

The effects of alcohol and smoking on serum, saliva, and urine sialic acid levels

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ABSTRACT

Objective: To investigate the effect of smoking and alcohol on serum, saliva, and urine total sialic acid (TSA) levels, and on serum gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) enzyme activities.

Methods: Serum, urine and saliva samples obtained from smokers, drinkers, and nonsmokers-nondrinkers (control) subjects. Total sialic acid was measured with the Warren's colorimetric method, modified by Pönnö et al. The study was performed at the Department of Chemistry, Division of Biochemistry, University of Kahramanmaraş Sutcu Imam, Turkey, in 2002.

Results: Serum and saliva TSA levels of alcohol drinkers and serum TSA levels of smokers were higher than those in control subjects. Urine TSA levels were much higher in alcohol drinkers than those in healthy subjects and smokers. Serum GGT activities were high

in smokers and alcohol drinkers and there was no statistically significant difference in serum AST levels between smokers and non-smokers and also serum ALT levels were higher in alcohol drinkers than those in control subjects and smokers. Serum ALT levels were higher in smokers and alcohol drinkers than those in controls.

Conclusion: Our results indicate that serum TSA were affected by, and possibly related to, smoking, and that serum GGT, AST, ALT and serum TSA can be used as a marker for monitoring of alcohol abuse. Our study indicate that urine, and saliva TSA can be used as non-invasive markers for alcohol abuse. However, further studies are necessary to evaluate the concentrations of TSA on a greater number of serum, saliva, and urine samples from smokers and drinkers.

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Cigarette smoking and alcohol abuse is very common all over the world^{1,2} and becomes health risk that can lead to a broad range of medical and social problems associated with a high cost for society.³⁻⁶ Many biochemical markers have been used for detecting and monitoring the alcohol abuse, such as gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).^{5,7} Recently, serum total sialic acid (TSA) has been suggested as a new marker of alcohol consumption. Moreover, Sillanauke et al⁸ showed that TSA in serum had the

highest efficiency for detecting the alcohol abuse when compared with the traditional alcohol markers (GGT, ALT, and AST).^{5,7} Elevated serum TSA has been reported in alcoholics^{5,7} and smokers⁸ as well as in patients with cancer, cardiovascular disease (CVD) and diabetes, indicating interest to serum TSA in a number branch of medicine.⁹⁻¹¹ Sialic acid (SA) is the common name for compounds of N-acetylated derivatives of neuraminic acid, which mainly occurs as non-reducing terminal residues of carbohydrate chains of glycoproteins (GPs) or glycolipids (GLs) in biological fluids and cell

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membranes. Sialic acid is also an important component of salivary GPs. Sialic acids have a central role for the function of biological systems: stabilizing the conformation of GPs and cellular membranes; assisting in cell-cell recognition and interaction and serving as chemical messengers in tissue and body fluids; affecting the function of membrane receptor molecules by developing binding sites for ligands, enzymes, and so forth or by blocking such affecting the functioning, stability, and survival of GPs in blood circulation; and regulating the permeability of basement of glomeruli.^{5-7,11,12}

Previous reports concerning serum TSA levels in smokers are somewhat controversial. Lindberg et al¹⁰ found that serum TSA levels were increased in smokers compared with non-smokers, whereas Patel et al¹³ reported that TSA levels were not affected by smoking habit. A study showed that SA levels were significantly higher in serum among alcoholics as compared with social drinkers.⁵ Another study also showed that SA concentrations in alcoholics were significantly higher in serum and saliva but not in urine, compared with social drinkers.⁷ But, there is no report available on SA concentrations in saliva and urine in smokers. No previous study was encountered on serum, urine, and saliva SA levels in smokers and alcoholics. Therefore, the present study was undertaken to carry out such a study. The purpose of this study was to search the effect of consumed alcohol and cigarette on serum, saliva, and urine SA levels. We also wanted to determine the serum AST, ALT, and GGT activities in smokers and drinkers.

