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TRAFİK KAZASI GEÇİREN TRAVMALI HASTALARDA TRİSS SKORLAMASI İLE NO VE TBARS SEVİYELERİ ARASINDAKİ İLİŞKİ

THE CORRELATION BETWEEN THE TRISS TRAUMA SCORE AND PLASMA LEVELS OF NO AND TBARS IN MULTIPLY TRAUMATIZED PATIENTS FOLLOWING TRAFFIC ACCIDENT

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ÖZET

Amaç: Çoğul travmalı hastalarda travma şiddetinin belirlenmesi ve travma derecesinin organizma üzerine etkilerinin belirlenebilmesinde sorunlar vardır. Trafik kazalarına bağlı çoğul travmaların oldukça yüksek olduğu ülkemizde bu sorunların çözümlenebilmesi önemlidir. Çalışmamızda trafik kazası geçiren çoğul travmalı hastalarda travma şiddeti ile plazma NO(Nitrik oksit) ve Thiobarbituric Asid Reactive Substance (TBARS) seviyeleri arasındaki ilişkiyi belirlemeyi hedefledik.

Gereç ve Yöntem: Trafik kazası nedeniyle acil servisimize getirilen çoğul travmalı hastalardan TRISS puanı 30'un üzerindeki kişileri çalışma grubu olarak , 30'un altındaki hastaları kontrol grubu olarak belirleyip, her iki gruptan aldığımız plazma örneklerinden NO ve TBARS seviyeleri arasındaki ilişkiyi değerlendirdik. Çalışmamızda, TRISS (Trauuma Score, Injury Severity Score, Age Combination Index) travma skorunu kullandık.

Sonuç: Travma derecesinin artması ya da azalmasının plazma NO seviyeleri üzerine herhangi bir etkisinin olmadığını tesbit ettik ($p<0.05$). TRISS puanı ile plazma TBARS seviyeleri arasında pozitif yönde istatistiksel olarak anlamlı bir ilişki olduğu ($p<0.05$), travma şiddetinin artmasının plazma TBARS seviyesini arttırdığı tesbit edildi.

Anahtar Kelimeler: Çoğul Travma,TRISS Travma Puanı, Nitrik Oksit, TBARS

SUMMARY

Background: There are controversies on determination of trauma severity and on predicting effects of trauma severity on organism in patients with multiple trauma. It is of great importance to rule out these controversies in Turkey where incidence of multiple trauma due to traffic accidents is considerably high. The aim of our study was to investigate whether a correlation exists between trauma severity and plasma levels of nitric oxide and thiobarbituric acid reactive substance in patients with multiple trauma.

Methods: The patients with multiple trauma were divided into two groups as the study group (TRISS score 30 or above) and the control group (TRISS score below 30) and the relationship between plasma NO and TBARS levels was evaluated. In our study, we used the TRISS trauma score (Revised Trauma Score, Injury Severity Score and Age Combination Index) to evaluate trauma severity.

Results: There was no effect of trauma severity on plasma NO levels ($p>0.05$). There was a positive correlation between TRISS scores and plasma TBARS levels ($p<0.05$).

Conclusions: It can be suggested that measurement of plasma NO levels is not useful to indicate trauma severity because change in TRISS is not associated with a correlated change in plasma NO levels. On the other hand, there is a significant correlation between plasma TBARS levels and increase in trauma severity

Key Words: Multiple Trauma, TRISS Trauma Scoring, Nitric Oxide, TBARS

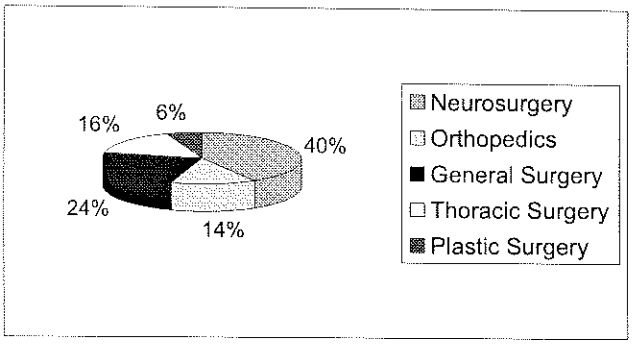
INTRODUCTION

Since traffic accidents affect mostly the working young and middle-aged groups, they bring about financial and moral losses, which persists to be a serious public health problem in our country. Therefore, trauma evaluation and management in terms of mortality and morbidity should be given more emphasis (1).

