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Plasma Semicarbazide-Sensitive Amine Oxidase Activity in Type I and II Alcoholics

[Tip I ve Tip II Alkoliklerde Plazma Semikarbazid-Duyarlı Amin Oksidaz Aktivitesi]

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ABSTRACT

Semicarbazide-sensitive amine oxidase (SSAO) which catalysis the deamination of primary amines is involved in vascular endothelial or tissue damage through the formation of reactive aldehydes, hydrogen peroxide and ammonia from endogenous substrates. In the present study, plasma SSAO activity, plasma and erythrocyte lipid peroxidation status, glutathione levels and the correlation between the plasma SSAO activity and lipid peroxidation were determined to clarify the mechanism of liver injury related to the oxidative stress in early- and late-onset (Type I and II) alcoholic subtypes. Plasma SSAO activity and , plasma and erythrocyte malondialdehyde (MDA) levels as the indicators of lipid peroxidation status of alcoholics were found to be significantly higher than those of the control group. Increased plasma SSAO activity was strongly correlated with the elevated plasma and erythrocyte MDA levels and decreased reduced/oxidized glutathione (GSH/GSSG) ratio in alcoholics suggesting that enhanced plasma SSAO activity might contribute to the production of reactive oxygen species (ROS) detected in the liver of the alcoholics. Plasma SSAO activity and blood MDA level of type II alcoholics were found to be significantly higher than those of of the type I alcoholics and a strong positive correlation was detected between these parameters in the alcoholic subgroups. Data demonstrated that the age-of onset and the duration of alcohol intake were important factors leading to the liver injury induced by oxidative stress might due to the increased plasma SSAO activity in alcoholics.

Key Words: semicarbazide-sensitive amine oxidase (SSAO), lipid peroxidation, glutathione, alcoholism

ÖZET

Primer aminlerin deaminasyonunu katalizleyen semikarbazid-duyarlı amin oksidaz (SSAO), endojen substratlardan reaktif aldehitler, hidrojen peroksit ve amonyak oluşturmak suretiyle damar endotelinde ve dokularda hasara yol açan bir enzimdir. Bu çalışmada, alkole erken ve geç başlayan (Tip I ve II) alkolik gruplarda karaciğer hasarına yol açan mekanizmaya açıklık getirmek amacıyla plazma SSAO aktivitesi, plazma ve eritrositlerde lipid peroksidasyonu ve glutatyon düzeyleri ölçüldü; plazma SSAO aktivitesi ile sözkonusu parametrelerin korelasyonu incelendi. Alkoliklerde plazma SSAO aktivitesi, lipid peroksidasyonunun göstergesi olarak tayin edilen plazma ve eritrosit malondialdehit (MDA) düzeyi kontrol grubuna oranla önemli derecede yüksek bulundu. Alkolik grupta artmış plazma SSAO aktivitesi ile artmış plazma ve eritrosit MDA düzeyleri; artmış plazma SSAO aktivitesi ile azalmış redükte /okside glutatyon (GSH/GSSG) oranı arasında kuvvetli bir ilişkinin görülmesi alkoliklerde sıklıkla gözlenen karaciğerde aşırı reaktif oksijen türleri (ROS) oluşumunda artmış plazma SSAO aktivitesinin de rolü olabileceğini düşündürdü. Çalışmada tip II alkoliklerin plazma SSAO aktivitesi ve kan MDA düzeyleri, tip I altgrubuna göre daha yüksek bulundu ve her iki altgrupta da bu iki parametre arasında kuvvetli pozitif bir ilişki saptandı. Alkoliklerde alkole başlama yaşı ve alkol alma süresinin, plazma SSAO düzeyi, SSAO ile indüklenen oksidatif stres ve ilgili karaciğer hasarının oluşumu açısından önemli faktörler olabileceği öne sürüldü.

Anahtar Kelimeler: semikarbazid-duyarlı amin oksidaz (SSAO), lipid peroksidasyonu, glutatyon, alkolizm

INTRODUCTION

The semicarbazide-sensitive amine oxidases (SSAO, E.C. 1.4.3.6.) are a group of heterogeneous enzymes widely distributed in plants, microorganisms, and in many organs of mammals (1,2). SSAO converts amines into the aldehydes, generating hydrogen peroxide and ammonia. It contains Cu^{2+} and a functional carbonyl group. SSAO and FAD-containing monoamine oxidase (MAO) are distinct from each other with respect to substrate specificity and inhibitor sensitivity. The physiological substrates include aminoacetone, methylamine, 2-phenylethylamine, tyramine and dopamine whereas benzylamine is a good non-physiological substrate for the mammalian SSAO (3,4). A common feature is that the SSAO is not inhibited by MAO inhibitors such as clorgyline and pargyline, but instead by semicarbazide and similar compounds (2,5).

