ORIGINAL ARTICLE

# The utility of serum miRNA-93 and miRNA-191 levels for determining injury severity in adults with multiple blunt trauma

Özgür Söğüt, M.D.,[1] Merve Metiner, M.D.,[2] Onur Kaplan, M.D.,[1] Mustafa Çalık, M.D.,[2] Sümeyye Çakmak, M.D.,[3] Tuba Betül Ümit, M.D.,[1] Hüseyin Ergenç, M.D.,[1] Fahri Akbas, M.D.,[4] Seda Süsgün, M.D.[4]

[1]Department of Emergency Medicine, University of Health Sciences, Haseki Training and Research Hospital, İstanbul-*Turkey*
[2]Department of Emergency Medicine, University of Health Sciences, Gaziosmanpaşa Training and Research Hospital, İstanbul-*Turkey*
[3]Department of Emergency Medicine, University of Health Sciences, Dr. Sadi Konuk Training and Research Hospital, İstanbul-*Turkey*
[4]Department of Medical Biology, Bezmialem Vakif University Faculty of Medicine, İstanbul-*Turkey*

## ABSTRACT

**BACKGROUND:** Various scoring systems have been developed to determine the trauma severity and prognosis of patients following multiple blunt trauma (MBT). However, these scoring systems do not provide exactly the desired severity assessment. In recent years, serum concentration of many specific microRNAs (miRNAs), especially for head trauma, has been shown to play an important role in determining the diagnosis, severity, and prognosis of injury. To date, however, no studies have investigated serum miRNAs in patients with MBT. Thus, this study measured the expression of miRNA-93 and -191 in the serum of adults with MBT and examined the correlations of Injury Severity Score (ISS) and Revised Trauma Score values with serum miRNA-93 and -191 levels in these patients with the aim of predicting trauma severity based on the miRNA levels.

**METHODS:** This prospective case–control study enrolled 50 consecutive adults with MBT and age- and sex-matched 60 healthy controls. The patients were divided into ISS >16 (Group 1, major or severe trauma) and ISS ≤16 (Group 2, minor or mild-moderate trauma) groups. Serum miRNA-93 and -191 levels were assessed using quantitative real-time reverse transcription-PCR. We evaluated whether the miRNAs were differentially expressed in major and minor MBT patients and determined their utility for assessing the severity of injury.

**RESULTS:** The mean serum miRNA-93 and -191 levels were significantly elevated in the patients compared to the controls and were higher in patients with ISS >16 compared to those with ISS ≤16, although the difference was not significant. In the patients with multitrauma, ISS was significantly, negative and weak correlated with serum miRNA-191 level (rho=−0.320, p=0.023) but not with the serum miRNA-93 level. No optimal cutoff for the serum miRNA-93 level was found with respect to trauma severity (AUC 0.617, [0.455–0.779]). However, an optimal cutoff value for serum miRNA-191 was identified, with values <1.94 indicating severe trauma (AUC 0.668 [0.511–0.826]; 65.6% sensitivity, 77.8% specificity).

**CONCLUSION:** miRNA-191 and -93 levels were significantly upregulated in multitrauma patients compared to controls. The level of miRNA-191 in conjunction with ISS, but not that of miRNA-93, may be a useful biomarker for determining injury severity in patients with multitrauma.

**Keywords:** Injury severity score; miRNA-93; miRNA-191.

Cite this article as: Söğüt Ö, Metiner M, Kaplan O, Çalık M, Çakmak S, Ümit TB, et al. The utility of serum miRNA-93 and miRNA-191 levels for determining injury severity in adults with multiple blunt trauma. Ulus Travma Acil Cerrahi Derg 2021;27:631-638.

Address for correspondence: Özgür Söğüt, M.D.
Sağlık Bilimleri Üniversitesi, Haseki Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği, İstanbul, Turkey
Tel: +90 212 - 453 20 00 E-mail: ozgur.sogut@sbu.edu.tr

Ulus Travma Acil Cerrahi Derg 2021;27(6):631-638 DOI: 10.14744/tjtes.2020.45470 Submitted: 02.05.2020 Accepted: 25.07.2020

## INTRODUCTION

Multitrauma is obvious trauma involving more than one large organ system. For example, patients may have head injuries, multiple fractures, and injuries to the internal organs of the chest, limbs, pelvis, and abdomen. The severity of trauma is closely related to the number of affected body areas.[1] Trauma is the leading cause of death in healthy young adults aged 18–44 years. The most common causes of trauma are traffic accidents, falls, and assaults. Trauma risk scores have been developed to assess the severity, mortality, and morbidity of trauma in these patients.[2]

