amines levels (TSNA), causes chronic inflammatory changes in various cells, organs and systemic circulation.

Key Words: Hematological values, Maraş powder, Smokeless tobacco.

ÖZET

Maraş tozunun (dumansız tütün) hematolojik parametreler üzerine etkisi

Nikotin dumansız tütün dahil olmak üzere çeşitli şekillerde kullanılır. Dumansız tütünün özel bir şekli olan Maraş tozu başta Kahramanmaraş, Gaziantep ve diğer Güneydoğu illeri olmak üzere Güney Doğu Anadolu bölgesinde yaygın olarak kullanılır. Bu çalışmanın amacı dumansız tütünde bulunan nikotinin hematolojik parametrelere olan etkisini araştırmaktır. Kahramanmaraş ve çevresinden 92 Maraş tozu kullanıcısı ve 68 tütün kullanmayan kontrol çalışmaya alındı. Demir ve lökosit düzeyleri Maraş tozu kullananlarda yüksek bulunurken ($p< 0.001$), monosit ve trombosit düzeyleri düşüktür ($p< 0.05$ ve $p< 0.01$). Maraş tozu kullananlarda lökosit sayasındaki yükseklik çeşitli dokulardaki inflamatuvar etkiye bağlanabilir. Buna göre Maraş tozu, gerek içerdiği nikotin, gerekse tütüne özgül nitrozamin düzeylerinin çeşitli hücre, organ ve sistemik dolaşımda kronik inflamatuvar değişikliklere yol açtığı öne sürülebilir.

Anahtar Kelimeler: Hematolojik değerler, Maraş tozu, Dumansız tütün.

INTRODUCTION

The use of tobacco as a drug substance has been used throughout the world although it has dangerous effect on human health[1]. An interesting kind of smokeless tobacco (ST) known as Maraş powder (MP) is very common almost in all regions of Turkey, especially in the cities of southeast Anatolia (Kahramanmaraş, Gaziantep and Adıyaman)[2,3]. This kind of ST has been called as Toombak in Sudan and it has similarities in structure and the way of use[4]. It is assumed that Toombak has been imported from Turkey or Saudi Arabia to Sudan[5]. Basically, Nicotina rustica L. (NRL), a tobacco species, is used to prepare MP. Their leaves are powdered, and then mixed with ashes of oak or grapevine woods in 1:2 ratios. This mixture is packed in 10 g-nylon bags and sold in local groceries. The ready availability and the inexpensive price give rise to high consumption of ST. Although never been studied before, prevalence of MP is thought to be highly common. MP is simply applied between lower labial mucosa and gingiva for about 5-10 min, and then spit out. The mixture is used 3-6 times/a day in the amount of 1-2 g for each time. Smokers do not usually want to smoke cigarettes after they start using MP because of high nicotine amount which induce dependence among users.

NRL plant contains tobacco-specific alkaloids, including 4-(nitrosomethyl-amino)-1-(3-pyridil)-butanon (NNK), N' nitrosonornicotine (NNN), N' nitrosoanabasine (NAB) and nitrosoanatabin (NAT)[5]. The use of mixture around 10 g/day leaves approximately 64-92 mg of tobacco-specific nitrosamine (TSNA) inside the mouth[1]. The effect and amount of carcinogenic substances found in tobacco is dependent upon the age of tobacco, and the temperature and humidity of the place in which tobacco is stored. The amount of TSNA reaches to 100% after six month keeping time. The pH value is important at absorption function by lip cells. At pH 5.84, only 10% of nicotine is found as a free base, while at pH 7.99, 59% of nicotine is present in unprotonated form, that is quickly absorbed through the mucosal membranes of the oral cavity[1]. Nicotine absorption also increases when alcohol and alcohol beverages are consumed with the use of tobacco[6]. Snus, which is another kind of snuff, used in Sweden has nicotine of 5-11 mg/dry matter and NRL contains nicotine of 8-102 mg/g dry matters[5]. NRL has nicotine 5-8 times more than that of nicotina tabacum L (NTL), which also gives rise to high TSNA compared with cigarettes[2]. Tobacco specific N-nitrosamines are the group of carcinogens, derived from the tobacco alkaloids, which are likely the causative factors for cancers of lung, esophagus, pancreas and oral cavity in people using tobacco products[4].

Cigarette smoking is known to be associated with peripheral and coronary arteries disease, chronic lung disease and cancer mortality[6,7]. Previous studies showed that nicotine increased the peripheral blood leukocyte count. Peripheral blood leukocyte count is important predictor of many disease[6-9].

The aim of this study is to evaluate the effect of MP and tobacco on haematological parameters.