In mammals, SSAO is located in many organs and tissues, most prominently in vascular smooth muscle, adipocyte, gut, lung, liver, retina, kidney, placenta, pancreas and plasma (6). Although SSAO has been often regarded as being involved in the detoxification of amines, the products of the reaction are more toxic than the amine substrates themselves (2). The functional role of SSAO is not yet quite clear, but in recent years some functions have been described as protection against exogenous amines, role in glucose transport into the cells via GLUT4 transporter, role in apoptosis and atherogenesis, local generation of hydrogen peroxide as signal molecule, cross-linking of proteins and leucocyte trafficking (7). The origin of soluble plasma SSAO is not known, nor whether it is exactly the same as the membrane-bound SSAO or a form of transmembrane fragment (7).

Interest in the role of SSAO in human pathophysiological conditions has increased in recent years since it was shown that plasma SSAO is raised in both insulin-dependent and non-insulin-dependent diabetes mellitus, congestive heart failure, Alzheimer's disease and some inflammatory diseases (8-11). SSAO-mediated deamination of methylamine and aminoaceton produces formaldehyde and methylglyoxal, which have been proposed to be cytotoxic to the various tissues and might be involved in the pathogenesis of some disesases such as atherosclerosis, aging, cancer, and skin disorders (7). Hydrogen peroxide is also a product in SSAO-mediated oxidative deamination of methylamine and aminoaceton. It was reported that formaldehyde and hydrogen peroxide were simultaneously formed during deamination of metylamine which could lead to increased oxidative stress (3).

Excessive alcohol consumption and alcoholism are widely observed risk factors for health damage and social problems. Two subtypes of alcoholism was defined by Cloninger et al.(12), based on the implication that age-of-onset for symptoms of alcoholism might be a discriminative marker. According to these authors, ***type I alcoholics*** have a later onset of alcohol abuse, less psychiatric disturbance, a more benign alcohol related problem profile and a better prognosis. ***Type II alcoholics*** manifest alcohol problems at an early age, are socially disruptive when drinking, having more psychiatric disturbance, greater symptom severity, and a poor prognosis. Although the existence of type I and II alcoholism was confirmed by many researchers, additional criteria such as more discriminative biological markers or abnormalities are needed in order to determine the novel medical strategies for the treatment of the alcoholic subgroups.

Recent studies indicated that reactive oxygen species (ROS) such as superoxide, hydroxy, peroxy, α-hydroxyethyl radicals and hydrogen peroxide are implicated in ethanol-induced oxidative tissue damage (13-15). Although it is generally accepted that increased lipid peroxidation is a major pathogenetic mechanism in ethanol-induced hepatotoxicity (15), the mechanism is not clear yet.

The present study was undertaken to determine the plasma SSAO activity and the correlation between the plasma SSAO activity and lipid peroxidation in alcoholic subtypes which could help to clarify the mechanism of oxidative stress and liver injury detected in alcoholics. The role of age of-onset and the duration of the alcohol intake on these parameters were also assessed.

MATERIALS AND METHODS

Subjects

Patient group consisted of 33 voluntary male alcoholic patients who satisfied the diagnostic criteria of ICD-10 for alcohol dependence (16). All the patients were receiving treatment for their withdrawal symptoms at an in-patient treatment centre during the time of the investigation. *Alcohol and Drug Modules of Schedules for Clinical Assessment in Neuropsychiatry (SCAN)*(17) were administered to each patient in order to gather data on alcohol intake variables and drinking related behaviour. The lifetime severity of drinking problems was assessed by the *Michigan Alcoholism Screening Test (MAST)* (18).

17 type I and 16 type II male chronic alcoholic patients were included in the study. The patients whose subjective alcohol problems had started before reaching 21 years of age (≤ 20) were categorized as "Type II". For inclusion in the type II group, the reporting of at least two instances of social complications related to alcoholism before the age of 20 was required (such as job loss, alcohol related absence from school or work, arrest for intoxicated behaviour, or violence while intoxicated). Those who did not meet these criteria were categorized as type I dependent. The time when alcoholic patients satisfied the rule for the social complications of alco-

holism was considered the beginning of their alcohol misuse. In the type II group, ages ranged from 27 to 62 years with an average of 41.6 ± 9.6. In the Type I group, ages ranged from 34 to 56 years with an average of 45.1 ± 6.3.