The main scoring systems used to evaluate trauma severity and prognosis in patients with multiple blunt trauma (MBT) are the Injury Severity Score (ISS), Revised Trauma Score (RTS), and Trauma Score.[3,4] The ISS is the most predictive and reliable score for clinical development and prognosis.[4,5] It is a scaled measurement indexed to anatomical injury. By scoring the damage done by trauma to each organ (with the Abbreviated Injury Score), the squares of the three highest scores, that is, the most severely injured regions, are added to give ISS.[5,6] Major trauma is considered present with ISS >15.[6] Bolorunduro et al.[7] validated the ISS and categorized the injuries as mild (<9), moderate (9–15), severe (16–24), and profound (≥25). These scoring systems measure the static condition of an injury and are widely used in epidemiological studies. However, when used alone, these scoring systems inadequately describe injury severity and clinical outcomes.[5,7] Recent studies have shown the utility of serum microRNA (miRNA) levels for determining injury severity, diagnosis, and prognosis in head trauma.[8–10]

miRNA is a post-transcriptional non-encoding RNA class that acts as the target protein in neuron development and plays a role in intercellular signal transduction.[9] Numerous human miRNA molecules represent similar properties, such as RNA stability and size and are abundantly expressed. However, their expression levels can change under different experimental conditions and may be affected in various diseases.[11,12] miR-191 was the most consistently expressed miRNA across different human tissues, followed by miR-93, miR-106a, miR-17–5p, and miR-25.[13] Although many studies have investigated miRNA levels in rats with experimental traumatic brain injury (TBI),[14–17] few human studies have measured serum miRNA levels and predicted trauma severity and prognosis in the early post-trauma period (within the first 24 h).[18,19] Indeed, the applicability of these markers to MBT remains unknown. Therefore, this study quantified the serum expression of miRNA-93 and -191 in adults with MBT following the early post-trauma period and examined the correlations of ISS and RTS with the serum miRNA-93 and -191 levels in these patients with the aim of predicting trauma severity and prognosis using these serum miRNA levels.

## MATERIALS AND METHODS

This prospective case–control study was conducted in accordance with the 1989 Declaration of Helsinki and was approved by the Ethics Committee of Haseki Training and Research Hospital (trial registration no. 592). The study was funded by the Health Sciences University Board of Scientific Research Projects (grant no. 2019/002). Venous blood was collected within 24 h of trauma onset from consecutive patients with MBT admitted to our tertiary care university hospital emergency department (ED) between June 2017 and December 2018. Participants included 50 consecutive adults with MBT (8 females, 42 males; age 18–64 years) due to vehicle accidents, vehicle–pedestrian accidents, falls from a height, or assaults and age- and sex-matched 60 healthy controls who exhibited no evidence of disease or acute trauma.

Patients were provided basic life support at presentation and advanced trauma life support (ATLS) with the current 2018 ATLS-10 guideline if required.[2] After monitoring vital functions, informed consent was obtained directly from the patient or an authorized representative. Healthy volunteers were informed about the study protocol, and consent was obtained from all participants before their enrollment in the study. Inclusion criteria were adults (≥18 years of age) with MBT. Three patients who had traumatic cardiac arrest due to severe TBI that may affect the miRNAs expression levels were excluded from the study. The RTS and ISS were calculated for each patient. The patients were divided into two groups: Those with ISS >16 (Group 1; major or severe trauma) and those with ISS ≤16 (Group 2; minor or mild-moderate trauma).

The serum miRNA-93 and -191 levels were assessed in MBT patients and healthy controls using quantitative real-time polymerase chain reaction (qRT-PCR). For blood sampling and miRNA isolation and quantification, the examiners were blinded to the subjects' histories and diagnoses. Blood samples were drawn from each participant's antecubital vein immediately after presentation to the ED; samples were collected in vacuum gel tubes and immediately placed on ice at 4°C without the use of medications or serum infusions. Plasma was separated from the cells by centrifugation at 2515× g for 10 min (Electro-mag M615E, Istanbul, Turkey) and immediately stored at −80°C until determination of the miRNA-93 and -191 levels.

Total RNA in serum samples was isolated using the Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's protocol. An equal volume of room-temperature (RT) 2× denaturing TRIzol reagent was added to 700 µL of a 32-unit sample and immediately vortexed. The vortexed samples were then centrifuged at 12,000 rpm for 10 min at 4°C to separate the organic phase. The upper clear phase was transferred to a new tube. Next, RT 100% ethanol at a volume 1.25× the

sample volume was added to the tube and mixed well. This mixture (ethanol/lysate) was then filtered. The filter was washed with 700 µL of miRNA Wash 1 Solution. This procedure was repeated twice with 2/3 Solution. Finally, RNA was collected from the filter in 50 µL RNase/DNase-free water. The concentration and quality of the obtained RNA were assessed by spectrophotometry (Thermo Scientific Multiskan GO, Vantaa, Finland). The samples were considered adequate if their A260/A280 ratio was between 1.8 and 2.0. The RNA isolation kit (Direct-zol RNA MiniPrep Kit, Zymo Research) was used in compliance with the manufacturer's instructions for isolating total RNA from isolated and stored cardiomyocytes.