In order to assess success in trauma management, it is necessary to determine objectively the severity of injury. The information about the severity of trauma will help to determine any possible changes that might arise in the patient and to apply a planned treatment program (2,3).

In evaluating multiple trauma cases, grading and scoring of the trauma are important. In our study, therefore, we used

Fig1.The Departments where the patients were referred



TRISS scoring system in which the physiologic and anatomic scores were used together (1). The TRISS trauma score consists of ISS (Injury Severity Score), RTS (Revised Trauma Score) and A (Age) combinations. Survival probability of trauma patients can be calculated by using the TRISS scoring system with its neutral methods (1,4,6). It is also possible for the hospitals to evaluate their own abilities and to compare their results to those of the other hospitals in determined time intervals (6,7).

The free radicals play a role in pathogenesis of the clinical situations with their structure, physical and chemical characteristics, cell sources, chemical reactions and their effects. Due to free radicals, the hydrogen atom leaving the unsaturated fatty acids in the cell membrane starts lipid peroxidation. The relations between the harmful effects of lipid peroxidation in the organism and the formation of various diseases have been studied extensively (5).

In this study, we studied the relationship between plasma NO and TBARS levels in patients with multiple trauma and the severity of trauma.

Our aims were as follows: 1- to determine effects of increased trauma severity on plasma levels of NO and TBARS and on clinical management of the patients, 2- to investigate whether possible correlations between these parameters could be consistently used in predicting prognosis.

MATERIAL AND METHOD

Patients with multiple trauma who were brought to the emergency department following traffic accidents were evaluated according to TRISS trauma scoring. The patients with a TRISS score of 30 or more were included in the study group (n:52), and those with a score below 30 in the control group (n:26). The TRISS form was used in the scoring of the patients . Of the study group, 36 were men (69%) and 16 were women (31%). Their average age was 35 years (range 18 and 71). Severely injured patients in this group were hospitalized in various departments of surgery.

Blood samples were collected using a syringe washed with heparin from a peripheral vein on the day of arrival at the emergency unit, and 2nd and 7th days of the trauma. The samples were centrifuged for 10 min at 2000 rpm. After separating from the blood, the serum was transferred to another tube, which was sealed with paraffin and kept at 70°C. NO and TBARS were studied in these plasma samples.

The control group contained victims of traffic accidents who had TRISS scores lower than 30.There were 18 male (70%) and 8 female (30%) patients in the control group. These patients were observed for a period less than 24 hours, so only the first day blood samples were obtained. The control group contained the patients who were referred to the emergency department (ED) following a traffic accident and had a TRISS below 30. Blood-samples were drawn from these patients immediately after their arrival to the ED. All clinical and laboratory evaluations of the control group patients were performed in the ED. All of the patients in this group were observed in the ED during a period shorter than 24 hours and then discharged. The group contained 26 patients, 18 of whom were male (70 %) and 8 were female (30 %). The age range was between 19 and 70 years (mean: 38 years).

Blood samples could not be drawn from the patients of the control group on the 2nd and 7th days because they were discharged following an observation period shorter than 24 hours. NO and TBARS levels obtained immediately after the arrival of the control group patients to the ED were taken into consideration in statistical comparisons.

NO Measurement

Total nitric oxide assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of Griess reaction. The Griess reaction is based on the two- step diazotization reaction in which acidified NO_2^- produces a nitrosating acent which reacts with sulfanilic asid to produce the diazonium ion. We used commercial kit from (R&D Systems, Nitric Oxide Assay, Catalogue Number DE 1500).