17 healthy males selected on the basis of their similarity to the patients in terms of age, years of education and smoking status were taken as control group. Exclusion criteria included neurological, psychiatric and major medical illnesses, a history of alcohol/drug dependence. Their ages ranged from 29 to 56 years (mean±SD: 40.3 ± 8.4).

All subjects provided written informed consent for participation in the study, which was approved by the local ethics committee (99-7).

Reagents and equipment

All chemicals were obtained from Sigma Chemical Co. (Germany). Spectrophotometric measurements were performed using Shimadzu UV-160A spectrophotometer.

Determination of plasma SSAO activity

Blood was collected into heparinized tubes containing glutathione (1.2 mg.ml^{-1}) and centrifuged at 3000 x g for 15 min. Plasma SSAO activity was measured according to the method of Tabor (19) with some modifications. The reaction mixture contained 50 mM potassium phosphate buffer, pH 7.2, and plasma (100 μl) in a final volume of 1 ml. Plasma was preincubated with clorgyline (1 μM) at 37°C for one hour to inactivate any monoamine oxidase (MAO) possibly present in plasma. The reaction was initiated by the addition of the substrate benzylamine (5mM) and the absorbance change was monitored at 250 nm. at 37°C. The molar extinction coefficient of benzaldehyde was taken as 13,800 M^{-1} cm^{-1}. Plasma SSAO activity was expressed as pmol of benzaldehyde formed per minute per ml of plasma or mU.l^{-1}.

Preparation of erythrocyte lysates

Venous blood samples (approx. 20 ml) were taken after an overnight fast, collected in stoppered vacutainer tubes containing sodium citrate as anticoagulant. Erythrocyte lysates were prepared from the human peripheral blood according to the method of Ceballos-Picot et al. (20). Briefly, erythrocyte pellets were obtained from the blood samples by centrifugation at 500 x g for 15 min. at room temperature following the determination of hemoglobin content of the blood sample. The plasma and buffy coat were then removed, and the erythrocytes were washed twice in 100 ml of saline and stored at –80°C until used. Lysed erythrocytes were prepared by freezing and thawing two times and by addition of three volumes of ice-cold distilled water. Cell membranes were removed by centrifugation at 1000 x g for 20 min., and the supernatant was used as the erythrocyte lysate. The hemoglobin content of the lysates was determined by the standard cyanomethemoglobin method .

Determination of lipid peroxidation in plasma and erythrocyte lysates

Lipid peroxidation in plasma and lysates was determined by the measurement of malondialdehyde (MDA) levels in plasma on the basis of MDA reacted with thiobarbituric acid at 532 nm, according to the method of Ohkawa et al.(21) with some modifications. A mixture of 8% sodium dodecyl sulfate (SDS) (0.2 ml), 20% acetic acid (1.5 ml) and 0.9% thiobarbituric acid (TBA) (0.2 ml), was added to 0.2 ml lysate or plasma, and distilled water was added to the mixture to bring total volume up to 4 ml. The mixture was boiled at 95°C for one hour and cooled. 1 ml water and 5 ml n-butanol/pyridine (15:1, v/v) was added, mixed and centrifuged at 1000 g for 15 min. Absorbance of the supernatant was measured at 532 nm. 1,1,3,3-tetraethoxypropane was used as standard. Values were expressed as nmol. mg protein^{-1} for MDA in lysates and nmol. ml^{-1} for MDA in plasma.

Determination of total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in plasma and erythrocyte lysates

Total glutathione content, as the sum of GSH and GSSG, and GSSG were determined in the samples according to the method described previously (22). The sum of the reduced and oxidized forms of glutathione was determined using a kinetic assay in which amounts of GSH or GSSG and glutathione reductase brought about the continuous reduction of 5,5'- dithiobis(2-nitrobenzoic acid) (DTNB) by NADPH according to:

$$\mathrm{NADPH + H^+ + DTNB \xrightarrow[\text{GSSG reductase}]{\text{GSH/GSSG}} 2\ TNB + NADP^+}$$