MATERIALS and METHODS

Ninety-two male MP users and 45 cigarette smokers from Kahramanmaraş city and its provinces, and 68 healthy males who did not use MP or cigarette or any other medicine were included in the study. The average year of subjects for powder use was 15.78 ± 8.98 years (1.0-33.0 years). Some subjects were using cigarettes also. Their average time for using cigarettes were 6.03 ± 8.59 years (1.0-30.0) and the amounts were 0.28 ± 0.48 pocket/day. About 4-5 mL venous blood samples were collected from the MP user, cigarette smokers and the control group. The blood samples were obtained from the subjects after overnight fasting. Blood samples were collected in plastic tubes containing EDTA and immediately taken to the laboratory. After the

haematological parameters were measured than the blood samples were centrifuged at 2000 rpm for 20 minutes and plasma was then separated. Haematological parameters such as haemoglobin (Hb), hematocrit (Hct), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) and leukocyte types percents (neutrophil, lymphocyte, monocyte, eosinophil, basophil) and platelet counts were studied with an automatic electronic blood count analyser (Cell Dyne 3700, Abbott diag. USA).

Transferrin (Tf) and soluble transferrin reseptor (sTfR) (measurement by latex agglutination) measured with nephelometer (Dade Behring BN 100, Germany)[10]. Ferritin was measured with hormon analyser (Chemiluminescent, Access, Beckman, USA).

For the analysis of the mean, standard deviation and differences of the data Epi Info 6.0 computer program was used.

RESULTS

MP user, cigarette smokers and control group's erythrocyte parameters and leukocyte cells counts are shown in Table 1 and Table 2.

Table 1. Erythrocyte parameters in smokers and Maraş powder (MP) user and controls

	Smokers (n= 45)	MP user (n= 92)	Control group (n= 68)
Age (year)	32.42 ± 4.90	36.58 ± 9.66	33.96 ± 6.09
RBC (M/µL)	5.37 ± 0.36 **b**	5.04 ± 0.48	5.12 ± 0.31
Hgb (g/dL)	15.32 ± 1.21	15.03 ± 1.27	15.08 ± 0.90
Hct (%)	46.36 ± 7.28 **c**	43.70 ± 3.67	44.25 ± 2.68
MCV (fL)	83.37 ± 10.33 **c**	86.84 ± 4.28	86.54 ± 2.58
MCH (pg)	28.69 ± 2.41 **c**	29.89 ± 1.92	29.46 ± 1.16
MCHC (g/dl)	33.36 ± 2.86	34.42 ± 1.25 **d**	33.95 ± 1.32
RDW	15.10 ± 1.02 **a**	14.60 ± 0.91	14.25 ± 0.78
STfR (mg/L)	2.07 ± 0.55 **ab**	1.39 ± 0.32	1.45 ± 0.34
Tf (mg/L)	2.64 ± 0.44	2.65 ± 0.39	2.74 ± 0.45

Significant difference between smokers and control groups; **a**: $p< 0.01$, smokers and MP users, **b**: $p< 0.01$ and, **c**: $p< 0.05$, MP users and control group, **d**: $p< 0.05$ were found.

Table 2. Comparison of leukocyte cells in smokers and MP user and controls

	Smokers (n= 45)	MP (n= 92)	Control group (n= 68)
WBC (K/µL)	9.04 ± 2.32 **ab**	8.43 ± 1.87 **c**	6.69 ± 1.29
Neutrophil (%)	59.26 ± 7.57 **ab**	53.80 ± 9.15	54.67 ± 8.68
Lymphocyte (%)	28.77 ± 7.39 **ab**	35.73 ± 8.76 **d**	32.99 ± 7.24
Monocyte (%)	8.08 ± 1.54	6.88 ± 2.01 **d**	7.58 ± 1.29
Eosinophil (%)	2.50 ± 1.38	2.80 ± 1.59	3.38 ± 2.26
Basophil (%)	0.76 ± 0.24 **b**	0.86 ± 0.31 **c**	1.01 ± 0.38
Platelet (K/µL)	207.74 ± 50.47 **b**	227.18 ± 60.29	240.45 ± 42.74

Significant difference between smokers and control groups as; **a**: $p< 0.01$, smokers and MP users, **b**: $p< 0.01$, MP users and control groups, **c**: $p< 0.01$ and, **d**: $p< 0.05$ were found.

In MP user iron, monocyte and platelet counts were significantly lower than the controls' but WBC inverse significantly higher. Comparison of total leukocyte, neutrophil leukocyte and lymphocyte counts in smokers, MP users and control group are shown as a bar graphic in Figure 1.

Significant correlations between the duration of habitual use and WBC, neutrophil, lymphocyte were found to be r= 0.282, r= 0.339, r= 0.373 (p< 0.01) respectively. Negative correlations between the duration of habitual use and RBC, Hgb, Hct were found to be r= -0.444, r= -0.359, r= -0392 (p< 0.01) respectively.

DISCUSSION

NTL is the cultivated tobacco variety used for making cigarette. Alkaloid contents of the NTL and NRL are similar but nicotine quantity of NRL (MP) is 6 to 10 times higher than that of NTL. It has been accepted that the ash mixture transforms the alkaloids into the base form and provides their absorption from the buccal mucosa[11]. Previous cigarette smokers do not need cigarette any more after beginning the MP use, the reason of which is thought to be the physiological effect of nicotine compound of powder. The effects of MP on human health are not studied in detail.

