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COMPARATIVE ASSESSMENT OF DIAGNOSTIC ACCURACY OF SERUM SIALIC ACID AND SEVERAL CONVENTIONAL BIOMARKERS IN ALCOHOL-DEPENDENT INDIVIDUALS

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Abstract:

Identification of alcoholism is crucial in preventing adverse health effects and social consequences of excessive alcohol consumption. The aim of this study was to determine concentrations of sialic acid (SA) in serum samples of alcoholics and to compare its diagnostic value with some conventional markers. Samples were obtained from alcohol-dependents (n=25) and healthy controls (n=23). To assess the possible effect of amount of alcohol consumed, alcohol-dependent subjects were divided into three subgroups [<200g ethanol per day (n=9), 200-300g ethanol per day (n=9), and >300g ethanol per day (n=7)]. Additionally, alcohol-dependents were also split into two subgroups according to presence (n=11) or absence (n=14) of hepatosteatosis. As evaluated according to the amount of alcohol consumption, SA levels were elevated in the 200-300 and >300g ethanol per day subgroups ($p<0.05$); on the other hand, interestingly, SA levels of alcohol-dependents with and without hepatosteatosis were significantly increased as compared with controls ($p<0.05$). When sensitivities were evaluated, SA was superior to others in overall, in the absence of hepatosteatosis, and in >300g ethanol per day subgroup (64%, 57.1%, and 85.7%, respectively). SA may be of value in identifying alcohol-use-disorder in presence and absence of hepatosteatosis, as well as reflecting the amount of alcohol consumed.

Key words: *Alcohol dependency, sialic acid, ROC curve, sensitivity, specificity, biomarker.*

Alkol Bağımlılarında Serum Sialik Asit ve Bazı Geleneksel Biyolojik Göstergelerin Diagnostik Doğruluğunun Karşılaştırmalı Değerlendirmesi

Aşırı alkol tüketiminin sağlık üzerindeki olumsuz etkileri ve sosyal sonuçlarının önlenmesi için, alkolizmin belirlenmesi zorunludur. Bu çalışmanın amacı, alkoliklerden alınan serum örneklerinde sialik asit (SA) konsantrasyonlarının belirlenmesi ve diagnostik değerinin bazı geleneksel biyolojik göstergelerle karşılaştırılmasıdır. Örnekler, alkol bağımlıları (n=25) ve sağlıklı kontrol bireylerden alınmıştır (n=23). Tüketilen alkol miktarının olası etkisini değerlendirmek için, alkol bağımlıları üç alt gruba ayrılmıştır [günde <200g alkol tüketen (n=9), 200-300g alkol tüketen (n=9) ve >300g alkol tüketen (n=7)]. Bunun yanı sıra, alkol bağımlıları, hepatosteatosis görülen (n=11) ve görülmeyen (n=14) iki alt grupta da değerlendirilmiştir. Alkol tüketimine göre değerlendirildiğinde, SA düzeyleri, günde 200-300 ve >300g alkol tüketen alt gruplarda ($p<0.05$) artmıştır; diğer taraftan, hepatosteatosis görülen ve görülmeyen grupların her ikisinde de ($p<0.05$) SA düzeyleri yükselmiştir. SA, tüm alkol bağımlılarında, hepatosteatosis görülmeyen ve günde >300 g alkol tüketen gruplarda, diğer göstergelerden daha hassas bulunmuştur (sırasıyla, %64, %57.1 ve %85.7) . Tüketilen alkol miktarını yansıtmasının yanı sıra, SA, hepatosteatosis görülen ve görülmeyen bireylerde alkol kullanım bozukluğunun tanımlanmasında yararlı olabilir.