TBARS Measurement

Plasma lipid peroxidation was estimated by TBARS assay determination of TBARS were made by Okhawa method (8).The plasma sample was incubated for 1 hour at 95°C with thiobarbituric asid, which a TBARS-MDA(malondialdehyde) adduct was measured by absorption at 530 nm. A standard curve for absorption and MDA concentration was then determined, the amount of lipid peroxidation was reported as micro molar MDA equivalents. The run-to-run CV for TBARS at our laboratory was 5,9%.

The statistical calculations of the data in a group were performed Scheffe by Annova test for repeated measures and scheffe tests and those between the separate groups were performed through Mann-Whitney-U test and

correlation analysis. The statistics program SPSS was used for calculations in the computer.

RESULTS

While mean TRISS score in the patient group was 36 (range, 30-48) , that in the control group was 17.5 (range, 11-26). There was a statistically significant difference in terms of TRISS scoring between the study and control groups ($p<0.05$) (Table I.)

All the patients were multiply traumatized and brought to emergency department following a traffic accident. They were referred to the departments relevant to their major injuries (Figure 1). Twelve of the patients with a TRISS score greater than 30 were in hypovolemic shock state. These patients were administered intravenous fluid and blood in ED. Fourty two of the patients (87 %) were operated on immediately or in the first week following the admission to the relevant departmens. All the operations were performed under general anesthesia and postoperative care was carried out in the surgical intensive care unit. The patients who developed infection during the follow up were excluded from the study.

The TBARS and NO levels in the plasma collected from the study group on the day of arrival at the emergency service and 2nd and 7th days following trauma were evaluated (Table I).

Table 1. Relationship between the TBARS and NO levels on 1st ,2nd and 7th days in patients with multiple traumas

Days	1st (X± SD)	2nd (X±SD)	7th (X±SD)	F	p
TBARS (µmol/ L)	2,43±1,18	2,56±0,94	3,51±1,42	12,56	<0,05
NO (µmol/ L)	33,9 ± 40,5	33,69±51,7	26,7 ± 33,6	0,48	>0,05

It was determined that there was a statistically significant difference between the TBARS levels of the patients measured on days 1, 2 and 7 ($p<0.05$). It was noted that this significant difference was caused by the 7th day plasma samples, and that 7th day plasma samples increased more compared to those on days 1 and 2 (Table I).

When the plasma TBARS levels of the study and control groups were compared, the difference was statistically significant ($p<0.05$) (Table II). However, the difference between NO levels was not statistically meaningful ($p>0.05$)(Table III).When the plasma TBARS levels of the patients group according to the TRISS scores using the correlation method, the difference found was statistically significant ($p<0.05$). There was a positive correlation between these two parameters. As the TRISS scores increased, the plasma TBARS level also increased (Table IV).

Table 2: The comparison of TBARS levels according to study and control groups

Days	1st	2nd	7th
Study (X ± SD)	2,43 ± 1,19	2,43 ± 1,19	3,52 ± 1,42
Control (X ± SD)	1,82±0,99	1,82±0,99	1,82±0,99
T	2,28	3,25	5,46
P	<0,05	<0,05	<0,05

The comparison was found between the TRISS trauma score and the daily TBARS levels. Similarly, when a correlation was made between the TRISS scores and NO levels, it was determined there was no relationship between them either positively or negatively ($p>0.05$).

DISCUSSION

Following a trauma many metabolic and endocrine changes occur in the organism. The individual response to the trauma and the metabolic changes affect the rate of mortality and morbidity. Following trauma and bleeding, there is inconsistency in NO formation. Although it is claimed that the NO synthesis is inhibited in trauma patients, the inhibition mechanism is unknown. Ischemia may be responsible for this, because molecular oxygen is needed for the NO formation. On the other hand, protection from vasodilatation after trauma seems to be important (10,13).

