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Oxidative stress status of individuals involved in traffic accidents*

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Aim: To investigate the role of the oxidative stress status of drivers involved in traffic accidents as a risk factor.

Materials and methods: Twenty-six drivers and 28 passengers involved in traffic accidents and 31 healthy control drivers and 30 healthy control pedestrians were enrolled in the present study. The total antioxidant status (TAS) and total oxidant status (TOS) were measured in blood samples.

Results: The mean value of the TOS level in drivers involved in traffic accidents was significantly higher than that of the control pedestrians ($P \leq 0.05$). The mean value of the TAS level in passengers involved in traffic accidents was significantly lower than that of the control drivers and pedestrians ($P \leq 0.001$ and $P \leq 0.01$, respectively).

Conclusion: This is the first study assessing the oxidative markers of individuals involved in a traffic accident compared with a control group. The results reveal that involvement in a traffic accident has an effect on oxidative markers. However, further studies are required in order to use the oxidative stress status of drivers involved in traffic accidents as a marker for estimating the accident risk.

Key words: Total oxidant status, total antioxidant status, oxidative stress index, traffic accident, driver, forensic medicine

Trafik kazalarına karışmış kişilerde oksidatif stres durumu

Amaç: Bu çalışmanın amacı trafik kazasına karışmış sürücülerde oksidatif stress durumunun risk faktörü olarak rolünü araştırmaktır.

Yöntem ve gereç: Trafik kazalarına karışmış 26 sürücü ve 28 yolcu ile 31 sağlıklı kontrol sürücü ve 30 sağlıklı kontrol yaya çalışmaya dahil edildi. Kan örneklerinde toplam antioksidan kapasite (TAK) ve toplam oksidan seviye (TOS) çalışıldı.

Bulgular: Kazaya karışan sürücülerin ortalama TOS değeri kontrol yayaların ortalama TOS değerinden anlamlı olarak yüksek bulundu ($P \leq 0,05$). Kazaya karışan yolcuların ortalama TAK değeri ise kontrol sürücülerin ve kontrol yayaların ortalama TAK değerlerinden anlamlı olarak düşüktü (sırasıyla, $P \leq 0,001$ ve $P \leq 0,01$).

Sonuç: Çalışmamız kazaya karışan sürücü ve yolcularda oksidatif ve antioksidatif durumu değerlendiren ve kontrol grupları ile kıyaslama yapan ilk çalışmadır. Sonuçlarımız kazaya karışma durumunun oksidatif stres belirteçleri üzerine etkisi olduğunu göstermektedir. Ancak trafik kazasına karışmış sürücülerde oksidatif stres durumunun kaza yapma riskini tahmin etmede bir belirteç olarak kullanılabilmesi için daha fazla sayıda çalışmalar gerekmektedir.

Anahtar sözcükler: Toplam oksidan seviye, toplam antioksidan kapasite, oksidatif stres indeksi, trafik kazası, sürücü, adli tıp

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Introduction

Traffic accidents are an important public health problem associated with significant mortality, disability, and labor loss. The World Health Organization (WHO) has reported that more than 1.2 million people die on the roads and 20-50 million people suffer nonlethal injuries annually (1).

In order to reduce the number of traffic accidents and to prevent the unwanted consequences, it is essential to identify the causes of accidents and the associated risk factors (2,3). Driving at excessive speeds (4), obstructive sleep apnea syndrome (5), Alzheimer disease, Parkinson disease, epilepsy (6), and alcohol consumption (7) are among the risk factors for traffic accidents.

