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Diagnostic value of serum pentraxin-3 in deep vein thrombosis disease

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Abstract

Aim: We investigated the diagnostic value of Pentraxin 3 (PTX-3), a marker that increases in vascular and inflammatory pathologies, in patients admitted to hospital with deep vein thrombosis clinic.

Materials and Methods: 44 patients admitted to a tertiary medical faculty hospital and a tertiary training and research hospital, with suspect of deep vein thrombosis included in our study. Patients confirmed to have DVT by doppler ultrasonography named as DVT(+) group, and patients not confirmed to have DVT by doppler ultrasonography named as DVT(-) group. PTX-3 levels determined in blood samples and compared between these groups.

Results: Median levels of D-dimer in DVT(+) group was 3.92 μg/ml (1.57-6.05), and in DVT(-) group was 1.47 μg/ml (0.97-2.37), and a statistically significant difference was found between these groups (p<0.05). Median levels of Pentraxin-3 in DVT(+) group was 0.42 (0.36-0.49) μg/ml, and in DVT(-) group 0.40 (0.37-0.49) μg/ml, and there was no significant difference found between these groups (p>0.05).

Conclusion: According to our study, PTX-3 is not a suitable diagnostic marker for the diagnosis of deep vein thrombosis. We think that the value of PTX-3 in the diagnosis of deep vein thrombosis now need to be confirmed with broader, controlled studies.

Keywords: Deep vein thrombosis (DVT), D-dimer, Diagnosis, Pentraxin 3 (PTX-3)

INTRODUCTION

Deep vein thrombosis (DVT) is a systemic disease characterized by the formation of clot anywhere in the venous system. This disease and its sequelae are among preventable diseases. Factors that increase venous stasis such as prolonged immobilization, varicose veins, obesity and atrial fibrillation; factors that increase hypercoagulability such as factor V Leiden deficiency, homocystinuria, protein C or S deficiency, pregnancy, surgery, cancer and hyperlipidaemia and factors that cause endothelial damage such as history of surgery, intravenous drug addiction, insertion of central catheter increase the incidence of deep vein thrombosis. Clinical findings and detectable symptoms are mostly insufficient in diagnosing venous thromboembolism; therefore, there may be a need for objective diagnosis [1]. Accurate diagnosis is very important in individuals with a suspicion of DVT; untreated thrombosis may cause mortal pulmonary embolism. Since the diagnosis is correct only in one fourth of the cases with suspected
DVT, the presence of thrombus should be determined with non-invasive, rapid and inexpensive methods. Clinical examination, laboratory tests and imaging methods can be combined for this [2,3]. Pentraxin 3 (PTX-3) is an independent marker in the determination and diagnosis of prognosis and in the prediction of death due to cardiovascular diseases, mainly with its role in vascular inflammation. It has been stated that plasma PTX-3 levels increase and show a positive correlation with disease activity in ischemic heart diseases (angina pectoris, myocardial infarction) in which inflammation play an important role and in small vessel vasculitis [4,5].

Our research was carried out to investigate whether this biochemical parameter could be included in diagnostic algorithm in addition to physical examination and radiological methods in the diagnosis of deep vein thrombosis.

**MATERIAL AND METHOD**

In the external examination of 63-year-old male patient who was admitted to emergency service after an argument with the neighbours and who had previous heart disease, no ecchymoses, scratches or bleeding was seen. Cardiology consultation was asked because he had complaints of pain on the back and chest. In the cardiology consultation, hospitalization to coronary intensive care unit was recommended since troponin was with in normal limits and the patient had left bundle branch block. The patient did not agree to hospitalization.

In the examination at Forensic Medicine Clinic eight months after the incident, the patient stated that during the incident, he was hit hard on his back and he had pain in the chest and back after he fell on his knees, after the first intervention in the emergency service, the cardiologist told him that he needed to stay in the hospital, but he left the hospital of his free will, the doctor prescribed him Nextep and Ecopirin, he was still using these drugs, and he had used medication before due to heart disease. He still had pain on his left shoulder and neck from time to time; his examination did not show any external traumatic lesions.

**DISCUSSION**

The study is a multi-centred, prospective, time-limited, cross-sectional clinical study. Permission was obtained from local Clinical Research Ethics Committee for the study protocol (2015-159). After ethics committee approval was obtained, the study was conducted in 6 months between June and December 2016 in the Emergency service and Cardiovascular surgery (CVS) outpatient clinics of a tertiary medical faculty hospital and a tertiary training and research hospital.

44 patients aged 18 and older who were admitted to the clinic with suspected DVT according to the diagnostic algorithm in the Guide published by ACEP (American College of Emergency Physicians) in 2009. The patients who did not give consent for the study, those who had missing data in the study form and those who were found to have hemolysis in the serum samples taken were excluded from the study. Of the patients who were evaluated in terms of Wells score with a clinical suspicion of DVT and who were found to have low risk, those whose D-dimer test were negative were also excluded from the study since DVT was excluded according to diagnostic algorithm. Patients diagnosed with DVT on Doppler USG constitute the DVT positive group, and patients without DVT on Doppler USG constitute the DVT negative control group. Individuals younger than 18 years of age, those who had advanced liver, kidney and heart failure, acute coronary syndrome, acute pulmonary embolism, acute cerebral stroke, acute mesenteric ischemia, peripheral artery occlusion, pregnancy, malignancy, hematological or rheumatological diseases were excluded from the study. Demographic characteristics, symptom and physical examination findings, laboratory and radiology reports of the patients to be included in the study were recorded with study forms prepared by the researchers.

**Study protocol**

**Wells scoring**

The patients who referred to the related clinics with extremity pain, redness and swelling were evaluated by clinicians in accordance with the diagnostic algorithm created by Wells scoring (Figure-1). The patients whose low risk D-dimer test was positive according to this scoring (cut-off value 0.5μg/ml) and who had higher risk degree were included in the study by performing doppler USG.

**Doppler USG**

The patients included in the study underwent doppler examination of the veins in the extremity with symptoms with the use of Toshiba Aplio 500 USG by radiologists. Veins with loss of compression were thrombosed and reported. While the patients who were found to be DVT positive formed the DVT + group, those who did not have DVT were included in the control group.
Biochemical sampling
At the time of admission, approximately 5 cc of venous blood samples of the patients in both DVT and control group were taken with an injector from the brachial vein into a chemistry tube with a separator. The blood samples were kept for minutes at room temperature for coagulation. After the samples were centrifuged for 10 minutes at a speed of 1,800 × g, the resulting serum was separated and the analysis was kept at -80 C degrees. At the end of the study protocol, all samples were studied simultaneously by a researcher who was blind to the study data and patient groups.

Determination of Pentraxin-3 (PTX3) levels in human serum
PTX3 levels of the serum samples were determined by using enzyme-linked immunosorbent assay (ELISA) kit (Boster Biological Technology, Cat No: EK0861, Pleasanton, CA, USA) according to the recommendations of manufacturers.

Statistical analysis
SPSS (Statistical Package for Social Sciences for Windows) v.13.0 program was used for the statistical analysis of the study. Categorical variables were expressed as number (n) and percentage (%). The difference between the categorical variables was evaluated with Chi-square test. Since the data for numerical variables did not meet the parametric conditions, they were calculated as median (25%-75%) values. Bonferroni corrected Mann Whitney U test was used to compare the medians between groups. Spearman Correlation Analysis was used to see how the other variable was affected when the value of a variable changed. p< 0.05 level was taken as statistically significant difference in the results.

RESULTS
A total of 44 individuals, 19 patients who had acute DVT confirmed with doppler and 25 patients who had clinical DVT but no acute thrombus in the doppler USG, were included in the study. 6 patients were excluded because they did not want to participate in the study, 1 patient was excluded because he had missing data, 7 patients were excluded because they were found to be low risk with Well’s scoring and they had negative D-dimer value and 2 patients were excluded because they had acute renal failure.

Table 1 shows the demographic and clinical characteristics of the patients. When age and gender of the patients were compared, no significant difference was found between the groups (p>0.05). Of the vital findings, significant difference was found between groups only in systolic blood pressure and fever measurements (p<0.05). Table 2 shows the measurement values of D-dimer and PTX-3 biochemical parameters measured in the study. When the D-dimer and PTX3 values were compared between groups, D-dimer value was found as 3.92 μg/ml (1.57-6.05) in the DVT(+) group and as 1.47 μg/ml (0.97-2.37) in the DVT(-) group and statistically significant difference was found between the groups (p<0.05). However, Pentraxin-3 values were measured as 0.42 (0.36-0.49) ng/ml in the DVT(+) group and as 0.40 (0.37-0.49) ng/ml in the DVT(-) group and no statistically significant difference was found between groups (p>0.05). It was also found that PTX-3 levels were within normal limits in all patients included in the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DVT (+)</th>
<th>DVT (-)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>66</td>
<td>64</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender Male</td>
<td>9 (47.4%)</td>
<td>11 (44%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>10 (52.6%)</td>
<td>14 (56%)</td>
<td>&gt;0.05</td>
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<tr>
<td>Comorbid diseases n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (42.1%)</td>
<td>13 (52%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6 (31.6%)</td>
<td>4 (16%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>2 (10.5%)</td>
<td>6 (24%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>1 (5.3%)</td>
<td>1 (4%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Past SVO</td>
<td>1 (5.3%)</td>
<td>4 (16%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Vital findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic Pressure (mmHg)</td>
<td>120 (110-130)</td>
<td>128 (117-139.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic Pressure (mmHg)</td>
<td>77 (70-80)</td>
<td>80 (76.5-84.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Pulse</td>
<td>80 (67-96)</td>
<td>76 (66.5-88.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Respiratory rate (/min)</td>
<td>15 (14-19)</td>
<td>16 (13.5-18)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fever (°C)</td>
<td>36.6 (36.4-36.9)</td>
<td>36.9 (36.55-37.1)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Habits</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>8 (42.1%)</td>
<td>8 (32%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Wells score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>3</td>
<td>4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Moderate</td>
<td>9</td>
<td>16</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>High</td>
<td>7</td>
<td>5</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*Categorical variables number (n) and percentage (%), ** The difference between categorical variables Chi-square test, SVO: cerebra vascular disease
DISCUSSION