Methods. The study was performed at the Department of Chemistry, Division of Biochemistry, University of Kahramanmaraş Sutcu Imam, Kahramanmaraş, Turkey in the year 2002. Serum, urine and saliva samples obtained from smokers (Group I), drinkers (Group II), and healthy nonsmokers-nondrinkers (Group III), with the latter having never smoked and being not exposed to any passive smoking in their environment. In groups I and II, there were any subjects who were both smokers and drinkers. There was no significant difference between the ages of participants. Their demographic data are presented in **Table 1**.

All subjects in this study were healthy volunteers recruited from environment and university students or their friends and acquaintances. Informed consent was obtained from all subjects, who were not suffering from any disease and were not on any medications including oral contraceptives. Venous blood was collected for the determination of serum TSA. Saliva and urine samples were simultaneously collected for the determination of saliva and urine TSA. All samples were stored at -20°C until analysis. Total sialic acid was measured

with the Warren's colorimetric method¹⁴ modified by Pönniö et al.⁷ Glycoproteins and glycolipid-bound SA were dissociated by acid hydrolysis. Periodate was then used under strongly acidic conditions to oxidize N- acetylneuraminic acid to form formylpyruvic acid. The reaction was stopped by sodium arsenite, after the reaction with thiobarbiturate to obtain a red chromophore. The extraction of this into cyclohexane was used to intensify the color with a maximum absorbance at 549 nm. Gamma-glutamyltransferase, AST and ALT enzyme levels were measured by autoanalyzer (Humalyzer 2000-Germany) using commercial kits (Human-Germany). All chemicals in this study were of analytical grade and purchased from Sigma (Stockholm) and Merck Chemicals Co. (Germany). All solutions were prepared in deionized and distilled water.

Statistical analyses were performed with the Statistical Package for Social Sciences pocket program for windows. The data were expressed as mean values \pm standard deviation ($\bar{x} \pm SD$). The mean values in the groups were compared with Mann Whitney U test; significance was defined as $p < 0.05$. For correlation analysis, the Spearman correlation coefficient (r) was used.

Results. **Table 2** shows the mean TSA levels in serum, saliva, and urine for smokers, alcohol drinkers and controls. Also, AST, ALT and GGT activity levels in serum are presented in the same table. The TSA concentrations were significantly higher in the sera of smokers ($p < 0.001$) and alcohol drinkers ($p < 0.005$) than those of control subjects. There was no significant difference in saliva TSA levels between smokers and healthy subjects ($p > 0.05$), whereas we have observed that saliva TSA levels were higher in alcohol drinkers than those in healthy subjects ($p < 0.05$). We have determined that there was no significant difference in urine TSA levels between smokers and non-smokers ($p > 0.05$) but urine TSA levels were much higher in alcohol drinkers than those in healthy subjects and in smokers (for both $p < 0.001$). We have observed that serum GGT level was higher in smokers ($p < 0.005$) and alcohol drinkers ($p < 0.001$) and there was no significant difference in serum AST levels between smokers and non-smokers ($p > 0.05$) and serum AST levels were higher in alcohol drinkers than those in healthy subjects ($p < 0.001$) and smokers ($p < 0.005$). We have determined that serum ALT levels were higher in smokers ($p < 0.001$) and alcohol drinkers ($p < 0.01$) than those in healthy subjects. When one considered the data with respect to the gender of the subjects, no statistically significant differences were found in all analytes of all sample types between all groups. There were no females in Group II. The subjects in this group had higher levels of all

Table 1 - Demographic variables for the subjects.

Group	N	Gender female/male	Age (years) range (x ₂ SD)	Duration of smoking/drinking (years) range (mean)	Consumption of cigarette (cigarettes per day) and alcohol (gm per day) range (mean)
Group I	55	10/45	20-57 (26.67±7.62)	2-15 (7.87)	10-23 (16)
Group II	27	-/27	18-57 (32.59±11.49)	1-35 (16.25)	45-75 (62)
Group III	59	33/26	18-65 (28.83±12.34)	-	-

Table 2 - The levels of serum, saliva, and urine total sialic acid (TSA) and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) (x±SD = mean values ± standard deviation).