Anahtar kelimeler: *Alkol bağımlılığı, sialik asit, ROC eğrisi, hassasiyet, spesifisite, biyolojik gösterge.*

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INTRODUCTION

Alcohol abuse is a very common problem all over the world. A specific, sensitive, inexpensive, simple and rapid biomarker for the early identification of chronic heavy alcohol consumption could be valuable for clinical intervention and in preventing the socioeconomical consequences. Various screening questionnaires such as CAGE questionnaire are available to assist in the diagnosis of alcoholism. Questionnaires are ideally suited for population screening and can identify up to 80% of alcoholics, but they rely on the patient's truthfulness and memory which also cause them to be highly susceptible to deliberate concealment. Laboratory tests are useful to enhance suspicion since they may provide objective information about alcohol consumption (1).

There are several biomarkers available such as carbohydrate deficient transferrin (CDT), γ-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and mean corpuscular volume (MCV); however each comprise both benefits and limitations (1, 2). Therefore, research is underway to identify sensitive and specific biomarkers which can also be utilized easily and rapidly for early detection of chronic alcoholism.

Sialic acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction, contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions, and affecting the function, stability and survival of glycoproteins in blood circulation (3). Increased levels of total SA and/or lipid associated SA have been observed in various diseases including several types of cancer (4), diabetes (5), and renal disease (6). It has been previously reported that SA levels may be increased in biological fluids of alcoholics, and it has been suggested that SA can be valuable as a biomarker for excessive alcohol consumption (7-11).

The aims of the present study were to determine serum levels of SA and MCV and activities of GGT, AST, and ALT in blood samples obtained from alcohol-dependent individuals, and to evaluate the diagnostic accuracy of these markers in predicting alcohol abuse. In order to assess the possible effect of amount of alcohol consumed, alcohol-dependent subjects were divided into three subgroups; additionally, according to the presence or absence of hepatosteatosis, another evaluation was performed.

EXPERIMENTAL

Chemicals

All chemicals were of the highest grade available and purchased from either Sigma Co. (St. Louis, MO, USA) or Merck Co. (Darmstadt, Germany).

Ethics

The study protocol was approved by the Ethics Committee of Ankara University Hospital. The studies were conducted according to the Helsinki Declaration of Human Experimentation, and all participants included in this study were asked to give their informed consent.

Study Groups

Blood samples were obtained from alcohol-dependent males (n=25) who applied to Ankara University Psychiatry Clinic and from healthy controls (n=23, males) who attended a voluntary health screening. Each alcohol-dependent subject was asked to fill out a questionnaire that contained general information on drinking habits including type of alcoholic beverages consumed and frequency and also duration of drinking. The criterion for inclusion of alcohol-dependent individuals was diagnosis of alcohol dependency as determined by CAGE questionnaire; the exclusion criteria were diagnosis of diabetes, cancer, and presence of other substance related addiction.

To assess the possible effect of amount of alcohol consumed, alcohol-dependent subjects were divided into three subgroups, namely, <200 g ethanol per day (n=9), 200-300 g ethanol per day (n=9), and >300 g ethanol per day (n=7). Additionally, according to the presence (n=11) or absence (n=14) of hepatosteatosis, another classification was performed (Table 1).

Analytical Methods

Blood samples were allowed to stand in room temperature then centrifuged at 1000g for 10 minutes in order to separate serum and were stored at –70°C until analyses. Total SA measurements have been carried out according to the method described by Warren (12) and modified by Özben (6). GGT, AST, ALT and MCV measurements were conducted using commercially available kits (Olympus Diagnostika, Hamburg).

Statistical Analysis

All data were expressed as mean ± standard error (SE). Comparison of the levels of markers in serum of alcohol dependent individuals and control subjects were performed using Kruskal Wallis test, followed by Mann Whitney U test for the evaluation of subgroups. The accuracy of diagnostic markers was compared using receiver operating characteristic (ROC) analysis. Analyses were performed with SPSS for Windows, version 10.0 (SPSS Inc., IL, USA).

RESULTS

Table 1 depicts age, body mass index (BMI) and amount of alcohol consumption in the study groups. Serum SA and MCV levels and activities of GGT, AST, and ALT are presented in Table 2.