No significant result was found in the clinical study in which we investigated the diagnostic value of the biochemical study called PTX-3 in the diagnosis of DVT. DVT, which is the most common type of venous thromboembolism (VTE), is a disease characterized by clot formation in deep venous system and its annual incidence in the general population has been reported as 0.1-0.2%. Pulmonary embolism, which is a clinical situation that threatens life in the acute period, is the main cause of post-thrombotic syndrome that causes permanent damage in the lower extremity in the chronic period. Despite suitable medical treatment, it causes pulmonary embolism with a rate of 10% in the early period, while it causes serious complications such as postthrombotic syndrome development with a rate of 40% in the long term. Despite the risk of serious complications, patients with DVT mostly refer with nonspecific symptoms and this causes delays in the diagnosis and treatment [6,7]. A large number of biochemical parameters such as homocysteine, haemoglobin, leukocyte, monocyte, platelet levels, CRP, protein C, protein S have been worked in the diagnosis of VTE; however, they have not found a place in diagnostic algorithm since they are not specific or they are not worked easily [8]. In a study conducted with P-selectin, which is a member of the adhesion molecules family and which is released from active thrombocytes and endothelium, sP-selectin (soluble Pselectin) levels were found to increase in acute DVT [9]. In another prospective study in literature, 2.6 times increase was found in VTE development with the increase in sP-selectin levels [10]. D-dimer, which is a biochemical marker recommended to be studied according to DVT diagnostic algorithm, has a high sensitivity in excluding D-dimer DVT diagnosis and therefore it is used not as a diagnostic criterion, but for diagnostic exclusion. For this reason, objective radiological imaging is required for DVT diagnosis. These imaging methods are conventional angiography (venography), CT-MR venography, Doppler Ultrasonography and radionuclide imaging. [11-14]. Venography is considered as the gold standard in diagnosis; however, in recent years, colourful doppler USG, which has high accuracy, which is cheap, easily applicable and non-invasive, has begun to be preferred more in line with recent technological developments [15]. Therefore, doppler USG was used in our study as the diagnostic matters.

PTX-3 plays a significant role in primary inflammatory response. For this reason, it is included in diagnostic tests of many diseases, especially cardiovascular diseases, from ovarian torsion to pleural effusion [16,17]. With immunohistochemical studies showing that plasma PTX-3 amount is increased in atherosclerosis lesions, whereas it is not increased in non-atherosclerotic lesions and PTX-3 is an indicator of localized vascular immobilization and damage, investigating the relationship between clinical atherosclerotic incidents has become important. The high PTX-3 levels in patients with CT elevated myocardial infection and the higher level in patients with CT elevated myocardial infarctions has led to investigating plasma PTX-3 levels in this patient group [18,19]. In a study by Akira et al., PTX-3 was not found to increase in patients with acute pulmonary embolism, while PTX-3 level was increased in patients who developed pulmonary hypertension secondary to embolism in the chronic period [20]. In a study by Barbui et al. on patients with essential thrombocytopenia and the risk of thrombosis was found to decrease as PTX-3 levels increased [21]. Unlike other studies in literature, no increase was found in PTX-3 Thrombotic risk. In our study, PTX-3 levels were not found to increase in DVT, which is a thrombotic event. The mechanism of why PRX-level which increased in vascular incidents and which we are expecting to increase in acute deep vein thrombosis did not increase in our study cannot be explained fully.

Limitations

A large number of patients with DVT suspicion who referred to CVS outpatient clinic and Ahi Evran Hospital Outpatient clinics could not be included in the study due to the insufficient support of the related clinics. Since a great majority of the population consisted of patients who referred to the emergency service, it was not possible to reach the desired number of cases.

CONCLUSION

According to the results of our study, PTX-3 is not a suitable biochemical marker in terms of the diagnosis of deep vein thrombosis. Clinical studies with larger sample are required to determine the place of PTX-3 in DVT diagnosis.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

Ethics Permission was obtained from Karadeniz Technical University Clinical Research Ethics Committee for the study protocol (2015-159).

References

CASE REPORT

Preparing reports in cases with blunt trauma to the heart area: Three cases

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Abstract
This case series has been prepared to create a perspective to evaluate blunt heart trauma, in which physical and psychological effects of trauma are seen together, in clinical forensic medicine practices. Case 1: A 50-year-old female patient with a history of hypertension had a heart attack in the first 24 hours after blunt trauma, she had an angiography and a coronary stent inserted. Case 2: A 60-year-old woman with no known disease fell after one of the children who entered her garden without permission kicked her; she had angiography within 24 hours due to chest pain and stenocardia and found that she had coronary heart disease. Case 3: A 60-year-old man with a history of atherosclerotic heart disease had argued with neighbours and had a blow to his chest; in the emergency service, hospitalization was recommended to the patient due to left bundle branch block and troponin results; However, he refused hospitalization and left the hospital. Forensic medical evaluation of heart attack and rhythm disorders after blunt chest trauma or arguments is an important issue. Medical evaluation in terms of heart damage following blunt chest traumas is one of the factors that contribute to forensic reports prepared after the incident.

Keywords: Blunt chest trauma, heart damage, rhythm disorders, forensic report

INTRODUCTION
It is a well-known fact that in addition to the physical damage that trauma can cause to the body of the individual, it also causes psychological problems and the body has physiological responses to these problems. It is stated in the literature that complications due to psychological stress and physical exertion that develop during and after trauma in individuals with and without previous cardiac problems are a spectrum that can go up to cardiac arrest [1-3]. In the process of evaluating the stressor effects of trauma and reflecting these in reports, forensic medicine specialists may need to establish a causal link. First of all, it should be reminded that whether the perpetrator is at fault or not is a matter that should be evaluated by the court.

It has been reported that various rhythm disorders can be seen within 24-48 hours after trauma [4]. Clinical sensitivity of using CK-2 isoforms in the diagnosis of myocardial infarction is 90-95% [5]. The presence of contusion is most accurately detected with troponins [6]. In blunt chest traumas, cases of direct cardiac damage are reported as 5-15% [7]. This damage may belong to the pericardium, myocardium, endocardium and coronary arteries.

This study aims to attract attention to the importance of determining whether myocardial damage detected after trauma in clinical forensic medicine is due to trauma.

CITATION
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CASE 1

In the examination of the 50-year-old patient who was brought to the emergency service after a family argument and fight and who had previous hypertension disease, shapeless ecchymoses with pale edges in sizes of 1x1 cm., 1x0.5 cm. and 0.5x0.5 cm. in the middle 1/3 part of the right arm on the inner side, and 2.5x1 cm. on the right arm 1/3 part outer side, shapeless ecchymoses in sizes of 2x0.5 cm., 0.5x0.5 cm., 0.5x0.5 cm. and 1x0.5 cm. on the left arm 1/3 part outer side, a shapeless ecchymosis in a size of 5x3 cm. on the right elbow posterior side and a shapeless ecchymosis in a size of 4x2 cm. on the right thigh inner side in the middle 1/3 part were seen. In addition, high troponin was found in tests performed for the patient’s chest pain complaint. In the cardiology consultation, his electrocardiography showed T wave negativity at V3- V6 and dynamic T wave changes; ejection fraction was 55% in the echocardiography, left ventricular diameters were normal and pericardial effusion was not found. Thoracic CT was found to be within normal limits. The patient was referred to the intensive care unit indicating that intensive follow-up was appropriate with a suspicion of acute coronary syndrome.

In the coronary angiography performed a day later in the hospital B he was transferred to, a stent was applied to the patient who had total stenosis in the right coronary artery (RCA), retrograde filling of RCA, 40-50 % stenosis and 30 % stenosis in the distal of left descending coronary artery (LMCA).

In the examination at Forensic Medicine Clinic seven days after the incident, the patient stated that he had been injured after an argument in the family, he had taken a blow to the chest, he still had complaints of shortness of breath and chest pain, he had a heart attack after the incident, a coronary stent was inserted and his treatment was still continuing, and ecchymoses with pale lemon-yellow-pistachio green edges in places compatible with the first examination were found in the patient's body. It was found that he had ecchymotic needle marks around both elbows and inguinal region which were due to the medical interventions.

CASE 2

When a 60-year-old female patient warned the children sitting around and on the tree in her garden, one of the children kicked her chest. The patient, who realized that she could not breathe and she had low blood pressure, called an ambulance and went to the District State Hospital. In her examination, it was found that the patient described trauma-related sensitivity in her chest, while her thoracic CT did not show any findings. During her follow-up, she was transferred to the Cardiology Centre because her cardiac troponin was 1.02 and these values increased, her general condition worsened and there were significant ECG changes.

In the cardiology clinic of Hospital A, it was found that the patient had chest ache, her electrocardiography (ECG) showed ST elevation at AVL part; the patient was admitted to coronary intensive care unit; found LAD (left anterior descending coronary artery) plaque, CX (circumflex coronary artery) plaque and while RCA (right coronary artery) was normal.

In the cardiology clinic, it was found that the patient had chest ache, and her ECG showed ST elevation at the AVL part; she was admitted to coronary intensive care unit and found LAD (left anterior descending coronary artery) plaque, CX (circumflex coronary artery) plaque, and RCA (right coronary artery) was normal.

In the examination at Forensic Medicine Clinic three days after the incident, the patient stated that she came to the cardiology service of the hospital in the district centre after her first intervention was made in the District State Hospital, she had angiography, she was hospitalized in the intensive care for one night and in the normal service for another night, she still had shortness of breath and chest pain; her examination showed a 2x1 cm. shapeless ecchymosis with pale edges and purple coloured in the middle on upper 1/3 part of the right thigh.