In some studies, it has been reported that ST may have a mucosal carcinogenic effect[4,5,11]. Carcinogenic changes in oral mucosa of Toombak users have been reported to be largely due to chronic use of Toombak[4,5]. Carcinogenic N nitroso compounds are implicated as DNA damaging agents in cancers of digestive tract beginning from the oral mucosa[8]. Besides the carcinogenic effects of nicotine, one of its most important effects was on the endothelial cells. All subcellular organelles normally generate superoxide, hydrogen peroxide and a variety of free radicals[12]. Cell injury associated with free radical formation occurs either in situation of overwhelming scavenging systems or in disease states of depleting protective antioxidant systems[12,13]. One of the best indicators for cytological damage was lipid peroxidation, which could be demonstrated by MDA levels[14]. In our previous study on MP users we found that MDA levels in our subjects were higher than the control group[15]. One of the prominent risk factors for the increased lipid peroxidation was smoking[16]. In the literature, there have been many studies reporting that smoking increased lipid peroxi-

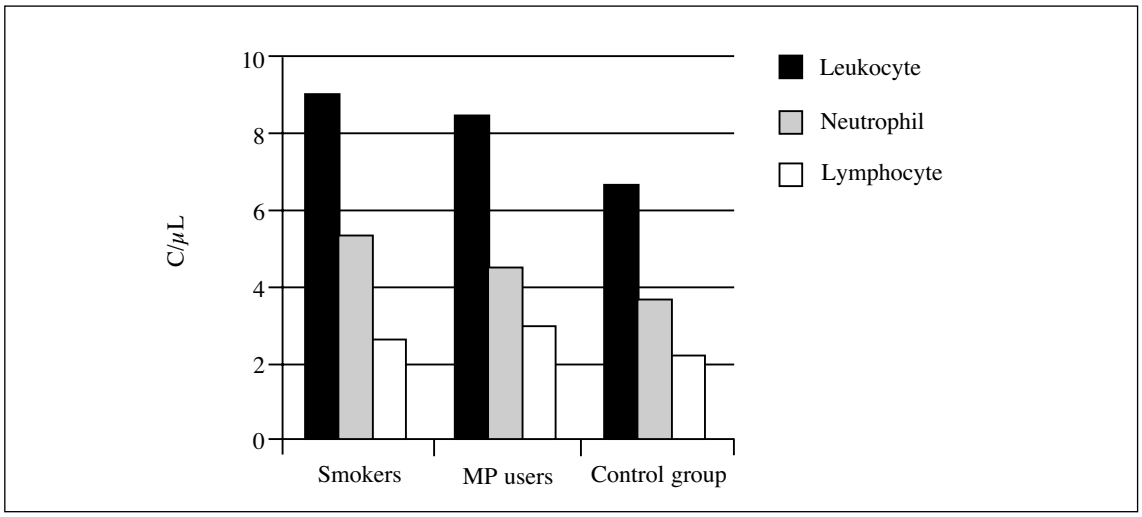

Figure 1. Comparison of total leukocyte, neutrophil leukocyte and lymphocyte counts in smokers, MP users and control group.

dation because of the presence of free radicals in cigarette smoke[16-20]. In some studies, leukocyte counts were found significantly high in cigarette smokers, which was reported to be due to chronic bronchitis or release of leukocytes from lymphoid organs to periphery[21-27]. In our study subjects demonstrated higher leukocytes counts than those of control group.

Another factor for increased leukocyte count is arteriosclerosis. Having a role in arteriosclerosis, oxidized LDL causes the release of proinflammatory lipids activating the endothelial cells, resulting in migration of mononuclear leukocytes into the atherosclerotic area[21,28]. Leukocytes, which enter the arterial intima, change into macrophages and these cells have an important role in atherosclerotic procedure. Increased leukocyte counts might cause the inflammatory event. Also, neutrophils contain metabolic active products like superoxide anion and H_2O_2. These products could be the cause of tissue damage[18]. Another factor for the arteriosclerosis is smoking. Smoking does reduce NO metabolites and causes arteriosclerosis[22].

Previous studies showed that in smoker RBC, Hgb, Hct and MCV counts were higher than the non-smokers'[23-26]. Our results of RBC, Hgb, Hct and MCV counts in MP users were higher than in control group but this did not reach statistical significance. In smokers MCV, MCH counts were lower but RDW and sTfR levels were higher than the controls. STfR is an important parameter of the iron deficiency anemia. It is different in some features from the ferritin. Since ferritin behaves as an acute phase reactant, it could be arisen to high levels in some situations.

As a result, in MP user while the WBC counts were higher, monocyte and platelet counts lower than the controls'. These data show that high WBC levels may be sign of inflammation in smokers and MP users and high sTfR level could be an indicator of iron deficiency in smokers. Consequently, the present study has shown that nicotine increases the inflammation, which may be responsible for the carcinogenic effects on oral mucosa and gastrointestinal tract, atherogenic effects on endothelial cells, and any other pathological events. Further comparative studies to be carried out on this subject will clarify the harmful effects of the powder on human health in detail.

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