NO made by neutrophils, macrophages, microglia and vascular smooth cells, astrocytes and other cells passes to the extracellular fluid following trauma and is oxidised to nitrite and nitrate. As the half-life of NO is very short, there are no studies measuring the direct nitric oxide level. Peroxynitrite, which is the product of the reaction of NO with superoxide, is a potent oxidant with a relatively

Table 3: The comparison of NO levelaccording to study and control groups

Days	1st	2nd	7th
Study (X ± SD)	33,97 ± 40,6	33,97 ± 40,6	33,97 ± 40,6
Control (X ± SD)	21,9±27,1	21,9±27,1	21,9±27,1
T	1,37	1,09	0,63
P	>0,05	>0,05	>0,05

Table 4 The relation between the TRISS scores and daily TBARS levels

Days	r	p
1st. day	0,30	p<0,05
2nd day	0,31	p<0,05
7th day	0,51	p<0,05

longer life than NO. During its decomposition excessive NO is formed which starts lipid peroxidation (9,10,11,12). It is possible to have an idea about the serum NO level by using the serum nitrite and nitrate values. Even though various clinical studies have used nitrite and nitrate measurements as a measure of the endogenous NO formation, this method has its limitations. Systemic plasma levels can be affected by the nitrogen equilibrium and renal excretion (13,14).

Timoty et al (13) conducted a study and examined the NO metabolism in the trauma patients. Starting the 5th day following the trauma, a significant decrease was observed in the plasma nitrate levels, which were reported to have remained constant until the 14th day. This decrease in the nitrate levels could not be explained with urinary excretion. The trauma patients are unable to regulate NO production. It is reported that the reason for this decreased production is not the substrate unsuitability (13).

In a study conducted by Goodman (14) in 1995 normal and decreased plasma nitrate levels were observed following head trauma. Ochoa et al (15) reported a decrease in plasma nitrate concentrations in patients with general body trauma. It was evaluated whether the decrease was caused by the increase in the excretion and was found that there was a direct relation between the decreased plasma levels and unchanged urine levels. The question whether the pressure is a normal physiologic anomaly or a necessity of the conformity mechanism could not be answered clearly.

The plasma NO levels of the patients admitted to the intensive care unit because of multiple trauma were studied. NO levels of the patients who died on day 3 following trauma were found high. It was determined that the NO levels increased in proportion with sepsis (15,16). In our study, there was no decrease in the plasma NO levels of the multiple trauma patients with a high degree of trauma. When serial plasma samples were evaluated, there was a significant decrease in the average NO levels on day 7 following the trauma compared to the first two days. However, this decrease was not statistically significant (Table I). When these results were compared to the control group, it was determined that the trauma degree did not affect the plasma NO level (TableIII).

Our results are parallel to the previous literature studies. Although the reason for the decrease in the plasma NO levels on day 7 following trauma is not clear, the individual response to the trauma, nitrogen imbalance in the units where the patients were followed up , post operative anaesthesia, and an increase in the renal clearance may be responsible. Further studies may help to clarify this issue.

In patients with multiple trauma, hypoperfusion and subsequent hypoxia of the tissues can occur from inadequate resuscitation either immmediately after the trauma or in the days of recovery. Damage occurs at the cellular level with lipid peroxidation and thus begins a dangerous chain reaction begins. Once the membrane structure has been altered, reactive aldehydes are produced which result in tissue damage and many diseases (19).

It is known that free oxygen radicals and toxic metabolites formed cause cellular damage by disrupting the cellular nucleic acid, membrane lipids, enzymes and receptors (17,18). This increase in free oxygen radicals induces lipid peroxidation. As a result of this there is damage in the cellular membrane and cellular dysfunction is formed. Lipid peroxides formed as a result of membrane damage negatively affect the mitochondrial functions. TBARS which is a final product of the lipid peroxidation, is a highly toxic metabolite and increases tissue damage by denaturing intracellular enzymes and proteins (20). During the peroxidation of the fatty acids containing three or more double stands TBARS measurable thiobarbituric acid (TBA) is formed. This method is frequently used in the measurement of lipid peroxide levels. TBARS is not a specific or quantitative indicator of the fatty acid oxidation degree (21).