CASE 3

In the external examination of 63-year-old male patient who was admitted to emergency service after an argument with the neighbours and who had previous heart disease, no ecchymoses, scratches or bleeding was seen. Cardiology consultation was asked because he had complaints of pain on the back and chest. In the cardiology consultation, hospitalization to coronary intensive care unit was recommended since troponin was within normal limits and the patient had left bundle branch block. The patient did not agree to hospitalization.

In the examination at Forensic Medicine Clinic eight months after the incident, the patient stated that during the incident, he was hit hard on his back and he had pain in the chest and back after he fell on his knees, after the first intervention in the emergency service, the cardiologist told him that he needed to stay in the hospital, but he left the hospital of his free will, the doctor prescribed him Nextep and Ecopirin, he was still using these drugs, and he had used medication before due to heart disease. He still had pain on his left shoulder and neck from time to time; his examination did not show any external traumatic lesions.

DISCUSSION

Blunt trauma can be affected the heart in several different ways; 1) through direct energy transfer of the force on the rib cage, 2) through slower heart rate, 3) through compression of the heart between the spine and the sternum. Theoretically, it describes a histologically detectable situation that cannot be detected by myocardial commode imaging methods. It can frequently be seen in cases that peak at T-wave occurring after a low energy force during sports events (football, baseball, golf, etc.), causing ventricular fibrillation afterward or cardiac arrest by leading to complete heart block in which QRS complex is affected [8]. Myocardial contusion is a state which occurs in traumas with higher energy and it is characterized by myocardial lesions (haemorrhage, increased edema formation, necrosis, polymorphonuclear infiltrates) and increased extravascular resistance and decreased coronary blood.
flow and cardiac functions due to these myocardial lesions [9,10]. Even if severe myocardial contusion reduces heart functions, a significant cardiac shock may rarely develop [11].

When the clinical appearance of blunt heart trauma is examined, it is stated in the literature that it may show findings such as minor ECG or enzyme anomaly, arrhythmia, free wall rupture, septal rupture and heart failure [12,13]. In addition, following blunt heart injuries, lesions can be seen such as hemopericardium, myocardial contusion, free wall rupture, rhythm or conduction disorders, valve injuries and myocardial infarctions (MI) [14]. Since clinical findings may be mild-transient and difficult to understand in blunt heart traumas, especially in cases of pericardial and myocardial contusion, diagnostic problems may often arise. It is also a medical necessity to avoid possible and significant late complications. In a study conducted by Parmly et al. on cases with blunt trauma, which make up 1% of autopsies, it was reported that isolated myocardial contusion and laceration was the second most common injury pattern after ruptures with a rate of 23.6% [15].

Although ECG changes can occur frequently in myocardial contusion, a normal ECG should not exclude trauma. It should be kept in mind that ECG mostly shows left ventricular functions and may show normal findings in trauma cases associated with the right ventricle [16]. In order of frequency, cardiac anomalies after blunt trauma include sinus tachycardia, extra systoles, right bundle branch block, and repolarization anomalies (ST segment and T waves). Q waves and life-threatening severe arrhythmia are very rare [16]. T wave negativity at V3- V6 and dynamic T wave changes in the ECG of our first case and ST elevation in the second case were evaluated as ECG findings in parallel with the literature supporting myocardial infarctions and it was decided that the injuries endangered individuals’ lives.

In the determination of traumatic myocardial damage, Troponin I is preferred since it is more specific when compared with Troponin T. Troponin levels should be followed swiftly and they should be evaluated with the clinical state of the patient. In our second case, a swift follow-up of post-traumatic troponin levels and a continuous increase in this value led to the patient’s being transferred to a more advanced centre. It was understood that the case did not develop a serious clinical complication, such as cardiac rupture, etc. Since the test results supported myocardial infarction in this patient, it was decided that the injury endangered individual’s life.

The echocardiographic finding was present only in our first case and this eco result was reported as within the normal boundaries. Echocardiography is a significant diagnostic tool in terms of the definitive diagnosis of other cardiac lesions and in preventing complications, especially in severe traumas. In a case report by Tsokuas et al., the echocardiography of a 46-year-old male case who developed acute myocardial infarction and congestive heart failure showed anterior myocardial infarction findings. Although echocardiography showed akinesia in the interventricular septum, dyskinesia on the apical anterior wall and severe deterioration in the left ventricle general systolic function, coronary angiography showed to be within normal limits [17].

Our first two cases had ecchymosis as a traumatic finding outside the chest area. This can be evaluated as the fact that the individual was exposed to a trauma. On the other hand, although our third case did not have the other findings of trauma, it is a medically known fact that the psychological effect of the argument that took place, adrenaline discharge, and other physiological incidents may cause arrhythmia in the heart. In a case report by Allemheersch et al., a 41-year-old patient who was exposed to a high-energy traffic accident was found to have fracture in the skull base, fractures in the bilateral ribs in the chest wall, pericardial haemorrhage in addition to subdural and subarachnoid haemorrhage and complete dissection in the right coronary artery [18].

The incidence rate of common clinical myocardial infarction findings in cases younger than 45 years of age was between 6 and 10% in the literature. In a study by Christensen et al., a total of 77 cases, 64% of which were associated with traffic accidents and 4% of which were associated with fights, were evaluated as myocardial infarctions that developed after blunt chest trauma [19]. In a study by Park et al., it was found that in a 16-year-old male patient who had chest trauma by hitting the handrails while riding a motorcycle, ST elevation was found in ECG, an increase was found in cardiac enzymes and the left ventricular function was suppressed. In the diagnostic coronary angiography performed three weeks later, a complete occlusion including collaterals in the proximal left anterior descending artery (LAD) right coronary artery and left circumflex artery, and percutaneous coronary intervention was applied to the LAD lesion [20]. In another case report, a 37-year-old male with severe mental retardation was evaluated in the emergency service he presented with a complaint of substernal chest pain after physical assault to his head, face and chest area. Electrocardiography showed ST segment elevation in V2, V3 and aVL and normal sinus rhythm with Q waves in aVL. Coronary artery also showed total occlusion in the left descending coronary artery. The patient was discharged four days later without any complications [21].

The common characteristics of our three cases was the presence of atherosclerotic heart disease, which was detected after trauma in the first two and which was previously present in the third case. There is a case of exacerbation in the disease here. According to the Article 23 of the Turkish Penal Code (TPC); if an act causes a more severe or another result than intended, the individual must act with negligence at least in terms of this result.

Article 86 of TCK includes the penalties for deliberate injuries, while Article 87 includes the penalty for “aggravated crime by result”. “Causing life-threatening danger” is one of the “aggravated crime by the result” cases in subparagraph (d) of paragraph 1 of Article 87.

In the Guide to the Evaluation of Injury Crimes Defined in the Turkish Penalty” code which was prepared by the Forensic Medicine Institute and Forensic Medicine Specialists and Forensic Medicine Association defined by the Turkish penalty code, Table
I gives a list of injuries that cause a life-threatening situation. “Myocardial infarction triggered by the stressors that come out within 24 hours after trauma” is also on the list and it is one of the “aggravated crime by result” cases [22].

In legal evaluations, for people who injure others to be punished for “aggravated crime by the result”, issues such as whether they knew the injured person had heart disease previously, whether they predicted those people may have a heart attack after the argument-fight and trauma are important.

Although the risk of developing acute MI is low in cases with blunt chest trauma, cardiac evaluation of patients with chest ache in emergency services is judicially, medically and legally important in terms of detecting late complications of trauma.

The important thing in forensic medical evaluation is the establishment of a cause-effect relationship between the trauma experienced and the myocardial infarction that occurs after the trauma experienced. In terms of establishing this cause-effect relationship, the hour the individual experienced the trauma, the first admission hour to the hospital, admission complaints, tests conducted, diagnosis and medical and interventional procedures should be examined in detail and consultation should be demanded from the specialists in the related branch.

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RNA-Approached technology applications in forensic genetics

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Abstract

Ribonucleic acid (RNA) is a nucleic acid which is structurally different from DNA. DNA is the most used and approved nucleic acid in routine applications in forensic caseworks. In this article, giving an information about which RNA types are chosen for which forensic applications is aimed. RNA technologies are developing in the concept of forensic genetics and they can be adapted into routine case works in the case of well conditions are supplied. Both coding and non-coding RNAs are investigated for forensic purposes and most examined ones are messenger RNAs, and microRNAs. There are some researches on circular RNAs and piwi-interacting RNAs but they are in low number when compared with first two RNA types. Forensic studies based on RNA technologies are body fluid identification, post mortem interval determination, determination of stain age, estimation of an individual's age and sex, identification of organ tissues, wound age estimation, and determination of drug abuse. Lastly, different RNA based technologies can be used in these studies and some of them are micro-array, Nano-String technology, real time PCR, end point PCR, high resolution melt (HRM) analysis and next generation sequencing (NGS) technology.

Keywords: RNA technologies, Messenger RNA, MicroRNA, Circular RNA, Piwi-interacting RNA, Forensic Genetics

Ribonucleic Acid (RNA)

Ribonucleic acid (RNA) is a nucleic acid which consist of phosphodiester bond linked nucleotide subunits. DNA and RNA have some differences structurally. DNA has deoxyribose sugar in its structure while RNA has ribose sugar. Furthermore, DNA and RNA contains adenine, guanine, and cytosine bases in common but fourth base in DNA is thymine while RNA has uracil. There are diverse types of RNAs and messenger RNAs (mRNA), ribosomal RNAs (rRNA), and transfer RNAs (tRNA) are the main types of RNAs which play important roles in protein synthesis [1]. The main types and the structure of RNA can be seen in Figure 1 below [2]. Messenger RNAs are in charge of protein coding while ribosomal RNAs catalyzes the synthesis of proteins and responsible from forming the ribosome’s basic structure. Transfer RNAs work as adaptors between amino acids and mRNA in protein synthesis. In addition to these type of RNAs there are small nuclear RNAs (snRNAs, have role in nuclear processes) and small nucleolar RNAs (snoRNA, have role in the modification of rRNAs). Except these RNAs, there are other noncoding RNAs which play role in distinct processes in the cell [1] and some of them (especially microRNAs) are important in studies conducted in forensic sciences.
stated that endpoint PCR and real-time PCR can be used as an alternative for conventional methods in order to determine body fluids [7]. In the same study, up to 2-year-old stains, mRNA stability in biological stains was shown.