Total antioxidant status (TAS) and total oxidant status (TOS) have been investigated for many medical situations and health problems. Oxidative stress is a state of imbalance between free radicals, formed during normal aerobic metabolism or intra- and extracellular pathologic events and antioxidants, in favor of the oxidants. Oxygen radicals are the major free radicals formed within biological systems (8). Free radicals lead to oxidative tissue injury in many central nervous system pathologies. Free oxygen species result in oxidative tissue injury through multiple mechanisms, including excitotoxicity, metabolic dysfunction, and impairment of calcium intracellular homeostasis (9). They may lead to cell injury or death by both themselves and by inactivating several enzymes and proteins. Several defense mechanisms exist to keep the levels of reactive oxygen species under control and prevent potential harm. The greatest contribution to the total antioxidant capacity comes from the antioxidant molecules in the plasma. In addition to bilirubin, free iron-binding, transferrin and ceruloplasmin, uric acid, vitamin E, and vitamin C, there are also chain-breaking antioxidants that neutralize free radicals in plasma. Albumin, uric acid, and ascorbic acid constitute >85% of the total antioxidant capacity in human plasma. Rather than the individual antioxidants, measurement of the TAS is preferred to detect the antioxidant status of blood (10).

It is possible to measure the blood levels of antioxidants individually; however, it is time-consuming and expensive. Moreover, measurement of a single antioxidant or several antioxidants may not reflect the entire status. Therefore, several methods have been developed to evaluate the oxidant/antioxidant status, which measure the TAS and TOS (11,12). The TAS and TOS have not been investigated as individual risk factors for traffic accidents until now.

The aim of the present study was to determine whether or not the TAS and TOS levels can be considered as risk factors for traffic accidents and whether or not the TAS and TOS levels can be used to determine the driver of the vehicle involved in a traffic accident. The parameters that are the subject of this study may contribute to the medical literature and support methods that can be used to elucidate suspicious traffic accidents, which have a significant place in forensic medicine.

Materials and methods

Study groups

Male drivers and passengers who were admitted to the Emergency Department (ED) of Harran University's Medical Faculty between January 2010 and May 2010 as a result of a traffic accident were included in this prospective, controlled study. Two separate control groups were formed; the first group involved healthy male drivers whose occupation was driving, and the second group involved healthy males without a driver's license. The study was approved by the Harran University School of Medicine's Ethical Board, and written informed consent was obtained from all of the participants.

Drivers with measurable blood alcohol concentrations, passengers with a history of consumed alcohol, subjects who had a history of sedatives/hypnotics or stimulating substances, subjects who had acute traumatic injuries, subjects with a history of medical conditions within the last 3 months, current smokers, and subjects who had X-ray examinations and vaccinations were excluded from the study. None of the subjects were taking drugs known to affect lipid or lipoprotein metabolism. Special care was taken to exclude subjects who were taking anabolic drugs, vitamins, or other antioxidants. None of the subjects were following a special diet.

Blood samples of the study group were taken in a period of a maximum of 2 h (1.49 ± 0.76 h) after a traffic accident at the ED. Drivers in the control groups had been driving for 3-8 h (taxi/car, bus, or truck) every day. Blood samples of drivers in the control group were taken in the same period after the driving had stopped. Likewise, the samplings for pedestrians were included in the control group of subjects who never drive.

Age, height, weight, and educational status of the participants and accident-related information were recorded.

Laboratory measurements

Blood samples were drawn from an antecubital vein into heparinized tubes and were immediately placed on ice. Plasma was separated from cells by centrifugation at 4000 rpm for 5 min. The plasma samples were stored at –80 °C until analysis.

The TAS levels were determined using a novel automated measurement method developed by Erel (11). With this method, hydroxyl radicals, which are the most potent biological radicals, are produced. The assay measures the antioxidative effect of the sample against potent free radical reactions that are initiated by the hydroxyl radical produced. The precision of the assay is excellent, at <3%. The results are expressed as mmol Trolox equivalent/L.

The TOS levels were determined using a novel automated measurement method, as described previously (12). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by the glycerol molecules present in the reaction medium. The ferric ion produces a colored complex with xylenol orange in acidic solution. The color intensity, which is measured spectrophotometrically, is proportional to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 equivalent/L) (12).