Total RNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples with concentrations between 200 and 700 ng/µL were used for microarray analysis and qRT-PCR.

cDNA was synthesized using 1 µg of the obtained total RNA in accordance with the information in the literature and with the manufacturer's protocol (Qiagen miScript Reverse Transcription Kit, Qiagen, Venlo, Netherlands).[19–21] In brief, 3.5 µL RNA was mixed with the solution provided in the kit containing poly-A polymerase and reverse transcriptase to reach a final volume of 20 µL. cDNA was synthesized from total RNA after the resulting solution was incubated at 37°C for 60 min and then at 95°C for 5 min.

cDNA samples (3 µL) were mixed with SYBR dye, universal primer, the miRNA-specific primer provided in the kit, and water to reach a final volume of 20 µL, and the mixture was loaded into the RT-PCR instrument. Fluorescence measurements were recorded during the lag phase of the reaction. Primers specific to miR-93 and miR-191 were used in the reactions along with the U6 primer as the reference gene. The crude miRNA data obtained by RT-PCR were analyzed using the delta delta CT (ΔΔct) method.[19,21]

miRNA levels were calculated using Δct values. Nominal miRNA levels below the clinical norms indicated upregulation, and higher levels indicated downregulation. The result was determined using the absolute value of the fold change. Because a negative nominal value, rather than a reduction, results from the relative expression of miRNA, it corresponds to upregulation, that is, an increase compared to normal levels.

**Table 1.** Δct-based quantification of miR-191 in random patient and control samples

| | Δct | Standard deviation | | |
|---|---|---|---|---|
| Patient | 3.14 | 2.75 | −2.53 | 0.904608 |
| Control | 0.61 | 2.49 | 2.53 | |

Δct: Delta CT.

Δct = Target gene mean ct−Control gene mean ct
Fold change = 2−Δct

When calculating the fold-change value, a negative value is obtained as a constant, which prevents the miRNA value from being negative, indicating that a smaller numerical value will, in fact, correspond to upregulation (i.e., an increase) (Table 1).

The required sample size was calculated by power analysis before data collection based on information from previous studies.[18,19,22] An estimated 50 patients and 60 controls were required to detect significant differences between the groups with a power of 95% and an alpha error of 5%. The data collected in the study were analyzed using SPSS 15.0 for Windows (IBM, Armonk, NY, USA). Descriptive statistics are expressed as numbers and percentages for categorical variables. Quantitative variables are expressed as the mean, standard deviation, median, and interquartile range. The miRNA-93 level and gender distribution in two independent groups was compared using Student's independent t-test and Pearson's Chi-squared test, respectively, when the data were normally distributed. Otherwise, the Mann-Whitney U-test was used to compare patient groups for non-normally distributed data (e.g., miRNA-191 level and age). Pearson's correlation was used to evaluate correlations of ISS and RTS with data on the normally distributed serum miRNA-93 and -191 levels. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff value of miRNA-93 and miRNA-191 levels for predicting the severity of trauma according to ISS scores. The decisive factor was analyzed using forward logistic regression analysis to determine the cutoff value for miRNA-191. The significance level was set at $p<0.05$.

## RESULTS

The mean age of the 50 patients with MBT was 34.22±13.53 (range 18–64) years, and 42 (84%) were male. The mean age of the 60 healthy volunteers was 35.97±10.74 (range 19–65) years, and 52 (86.7%) were male. Age (p=0.171) and gender (p=0.444) did not differ significantly between the patients and controls. The mean serum miRNA-93 and -191 levels were significantly elevated in patients compared to controls (both $p<0.001$; Table 2). The mechanisms of injury in the patients were falling from heights (38%), motor vehicle accidents (24%), assaults (16%), motor vehicle-pedestrian collisions (14%), and motorcycle accidents (8%) (Table 3).

Seventeen patients (34%) were hospitalized, and 33 (66%) were discharged from the ED. The mean hospital stay was 3.5 days (range 2–7 days). There was no significant difference between the hospitalized and discharged patients in terms of age or gender (p=0.448 and p=0.442, respectively). The mean serum miRNA-93 and -191 levels of the hospitalized patients were upregulated compared with those of dis-

**Table 2.** Comparison of age, gender, and serum quantities of miRNA-93 and miRNA-191 between the patient and control groups

| Characteristic | Patient group | | Control group | | p* |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | |
| Age | 30.50 | 23–43.50 | 34 | 27.25–41 | 0.171 |
| Gender** | | | | | |
| Male | 42 (84%) | 52 (86.7%) | 0.449 | | |
| Female | 8 (16%) | 8 (13.3%) | | | |
| miRNA-93** | 3.23±2.39 | 1.71–10.83 | 6.04±2.43 | -1.91–9.07 | <0.001 |
| miRNA-191 | 1.94 | 0.67–4 | 3.43 | -1.92–5.36 | <0.001 |

Non-normally distributed data are expressed in median and IQR.
*The Mann-Whitney U-test was used to compare age and miRNA-191 level, and Pearson's Chi-squared test was used to compare the gender distribution between groups.
**Normally distributed data are expressed in numbers, percentages, and mean±standard deviation.
Student's t-test was used to compare the serum miRNA-93 level between groups. IQR: Interquartile range, miRNA: microRNA.