As RNA-based studies increase, comparison between biomarkers are becoming seen. Both mRNAs and miRNAs are tested for body fluid identification. When compared with miRNAs, mRNAs are thought less ideal for forensic samples which are found degraded frequently. Because of their amplification sizes and tissue-specific features, interests on miRNAs are increasing [8]. In a study which simultaneous examination of miRNA and mRNA biomarkers in body fluids, study group stated that miRNA biomarkers are more advantageous when compared with mRNA biomarkers in degraded samples [9].

In addition to mRNAs and miRNAs, there are some regulatory non-coding RNAs examined for RNA-based approaches in forensic sciences but they are low in number when compared with these two biomarkers. Circular RNAs (circRNAs) are pre-mRNA back-splicing products and they are found in high amounts in human cells. They show cell-type-specific expressions and high stability [10]. It can be said that they have similar features like miRNA which is another type of non-coding RNA. In a study, inclusion of circRNAs in mRNA profiling was investigated and researchers concluded that this application improved the detection of mRNA biomarkers in bodily fluids for forensic purposes [10]. In another study, distinguish based on circRNA expression profiles of body fluids was performed and method was successful for distinction of venous blood, semen, and saliva. In addition, method was not able to differentiate menstrual blood from vaginal secretion [11].

Another non-coding RNA tested for forensic applications is piwi-interacting RNAs (piRNA). They also show tissue-specific expressions and have short length. In a study, four piRNAs were examined in forensically relevant venous blood, menstrual blood, saliva and semen samples. One piRNA (piR-55521) was found in high expression levels for semen. Additionally, according to stability tests, they stated that piRNAs from dried samples could be detected both in laboratory and outdoor conditions for at least six months [12]. In another study, potential piRNAs for distinction of venous and menstrual blood were stated. In the same study, potential piRNAs for vaginal secretion and saliva distinction were also stated [13].

As seen in previously mentioned studies, more information about RNA technologies is obtained due to use of different biomarkers. Furthermore, with the comparison of diverse RNA biomarkers in different studies in the future, more accurate results may be obtained and more knowledge about human body may be collected in order to use for forensic purposes.

Because mRNA analysis may give information about the occurring events in a specific tissue types at a certain point of time, some researchers stated that mRNA analysis may be used for forensic purposes such as understanding the mechanisms leading to death or estimating the death time [14]. Determination
of the exact time of death is quite important for forensic caseworks and it may provide significant information for solving the case. In a study, mathematical model was developed for PMI determination by using RNA degradation [15]. Researchers conclude that developed model may be used for complementary tools for conventional methods. Another study investigated the human dental pulp samples for estimating PMI based on RNA degradation and they found promising results too [16]. In a systematic review, miRNAs were assessed as potential biomarkers for PMI estimation thanks to their tissue-specific expression nature and low molecular weight features [17]. Additional RNA based technology for forensic use is age determination of stains. For some specific cases such as sexual assault, determination of stain age may be quite important. In order to prove the relationship of a stain with crime, stain age determination method is quite important. In a study which blood and saliva stains were analyzed, results showed that mRNA can be extracted from biological stains up to two years old [18].

According to another study, distinct type of stains was analyzed and they found that global abundance of mRNA transcripts decreased with time. Additionally, no relationship between storage time and length of transcript was found [19]. RNAs are also be used for age prediction in the concept of forensic sciences. There are some age-related miRNAs and in a study, age prediction models for bloodstains were established [20]. In the same study, mean absolute error for males was found as 5.52 years while it was 7.46 years for females. In another research, two new isoforms of gamma hemoglobin mRNA were discovered and they found that these mRNA isoforms exhibit a gene expression pattern limited for newborns [21]. This information may be useful in newborn included forensic cases. When researches increase in the future, sex determination of individuals may be done by RNA technologies. To illustrate, two miRNAs (miR-130b and miR-18b) showed concentration dissimilarity among genders. They showed slightly higher concentration in male serum samples when compared with female serum samples [22]. Furthermore, mRNA content of some fat oxidation related genes showed difference between genders and they were found higher in females when compared with males [23]. When these type of studies improved, obtained data may be assessed more accurately and used for forensic purposes.

Another forensic genetic application of RNAs is identification of organ tissues. This application may be useful for especially in shooting-related crimes because of tissue scattering. To illustrate, crime tool can be identified or exact crime scene can be determined more accurately thanks to this method. By using mRNA profiling, an assay was developed in order to identify 10 distinct organ/tissue types [24]. In addition to mRNA based methods, miRNA based methods on organ tissue determination were also studied [25].

Drug abuse is a serious problem which includes in many forensic cases. Cocaine is one of the abused drugs and their behavioral effect was related with circRNA and miRNA interaction [26]. Additionally, non-coding RNAs (e.g. miRNAs) were stated as important biomarkers for addiction-related behaviors [27]. According to this information, it can be inferred that RNA based applications may be used for drug abuse determinations in the future. Lastly, wound age determination are examined with RNA methods. In a study, wound age was found related with seven genes and their mRNA expressions were analyzed for this information [28]. Wound age estimation is important in forensic sciences because they help to assess injury-crime relationship. The mRNA expression patterns of six genes were found related with the age of human dermal injury [29].

In all of these mentioned studies and the other RNA studies, different analyzing techniques can be chosen according to the needs of a study. Some of the RNA-based technologies used in forensic science studies can be listed as micro-array, Nano-String technology, real time PCR, end point PCR, and high resolution melt (HRM) analysis [30]. In addition to these technologies, next generation sequencing (NGS) is a useful method. NGS technology may be used for different forensic purposes and it provides simultaneous analyzes [31].

CONCLUSION

When compared to use of DNA profiling, RNA based approaches are less common in forensic science community. However, increasing number of studies and their results on this topic are promising for the future applications. With the application of novel technologies developed for scientific use, RNA based technologies can be applied in routine caseworks. As mentioned before, there are distinct types of RNAs. Both non-coding and coding RNAs are tested for forensic potentials but mRNAs and miRNAs are the most investigated biomarkers in the literature. In addition, circRNA and piRNA included studies in different topics were also mentioned in the article.

Applications of diverse RNAs in the field of forensic genetics are quite abundant and different studies are being conducted. Research topics can be listed as body fluid identification, post mortem interval determination, determination of stain age, estimation of an individual’s age and sex, identification of organ tissues, wound age estimation, and determination of drug abuse. With developing technologies, more than one techniques may be chosen by different research groups. In other words, there is no single method for analyzing RNAs. Different RNA based technologies can be used in RNA studies and some of them may be listed as micro-array, Nano-String technology, real time PCR, end point PCR, HRM analysis and NGS technology.

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Usage areas of microRNA (miRNA) in forensic genetics

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Abstract

Interest in molecular based techniques increases in every field of forensic sciences. Use of microRNAs (miRNA) in forensic genetics is one of them. The aim of this article is giving a brief information about microRNAs and their use in forensic genetic applications. MicroRNAs are small noncoding RNAs and because of their important features such as tissue-specificity and high stability in specific conditions, they are used in various studies for forensic purposes. In the article, usage areas of miRNAs and promising results obtained from these studies are mentioned. Body fluid identification, organ tissue identification, determination of stain age, wound age determination, post mortem interval (PMI) identification, age estimation of an individual, lesion assessment in strangulation cases, identification of brain damage and determination the abuse of anabolic androgenic steroids are the subjects which potential use miRNA are examined. The limitations of miRNAs in the forensic genetics applications are also mentioned in the article.

Keywords: MicroRNAs, Forensic Sciences, Forensic Genetics

INTRODUCTION

In molecular biology, central dogma is a very important phenomenon and it indicates the flow of genetic information in cells. According to this phenomenon, information is transferred from DNA to RNA (mRNA) and this whole transfer is named as transcription. Secondly, the information is transferred from RNA to protein. Protein synthesis from mRNA is called translation. In protein synthesis, three nucleotide found in mRNA indicates one amino acid in produced protein. Therefore, even a single change in mRNA can affect the structure of produced protein [1]. There are many regulatory mechanisms that play role in central dogma and some of them can be seen in Figure 1 below.

Primary role of RNA is playing a role in protein synthesis. During this synthesis, there are three main types of RNAs which are messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). Additional to these RNA types, there are regulatory RNAs which one of them is microRNA.

MicroRNAs (miRNAs)

MicroRNAs are noncoding RNAs which their length is approximately 19-24 nucleotides. Lin-4 was the first discovered miRNA in 1993 by Lee et al [3]. Second miRNA (let-7) was discovered 7 years later [4]. After that, researches were accelerated and their regulatory mechanisms were started to understand. Today, approximately after 30 years from the first
discovery of them, there are undiscovered miRNAs. Primary mission of miRNAs is interfering to translation mechanism by binding the target sequences on messenger RNA. By doing that, production of proteins is prevented or changed [5].

**Forensic Genetics Applications of MicroRNAs**

In forensic sciences, applications of molecular techniques are becoming widespread owing to developing technologies. Use of miRNAs is one of the new technologies which adapted for forensic sciences. Before going through the details of miRNA use in forensic sciences, brief explanation will be given about the importance of evidences recovered from crime scene and the answers of who/what/how questions, for forensic cases. As seen in Figure 2, each question which may be answered by evidences are linked together and obtained answers lead to solve of the crime. To illustrate, knowing the identity of a person by analyzing the evidence can lead to arrest of a criminal or identification of a victim. Additionally, in the case of novel technology use, information of individuals’ characteristics such as biogeographical ancestry, hair color, age may be obtained. By using that information suspect pool can be narrowed. Secondly, having an information about the nature of evidence is very important in cases. For example, identification of a stain from crime scene can change the way of case especially in sexual assault cases. Lastly, obtained evidences can give some information about the way that criminal activity takes place [6]. MicroRNAs can be adapted to solve of all three questions in distinct ways.