Table 1. Characteristics of the control group and alcohol-dependent subjects*

	n	Age **(years)**	BMI **(kg/m^2)**	Alcohol consumption **(g ethanol per day)**
Control Subjects	23	41.5±2.0	26.2±0.5	-**
Alcohol-dependent individuals (overall)	25	44.0±1.5	24.9±0.7	270.7±31.3
<200g ethanol per day	9	44.0±2.5	25.5±1.1	136.8±10.3
200-300 g ethanol per day	9	42.0±2.0	24.0±1.0	255.6±4.4
>300 g ethanol per day	7	46.0±4.0	25.5±1.3	462.4±61.6
Alcohol-dependent individuals without hepatosteatosis	14	41.0±2.0	24.1±0.9	229.9±26.9
Alcohol-dependent individuals with hepatosteatosis	11	47.0±2.0	26.1±0.8	322.7±60.6

* Data are expressed as mean ± SE.
**Alcohol consumption of control group was either no consumption pattern or limited social drinking.

Table 2. Serum SA and MCV levels, GGT, AST, ALT activities and AST/ALT ratio in study groups

	SA **(mg/dL)**	MCV **(fL)**	GGT **(U/L)**	AST **(U/L)**	ALT **(U/L)**	AST/ALT
Control subjects	56.12±2.05	86.96±0.46	23.30±2.41	20.26±1.04	24.26±2.29	0.91±0.05
Alcohol-dependents (overall)	66.70±2.29*	90.84±1.65*	170.84±44.61*	57.04±11.01*	35.68±4.82	1.55±0.16
<200g ethanol per day	61.47±3.60	88.52±3.81	155.89±65.81*	60.22±23.59*	31.89±4.71	1.72±0.43
200-300 g ethanol per day	66.40±3.93*	92.01±2.38	157.00±78.15*	63.11±19.36*	42.00±11.12	1.45±0.12
>300 g ethanol per day	73.80±3.44*	92.31±1.65*	207.86±101.06*	45.14±10.03*	32.43±8.27	1.44±0.14
Alcohol-dependents without hepatosteatosis	66.34±3.33*	89.88±2.01	50.14±8.60*	33.64±3.88*	26.00±3.93	1.37±0.09
Alcohol-dependents with hepatosteatosis	67.17±3.18*	92.06±2.82*	324.45±80.73*#	86.82±21.85*#	48.00±8.60*#	1.77±0.34

Values are expressed as mean±SE.
*$p<0.05$ as compared with control group
# $p<0.05$ as compared with alcohol-dependents without hepatosteatosis

The results have shown that when hepatosteatosis status or alcohol consumption were not taken into account and all alcohol-dependent subjects were taken together (alcohol dependent subjects, overall); levels of SA and MCV and also activities of GGT and AST were elevated ($p<0.05$) as compared with controls. Although serum ALT level was also increased, the change was statistically insignificant ($p>0.05$).

When subgroups were evaluated, serum SA levels as well as GGT and AST activities in alcohol-dependent subjects with and without hepatosteatosis were significantly elevated as compared with controls ($p<0.05$). On the other hand, mean serum ALT activity and MCV level of subgroup with hepatosteatosis were significantly higher as compared with controls ($p<0.05$), whereas in individuals without steatosis no statistical difference was found. Evaluation on the basis of alcohol consumption revealed that GGT and AST activities were significantly elevated in the three subgroups ($p<0.05$), whereas SA concentrations were increased in the 200-300 and >300 g ethanol per day subgroups ($p<0.05$). The only significance found in MCV levels was in the >300 g ethanol per day subgroup. Interestingly, ALT was slightly elevated in the subgroups; however no statistical significance existed.

When parameters were evaluated with respect to hepatosteatosis, mean GGT, AST, and ALT activities in alcoholics with hepatosteatosis were found significantly different from those of alcohol-dependents without steatosis (Table 2). However, no significant difference was observed in SA (67.17±3.18 mg/dL and 66.34±3.33 mg/dL, respectively) and MCV levels (92.06±2.82 fL and 89.88±2.01 fL, respectively) in the presence or absence of hepatosteatosis.