Group	Serum TSA µg/mL (n)	Saliva TSA mg/mL (n)	Urine TSA mg/mL (n)	Serum ALT U/L (n)	Serum AST U/L (n)	Serum GGT U/L (n)
Group I (smoking)	763 ± 225 ^a (48)	61.8 ± 26.4 (40)	36.8 ± 14.4 (33)	13.2 ± 2.7 ^a (25)	12.3 ± 2.9 (25)	22.5 ± 7.4 ^a (25)
Female	709 ± 222 (9)	63.2 ± 28.7 (8)	36.4 ± 4.5 (5)	13.0 ± 0.0 ^a (12)	11.0 ± 1.4 (12)	19.0 ± 4.2 (12)
Male	775 ± 226 ^a (39)	61.5 ± 26.3 (32)	36.9 ± 15.5 (28)	13.2 ± 2.9 ^a (13)	12.5 ± 3.0 (13)	23.0 ± 7.7 ^a (13)
Group II (alcohol)	728 ± 132 ^b (25)	80.0 ± 40.6 ^b (18)	68.9 ± 27.8 ^b (14)	14.3 ± 4.8 ^a (25)	19.2 ± 7.0 ^a (25)	21.0 ± 4.2 ^a (25)
Female	709 ± 222 (9)	63.2 ± 28.7 (8)	36.4 ± 4.5 (5)	13.0 ± 0.0 ^a (12)	11.0 ± 1.4 (12)	19.0 ± 4.2 (12)
Male	728 ± 132 ^b (25)	80.0 ± 40.6 ^b (18)	68.9 ± 27.8 ^b (14)	14.3 ± 4.8 ^a (25)	19.2 ± 7.0 ^a (25)	21.0 ± 4.2 ^a (25)
Group III (control)	616 ± 156 (46)	54.6 ± 19.9 (28)	35.9 ± 13.6 (38)	10.4 ± 0.5 (35)	11.0 ± 1.4 (35)	15.0 ± 3.4 (35)
Female	657 ± 94 (20)	57.2 ± 23.7 (8)	33.6 ± 14.9 (17)	10.2 ± 0.46 (18)	11.2 ± 1.9 (18)	15.0 ± 3.6 (18)
Male	585 ± 186 (26)	53.5 ± 18.7 (20)	37.7 ± 12.5 (21)	10.5 ± 0.53 (17)	10.8 ± 0.89 (17)	15.1 ± 3.5 (17)

^ap<0.001 versus controls, ^bp<0.001 versus smokers, ^cp<0.005 versus controls, ^dp<0.005 versus smokers, ^ep<0.01 versus controls, ^fp<0.01 versus smokers, ^gp<0.05 versus controls

analytes measured than the males in Group III. Group II had higher urine TSA and serum AST level than the male smokers. The male smokers' serum TSA, ALT, and GGT levels were higher than those in control males. However, no statistically significant differences were found in all analytes measured between the females of the cases and controls.

In smokers, serum TSA levels showed a correlation with those of GGT ($r=0.524$; $p<0.05$), ALT ($r=0.614$; $p<0.05$) and urine TSA ($r=0.498$; $p<0.01$). The GGT levels of the smokers exhibited a correlation with ALT ($r=0.790$; $p<0.01$) and AST ($r=0.706$; $p<0.01$). Additionally, a correlation was present between ALT and AST in this group ($r=0.669$; $p<0.01$). Group II had no correlations in their analytes except present between ALT and AST ($r=0.704$; $p<0.01$).

Discussion. This study was aimed to search for the effects of alcohol drinking and cigarette smoking on serum, saliva, and urine TSA levels.