The percent ratio of TOS to TAS was accepted as the oxidative stress index (OSI) (10). The index was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equiv/L) / TAS (mmol Trolox equiv/L) (13).

Statistical analysis

Study data were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Intergroup comparisons were performed using one-way ANOVA, and intragroup pairwise comparisons were performed with the Bonferroni test. The Mann-Whitney U test was used for nonnormally distributed variables, identified with the Kolmogorov-Smirnov Z test, for variables below 30 observations, and a chi-square test was used to evaluate the distribution of individuals within the study groups. $P \leq 0.05$ was considered statistically significant.

Results

Included in the study were 115 individuals. The distributions of the individuals to the study groups were: Group 1 (n = 26), drivers who had experienced a traffic accident; Group 2 (n = 28), passengers who had experienced a traffic accident; Group 3 (n = 31), healthy male drivers who had not had a traffic accident; and Group 4 (n = 30), healthy male pedestrians with no driver's license.

There was no significant difference among the groups in terms of age, body mass index (BMI), and marital status. Most of the drivers and passengers who had experienced a traffic accident and the drivers in the control group were primary school graduates. The demographic characteristics of the study groups are presented in Table 1.

The vehicles mostly involved in the traffic accidents were cars, and motorcycles ranked second. Most of the drivers in the control group were driving a car, followed by a minibus. The accidents mostly occurred between 16.00 and 23.59 hours, and accidents were rarest between 00.00 AM and 08.00 hours. Of the drivers involved in a traffic accident, 12 did not have a driver's license. The duration of having a driver's license was significantly shorter in drivers who had experienced a traffic accident compared to drivers in the control group. There was no significant difference between drivers and passengers who were involved in a traffic accident in terms of location of the accident, time of the accident, and the time between the accident and blood sampling at the ED. The traffic accident-related information is summarized in Table 2.

Table 1. Demographic characteristics of individuals in the study groups.

	Group 1 (n = 26)	Group 2 (n = 28)	Group 3 (n = 31)	Group 4 (n = 30)	P
Age (years)	29.08 ± 11.97	31.29 ± 14.68	34.35 ± 6.48	30.07 ± 7.77	0.252
BMI (kg/m^2)	24.37 ± 1.97	23.55 ± 1.78	24.77 ± 2.63	24.75 ± 3.40	0.235
Education					
Literate	1	11	0	0	
Primary	17	12	12	2	<0.001
High school	6	5	11	12	
University	2	0	8	16	
Marital status (bachelor/married)	15/11	17/11	26/5	17/13	0.085

Data presented as mean ± SD or n of individuals.
BMI: body mass index.

Table 2. Data regarding the vehicle, driver, and traffic accidents.

	Group 1 (n = 26)	Group 2 (n = 28)	Group 3 (n = 31)	P
Type of vehicle				
Car	14	14	17	
Bus	0	0	1	0.001
Minibus	1	11	13	
Motorcycle	10	3	0	
Bicycle	1	0	0	
Driver's license				
Present	14		31	0.001
Absent	12		0	
Duration of driver's license (years)	4.42 ± 6.40		11.32 ± 6.81	0.011
Location of the accident (within city limits/outside city limits)	20/6	18/10		0.310
Time of accident				
08.00-15.59 hours	12	13		0.574
16.00-23.59 hours	13	15		
00.00-08.00 hours	1	0		
Time period between the accident concerned and the taking of a blood sample (h)	1.49 ± 0.76	1.93 ± 0.90		0.072

The mean value of the TOS level in drivers involved in traffic accidents was significantly higher than that of the control pedestrians (mean ± standard deviation (SD), 13.44 ± 3.90 vs. 10.84 ± 3.20; $P \leq 0.05$). The mean value of the TAS level in passengers involved in traffic accidents (0.78 ± 0.19) was significantly lower than that of the control drivers (0.96 ± 0.17) and pedestrians (0.94 ± 0.16) ($P \leq 0.001$ and $P \leq 0.01$, respectively). The oxidative stress parameters in the study groups are presented in Table 3. The OSI of the passengers who experienced a traffic accident was significantly higher than that of the control drivers and pedestrians (Figure; Table 3).