Although the first miRNA was discovered in 1993, application of them into forensic sciences took 16 years. First research study for forensic purposes was done by Hanson et al. about the identification of body fluids [7]. After that, studies in different topics in the concept of forensic sciences were started to conduct. In this article, body fluid identification, organ tissue identification, stain age determination, wound age determination, post mortem interval (PMI) identification, prediction of an individual’s age, lesion assessment in the case of strangulation, brain damage identification and determination of anabolic androgenic steroids abuse are investigated forensic applications.

![Figure 1. Central dogma and regulatory mechanisms which play role in protein synthesis [2].](image)

![Figure 2. Relationship of who/what/how questions with evidences recovered from crime scene for solving the crime [6].](image)

Firstly, body fluid identification by analyzing miRNAs is the topic which most of the studies are being conducted in the concept of forensic sciences. Because of the tissue-specific characteristics of miRNAs, the first idea was identifying the body fluids [7]. In some cases, determination of body fluids in the crime scene is very important for completing the story of case. To illustrate, in sexual assault cases, determining the origin of blood (venous or menstrual) is a serious issue. If this determination can be accomplished by miRNAs as a routine procedure, it will be helpful for forensic cases. Furthermore, reliability of taken statements can be questioned with the help of this technique. When compared with routinely used techniques, miRNA profiling is less harmless and requires less sample sizes [8]. In an interesting study which conducted in University of New Heaven, DNA and RNA isolation were performed simultaneously from the same sample [9]. Study was conducted in small sample group (each of blood, semen, saliva and urine samples from five voluntaries). From all the samples, DNA and miRNA profiles were obtained carefully. They conclude that this method is promising. If this technique can be developed by using larger sample groups and adapted into routine, both the individual’s identity and the nature of body fluid can be identified at the same time. Age of stains which obtained from crime scene can provide significant information about order of actions or relationship of stain with the crime. At the same time, estimating the individuals age from biological evidences can provide valuable data. In one study, blood samples were investigated for age prediction and massive parallel sequencing technology was used for this purpose. Six age related miRNAs were tested and mean absolute error was found as 5.52 years for male, 7.46 years for female [10]. By increasing the number of tested miRNAs and sample group size, promising results can be obtained.
In another study, a team worked with both young and old donors and they found that most of the miRNAs (total 800 miRNA was investigated) are decreasing with age from 800 investigated miRNA profiles [11]. Organ tissue identification is another promising field in the application of miRNAs in forensic science. Especially in violence related crimes, identification of organ tissues can be quite valuable. In order to identify the crime tool, this application may be used because during violence related crimes, tissues of internal organs may scatter to crime tool or crime scene. In addition to verification of crime tool, relocation of dead body can be identified in the case of organ tissue findings [8]. Robustness of miRNAs to degradation may be helpful in extreme conditions in the event of relocation.

In addition to these topics, estimation of post mortem interval (PMI) is one of the research area of forensic miRNA studies. PMI represents the time interval passed from the death. Like in other areas, high stability and durability of miRNAs when compared with other biomarkers made research groups to think as ideal biomarkers for PMI estimation [8]. Determination of PMI especially important in bodies which are in unrecognizable states. In this case miRNA analysis in skeletonized remains may be helpful to determine PMI. In one study, expression level of two specific miRNAs in bone tissue with PMI was associated. With the increasing PMI, negative correlation was monitored with expression of miRNAs [12]. Another interesting study related with application of miRNAs in forensic science is lesion assessment in hanging cases. In this study, miRNA profiles of skin samples belong to bodies died because of hanging were analyzed [13]. When compared with control group, significant differences were observed with inflammatory response related miRNAs. By increasing the sample group size and investigated miRNA number, more reliable results may be obtained and time of hang marks (before or after death) may be determined. Like in many scientific fields, molecular markers are becoming more important. If more accurate results may be obtained, miRNAs can be used for determination of vitality in hang marks in the future [14].

Another study area related with application of miRNAs in forensic science is wound age determination. In one of the review articles, it has been indicated that wound vitality studies are low in number. In order to differentiate antemortem and postmortem wounds, researchers stated that role of miRNAs in inflammation should be understood in depth [15]. In terms of forensic pathology, age of the wounds is one of the most important questions which should be answered. Studies about use of biomarker in this area are premature. With the collection of adequate and reliable samples, more accurate and helpful studies may be achieved [16].

Because miRNAs are useful biomarkers thanks to their features, brain injury diagnosis is one of the research areas which miRNA studies are conducted. In one of the studies about brain injury, specific miRNAs were found related with cocaine consumption, age related cognitive impairment and ischemic damage [17]. The study group stated that various biological samples could be investigated in order to obtain more informative results. Additionally, larger sample size could be beneficial too. By using this technique, determination of brain injury may be achieved by molecular techniques. In another study, researchers mentioned that miRNAs are promising biomarkers for forensic applications but obtained results are still insufficient for necessary specificity for traumatic brain injuries [18].

Another interesting study related with application of miRNAs in forensic science is detection of anabolic androgenic steroid (AAS) abuse. In the case of AAS abuse, some adverse effects can be observed in different systems (e.g., decreased glucose in endocrine system, irregular menstrual cycle in female reproductive system, and so on) in our body. These adverse effects lead to organ damages. As a result of organ damages, dysregulations of miRNAs occur. In the forensic concept, these dysregulations were investigated. Some important features such as high stability in tough conditions and long detection time of miRNAs make them potential anti-doping testing method [19]. In one of the systematic reviews, it has been mentioned that use of miRNA for identifying AAS related sudden death cases are low in number. They stated that this application not only increase the reliability of diagnosis the death cause but could support the scientific field for revealing the AAS abuse related conditions [20].

Academic Research Studies Conducted in Türkiye

When academic research studies conducted in Türkiye are examined, according to YÖK National Thesis Center [21], miRNA studies are mostly conducted in medical field especially for treatment aimed usages. In addition to these, there are studies which examines drug and alcohol addiction relationship with miRNAs. When forensic science related studies are searched (miRNA studies were filtered according to research area as 'forensic'), there were only two theses. One of them is about body fluid identification and the other one is about investigation of miRNA expression profiles in their post mortem brain tissues of people who are MDMA addicts.

Limitations of MicroRNA Applications in Forensic Sciences

Like every forensic analysis, degraded and/or mixed samples are the most challenging issue in miRNA analysis. When compared with other biomarkers, miRNAs’ short sizes can be advantageous in this case. In addition, second limitation is insufficient funds in this area. Because DNA profiling is one of the most used techniques in forensic sciences, RNA technologies are approached cautiously. Moreover, use of novel technologies such as new generation sequencing (NGS) for investigating the miRNAs is costly unlike conventional methods. As parallel to funds, expertise on RNA profiling is inadequate when compared with DNA profiling. Lastly, sufficient information about miRNA damage did not be gathered unlike DNA damage until now [8].
CONCLUSION

As seen in literature, miRNA studies are novel study area when compared with DNA studies. With the correct standardization and validation processes, number of trustable miRNA biomarkers can be increased in the future. Additionally, developing technology in our time should be evaluated in this research field. By the evaluation of novel technologies, unknown information can be obtained and/or known information can be understood deeply. RT-qPCR which is a RNA profiling method used widely right now, can be replaced with the novel methods as new generation sequencing (NGS) technologies when proper conditions are supplied. NGS is a costly technology but when compared with other sequencing technologies, it provides huge amount of data. In the case of miRNA studies which has many unexplored features, NGS technology can be very useful. Very efficient results which obtained from NGS can tolerate the cost of it. Because biosynthesis pathways of miRNAs are not unique, undiscovered features of them can be lightened by new technologies. With the addition of specialists in this field, analyses can be performed more accurate and faster.

Being tissue specific characteristic of miRNAs make them potential biomarkers in body fluid identification but except from this purpose they have potential in other forensic applications such as organ tissue identification, stain age determination, wound age determination, post mortem interval (PMI) identification, prediction of an individual's age, lesion assessment in the case of strangulation, brain damage identification and determination of anabolic androgenic steroids abuse.

With the increasing number of studies in this research fields can provide very useful information in forensic sciences. Additionally, as mentioned in body fluid identification, simultaneous extraction of both DNA and RNA may be very useful technique in forensic sciences. Because forensic evidences obtained from crime scenes are usually very limited in size or degraded, useful information can be acquired by using this method. Application of simultaneous RNA/DNA extraction into routine can be very beneficial for forensic purposes.

In conclusion, microRNAs are very significant biomarkers in legal medicine and forensic sciences fields. In addition to conventional methods, they can be very supportive and information providing methodologies. Especially in the case of inadequate results which obtained with using conventional methods, accurate results may be obtained by using molecular based methods (for instance miRNA biomarkers) in the future.

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Psychotherapy of post-traumatic stress disorder in the context of process and outcome studies

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Abstract

Therapeutic methods and techniques are quite diverse in the treatment of cognitive, emotional and behavioral problems that emerge as a result of traumatic experiences. Post-Traumatic Stress Disorder (PTSD) is a syndrome often triggered by extreme traumatic events. PTSD has been studied with various groups using different therapy techniques. The aim of the current study is to systematically review the extant literature on PTSD with a focus on efficacy of distinct forms of therapies including cognitive behavior therapy (CBT), eye movement desensitization and reprocessing (EMDR), group psychotherapy integrated with CBT (CBGT), hypnosis, cognitive behavioral writing therapy (CBWT), virtual reality exposure (VR), also group music therapy, narrative revealing therapy (NET). This article presents a review of the literature on effectiveness of various some therapies widely used in treating PTSD in studies with process and outcome research designs. It includes discussion of methods and results of studies as well as suggestions for future research avenues.