**Table 3.** Causes of multiple blunt trauma in patients admitted to the emergency department

| | n | % |
|---|---|---|
| Cause of multiple blunt trauma | | |
| Fall from a height | 19 | 38 |
| Motor vehicle crash | 12 | 24 |
| Assault | 8 | 16 |
| Motor vehicle–pedestrian crash | 7 | 14 |
| Motorcycle crash | 4 | 8 |

charged patients; this difference was significant for the mean serum miRNA-191 level (2.90±2.46 vs. 1.41±1.63; p=0.004) but not for the mean serum miRNA-93 level (5.54±2.21 vs. 6.30±2.53; p=0.300). Table 4 compares the age, gender, and serum miRNA-93 and -191 levels between the hospitalized and discharged patients.

When patients were evaluated using the ISS score to assess trauma severity, there was no significant difference in age or gender between patients with ISS ≤16 (minor trauma) and ISS >16 (major trauma) (p=0.578 and p=0.391, respectively; Table 5). No deaths occurred among those with major or minor trauma.

The mean serum miRNA-93 and -191 levels were increased in patients with ISS >16 compared to patients with ISS ≤16 (5.41±2.21 vs. 6.40±2.50 and 1.78±1.84 vs. 2.95±2.48, respectively), although the differences were not significant (p=0.169 and p=0.087, respectively, Table 5). There was no significant correlation between the serum miRNA-93 level and ISS value in patients (rho=−0.207 and p=0.149; Table 6) with multitrauma. However, there was a significant negative but weak correlation between the serum miRNA-191 level and ISS (rho=−0.320 and p=0.023; Table 6). In addition, there was no significant correlation between the RTS value and serum miRNA-93 or miRNA-191 levels (miRNA-93:

**Table 4.** Comparison of age, gender, and serum quantities of miRNA-93 and miRNA-191 between the hospitalized and discharged patient groups

| Characteristic | Hospitalized | | Discharge | | p* |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | |
| Age | 30 | 24–51 | 31 | 23–42.5 | 0.448 |
| Gender** | | | | | |
| Male | 15 (88.2%) | | 27 (81.8%) | | 0.442 |
| Female | 2 (11.8%) | | 6 (18.2%) | | |
| miRNA-93** | 5.54±2.21 | | 6.30±2.53 | | 0.300 |
| miRNA-191 | 0.86 | 0.49–2.94 | 2.16 | 0.72–4.80 | 0.004 |

Non-normally distributed data are expressed in median and IQR.
*The Mann-Whitney U-test was used to compare age and miRNA-191 level, and Pearson's Chi-squared test was used to compare the gender distribution between groups.
**Normally distributed data are expressed in numbers, percentages, and mean±standard deviation. Student's t-test was used to compare the serum miRNA-93 level between groups. IQR: Interquartile range, miRNA: microRNA.

**Table 5.** Comparison of age, gender, and serum quantities of miRNA-93 and miRNA-191 between the ISS ≤16 and ISS >16 patient groups

| Characteristic | ISS ≤16 | | ISS >16 | | p* |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | |
| Age | 32.5 | 26–41.75 | 29.5 | 23.75–48.50 | 0.578 |
| Gender** | | | | | |
| Male | 26 (81.3%) | | 16 (88.9%) | | 0.910 |
| Female | 6 (18.8%) | | 2 (11.1%) | | |
| miRNA-93** | 6.40±2.50 | | 5.41±2.21 | | 0.169 |
| miRNA-191 | 2.89 | 1.71–5.20 | 1.03 | 0.50–2.42 | 0.087 |

Non-normally distributed data are expressed in median and IQR.
*The Mann-Whitney U-test was used to compare age and miRNA-191 level, and Pearson's Chi-squared test was used to compare the gender distribution between groups. **Normally distributed data are expressed in numbers, percentages, and mean±standard deviation. Student's t-test was used to compare the serum miRNA-93 level between groups. ISS: Injury severity score, IQR: Interquartile range, miRNA: microRNA.

**Table 6.** Correlations of serum miRNA-93 and miRNA-191 levels with RTS and ISS

| Characteristic | ISS | RTS | miRNA-93 | miRNA-191 |
|---|---|---|---|---|
| ISS | | | | |
| rho* | 1 | -.337* | -.207 | -.320* |
| P | | .017 | .149 | .023 |
| RTS | | | | |
| rho* | -.337* | 1 | .166 | .047 |
| P | .017 | | .251 | .747 |
| miRNA-93 | | | | |
| rho* | -.207 | .166 | 1 | .530** |
| P | .149 | .251 | | .000 |
| miRNA-191 | | | | |
| rho* | -.320* | .047 | .530** | 1 |
| P | .023 | .747 | .000 | |

*ISS, RTS, serum miRNA-93 and miRNA-191 values were calculated by Pearson correlation test. ISS: Injury severity score; RTS: Revised trauma score; miRNA: microRNA.

rho=0.166, p=0.251; miRNA-191: rho=0.047, p=0.747; Table 6).

ROC analysis identified a cutoff serum miR191 level of 1.94 for determining the severity of trauma according to ISS scores in MBT patients, with 65.6% sensitivity and 77.8% specificity (area under the curve [AUC] 0.668, 95% confidence interval [CI] 0.511–0.826; Fig. 1). Serum miR191 values below 0.15 indicated that severe trauma was present in patients with MBT. However, no optimal serum miRNA-93 cutoff value was found for determining the severity of trauma according to ISS scores in patients with multitrauma (AUC 0.617, CI 0.455–0.779; Fig. 2).