To assess the value of these biomarkers in distinguishing between alcohol-dependent individuals and healthy controls, ROC curve analyses were performed (Table 3). The areas under ROC curve of GGT and AST were superior in overall (all alcohol-dependents). Sensitivity and specificity of the markers were summarized in Table 4. Data suggest that despite its low specificity, SA has a sensitivity superior to other markers in overall, in alcohol dependents without hepatosteatosis, and >300 g ethanol per day subgroup.

Table 3. Area under ROC curves for diagnostic accuracy of SA and conventional markers

	SA	**MCV**	**GGT**	**AST**	**ALT**
Alcohol-dependent individuals (overall)	0.750	0.744	0.904	0.903	0.638
<200 g ethanol per day	0.633	0.705	0.899	0.937	0.664
200-300 g ethanol per day	0.758	0.700	0.911	0.920	0.674
>300 g ethanol per day	0.891	0.851	0.904	0.835	0.559
Alcohol-dependents without hepatosteatosis	0.717	0.661	0.843	0.890	0.492
Alcohol-dependents with hepatosteatosis	0.792	0.850	0.982	0.919	0.824

Table 4. Sensitivities and specificities of SA and conventional markers*

		SA	MCV	GGT	AST	ALT
SENSITIVITY (%)	Alcohol-dependent individuals (overall)	64.0	44.0	56.0	48.0	28.0
	<200 g ethanol per day	55.6	44.4	55.6	33.3	33.3
	200-300 g ethanol per day	55.6	33.3	55.6	66.7	22.2
	>300 g ethanol per day	85.7	57.1	57.1	42.9	28.6
	Alcohol-dependents without hepatosteatosis	57.1	42.9	28.6	21.4	7.1
	Alcohol-dependents with hepatosteatosis	72.7	45.5	90.9	81.8	54.5
SPECIFICITY (%)		69.6	100	95.7	100	91.3

*The following cut-off values were used: 63 mg/dl for SA, 92fL for MCV, 50 U/L for GGT, 40 U/L for AST and ALT.

DISCUSSION

Alcoholism represents a serious health issue with major socio-economic consequences. The diagnosis is often based on the patient's self-reporting of alcohol consumption which requires a high degree of clinical suspicion. The further development and progression of alcohol problems may be prevented, if they are recognized at an early stage. Laboratory markers are useful in both raising the suspicion and confirming the diagnosis of alcohol abuse; they are also helpful in the follow-up of patients undergoing treatment and in monitoring abstinence. However, the sensitivities and specificities of the different laboratory markers vary considerably and depend on the population concerned (1).

The present study was undertaken to evaluate the clinical significance of serum total SA as a biomarker for alcohol abuse and to compare this potential biomarker with several conventional markers. In this context, to assess the possible effect of the amount of alcohol consumed, alcohol-dependents were divided into three subgroups, namely <200g ethanol per day, 200-300 g ethanol per day, and >300g ethanol per day. Additionally, another evaluation was also made according to presence or absence of hepatosteatosis.

Recent studies have reported that SA levels may be increased in biological fluids of alcoholics, and it has been suggested that SA can be valuable as a biomarker for excessive alcohol consumption (7-11). Our results confirm that serum SA levels are elevated in alcohol dependents as compared with controls (Table 2). The elevation of SA levels due to alcohol abuse may be explained by several mechanisms: Chronic alcohol consumption has been found to decrease sialyltransferase and increase sialidase activities (13, 14). In addition, when evaluated in regard to the alcohol consumption, SA concentrations followed an increasing pattern (61.47±3.60 mg/dL, 66.40±3.93 mg/dL, and 73.80±3.44 mg/dL, respectively), in agreement with a previous report (11).