Keywords: Post-traumatic stress disorder, Process and outcome research, Psychotherapies

INTRODUCTION

The history of PTSD diagnosis and treatment approaches dates back to the late 1800s and early 1900s. Pierre Janet was the first psychologist to formulate a systematic therapeutic approach to post-traumatic psychopathology and to argue that treatment should be adapted to the distinct phases of post-traumatic stress reactions. Developing an eclectic treatment approach based on clinical experience for patients with hysterical (dissociative) or psychasthenic (obsessive-compulsive) post-traumatic stress symptoms in the early 1880s, Janet’s publications spanned 50 years and focused on the treatment of PTSD [1].

Exposure to war, sexual assault and other types of traumas can cause traumatic stress syndromes. These criteria used for diagnosis in Diagnostic and Statistical Manual of Mental Disorders (DSM III) have led to further research seeking answers to PTSD. Consequently, research has focused on an array of traumas such as criminal victimization, sexual assault, natural disaster and exposure to war in detail. Furthermore, the empirical knowledge and conceptual adjustments produced by the studies conducted in this context have significantly improved the general understanding of PTSD and led subsequently revisions in the PTSD criteria in DSM III-R. The symptoms of PTSD were listed as the exposure to a traumatic event other than a normal human experience, having nightmares, flashbacks or repeated intrusive thoughts, avoidance, and hypersensitivity to stimuli reminding the trauma, all of which would necessarily continue for at least one month [2].

CITATION


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Continuous experience of the traumatic event, the tendency to avoid traumatic events, and high level of stimulation-hypersensitivity constitute the three main symptom schemes of PTSD in DSM-V. PTSD, which is approached with a single psychiatric diagnosis in terms of its origin, is a clinical case that can be explained by psychological processes rather than physical. Many psychotherapy methods have been developed for the treatment of the common complaints for PTSD and using process and outcome research studies. Process research usually examine how the counseling or psychotherapy processes, namely intervention styles and approaches, are structured. Whereas outcome studies focus on and examine the long or short-term changes that occur after the completion of the counseling or psychotherapy process [3-4].

Using document analysis method, I reviewed the existing literature up to 2017 of process and outcome studies, specifically cognitive behavioral therapy (CBT), eye movement desensitization and reprocessing (EMDR), CBT enriched group psychotherapy (BDGT), hypnosis, cognitive behavioral writing therapy (CBWT), virtual reality exposure therapy (VR), group music therapy apart from CBT and CBT-based therapies, and narrative exposure therapy (NET) which are widely used in the treatment of PTSD [5]. I aimed to create the pattern by making a connection between process and outcome research while explaining the methods used in these two types of research on PTSD using comparison. In the next sections, I focused on the level of effectiveness of therapies in the treatment of PTSD.

**Cognitive Behavioral Therapies for PTSD**

In the treatment of PTSD, Foa and Kozak drew attention to a context emphasizing cognitive processes and defined as fear constructs. The structures are separated from other mental structures by their emotional intensity and high number of stimuli. Moreover, they pointed out that the extreme sensitivity, tolerance and reactivity to fear stimuli, avoidance and resistance to change are the source of these structures. They suggested the method of emotional processing (transformation of memory that feeds emotions) for treatment of PTSD. This can be possible by initially transforming the cognitions that activate the fear structures and then weakening the connection between these structures. At this phase, emotional processing method suggests in-session habituation. In-session habituation or in-session experiences based on exposure to a fear-inducing situation cause the threatening memory to change, thus weakening the traumatic event and the cognitive context developed for it [6].

Traumas may evolve into cognitive schemas involving cognitive distortions and maladaptive beliefs about self and interpersonal life. Traumatic phenomena, in which emotions such as negative self-evaluation, guilt, helplessness, hopelessness, isolation, powerlessness, and anger cluster, can be easily integrated into the information processing model that enables the restructuring of underlying beliefs. Thus, the curative intervention is to be able to transform the schemas that lead to fear and avoidance into a different form via assimilation into new experiences containing information that is inconsistent with existing cognitions. In order to achieve it, effective therapeutic interventions such as recording automatic thoughts, examining hypothetical processes, and creating imagery (imaginary exposure) to identify core beliefs can be used [7].

The interpretation of the trauma and the deteriorated-decreased self-perception after the response to the traumatic event can negatively affect the behavior, lifestyle and human relations of the person. In this context, cognitive restructuring is the treatment approach that is recommended. Then, the treatment involves exposure to a harmless but triggering stimulus that does not actually cause trauma and continues with providing confirmatory information about this stimulus. Related to this Rothbaum and Foa developed a three-stage prolonged exposure therapy. The first stage involves exposure to stimuli that activate fear and in-session exposure to be monitored systematically. In second stage; repeated and prolonged exposure follows which is based on altering fear structures by initiating corrective information processing, thereby relieving anxiety. The third stage starts with in-session habituation and continues as inter-session habituation, all of which aim for reducing anxiety gradually [8-9].

In studies that focused on the effect of cognitive behavioral therapies (CBT) in the treatment of post-traumatic stress disorder, the specific contributions of the treatment components are not fully unveiled although CBT is among the most effective treatments for PTSD. However, prolonged exposure (PE) and cognitive restructuring (CR) were found to be highly effective in reducing PTSD symptoms. Imaginary exposure (IE) enabled the reprocessing of emotional information via prolonged activation of traumatic memories. Treatment applications that combine the two, rather than PE or CR alone, are found to be more effective [10].

In another study conducted on cognitive behavioral therapy (CBT), a three-stage prolonged exposure therapy, exposure to a harmless but triggering stimulus that does not actually cause trauma and then providing confirmatory information about this stimulus (cognitive restructuring-CR), creating treatment rationale by detailing and evaluating coping styles for thought fragments in trauma memory with cognitive interventions (cognitive restructuring-CR) in the form of CBT techniques, initiating in-session re-experiencing for cognitive restructuring and evaluation of avoidance behavior and traumatic memories in this process, approaching the stressful event in a more realistic way, using metaphors, in vivo or inter-session exposure are found to be highly effective in the treatment of PTSD [11].

Similarly, another study found that the treatment methods and efficacy of CBT as well as the interventions made in this context were quite effective in reducing PTSD symptoms. In this regard, Identifying the conceptualizations arising from client’s specific-distorted-dysfunctional thoughts, identifying the assumptions that impede compliance, identifying and testing them in which
the client can see them, and using the ABC method, which will be used to examine the mental process between the stimulus and the reaction with the client, forming therapeutic alliances, questioning hypotheses, and processing the information generated from the traumatic experience into the human mind as "fear constructs" in the form of verbal, behavioral and physiological responses through an exposure-based approach, using cognitive and behavioral processes and thus extinguishing the avoidance caused by cognitive distortion are the main focus and pillars of the cognitive behavioral therapy approach [12].

For treatment of PTSD, it is important to investigate new methods and approaches as well as proven techniques (e.g., exposure therapy). Acceptance and commitment therapy (ACT), as an alternative to cognitive-behavioral treatments that can facilitate exposure by reducing avoidance behavior, is a contextual behavioral therapy that targets the effects of symptoms in a wide spectrum of psychopathology on emotions, thoughts, memories and all other special experiences, avoidance efforts and suppression behaviors [13].

In another study, when the effect sizes of the treatments considered, one third of the participants did not respond to EMDR and CBT or stopped the treatments. Therefore, alternative treatments were needed in the treatment of PTSD. In this regard, Acceptance and Commitment Therapy (ACT), which is in the CBT group but has its own treatment methods, can lead to highly remarkable treatment. Thanks to its unique treatment methods, ACT indeed addresses processes that are not directly targeted by other CBT treatments. In addition, it does not focus on the form and frequency of inner experiences, but rather on their effects on behavior. ACT uses mindfulness-based cognitive therapy techniques to increase the quality of the individual’s life. While it uses acceptance and mindfulness procedures for operation of the emotions, thoughts, and bodily functions, it uses traditional behavioral procedures for behavior changes [14].

Since repeated exposure therapies in a classical style do not meet the expected level of clients' participation, maintenance and completion of therapy, habituation-focused exposure therapies, which are structured on the emphasis on anxiety, fear and related reactions are not normal, are found to cause some of the clients to focus more on anxiety-causing stimuli and to develop further avoidance behavior or consequently to abandon the treatment altogether. Therefore, considering that experiential avoidance is one of the most key factors forming the psychopathological case, acceptance and determination therapies (ACT) which aim to plan behavior in line with mindfulness, acceptance and values, are promising to eliminate some of the drawbacks of other therapies [11].

**Eye Movement Desensitization and Reprocessing**

People are born with an information processing system which stores experiences through physiological processes. The purpose of storing information is to ensure the continuity of an emotionally healthy life. However, with the deactivation of this system, pathologies can occur. The process of re-accessing information and processing that information causes neural changes in the balance between the stimulating and suppressive systems of the brain, and this distorted balance can be re-established with eye movements for the recovery. This treatment, which is based on the monitoring of therapist's bilateral hand movements while focusing on traumatic internal representations, is the Eye Movement Desensitization and Reprocessing (EMDR) method developed by Shapiro [8].

Shapiro structured the EMDR process on a three-step protocol, namely a) past experiences and their manifestations that underlie the pathology, (e.g., nightmares, physical sensations), b) conditions that trigger or exacerbate the current situation, c) establishing and incorporating templates for appropriate future actions. This protocol is integrated into an eight-step treatment approach. These stages are: 1. History taking, 2. Preparing the client, 3. Processing the traumatic memory-experience, 4. Desensitization, 5. Recollecting the target memory without any distress, 6. Body scanning; probing whether there is a physical tension, 7. Closing: When faced with new feelings, thoughts, memories and dreams, focusing on these and current feelings, 8. Re-evaluation [15].