## DISCUSSION

miRNAs are important post-transcriptional regulators of complementary mRNA targets and have been implicated in the pathophysiology of TBI.[10] Clinical and experimental studies have identified miRNAs in serum/plasma (e.g., miRNA-425-p, -21, -93, -191, and -499) as useful biomarkers for assessing TBI diagnosis, prognosis, and severity.[10,23] Several reports have indicated upregulation of miRNA-93, -191, and -499 in the cerebral cortex and dorsal hippocampus tissues in rats after experimentally created TBI.[14,17,24,25] However, whether these three miRNA subtypes are expressed in human serum after MBT is unclear. Therefore, we examined the expression of miRNA-93 and -191 and their associations with MBT using qRT-PCR. The key findings are as follows: (1) The patients with MBT were mostly male and falls from heights represented the most frequent cause of injuries; (2) compared with the control group, the serum miRNA-93 and -191 levels were significantly upregulated in MBT patients, and were higher in patients with ISS >16 compared to those with ISS ≤16, although the difference was not significant; (3) a significant negative correlation was observed between the serum miRNA-191 level and ISS in patients with MBT; and (4) in the ROC analysis, significant efficacy of the miRNA-191 cut off value of <1.94 had a 65.6% sensitivity and 77.8% specificity in predicting the severity of trauma according to ISS scores.

Recent clinical data implicate that circular RNAs have highly expressed in patients with neurological diseases, which indicates their neurospecificity.[26] A few human studies have investigated the severity of trauma and the relationships of miRNA levels with the prognosis after TBI.[18,19,27] In a clinical study of 76 patients with TBI treated either conservatively or surgically and 38 controls, Yang et al.[19] quantified the serum miRNA-93, -191, and -499 levels over 21 days and found that the serum levels of these three miRNA subtypes were significantly higher in patients than in controls. The se-

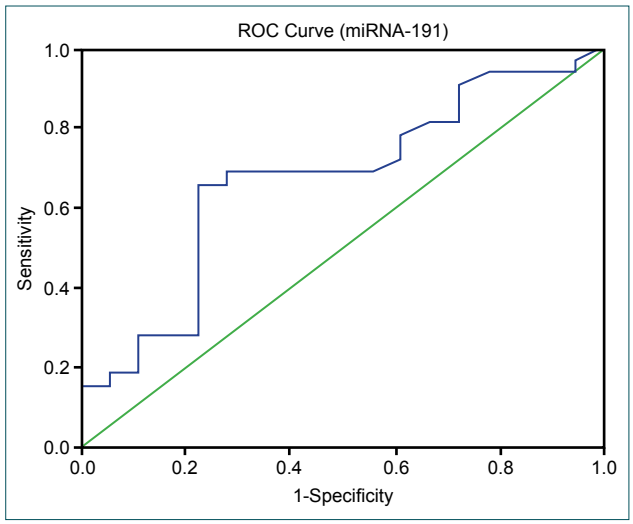


**Figure 1.** Specificity and sensitivity of the serum miRNA-191 level for determining the severity of trauma according to ISS using ROC curves (area under the curve 0.668; 95% confidence interval 0.511–0.826; 65.6% sensitivity, 77.8% specificity). ISS: Injury severity score, ROC: Receiver operating characteristics.

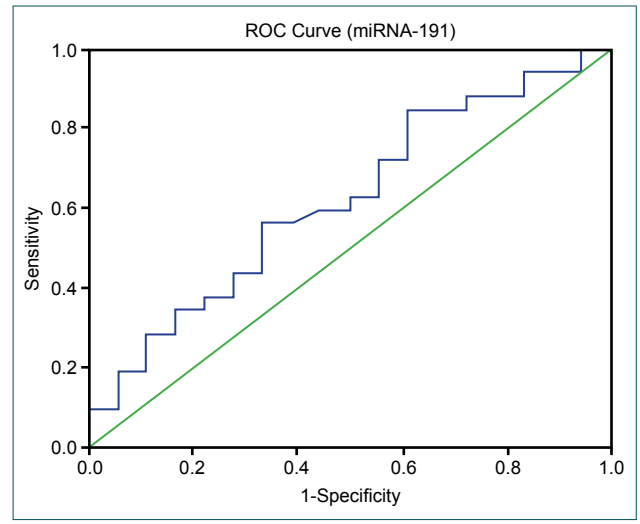


**Figure 2.** Specificity and sensitivity of the serum miRNA-93 level for determining the severity of trauma according to ISS using ROC curves (area under the curve 0.617; 95% confidence interval 0.455–0.779). ISS: Injury severity score, ROC: Receiver operating characteristics.

rum levels of these three miRNA subtypes were also significantly associated with the severity of trauma and with poor outcomes. Tas et al.[28] found that the mean serum levels of miRNA-93 and -191 were 4.3- and 6.2-fold higher, respectively, in patients with minor head trauma than in controls. Using cranial computed tomography (CCT), they found that the mean serum miRNA-93 and -191 levels were significantly higher in patients with abnormal CCT findings compared to those with normal CCT findings. In the present study, the serum miRNA-93 and -191 levels were quantified within 24 h of ED admission after trauma before any treatment to investigate their ability to predict the severity of trauma and their correlations with trauma scores. Among the miRNA subtypes examined, we found significant increases in the serum miRNA-93 and -191 levels in MBT patients within the first 24 h after trauma compared to controls, consistent with Yang et al.[19] Taken together, these findings suggest that the serum miRNA-93 and -191 levels are upregulated and associated with pathophysiological processes in patients with MBT. Thus, elevated these miRNAs levels might be attributable to the development of organ damage in MBT patients.