This is the first study specifically meant to compare the serum, saliva, and urine TSA levels in both smokers and drinkers. The results obtained indicate that serum, saliva, and urine TSA levels elevated in alcohol drinkers and only serum TSA levels increased in smokers compared with those of control subjects having no drink and cigarette. Recently, increased serum TSA concentrations have been reported in alcoholics,^{5,7} which confirm our results. However, there is a study reporting that the alcohol consumption showed no significant correlation with serum TSA concentration.¹⁵ The mechanisms that generate the elevated TSA levels in the serum of alcohol drinkers are unknown. It is reported that chronic ethanol consumption alters the microheterogeneity pattern of transferrin as a consequence of changes in the SA content.^{1,2,5} Based on earlier studies, one can mention that the excessive alcohol consumption affects the membrane proteins and the SA content of proteins such as transferrin.^{5,16} On the other hand, it is suggested that there is a connection between

alcohol-induced desialylation of transferrin and other GPs and an increased level of SA.⁵ Several reports suggest that both acute and chronic ethanol administrations in animal models impair the final steps of hepatic GP secretion after the accumulation of the terminal sugars mainly in the Golgi complex. Ethanol also interferes with the flow of membrane components from the Golgi apparatus to the surface of the plasma membrane.^{5,17,18} Acetaldehyde, the first metabolic product of ethanol oxidation, has been shown to inhibit the synthesis of secretory GPs and the glycosylation of proteins. Acetaldehyde has been suggested by several authors to be an important factor in the ethanol-induced impairment of hepatic GP formation and secretion.^{5,18} In addition, several reports have shown that alcohol intake decreases the activities of sialyltransferases in Golgi (and synaptosomes) and increases the activities of sialidase in both cytosol and plasma membranes.⁷ But the mechanism responsible for the elevated TSA concentration in serum of alcohol drinker is unclear. Furthermore, we have observed that serum GGT and ALT levels were higher in smokers than those in the healthy subjects, but there was no significant difference in serum AST levels between smokers and non-smokers. Steffensen *et al*¹⁹ showed that the daily smoking increased the risk of raised liver enzymes in women in Danish population. However, Maurel *et al*²⁰ found that GGT, AST, and ALT levels were not significantly different between the smokers and non-smokers. The present study show that the serum TSA did not correlate with GGT, ALT, and AST in alcohol consumers, also in smokers. These findings correspond to the results of a previous study, which indicated that traditional markers did not correlate with serum SA male alcoholics.⁵

We found elevated serum TSA in smokers compared with non-smokers. This finding is consistent with some previous studies. Lindberg *et al*⁸ reported the elevated serum TSA levels in smokers compared with non-smokers. Another study show that the smoking was associated with elevated serum TSA concentrations.¹⁵ In contrast, TSA levels were not affected by smoking habit.¹³ Lindberg *et al*⁸ failed to observe the effects of smoking on serum TSA in women. The study showed a weak but statistically significant positive correlation between the daily amount of tobacco smoked and serum TSA concentration in men but not in women. The present study shows that the male smokers had higher serum TSA levels when compared with those of controls, but the females in that group did not have, which may be due to less number of females in that group. However, several authors reported that gender did not appear to influence serum TSA levels in non-smoking group^{5,8,11} as in our study, which was mentioned above.