Discussion

Previous studies have reported that oxidative stress levels are elevated in several disorders, including

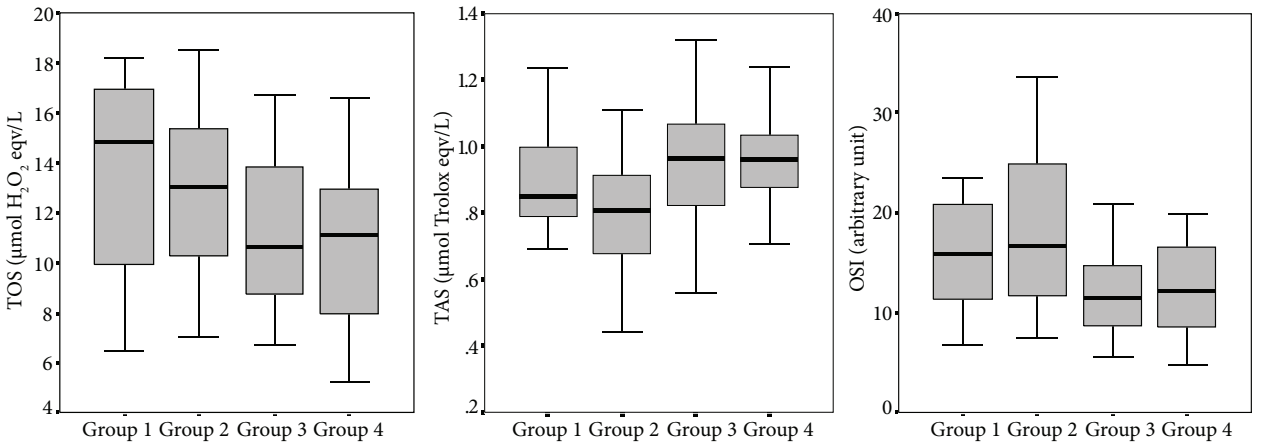

Figure. The OSI of passengers who experienced a traffic accident was significantly higher than those of the control drivers and pedestrians.

Table 3. Comparison of oxidative stress parameters among the study groups.

	Group 1 (n = 26)	Group 2 (n = 28)	Group 3 (n = 31)	Group 4 (n = 30)	P
TOS (µmol H_2O_2 equiv/L)	13.44 ± 3.90	12.82 ± 3.25	11.09 ± 3.10	10.84 ± 3.20[a*]	<0.001
TAS (mmol Trolox equiv/L)	0.89 ± 0.15	0.78 ± 0.19	0.96 ± 0.17[b***]	0.94 ± 0.16[c**]	0.009
OSI (arbitrary unit)	15.51 ± 5.48	17.92 ± 7.72	12.20 ± 4.98[b***]	12.06 ± 4.41[c***]	<0.001

Data presented as mean ± SD.
TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index.
[a]Difference between groups 1 and 4.
[b]Difference between groups 2 and 3.
[c]Difference between groups 2 and 4.
*P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.

obstructive sleep apnea syndrome, Friedreich ataxia, Alzheimer disease, parkinsonism, epilepsy, hyperactivity disorder, and chronic fatigue syndrome, which may potentially impair driving skills and increase the risk of traffic accidents (14-16).

To investigate the isolated effects of traffic accidents on oxidative status, subjects with conditions that may potentially affect oxidative markers, such as acute traumatic injury, medical conditions, and usage of medications, sedatives/hypnotics, or stimulating substances, were excluded from our study, along with smokers and subjects who consume alcohol or follow a special diet.