In the present study, when the patients with MBT were evaluated in accordance with hospital discharge, the mean serum miRNA-191 levels were significantly higher in hospitalized patients than in those discharged. Evaluation of the severity of trauma using the ISS showed that the serum miRNA-93 and -191 levels were higher in the patients with major trauma (ISS>16), but this increase was not significant. The serum miRNA-93 and ISS were not significantly correlated in patients with multitrauma. However, there was a significant correlation between the serum miRNA-191 and ISS (rho=–0.320, p=0.023). Regarding relationship of trauma severity and ISS scores, an optimal cut-off was established for the serum miRNA-191 level: Values below 1.94 indicated severe trauma (AUC 0.668 [0.511–0.826]; 65.6% sensitivity, 77.8% specificity). These results demonstrate that serum miRNA-19 level may reliably predict the severity of injury in MBT patients when used in combination with the ISS and therefore serve as a new biomarker in assessing trauma severity and prognosis in patients with MBT.

There are some limitations to this study, the most important being the small sample size and single center design. In addition, a single measurement of serum miRNA-93 and -191 was taken within the first 24 h after trauma. Hence, serum miRNA-93 and -191 levels, which may affect long-term outcomes, were not assessed in the patients with MBT following hospitalization. With repeated measurements, changes in serum miRNAs levels could be observed, but the surgical treatment and drug administration could also affect the expression levels of these miRNAs. Therefore, it was decided to evaluate serum miRNA-93 and -191 levels in the early post-trauma period after admission and to compare these with a matched control group to determine the expression of these miRNAs. The main strength of this study was that, to the best of our knowledge, this is the first clinical trial in the literature to examine the expression of miRNA-93 and -191 in a patient population with MBT, in comparison to a population of healthy individuals using qRT-PCR. Furthermore, this study investigated whether serum miRNA-93 and -191 levels predict injury severity in patients with MBT. Hence, the relationship between these miRNAs and injury severity deserves further study of large cohorts.

## Conclusion

The miRNA-191 and -93 levels were significantly upregulated in multitrauma patients compared to controls. There was

a significant negative correlation between the serum miRNA-191 and ISS. The optimal serum miRNA-191 cutoff for determining severe trauma was 1.94. We found that the level of miRNA-191 in conjunction with ISS, but not miRNA-93, might be a useful biomarker to predict injury severity in patients with MBT.

However, randomized, controlled studies with more cases are needed to validate the use of miRNAs as biomarkers and for clinical decision-making in trauma scoring.

**Ethics Committee Approval:** This study was approved by the Haseki Training and Research Hospital Local Ethics Committee (Date: 19.12.2017, Decision No: 2017-592).

**Peer-review:** Internally peer-reviewed.

**Authorship Contributions:** Concept: Ö.S.; Design: Ö.S.; Supervision: Ö.S.; Resource: M.M., H.E., Ö.S.; Materials: Ö.S., F.A., O.K., M.Ç., S.Ç., S.S., T.B.Ü.; Data: Ö.S., O.K., M.Ç., S.Ç., T.B.Ü.; Analysis: Ö.S., M.M., F.A., O.K., M.Ç., S.Ç., T.B.Ü.; Literature search: Ö.S., M.M., O.K., H.E., S.Ç., T.B.Ü.; Writing: Ö.S., M.M.; Critical revision: Ö.S., M.M.

**Conflict of Interest:** None declared.

**Financial Disclosure:** This study was funded by the Health Sciences University Board of Scientific Research Projects (grant no. 2019/002).