In one phenomenon study, an inmate who felt responsible the miscarriage of her wife, having nightmares and attempted suicide was studied to find out the effectiveness of EMDR in the treatment of PTSD in a correction facility. In order to monitor and evaluate the case scales were used, and evaluated four times as pre-therapy, after therapy, one month after, and finally four months after the therapy. There were five sessions in total. In the first session, inmate whose positive cognition was rated 4 (of 1-7 scale) was asked to focus on the worst memory, and to express the feelings and bodily sensation in the process. Moreover, inmate was asked to track the therapist’s hand your movements with his eyes, while the sensations and expressions were evaluated. As reached to stated high goodness in 6-7 scale, positive cognitions were in the focus and then continued with a new EM application. The application that lasted in total of 60 minutes sessions that are consecutive, finalized with body scan. In the three-month follow-up appointment (5th session), according to the monitoring and clinical evaluations, improvement was seen in recurrent nightmares, anger crises and complaint related to symptoms, and the inmate expressed to join new fun activities to brought joy [16].

In Kitchiner's study, nature of the prison environment, genetics predispositions, unable to adapt to new situations, real life trauma and loss can escalate the anxiety symptoms and disorders in individuals. As method that improves the cases in brief time, EMDR is an effective technique in controlled environments such as prisons. Therefore, EMDR can be used to improve the staff’s competency, to empower staff, to improve equipment and to address inmates’ needs [16].

EMDR was used in another clinical case for the PTSD’s...
treatment observed after traffic accidents. In the first stage, the trauma history of the case was collected and information related to EMDR was provided. EMDR has been initiated on images of the traffic accident and the moment of the accident; the moment of being removed from the vehicle, the images of his relatives at the moment of the accident and the accompanying thinking styles of "I am in danger, I am guilty, I am lonely". In the first two sessions of the EMDR treatment, it was found that the client's complaints decreased to a great extent, the tendency to avoid and self-blame over time, the anxious cognitions created by the belief against the bad things that he and his relatives could experience disappeared and were replaced by a positive statements that “it is over, done, it was not my fault” [17].

In EMDR, the information stored in the memory as images; the sensations and perceptions of the traumatic event were reprocessed and desensitization was achieved to the stimulus, the source of stress. Over time, new positive and adaptive cognitions are tried to be developed. Thus, appropriate attitudes and approaches have replaced the dysfunctional responses arising from “blunt thought patterns.” In the case as the focus of the study, negative thought patterns that first developed due to traumatic memories and were stored in the memory were reprocessed, after the depersonalization, an improvement was observed in destructive thoughts about the self through the development of positive-functional cognitions. [17].

In a similar study, a case study was conducted with therapist-client dialogues through detailing the first session of EMDR. The condition of the person who had a history of traffic accident and lost his father met the DSM IV-R diagnostic criteria and was determined as PTSD according to the results of the Post Traumatic Stress Disorder Scale (PTSD-S). At the end of the treatment, which was combined with homework and lasted for six sessions, the PTSD-S score, which was 99 at the beginning, decreased to 14 in the post-treatment evaluation. It was observed that there were significant decreases in re-experiencing, avoidance, blunting and evoking, which were frequently observed before. Moreover, complaints such as repetitive dreams, inability to remember some aspects of the traumatic memory, alienation from people, limited affect, sleep problems and anger outbursts, difficulty in focusing, and hypersensitivity were completely cured. It is important to support case studies with proven efficacy with EMDR with literature. EMDR is an effective therapy option for PTSD, which takes a brief time to learn and apply, and results can be obtained in a brief time [18].

CBT-Based Alternative Therapies for PTSD

In this part of the research includes studies on CBT-enriched hypnosis, CBT-Based Group therapy, Cognitive Behavioral Writing Therapy, and Virtual Reality Exposure Therapy for PTSD.

Hypnosis, which is not defined as a stand-alone psychotherapy, is a treatment method that can be used together with different psychotherapy approaches and increase the effectiveness of these therapeutic processes. It can take place in psychoanalysis as well as in behavioral interventions. The knowledge and experience of the therapist in this regard is important in the success of hypnosis. Another key factor in hypnosis being a supportive treatment technique is the client's potential for hypnosis. Özer and by Özmen (1999) conducted a study in which they provided the effectiveness of the hypnosis on a case with a trauma history. They defined treatment groups with high levels of hypnotizability potential. Their study results support literature regarding hypnotherapy’s effectiveness on PTSD cases [19].

Previous research has examined the relation between hypnotizability and dream power and found direct correlation between the indicators of PTSD and hypnotizability and dream power of indicators. The likelihood of high hypnotic predisposition in PTSD cases were linked to dissociative symptoms. Dissociation was regarded as defense mechanism that was developed during or after a trauma. Relevantly, hypnotherapy was found to be effective in the treatment of the PTSD like other dissociative cognitions, and these dissociative cognitions can be positively reconfigured via hypnotherapy [19].

Along with medication, five sessions of hypnotherapy were conducted with a 23-year-old female whose vital life functions were deteriorated after being fired abusively and in a threatening manner by bank management. In the first session, anxiety was alleviated and relaxation followed it. In the second session, the things she avoided and feared were repeated over and over and the things she avoided were administered with a controlled manner. She was suggested that she could do things she feared without hypnosis, and behaviorist treatment methods were used for the development of possible fear related avoidance in post-hypnosis. In the third session, experiences were planned in a less-controlled environment to face her fears. In the 4th session, suggestions were given to go to sleep smoothly and to sleep without dreaming, and taught autohypnosis in order to sleep seamlessly at home. In the fifth, the last, session, other processes in previous sessions were repeated and she did not have any of her previous complaints or reported any further complaints. Moreover, in the re-evaluation made after one year, she maintained her well-being which aids to the effectiveness of the hypnosis in the treatment of PTSD [19].

Although there is no generally accepted theoretical explanation about the processes/mechanisms of hypnosis as a specific treatment method, it is seen that the literature clearly supports the clinical effectiveness of hypnosis in treatment of PTSD. Hypnosis can also be used in combination with many treatment methods such as psychoanalytic- psychodynamic therapy, behavioral therapy, cognitive behavioral therapy, EMDR, ego state therapy. There are empirical studies showing that any treatment combined with hypnosis is clinically more effective than the same treatment without hypnosis [20].

An experimental study that focused on the long-term impact and effectiveness of CBT only versus CBT combined with hypnosis and other supportive consultations in preventing evolution of
acute stress to PTSD, recruited 30 female and 28 male participants with a few weeks of trauma history, who met autism spectrum disorder (ASD) criteria, experienced non-sexual attack and involved in motorized vehicle accident. In order to evaluate the participants’ status 6 months and 3 years follow-up periods were scheduled. PTSD Scale (CAPS) for research treatment post and follow-up evaluations, ASDI (structural clinical interview based on DSM IV criteria ) for ASD diagnosis, impact of event scale (IES), Beck Depression Inventory, state-trait anxiety inventory (STAI), Stanford scale for hypnosis susceptibility (SCHS) were used. Sessions were spanned to eight weeks with once a week 90-minute therapy which consisted of cognitive therapy, anxiety management, imaginary and in vivo exposal. During CBT/ hypnosis, there were 15-minute-long hypnotic induction tapes before each hypnotic exposure session along with CBT procedures. This tape included suggestions for focusing, muscle relaxation exercise, immersion-concentration suggestions [21].

CBT integrated hypnosis was found to be significantly more effective than supportive counseling (SC) in reducing symptoms, more effective than CBT alone in re-experiencing symptoms after treatment, but there was no significant difference at 6-month follow-up. It was reported that no significant difference was observed in the 3-year follow-up evaluations. In this research, hypnosis was limited to being only a stage of imaginary exposure. Anxiety management was used in combination with cognitive therapy and prolonged or in vivo exposure therefore, limited use of hypnosis may not have influenced its effectiveness. However, the most significant finding from this study is that the early provision of CBT within a few weeks after trauma is highly effective in improving PTSD symptoms [21].

Imaginary exposure is highly effective in the treatment of PTSD, as it allows the patient to confront and reprocess the emotions and memories that have been avoided about the trauma. However, many patients can be unwilling and unsuccessful in producing and re-experiencing painful images on their own. In a case study, status of a patient in this group who developed acute PTSD as survivor of the September 11, 2001, World Trade Center attack was evaluated in a structured clinical interview for the PTSD scale (CAPS), DSM-IV. A trauma history checklist was used in clinical interviews. Beck Depression Inventory and Post Traumatic Diagnostic Scales were used for standardized self-report measurement. These measurements were made one week before the beginning of Virtual Reality Exposure Therapy, and self-report measurements were repeated after each session of the treatment. The client showed emotional bluntness, limitation in emotional transfer, rejecting the situation he is in, speaking in a monotonous voice, avoiding all kinds of stimuli (newspaper news, TV, high towers, etc.) related to the event [22].

After having received four sessions of imaginary exposure therapy but did not show any change in the measures of PTSD and major depression, patient was included in virtual reality exposure therapy (VR). Over the course of six one-hour VR exposure sessions, the patient was exposed to slowly and systematically into virtual planes flying over the World Trade Center, jets crashing into the World Trade Center with animated explosions and sound effects, images of individuals’ deaths due to burning buildings, collapsing towers and dust clouds. A decrease in PTSD symptoms was observed in the self-report measurements made after each session of the patient who underwent six VR sessions. In the measurements made by an independent evaluator after completion of treatment, the individual did not meet the diagnostic criteria for PTSD, major depression, or any psychological disorder, thus systematic exposure therapy with VR was successful in reducing acute PTSD symptoms [22].

Difede and Hoffman’s research showed a major reduction in depression and PTSD symptoms after completing virtual reality exposure therapy, as measured by the Beck Depression Inventory and the Clinician-Applied PTSD Scale with 83 % decrease in depression and 90% decrease in PTSD symptoms. These strong findings reveal that virtual reality exposure therapy is promising for the treatment of acute PTSD although the case reports are not scientifically certain due to its nature. In an experimental study based on the implementation of a VR exposure-based software program, the survivors of the civilian bus bombing in Israel were treated with exposure therapy based on the illusion of entering a computer-generated virtual world in the treatment of their PTSD, and effectiveness of VR therapy was studied [22-23].