The reaction rate is proportional to the concentration of glutathione. The formation of 5-thio- 2- nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm.at 25°C. Assay mixture contained 1.0 ml 0.1 M potassium phosphate buffer/ EDTA, pH 7.0, 100 μl sample, 50 μl NADPH (4 mg/ml), 20 μl DTNB (1.5 mg/ml) and 20 μl glutathione reductase (6U/ml). Increase I absorbance at 412 nm was monitored. A blank assay, without glutathione, was run separately. For calibration, the procedure was repeated using 100 μl GSSG (10 μM). GSSG was determined with glutathione reductase according to:

$$\mathrm{GSSG + NADPH + H^+ \xrightarrow{GSSG\ reductase} 2\ GSH + NADP^+}$$

The reaction was monitored directly by measuring the conversion of NADPH at 340 nm at 25°C. Reaction mixture contained 1.0 ml of sample, 10 μl EDTA (100 mM), 0.1 M potassium phosphate buffer, pH 7.0, 10 μl

NADPH. The reaction was started by the addition of 5 μl glutathione reductase (20 U/ml). For the measurement of standard GSSG, sample was replaced by 1.0 ml buffer and standard GSSG with 5 nmol.

Total glutathione and GSSG were expressed as μmol. g hemoglobin^{-1} . GSH was calculated as [total glutathione]-2 x [GSSG].

Protein determination

Protein contents of erythrocyte lysates were determined according to the method of Bradford (23) and plasma protein contents were determined according to the method of Lowry (24).

Clinical chemistry

Plasma levels of some biochemical parameters were measured in autoanalyser (Roche Modular System) and the hematological parameters were determined using an electronic cell counter (Coulter STKS).

Statistical analysis

The results were expressed as the mean ± SD. Kruskall-Wallis analysis and Mann Whitney U-test of variance were used for comparison of the groups of the variables . Chi-square test was used for comparison of the demographic characteristics of the groups. Correlations between variables were assessed with Pearson's correlation coefficients (r). $p< 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Table 1 summarizes the demographic and clinical characteristics of the subjects included in the study. No statistically significant difference was found between the study groups in age and years of education. The average age of the onset of alcohol abuse symptoms was significantly lower in type II alcoholics. The difference was found to be statistically significant ($p<0.01$). Duration of alcohol dependence was found to be significantly higher in type II alcoholics ($p<0.05$). The lifetime severity of alcohol abuse symptoms, as measured by MAST, however was not statistically different between the alcoholic groups.

Table 1 Demographic and clinical features of the study group

	Type I alcoholics (n=17)	Type II alcoholics (n=16)	Control (n=17)
Age	45.1±6.3	41.6±9.6	40.3±8.4
Years of education	12.4±3.2	10.2±3.3	10.8±3.1
No.of smokers(%)	15/17(88.2)	15/16(93.8)	15/17(88.2)
Age of onset	30.4±5.5	19.4±0.5*	-
Duration of alcohol abuse/ dependence	14.1±6.9	22.3±9.7*	-
MAST	33.0±7.3	36.3±6.9	-

Results are expressed as mean ± SD

* $p<0.01$, versus to that of type I alcoholic group

Plasma SSAO activities and major biochemical parameters demonstrating the liver functions of the subjects were presented in Table 2. Plasma SSAO values of alcoholics were found to be 522.23±11.02 mU.l^{-1}, which was significantly higher ($p<0.001$) than the value of control group of 336.42±12.90 mU.l^{-1}. Previous studies have shown that plasma SSAO activity increased in patients with diabetes mellitus, cardiovascular disease, Alzheimer's disease, stress-related angiopathy and obesity (9-11,25-27) suggesting the idea that SSAO was somehow expressed in high amounts in blood vessels and in plasma to cause tissue damage via hydrogen peroxide produced as side-product of deamination reactions drived by SSAO. Although considerable researches suggest a role of oxidative stress in diabetic complications, cardiovascular damage or Alzheimer's disease by contribution of SSAO (11, 28), no data has been found the role of increased oxidative stress via elevated SSAO activity in alcoholic liver damage. It is unclear why and how plasma SSAO activity is increased in some pathological conditions, but it may be a consequence of initial vascular damage or/and an uncontrolled leakage into the blood circulation from the damaged liver tissue of alcoholics.

Type II alcoholics had higher plasma SSAO activity than that of the type I alcoholics ($p<0.001$) (Table 2). This result indicated that age-of onset and the duration of the alcohol intake might effect the SSAO activity in the plasma of alcoholics corresponding to the severity of the liver damage in this patient group. However, genetic markers effecting the liver or plasma SSAO activities as well as the hereditary tendency to alcoholism in these groups also had to be assessed.