The study groups were similar in terms of age, sex, and BMI, which are important parameters that may affect oxidative stress markers. It is known that oxidative stress levels may be variable in conditions such as pregnancy and lactation (17). Although different age groups have been reported to be affected in different countries and different regions of the same country, it has consistently been noted that men are significantly more affected in traffic accidents in terms of both drivers and accident-related casualties (18-20). Therefore, only males were included in this study. Individuals who consumed alcohol were not included. It has been reported that driving under the influence of alcohol increases the risk of accidents.

Although previous studies involved different age groups, most of the drivers under the influence of alcohol were reported to be men (3,19,21). It has been stated that alcohol reduces antioxidant levels by changing the oxidant/antioxidant balance and increasing free radicals (22). Current smokers were not included, as smoking has been associated with a reduction in antioxidant levels and an increase in free radicals (23). Several studies reported that oxidative stress levels are increased in patients with psychiatric conditions, including schizophrenia, autism, and bipolar affective disorder (24,25). Individuals who had a history of sedative/hypnotic or stimulating substance use were not included. It has been reported that the risk of a traffic accident is increased in individuals using hypnotics, benzodiazepines, muscle relaxants, and psychoactive drugs (26). As many studies reported increased levels of oxidative stress in individuals exposed to trauma, individuals with acute traumatic injury prior to or due to the traffic accident were not included (27,28).

The mean age of the drivers who had experienced a traffic accident was 29.08 ± 11.97 years. The remaining groups also involved males in the similar age group. The mean age of the study population was within the age range reported to be most affected by traffic accident-related deaths by the WHO (1).

In the present study, the TOS levels of the drivers who experienced a traffic accident were significantly higher than those of the pedestrians in the control group ($P \leq 0.05$). This might be attributed to the increased oxidative stress levels in drivers due to the stress induced by driving and by the recently experienced accident. Ascensão et al. (29) evaluated 10 elite male motocross riders in a 1.5-km motocross simulation competition. From each rider, 3 blood samples were drawn (1 sample during rest, 1 sample at baseline, and 1 sample 1 h after the race). The mean malondialdehyde level, which was 324.79 nmol/g protein at rest and 394.63 nmol/g protein at baseline, was reported to decrease to 303.43 nmol/g protein at 1 h. The mean TAS value, which was 1.51 mmol/L at rest and 1.83 mmol/L at baseline, was reported to increase to 1.86 mmol/L at 1 h. Thus, motocross racing was demonstrated to increase the level of plasma oxidative stress (29).

In the present study, no significant difference was found between the TOS values of the drivers and passengers involved in a traffic accident. This might be explained by the fact that both drivers and passengers had an equal amount of stress, which resulted in a similar amount of oxidative stress due to the traffic accident. The finding that there was no significant difference between the TOS values of the drivers involved in a traffic accident and the drivers in the control group might be attributed to the similar amount of stress experienced by the control drivers due to active driving.

Although it did not reach statistical significance, the TOS value of the passengers involved in a traffic accident was slightly higher compared to those of the drivers and pedestrians in the control groups. The grading of the TOS levels in the groups might be explained by the stress levels to which the groups were exposed. The study groups could be ranked in descending order in terms of potential stress levels experienced by the subjects as follows: drivers involved in a traffic accident, passengers involved in a traffic accident, drivers working constantly in traffic, and pedestrians in the control group. Thus, it may be suggested that being in traffic results in an increase in oxidative stress and that involvement in an accident leads to a further increase in this stress level. Previous studies have investigated the causes of traffic accidents and associated risk factors (2,3). Similarly, in several studies, it was shown that some disorders have an effect on the OSI (14-16).

Only drivers involved in a traffic accident were included in the present study. Drivers in the control groups consisted of drivers who had never been involved in a traffic accident. We could not compare the results with the initial values of these subjects before the traffic accident; hence, there is a limitation of the study in the impossibility of taking the blood samples of drivers before an accident. Moreover, despite this study being related to the metabolic consequences of stress in a traffic accident, we could not assess whether the drivers had undergone stress before the accident.