Smoking and alcohol are important causes of morbidity and mortality in many countries.^{3,4} Furthermore, smoking and alcohol consumption have currently been established to be cardiovascular risk factors.^{3,21} It is reported that increasing alcohol consumption is associated with increasing mortality from such causes as cancer, coronary heart disease, and cerebrovascular diseases.^{3,4} In contrast, it is well known from several reports that light and moderate alcohol intake diminishes the cardiovascular risk, but the heavy drinking increases it.²² On the other hand, elevated concentrations of serum TSA were suggested as a potent cardiovascular risk factor in the general population. Moreover, raised serum TSA concentrations have been shown to predict cardiovascular and cerebrovascular mortality.^{8,10,23} The reason for the association of TSA with cardiovascular disease is unclear. But, a plausible explanation is that a major quantity of TSA in serum is derived from the terminal oligosaccharide chain of several acute-phase proteins, such as fibrinogen, orosomucoid, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, and transferrin, which are all GPs.¹¹ It has been suggested that elevated serum TSA may reflect an acute phase response.^{3,11,23} An increased concentration of acute phase reactants is caused by an acute inflammatory disease or by an injury.^{5,10} As in the present study, Lindberg *et al*¹⁰ showed that the positive correlation between alpha-1-antitrypsin and smoking and haptoglobin but not orosomucoid.¹⁰ But, we did not measure the acute phase reactants, in this study. However, perhaps, elevated serum TSA levels might be reflect to CVD risk in smokers and drinkers. On the other hand, Latha *et al*²⁴ showed that prolonged exposure of rats to cigarette smoke resulted in significant alteration in the metabolism of GPs and glycosaminoglycans (GAGs) in different tissue, and they reported accumulation of GPs and GAGs in the lungs of the rats. The authors finally suggested that this finding may provide evidence that cigarette smoking is a risk factor for lung cancer. Smoking was reported to be a risk factor for oral cavity and esophageal cancers and liver cirrhosis as well as lung cancer.^{24,25} Moreover, alcohol consumption associated with mortality from cancer of the oral cavity, esophagus, and liver cirrhosis.^{3,4} Therefore, serum TSA has been used as a tumor marker for a number of different cancers including colorectal, prostate, and breast cancers.^{26,27} Cell surfaces and membrane components play a prominent role in neoplastic behavior. Neoplasms often have an increased concentration of SA on the tumor cell surface, and sialoglycoproteins are shed or secreted by some of these cells, which increase the concentration in blood. SA concentrations have been reported to be related not only to diagnosis, but also to staging, prognosis, and detection of early recurrence.²⁷ On

the other hand, cancer cells have been associated with increased activity of sialyltransferase, leading to an increased amount of SA on the cell surface, thus increasing the plasma concentration.^{5,28} Taking into consideration the studies we have mentioned above, increased serum TSA levels in smokers and drinkers might be related to various diseases such as various cancers and CVD, which also both often associated with elevated serum TSA levels and with cigarette smoke-alcohol consumption. Sialic acid is also an important component of salivary GPs. We observed increased salivary SA levels in alcohol drinkers but not in smokers. Our finding is in accord with a previous study having indicated that salivary SA levels increased in alcoholics.⁷ This result is possibly related to the effects of ethanol on salivary secretion and composition because it has previously been reported that chronic alcohol ingestion is associated with significant changes in parotid saliva secretion, the composition of saliva proteins, and its salivary electrolytes.²⁹ It is also suggested that another possible effect of alcohol is activation of saliva sialidase.⁷

We have demonstrated that there was no significant difference in SA levels in urine between smokers and non-smokers, whereas SA levels in urine were much higher in alcohol drinkers than those in healthy subjects and than in smokers. As contradictory to our finding, Pönniö et al demonstrated that urine levels of SA were not affected by alcohol consumption.⁷ There was no report available on SA concentrations in saliva and urine in smokers. Although we can explain the high levels of urine TSA with the high levels of serum TSA in drinkers, this is not true for the smokers on the basis of our results; that controversy is unexplainable. However, in this study, serum TSA levels were correlated with urine TSA in smokers. Perhaps, an explanation for elevated levels of TSA in urine may be that several SA-containing GPs originating from plasma are found in urine. The levels of them can be substantially elevated in patients suffering from proteinuria.⁵ Many relations have been established between smoking/drinking and proteinuria. For instance, Ramamurthi et al³⁰ showed that alcohol treatment increased urinary excretion of total GAGs, heparan sulfate and protein in normal mice. These and other related papers³¹ may explain increased urinary TSA in drinkers.

We can conclude from the results obtained that serum TSA affected by smoking and that serum GGT, AST, ALT and serum TSA can be used as a marker for monitoring of alcohol abuse. Our study indicates that urine, and saliva TSA can be used as non-invasive markers for alcohol abuse. However, further studies are necessary to evaluate the

concentrations of TSA on a greater number of serum, saliva, and urine samples from smokers and drinkers.

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