Lipid peroxidation determined as the malondialdehyde (MDA) was found to be elevated both in plasma

Table 2 Biochemical parameters and SSAO activity in plasma of the subjects investigated

Parameters	Type I alcoholics (n=17)	Type II alcoholics (n=16)	Control (n=17)
SSAO (mU.l^{-1})	572.23±10.56 ^{b}	488.75±11.13 ^{bc}	336.42±12.90 ^{a*}
AST (IU.l^{-1})	20.16±3.70 ^{b}	29.21±4.81 ^{b*}	17.16±4.70
ALT (IU.l^{-1})	25.10±4.80 ^{b}	34.11±4.33 ^{b*}	21.20±5.07
ALP (IU.l^{-1})	59.20±7.17	60.78±6.55	59.34±5.56
GGT (IU.l^{-1})	83.75±8.60 ^{a}	84.06±9.05 ^{a}	80.22±9.46
Total bilirubin (mg.dl^{-1})	0.58± 0.06	0.59±0.09	0.57±0.07
Total protein (g.dl^{-1})	6.93±0.80 ^{a}	6.77±0.60 ^{a}	7.68±1.23
Albumin (g.dl^{-1})	3.71±0.46 ^{a}	3.86±0.75 ^{a}	4.81±0.35
Glucose (mg. dl^{-1})	84.23±9.87^{a}	83.34±8.86^{a}	81.21±10.34

ALT: alanine aminotransferase AST: aspartate aminotransferase ALP: alkaline phosphatase GGT: γ-glutamyltransferase

Results are expressed as mean ± SD

[a] $p< 0.01$ versus control group

[b] $p<0.001$ versus control group

[c] $p<0.001$ versus type I alcoholic group

and erythrocyte lysates in alcoholic group (n=33) (0.299±0.011 and 0.305±0.012 nmol.mg protein^{-1}, respectively) when compared with those of the control group (n=17) (0.189±0.007 and 0.210±0.010 nmol.mg protein^{-1}, respectively, $p<0.001$). This data is in good agreement with the previous ones reported that lipid peroxides played a crucial role in the pathogenesis of ethanol-induced cell injury (29). MDA levels in plasma and erythrocyte lysates of type II alcoholics were dramatically reached to 0.302±0.010 and 0.362±0.014 nmol.mg protein^{-1}, respectively and this value was statistically significant when compared with 0.201±0.008 and 0.229±0.011 nmol.mg protein^{-1}, respectively ($p<0.001$) in type I alcoholics (Table 3). It has been shown that formaldehyde, which was produced by the deamination of methylamine and aminoacetone endogenously, caused the formation of adducts with proteins and nucleic acids, and appeared as quite cytotoxic and carcinogenic (30). Formaldehyde was previously shown to be normally metabolized in liver also could be oxidized by hydrogen peroxide to produce excited formaldehyde and singlet oxygen under physiological pH (31) leading to oxidative stress. Thus, it seems reasonable to suggest that the significantly increased plasma SSAO activity in type II alcoholics could enhance oxidative stress and contribute to alcoholic complications indicated with the certain parameters such as increased plasma ALT, AST and GGT levels in these patients (Table 2).

Glutathione contents of plasma and erythrocytes of the subjects were presented in Table 3. GSH content of plasma and erythrocyte lysates were significantly decreased while GSSG levels were significantly increased in alcoholics when compared with those of controls. Our data also indicated that GSH contents of plasma and erythrocytes of type II alcoholics were significantly lower than those of the type I subjects. Since it was reported earlier that the decrease in GSH content in liver or erythrocytes of alcoholics might be caused by increased utilization of GSH to scavenge the toxic intermediates formed by ethanol consumption (32,33), we suggest that disturbed antioxidant status in early-onset alcoholics could be possibly due to the increased ROS production in liver which might be resulted by the increased SSAO activity.

Since it was recently proposed that decreased GSH levels in alcoholics were not due to enhanced conversion to GSSG, but rather was due to a production failure in the liver of these patients (34), we calculated the GSH/GSSG ratio both in plasma and erytrocytes of the subjects. The ratio was found to be markedly lowered in alcoholic plasma and erythrocytes (Table 3). Significant positive correlations between the plasma and erythrocyte GSH/GSSG ratios and the levels of GSH ($r= 0.62$ and 0.60, $p<0.01$, respectively); negative correlations between the levels of GSH/GSSG ratios and the levels of GSSG ($r=0.59$ and 0.58, $p<0.01$, respectively) were found in the alcoholics. Data with decreased blood GSH/GSSG ratios together with decreased blood glutathione and increased blood MDA levels reflected the fact that decreased GSH levels as a consequence of enhanced ROS production in alcoholic subgroups rather than the production failure of GSH in their livers. GSH levels and GSH/GSSG ratios were negatively correlated with the age-of onset and the duration of the alcohol intake in alcoholics (for type I $r=0.51$ and 0.55, $p<0.01$; for type II $r=0.68$ and $r=0.70$, $p<0.001$, respectively) suggesting that the age-of onset and the duration of the alcohol intake strongly effected the degree of liver damage in alcoholics.