In the present study, the TAS value of passengers involved in a traffic accident was significantly lower compared to that of the drivers and pedestrians in the control groups ($P \leq 0.001$ and $P \leq 0.01$, respectively).

Although it did not reach statistical significance, the TAS value of drivers involved in a traffic accident was lower than the TAS values of the subjects in the control groups. The human body has developed an antioxidant defense system against oxidative stress, rapidly reacting with free radicals formed as a result of oxidative stress, thus preventing the progression of autoxidation and protecting the organism from damage. The stress, which is an inevitable consequence of a traffic accident, may lead to an increase in the oxidative stress level, and defense antioxidants may subsequently be consumed and lead to a decrease in the TAS level.

The OSI of passengers involved in a traffic accident was significantly higher than those of the drivers and pedestrians in the control groups (both $P \leq 0.001$). Although it did not reach statistical significance, the OSI of drivers involved in a traffic accident was slightly higher than those of subjects in both of the control groups. However, there was no investigation of the relation of the OSI with traffic accidents.

Identification of the driver of a vehicle in a traffic accident is an important issue in forensic medicine. In traffic accidents, juridical authorities may occasionally request the identification of the driver. Büken et al. (30) investigated a traffic accident in which the legal authorities requested the identification of the driver. All of the individuals in one of the vehicles died. There were 3 individuals in the other vehicle, but there were no eye witnesses. Bloodstain evidence did not help to solve the problem, since one of the individuals noted that he/she had entered the driver's seat to get a document. An initial opinion report was formed after the physical examination of the individuals, and it was reported that the case was closed after the individual confessed to the crime (30).

Our findings demonstrated that involvement in an accident resulted in oxidative stress, which was reflected in markers. A significant difference was found between the oxidative markers of accident survivors and the control subjects. In light of these findings, we suggest that oxidative markers may help to identify the driver because the detection of low antioxidant levels suggests high levels of oxidative stress, which correlates with the severity of stress depending on the active or passive state of the individuals in the accident. However, further studies are needed to confirm this finding before deciding to use oxidative markers to identify drivers.

In order to avoid risk factors for traffic accidents, including diseases and conditions affecting perception ability, attention, and reflexes, and to give appropriate warnings, we recommend that oxidative stress levels should be measured in the medical check-up of drivers who are involved in traffic. This measurement can be performed during the initial physical examination of drivers before licenses are issued. Although high results may result from a transient condition, they may also indicate an underlying systemic disease, sleeplessness, chronic fatigue syndrome, smoking, or alcohol consumption or illicit substance use. Drivers whose licenses have been suspended due to driving under the influence of alcohol should be subject to a psychotechnical examination. An OSI measurement during this examination may be helpful during the follow-up and psychological support and education of problematic drivers to understand the harmful effects of alcohol and other substances. Additionally, these individuals should be evaluated in terms of chronic fatigue and TAS measurements during all medical examinations, and they should use antioxidant vitamin C and E supplements in the case of low TAS levels. This study is important in that it draws attention to these issues and provides preliminary findings.

In conclusion, it is necessary that every piece of data be taken into account when evaluating the causes of traffic accidents and associated risk factors. We recommend that TAS and OSI measurements be made during routine medical check-ups of drivers, especially for professionals. Furthermore, studies focusing on the development of more simple techniques, such as urinary OSI measurement, are needed. We suggest that these findings will contribute to the medical literature and may support methods that can be used to understand suspicious traffic accidents, which have a significant place in forensic medicine. Further studies may reveal whether the studied parameters could be used to discriminate between drivers and passengers, or if these laboratory tests might be used routinely in the evaluation of risk factors of traffic accidents or in routine evaluations of motor vehicle drivers.

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