Interestingly, serum SA levels in the subgroups with and without hepatosteatosis were similar (67.17±3.18 mg/dL and 66.34±3.33 mg/dL, respectively) in the present study. Romppanen et al (9) found that when compared with the traditional markers (CDT, GGT, and AST), serum SA had a higher efficiency than serum CDT or AST, although it was lower than that of serum GGT, for detecting alcohol abuse in heavy drinkers and serum SA seemed to be less dependent on liver status as compared with other markers, a finding which was confirmed in the present study.

GGT is a glycoprotein enzyme which is normally released only in small amounts from the cell membrane into the circulation. However with repeated excessive alcohol consumption, there may be increased release of GGT (15). In line with earlier reports (7, 8), it was observed that GGT activities of alcohol-dependent subjects (overall) were significantly elevated as compared with controls in this study ($p<0.05$). In the subgroups, significant differences were observed in the serum GGT activities in alcohol-dependents with and without hepatosteatosis as well as in the subgroups divided according to the amount of alcohol consumed (Table 2), as compared to the control subjects. However, it is also known that the increase in serum GGT in response to different amounts and duration of alcohol consumption varies considerably between individuals (1).

Serum AST and ALT, which are sensitive indicators of liver cell injury (15), are often elevated in alcoholics, although generally not to more than 2-4 times upper normal limits (1). Another biomarker of heavy drinking is MCV, a measure of the average size of red blood cells. Heavy drinking may either directly affect MCV or may result in folic acid deficiency or liver disease, which in turn, causes red blood cells to enlarge (16). Similar to GGT, serum AST was significantly increased in all alcohol-dependent subjects (overall), alcohol-dependents with and without hepatosteatosis and also in the alcohol consumption subgroups (Table 2). On the other hand, mean ALT activity and MCV levels in the subgroup without hepatosteatosis were only slightly elevated. Moreover, no significant elevation was found in ALT in the subgroups divided according to the amount of alcohol consumed. Different from ALT, MCV was found significantly elevated in the >300 g ethanol subgroup. In view of the results, it seems that ALT and MCV could not be recommended as single markers for alcohol abuse.

An AST/ALT ratio exceeding 1.5 strongly suggests alcohol-induced liver damage, while a ratio above 2.0 is almost indicative of such a disease (1). In this study, the alcohol dependents with hepatosteatosis had an AST/ALT ratio above 1.5, whereas in the absence of steatosis the ratio was below this level (Table 2). Surprisingly, the ratio was 1.72 in the <200 g ethanol per day subgroup, whereas in the 200-300 and >300 g per day subgroups they were below 1.5. Nyblom et al (17) recently reported that most patients with high alcohol consumption but without severe liver disease did not have an AST/ALT ratio above 1 and suggested that high AST/ALT ratio showed advanced alcoholic liver disease. An analysis of ROC curve was performed in order to evaluate the ability of SA and other markers to discriminate between alcohol-dependent subjects and healthy controls. In the ROC curve analysis, while an area under curve of 1.0 is indicative of a perfect discrimination, a test with an area of 0.5 can discriminate no better than chance.

In the present study, the only area under curve below 0.5 was found out in ALT (0.492) in the alcohol-dependents without hepatosteatosis (Table 3). In the <200g per day and 200-300 g per day subgroups, GGT and AST were superior to others, whereas in the >300 g per day subgroup, SA was comparable to GGT. In line with the previous report (11), area under curve of SA showed a stepwise increase in the subgroups divided according to the alcohol consumption (0.633, 0.758, and 0.891, respectively). On the other hand, none of the other markers evaluated in this study followed such a pattern (Table 3).