Present study also showed that the erythrocyte count and the hemoglobin content of the blood in alcoholics were found to be decreased (Table 4). This result together with the previous one sowed that erythrocytes of alco-

Table 3 Lipid peroxidation status and glutathione contents of plasma and erythrocyte lysates in the study groups.

Parameters	Type I alcoholics (n=17)	Type II alcoholics (n=16)	Control (n=17)
Lipid peroxidation in plasma (nmol MDA.ml^{-1})	3.35±0.90[b]	4.85±0.99 [a,*]	2.80±0.75
Lipid peroxidation in lysate (nmol MDA.g hemoglobin^{-1})	2.99±0.01[b]	4.72±0.03[a,*]	2.10±0.01
GSH (μmol.ml^{-1}) in plasma	2.55±0.31 [b]	1.97±0.20 [a,*]	3.01±0.40
GSH (μmol.g hemoglobin^{-1}) in lysate	2.76±0.33 [b]	2.15±0.25 [a,*]	3.26±0.51
GSSG (μmol.ml^{-1}) in plasma	0.32±0.02 [a]	66.50±3.98 [a,*]	22.67±2.01
GSSG (μmol.g hemoglobin^{-1}) in lysate	0.36±0.03 [a]	0.80±0.05 [a,*]	0.22±0.01
GSH/GSSG in plasma	8.93±2.05 [a]	2.50±0.75 [a,*]	14.98±3.08
GSH/GSSG in lysate	8.26±2.11 [a]	2.41±0.81 [a,*]	15.23±3.07

Results are expressed as mean ± SD
[a] $p< 0.001$ versus control group
[b] $p<0.01$ versus control group
*$p<0.001$ versus type I alcoholic group

Table 4. Hematology of the subjects investigated

Parameters	Type I alcoholics (n=17)	Type II alcoholics (n=16)	Control (n=17)
Red blood cell count (10^6. μl^{-1})	4.80±1.19 [b]	3.90±1.01 [a,*]	4.91±1.70
White blood cell count (10^3. μl^{-1})	9.85±1.19[b]	10.05±1.80	10.11±1.76
Hemoglobin (g.dl^{-1})	11.20±1.50 [b]	9.05±0.95 [a,*]	12.99±1.05
Packed cell volume (%)	38.22±3.64 [b]	32.20±2.70 [a,*]	40.45±3.10
Platelets (10^4)	25.10±3.16	23.05±4.80	24.60±4.31
MCV (fl)	90.12±6.55 [b]	99.12±7.04 [a,*]	83.25±6.21

Results are expressed as mean ± SD
[a] $p< 0.001$ versus control group
[b] $p<0.01$ versus control group
*$p<0.01$ versus type I alcoholic group

holics were found to be highly fragile and susceptable to lipid peroxidation a consequence of failed antioxidant potential and greater oxidative burden (35) suggested that elevated lipid peroxidation might also be a cause of hemolysis and anemia often detected in alcoholics. The finding that erythrocyte count and the hemoglobin content in blood were negatively correlated with lipid peroxidation in plasma and erythrocytes of the subjects ($r= 0.45$ and 0.46 in type I and 0.43 and 0.51 in type II alcoholics, $p<0.05$, respectively) supported the previous findings suggesting that the end products of lipid peroxidation such as MDA could induce modification in structure, fluidity and permeability of erythrocyte membranes (36). Stronger correlation found between these parameters in type II alcoholics suggested that the age-of onset and the duration of the alcohol intake are also important factors leading to degradation of the erythrocytes in alcoholics.

This study demonstrated that elevated plasma SSAO activity was associated with increased lipid peroxidation and diminished GSH/GSSG ratio in the blood of alcoholic patients possibly related to the increased oxidative stress caused by the production of ROS formed by the increased activity of SSAO. However, further studies are needed to determine the exact role of SSAO in the pathogenesis of alcohol-induced oxidative stress in alcoholic subtypes.

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