In an experimental study, plasma TBARS levels following reperfusion were studied in dogs with multiple organ insufficiency and shock after gun-shot injuries; the results were compared to the dogs with gun-shot injury and without shock and multiple organ insufficiency as well as the plasma TBARS levels of the dogs before injury. The plasma TBARS levels in the first group were significantly higher when compared to the group who only had gun-shot injury without damage and shock (22).

Another study showed that lipid peroxidation increases TBA reactive materials following trauma and in the post-operative period (23). The formation of free radicals can be increased by various stress factors. Therefore, the patients' metabolism levels are important for the use of various anesthetics and the formation of respiratory support lipid peroxidation products. Enzymatic productive systems such as Superoxide dismutase, glutation peroxidase, catalase are beneficial

mechanisms because they prevent the formation of free oxygen radicals and their harmful effects. The production or mechanisms of actions of these enzymes depending on the increased need in stressful situations for the body such as trauma or surgery influence the production or activation of these enzymes.

In our study, when compared to the first two days, a statistically significant increase was determined on day 7 in TBARS levels of the patients with a TRISS trauma score of 30 and above ($p<0.05$), (Table II). When the results were compared to the control group, there was a statistically significant difference ($p<0.05$) (Table III). Multiple system injuries secondary to trauma, the onset of lipid peroxidation as a direct result of hypoxia formed due to hypo perfusion might have caused this increase. The fact that this increase is less in the early period, but more on day 7 following trauma can be explained with the insufficiency of the enzymatic or endogenous antioxidant defence mechanisms.

On the other hand, it can be possible to explain the increase of the lipid peroxidation products in patients with multiple trauma and post-operative patients by the ischemia reperfusion damage arising in such patients. The ischemia-reperfusion damage begins with a short period of ischemia. If ischemia lasts longer, cellular necrosis occurs and the effect on the tissues will be more profound such that restoring blood circulation will not revitalize the tissues (16,17, 18).

We had limitations in taking blood samples from the patients of the control group on the 2nd and 7th days. Only first-day blood samples were taken from the control group. The explanation of this limitation is as follows: the city where our hospital is situated, Kayseri, is very close to main highways forming crossroads. Most of the traffic accident patients, therefore, live in other cities than Kayseri. For this reason, it was not possible to keep or recall outpatients on the 2nd and 7th days. We had to use only first-day measurements of the control group for comparison. Because these patients had minor trauma and their TRISS score could be considered to be significant on the first day only with regard to trauma severity and also likelihood of complete recovery on the 7th day would be high, NO ve TBARS levels of the control group on the 7th day can be suggested to be close to those of healthy population. We had to make limited interpretations on the matter because only a few studies similar to ours are present and no studies or articles on measurement of levels of lipid peroxydase and free oxygen radicals in multiple trauma patients are present in the literature. Our study can be suggested to give ideas to new studies.

In the present study, it was determined that TRISS score had a positive correlation with plasma TBARS levels (Table IV), which means that as the trauma degree increases, the patients' plasma TBARS levels also increase. When the relation between the TRISS trauma scores and the plasma NO levels was evaluated, it was determined that the trauma degree had no effect on the plasma NO levels neither positively nor negatively. Therefore, the increase in the trauma degree neither increases or decreases the plasma NO level.

As a result of this study, it can be suggested that measurement of plasma NO levels is not a useful method to indicate trauma severity because increase in TRISS is not associated with increase in plasma NO levels. On the other hand, there is a significant correlation between plasma TBARS levels and increase in trauma severity. For this reason, detection of increases in plasma TBARS levels can be suggestive of a highly severe trauma.

More helpful is the significant positive correlation between plasma TBARS levels and trauma severity. A multiple trauma patient with a high TBARS level should be suspected for severe trauma and aggressive resuscitative measures and monitoring initiated to prevent unfavourable outcomes of multiorgan dysfunction.

It can be concluded that measurement of NO level in multiply traumatized patients with high trauma scores has no contribution on ED evaluation of the patients or planning of the resuscitative interventions and that measurement of TBARS level in these patients can be useful as an alert while patients in follow up period or under observation.

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