A screening marker should display high sensitivity and specificity and therefore discriminate between safe social drinking and heavy, hazardous drinking (1). Sensitivity (the percentage of correctly identified alcohol-dependent individuals) and specificity (the percentage of correctly identified control subjects) of the markers have been summarized in Table 4. When sensitivities were evaluated in all alcohol-dependents (overall), SA (64%) was superior to others. Interestingly, in alcohol-dependents without hepatosteatosis, SA also performed better (57.1%) than conventional markers. In line with this finding, in a very recent report by Anttila et al. it was indicated that SA and MCV show significantly higher sensitivities in the patients without liver disease than in those with liver disease and emphasized that serum SA measurements may be valuable especially when the effects of liver disease and alcohol consumption need to be differentiated (18). Furthermore, sensitivities of SA in <200 and 200-300 g ethanol per day subgroups were identical (55.6%), whereas in the >300 g ethanol per day subgroup a sensitivity of 85.7% was obtained (Table 4). A correlation has been previously shown between SA level and the amount of alcohol consumption (10, 11). A trade-off between sensitivity and specificity exists: If a lower cut off value is used in a test, the sensitivity increases, which means that more true cases will be correctly identified. On the contrary, if a higher cut off value is preferred, the specificity increases at the expense of sensitivity. Indeed, in the present study when a cut off value of 63 mg/dL for SA was used, the sensitivity (overall) and specificity were found 64% and 69.6%, respectively (Table 4). If the cut off was shifted to 65 mg/dL, the corresponding values were 52% and 78.3%. However, in order to obtain the maximum Youden's Index, a cut off value of 63 mg/dL was preferred. It has been reported that serum levels of GGT are elevated in about 75% of alcohol dependent individuals with a range in sensitivity of 60-90% (1). In the present study, the sensitivity of GGT in alcohol-dependent subjects (overall) was found 56%. Interestingly, in the subgroups divided according to the amount of alcohol consumed, GGT had very similar sensitivities in these subgroups (Table 4), whereas obviously very different sensitivities were found in the absence (28.6%) and presence of steatosis (90.9%). AST and ALT have been reported to demonstrate sensitivities between 25-60% and 15-40%, respectively (1). However, in this study, sensitivities of AST and ALT in some subgroups were found higher (Table 4). MCV has limited value as a single marker in screening because of its poor sensitivity, typically below 50% (15), as it was also observed in the present study, except for the >300 g ethanol per day subgroup (57.1%). When sensitivities of markers were evaluated on the basis of steatosis, it was obvious that GGT, AST, and ALT performed better in individuals with hepatosteatosis as compared to the dependents without steatosis. On the contrary, such a difference was not observed for the sensitivities of MCV and SA in the

presence and absence of steatosis: Sensitivities were almost identical in MCV (45.5% and 42.9%, respectively) while corresponding values for SA were 72.7% and 57.1% (Table 4). Indeed, GGT, AST, and CDT were reported to reflect the severity of liver disease and were found to be increased more often than serum SA (9).

MCV was reported to be more specific than GGT in most populations (15). However, the difference between the specificities of GGT (95.7%) and MCV (100%) was minor in the present study. It should also be mentioned that in the present study, specificity of AST was also found 100% which suggests that MCV and AST can identify all control (healthy) subjects correctly. On the other hand, specificity of SA was obviously less than those of conventional markers (Table 4), which may hamper its clinical usefulness as a marker of alcohol abuse. It is known that SA levels can be elevated not only in response to alcohol abuse but also in several other conditions (4-6). As mentioned earlier, laboratory tests are useful to enhance suspicion and must be combined with a clinical history, physical examination, questionnaires, and self reporting (1).

CONCLUSION

Present data confirm previous reports suggesting that serum SA is not influenced by liver disease which makes it a promising marker, particularly in the alcoholics without apparent hepatic disease. Therefore, SA could be recommended as a part of clinical tests for identifying alcohol abuse regardless of hepatosteatosis, as well as its value to demonstrate the amount of alcohol consumed. Introducing serum SA determination can be suggested as part of a basic suite of diagnostic tests for detecting and monitoring alcohol abuse. Moreover, SA determinations are inexpensive, simple and rapid, can easily be carried out in routine clinical laboratories.

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