## REFERENCES


1. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: An overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma 2006;60:S3–11. [CrossRef]
2. Galvagno SM Jr., Nahmias JT, Young DA. Advanced trauma life support® update 2019: Management and applications for adults and special populations. Anesthesiol Clin 2019;37:13–32. [CrossRef]
3. Wisner DH. History and current status of trauma scoring systems. Arch Surg 1992;127:111–7. [CrossRef]
4. Galvagno SM Jr., Massey M, Bouzat P, Vesselinov R, Levy MJ, Millin MG, et al. Correlation between the revised trauma score and injury severity score: Implications for prehospital trauma triage. Prehosp Emerg Care 2019;23:263–70. [CrossRef]
5. Deng Q, Tang B, Xue C, Liu Y, Liu X, Lv Y, et al. Comparison of the ability to predict mortality between the injury severity score and the new injury severity score: A meta-analysis. Int J Environ Res Public Health 2016;13:E825. [CrossRef]
6. Javali RH, Krishnamoorthy, Patil A, Srinivasarangan M, Suraj, Sriharsha. Comparison of injury severity score, new injury severity score, revised trauma score and trauma and injury severity score for mortality prediction in elderly trauma patients. Indian J Crit Care Med 2019;23(2):73–7.
7. Bolorunduro OB, Villegas C, Oyetunji TA, Haut ER, Stevens KA, Chang DC, et al. Validating the injury severity score (ISS) in different populations: ISS predicts mortality better among Hispanics and females. J Surg Res 2011;166:40–4. [CrossRef]
8. Yang Z, Zhong L, Xian R, Yuan B. MicroRNA-223 regulates inflammation and brain injury via feedback to NLRP3 inflammasome after intracerebral hemorrhage. Mol Immunol 2015;65:267–76. [CrossRef]
9. Bache S, Rasmussen R, Rossing M, Hammer NR, Juhler M, Friis-Hansen L, et al. Detection and quantification of microRNA in cerebral microdialysate. J Transl Med 2015;13:149. [CrossRef]
10. Martinez B, Peplow PV. MicroRNAs as diagnostic markers and therapeutic targets for traumatic brain injury. Neural Regen Res 2017;12:1749–61. [CrossRef]
11. Meyer SU, Pfaffl MW, Ulbrich SE. Normalization strategies for microrna profiling experiments: A "normal" way to a hidden layer of complexity? Biotechnol Lett 2010;32:1777–88. [CrossRef]
12. Mahdipour M, van Tol HT, Stout TA, Roelen BA. Validating reference microRNAs for normalizing qRT-PCR data in bovine oocytes and preimplantation embryos. BMC Dev Biol 2015;15:25. [CrossRef]
13. Peltier HJ, Latham GJ. Normalization of microRNA expression levels in quantitative RT-PCR assays: Identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA 2008;14:844–52. [CrossRef]
14. Sabirzhanov B, Zhao Z, Stoica BA, Loane DJ, Wu J, Borroto C, et al. Downregulation of miR-23a and miR-27a following experimental traumatic brain injury induces neuronal cell death through activation of proapoptotic Bcl-2 proteins. J Neurosci 2014;34:10055–71. [CrossRef]
15. Sun TY, Chen XR, Liu ZL, Zhao LL, Jiang YX, Qu GQ, et al. Expression profiling of microRNAs in hippocampus of rats following traumatic brain injury. J Huazhong Univ Sci Technol Med Sci 2014;34:548–53.
16. Han Z, Chen F, Ge X, Tan J, Lei P, Zhang J. miR-21 alleviated apoptosis of cortical neurons through promoting PTEN-Akt signaling pathway in vitro after experimental traumatic brain injury. Brain Res 2014;1582:12–20. [CrossRef]
17. Liu L, Sun T, Liu Z, Chen X, Zhao L, Qu G, et al. Traumatic brain injury dysregulates microRNAs to modulate cell signaling in rat hippocampus. PLoS One 2014;9:e103948. [CrossRef]
18. Di Pietro V, Ragusa M, Davies D, Su Z, Hazeldine J, Lazzarino G, et al. MicroRNAs as novel biomarkers for the diagnosis and prognosis of mild and severe traumatic brain injury. J Neurotrauma 2017;34:1948–56.
19. Yang T, Song J, Bu X, Wang C, Wu J, Cai J, et al. Elevated serum miR-93, miR-191, and miR-499 are noninvasive biomarkers for the presence and progression of traumatic brain injury. J Neurochem 2016;137:122–9.
20. Jarry J, Schadendorf D, Greenwood C, Spatz A, van Kempen LC. The validity of circulaing microRNAs in oncology: Five years of challenges and contradictions. Mol Oncol 2014;8:819–29. [CrossRef]
21. Chen YJ, Zhu JM, Wu H, Fan J, Zhou J, Hu J, et al. Circulating microRNAs as a fingerprint for liver Cirrhosis. PLoS One 2013;8:e66577. [CrossRef]
22. Gosho M, Nagashima K, Sato Y. Study designs and statistical analyses for biomarker research. Sensors (Basel) 2012;12:8966–86. [CrossRef]
23. Pan YB, Sun ZL, Feng DF. The role of MicroRNA in traumatic brain injury. Neuroscience 2017;367:189–99. [CrossRef]
24. Redell JB, Liu Y, Dash PK. Traumatic brain injury alters expression of hippocampal microRNAs: Potential regulators of multiple pathophysiological processes. J Neurosci Res 2009;87:1435–48. [CrossRef]
25. Redell JB, Zhao J, Dask PK. Altered expression of miRNA-21 and its targets in the hippocampus after traumatic brain injury. J Neurosci Res 2011;89:212–21. [CrossRef]
26. Qu X, Li Z, Chen J, Hou L. The emerging roles of circular RNAs in CNS injuries. J Neurosci Res 2020;98:1485–97. [CrossRef]
27. Redell JB, Moore AN, Ward NH, Hergenroeder GW, Dash PK. Human traumatic brain injury alters plasma microRNA levels. J Neurotrauma 2010;27:2147–56. [CrossRef]
28. Tas D. Validity of Serum microRNA-93 and microRNA-191 Levels to Reduce Unnecessary Computed Tomography in Adult Patients with Minor Head Trauma, Thesis. Turkey: Health Sciences University, Haseki Training and Research Hospital; 2018.

ORİJİNAL ÇALIŞMA - *ÖZ*

# Yetişkin çoklu travma hastalarında yaralanma şiddetinin belirlenmesi için serum mikroRNA-93 ve mikroRNA-191 seviyelerinin kullanımı

**Dr. Özgür Söğüt,[1] Dr. Merve Metiner,[2] Dr. Onur Kaplan,[1] Dr. Mustafa Çalık,[2] Dr. Sümeyye Çakmak,[3] Dr. Tuba Betül Ümit,[1] Dr. Hüseyin Ergenç,[1] Dr. Fahri Akbas,[4] Dr. Seda Süsgün[4]**

[1]Sağlık Bilimleri Üniversitesi, Haseki Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği, İstanbul
[2]Sağlık Bilimleri Üniversitesi, Gaziosmanpaşa Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği, İstanbul
[3]Sağlık Bilimleri Üniversitesi, Bakırköy Dr. Sadi Konuk Eğitim ve Araştırma Hastanesi, Acil Tıp Kliniği, İstanbul
[4]Bezmialem Vakıf Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, İstanbul

AMAÇ: Çoklu künt travma (ÇKT) sonrası hastaların travma şiddetini ve prognozunu belirlemek için çeşitli skorlama sistemleri geliştirilmiştir. Bununla birlikte, bu skorlama sistemleri tam olarak istenen şiddet değerlendirmesini sağlamaz. Son yıllarda, birçok spesifik miRNA'ların serum konsantrasyonunun, özellikle kafa travması için tanı, şiddet ve prognozun belirlenmesinde önemli bir rol oynadığı gösterilmiştir. Bununla birlikte, bugüne kadar, hiçbir çalışma ÇKT'li hastalarda serum mikroRNA'larını araştırmamıştır. Bu çalışmada, ÇKT'si olan yetişkinlerin serumunda miRNA-93 ve -191 ekspresyonu ölçülerek Yaralanma Şiddet Skoru (ISS) ve Revize Travma Skoru (RTS) ile korelasyonları incelendi. MiRNA düzeylerinenin bu hastalarda travma şiddetini tahmin etmesi amaçlandı.

GEREÇ VE YÖNTEM: İleriye yönelik, olgu-kontrollü olarak yapılan bu çalışmaya çoklu travma tanısı alan 50 erişkin hasta ile benzer yaş ve cinsiyette 60 sağlıklı kontrol grubu alındı. Hastaların önce ISS ve RTS'leri hesaplanıp ISS >16 (grup 1, majör ya da ağır travma) ve ISS ≤16 (grup 2, minör ya da hafif-orta travma) olmak üzere iki ayrı gruba ayrılarak değerlendirildi. ÇKT geçiren hastalar ile sağlıklı kontrol grubunun serum miRNA-93 ve mikroRNA-191 düzeyleri kantitativ real-time reverse transcription-PCR kullanılarak ölçüldü. MiRNA'ların majör ve minör ÇKT hastalarında farklı şekilde eksprese edilip edilmediği değerlendirildi ve yaralanma şiddetinin değerlendirilmesinde faydaları belirlendi.

BULGULAR: Kontrol grubu ile karşılaştırıldığında multitravmalı hastaların ortalama serum miRNA-93 ve miRNA-191 düzeylerinde anlamlı yükseklik tespit edildi ($p<0.001$). Ek olarak, ISS >16 olan hastaların ortalama serum miRNA-93 ve miRNA-191 düzeyleri, ISS ≤16 olan hastalarla karşılaştırıldığında, istatistiksel olarak anlamlı olmamasına rağmen, upregüle olarak saptandı. Hastaların ISS skorları ve serum miRNA-93 düzeyleri arasında anlamlı korelasyon bulunmazken, serum miRNA-191 düzeyleri arasında negatif yönde, anlamlı ve zayıf bir korelasyon saptandı (Rho=-0.320, $p=0.023$). ÇKT hastalarında ISS skoruna göre serum miRNA-93 düzeyinde anlamlı bir cut-off değeri saptanmadı (AUC 0.617, [0.455–0.779]). Bununla birlikte, şiddetli travmayı göstermede serum miRNA-191 <1.94 optimal bir kesim değeri olarak tanımlandı (AUC 0.668 [0.511–0.826]; %65.6 duyarlılık, %77.8 özgüllük).

TARTIŞMA: Kontrol grubu ile karşılaştırıldığında, ÇKT geçiren hastalarda miRNA-191 ve -93 düzeyleri önemli ölçüde yükselmiştir. ISS ile birlikte MiRNA-191 seviyesi, ancak miRNA-93'ün seviyesi değil, çoklu travmalı hastalarda travma şiddetini belirlemek için yararlı bir biyobelirteç olabilir.

Anahtar sözcükler: MiRNA-93; miRNA-191; multitrauma; yaralanma şiddeti skoru.

Ulus Travma Acil Cerrahi Derg 2021;27(6):631-638 doi: 10.14744/tjtes.2020.45470