In this research, subjects wore a screen made of VR devices designed to give the illusion of standing on a virtual sidewalk, next to a cafe, opposite a bus stop. On the screen, the virtual simulation of the bus bombing event is given in an increasingly real and progressive manner. The images given to the people are controlled from the keyboard by a therapist who has received special training for it. The most advanced stage includes scenarios consisting of explosion, sound effects, burning bus including screams and real photos of the event. The program consisted of 10 sessions, each lasting 90-120 minutes. The treatment program includes training about common responses to trauma (1st and 2nd sessions), breathing exercises (1st and 2nd sessions), in vivo exposure (2nd through 10th sessions), VR exposure to the traumatic event (3rd and 10th sessions), watching DVD and in vivo exposure assignments for at-home VR practices [23].

A treatment protocol was created to assess the effectiveness of the treatment process, and tools such as PTSD Scale (CAPS), DSM IV diagnostic criteria, Post Traumatic Diagnostic Scale (PDS), Beck Depression Inventory (BDI) were used in the treatment protocol. The analogue study of the research, which was developed via VR techniques based on original photographs, was completed by exposing 30 non-symptomatic people to various levels of bus bombing footage. Moreover, as a preliminary test of the program’s effectiveness, 5 people who developed PTSD as a result of their exposure to the suicide bus bombing were selected as participant for treatment. The study presented a VR-based therapy program demonstrating the effect of VR exposure therapy in reducing PTSD symptoms [23].
The purpose of cognitive behavioral writing therapy (CBWT), which is used to reduce the symptoms of PTSD by confronting with memories of the traumatic event, is to integrate the typically fragmented, gap-filled memories of traumatic experiences into a coherent narrative and to build a habit of emotional response to reminders of the traumatic event. With aim of determining the effectiveness of therapy (CBWT), an experimental study was conducted with 23 children (8-18 years old) who experienced a series of single and repetitive traumatic experiences initially in the Netherlands. A CBWT treatment protocol was prepared, a guidebook specially developed for the treatment was followed, and a series of scales were used to report the pre-test-post-test application and statistical analysis of the results for the selected sample group. These scales were the Child Behavior Checklist (CBCL), which was given as both a pre-test and post-test, and the Children's Response to Trauma Inventory (CRTI), which was used as a self-report form [24].

The sample group of the study consisted of children aged 8-18 years who were referred to a community mental health clinic in the Netherlands. After getting informed consent from the parents, interviews were conducted with both parents and children using the Anxiety and Related Disorders Interview Schedule (ADIS) parent-child version for the diagnosis of PTSD disorder. Children with IQ 80 and below, meeting different diagnostic criteria or living in a disrupted housing conditions were excluded from the study. The criteria for inclusion in treatment for each child were discussed in detail by a multidisciplinary team of psychologists, social workers and psychiatrists [24].

Before the CBWT begin, all therapists received specific training by a licensed CBT therapist (first and second authors) with a therapist with 20 years of experience in the field. They also collaborated with these individuals to evaluate each session. The therapists followed a guide covering the treatment procedure and traumatic cognitions as determined by the pediatric version of the Posttraumatic Cognitions Inventory developed during therapy. The CBWT guide content that includes a general treatment outline and specific sections (abuse, loss of a loved one, having a parent with a psychiatric disorder, etc.). The most crucial elements of treatment are psycho-education, exposure, cognitive restructuring, coping, and social sharing [24].

The treatment procedure was adjusted according to each specific child. Older children drafted the story on computer by themselves, while younger ones received the help of the therapist for writing. Child participants defined the most traumatic event and narrated this event in detail with the therapist. During the treatment, the traumatic event narration was shaped as story. The story began with introduction of the child and followed by child’s feelings, thoughts and behaviors during and after the traumatic event. In each stage, story included cognitive to distortions and cognitive restructuring related content, and Socratic questioning, exposure and verbal cognitive restructuring techniques used on the story. At the end of story, child and therapist developed coping strategies (e.g., what should I do when I encounter my abuser?), and these were included in the story. The next step was social sharing. Child and therapist decided on whom to read the story with appropriate criteria, and the story was read by chosen parent or any adult where child was not present [24].

This study evaluated the potential effectiveness of short, computer-assisted, cognitive-behavioral writing therapy for children with PTSD. The study yielded promising results: with an average of just 5.5 sessions, a significant reduction in children's existing PTSD and depressive symptoms was seen, and this effect was sustained at six months follow-up too. The effect size on the PTSD outcome measure was quite significant. The results showed that CBWT treatment can be performed in a clinical setting, is effective in a wide-range of ages (8-18) and in a comprehensive-wide context that includes specific episodes (e.g., abuse, loss of a loved one, having a parent with a psychiatric disorder, etc.) [24].

Moreover, empirical studies were reviewed to find out the effectiveness of cognitive behaviorist group therapies designed for individuals to focus on their current problems via benefits of group life on treatment of PTSD. Although there are studies focusing on the effectiveness of group therapy regarding treatment of PTSD, there is not enough study on the effectiveness of group therapies that are based on CBT. The studies on CBGT are fairly new covering recent six-seven years, increasing every year, effective in treatment of psychological distress arising in post-trauma, and useful on anxiety and depression that is common in PTSD. Moreover, the effects of CGBT continues even after group therapy and more effective than CBT [25].

In the reviewed literature, effectiveness of the setting and criteria such as therapy duration, number of therapists, session count on the treatment is incredibly difficult as the application process and details of the therapy are limited. Moreover, it was found that there are very few studies that classified the groups according to types of trauma, and the therapy types differ greatly between the studies [25].

**Other Effective Therapies for PTSD**

In this part of the study, the process and outcome studies of music therapy and narrative exposure therapy used for the treatment of post-traumatic stress disorder are included.

Music therapists have observed that music, with its accessibility feature, has the potential to evoke traumatic memories in the discussion and processing of the past, and the traumatic associations that arise with music are gradually reduced, especially in a therapy process based on group experience. Group music therapy is a social process that addresses the avoidance behaviors of patients with PTSD. Musical improvisation requires active participation not only in music but also in others’ music and lyrics. Music therapy addresses overstimulation in the physical, cognitive, and emotional domains. Hypersensitivity and startle responses can be regulated by promoting tolerance to silence and
of disconnectedness and contextlessness of numerous implicit experiences, and thus enables remembering the traumatic event to acknowledge the network of interconnected emotional biography with a consistent and step by step process helps ignoring chronological impacts. Working on an individual's complicates the complex context by oversimplifying and choose the worst can not only causes ethical issues but also other events during reexperiencing the trauma and aiming to situations in their authentic real life with a chronological order. Re-procession of the traumatic event via reimagining is done in this phase [27].

Narrative exposure therapy consists of three main phases. The first phase (one or two sessions, 90-120 minutes each) includes creation of event related control lists and doing a short psycho-training, which addresses trauma memory and symptoms. In the second phase, life is on the focus with a chronological order. The third phase is 4-12 sessions and aims for creating a biography with a chronological order related to trauma. Re-processing of the traumatic event via reimagining is done in this phase [27]. It was found that narrative exposure therapy is a robust treatment for individuals in real life and clinical environment as an in-patient or out-patient, for traumas with high levels of stress related to sexual and physical exploitation in childhood, immigration, suffering from political violence and torture, natural disasters. Trained counselors, psychologists, psychiatrists, health care workers can administer narrative exposure therapy in a relatively brief time period by reprocessing the memory via emotional re-exposure and narration of trauma [27].

CONCLUSION AND SUGGESTIONS

In light of the studies reviewed in this literature, I found that CBT and EMDR in treatment of PTSD were the most effective techniques in rehabilitation of dysfunctional thoughts, emotions, avoidance and decrease in life quality caused by trauma. CBT in experimental and comparative studies and EMDR in case studies were found to be effective in treatment of PTSD. In some cases, the concept of exposure, the emphasis on abnormality of reactions rooting from anxiety and fear impacts the expected engagement and involvement of patients in the therapy. Thus, acceptance and commitment therapy that uses mindfulness-based cognitive therapy techniques can give more effective results in treatment by easing the re-exposure and increasing the life quality via decreasing avoidance behavior. It was found that cognitive behaviorist therapy is found to be more effective when combined with hypnosis and group therapy unlike just using CBT. While group therapies for treatment of PTSD is common, group therapies based on CBT is significantly rare. Indeed, healing process can be faster and subsequent when CBT techniques and strong dynamics of group experiences used in combination. The studies that explained the structure and the contents of the
sessions are quite limited as the research focusing on PTSD via cognitive behaviorist group therapy is scarce. In this regard, a new and original study that aims to focus on particularly to process of the therapy can be planned to reveal the results of such process. There was only one experimental study that used CBWT and found effectiveness of using CBWT on treating children with PTSD. This relatively new study is promising for the future of the research direction in this matter. Experimental studies showed that exposure to virtual reality (VR) is effective in treatment on those individuals with no reaction to exposure therapy.

Music therapy, as an alternative to CBT and CBT based therapies, is nonverbal, life focused and effective technique which can decrease stimulation sensitivity via sound and harmony for the PTSD treatment. Music therapy can be implemented in experimental studies to examine the effectiveness in PTSD. In narrative exposure therapy (NET), patients are encouraged to keep the relation between now and here while reexperiencing the emotions while narrating the traumatic events in an historical order. NET decreases the avoidance and supports the restructuring of emotional cognition. In this regard, NET is one of the effective technique to treat PTSD that can be used in future studies. Considering the variety of PTSD cases in Turkish context, an experimental study design with implementation of the techniques reviewed in this paper can be unprecedented in in Turkish context.

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References
Cyber dissociative experiences and mass consciousness control: The age of cyber dissociation from the perspective of dissoanalytic theory

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Abstract
The mass control-oriented dynamics of digital communication networks and social media applications almost impose on individuals "adaptation by dividing" through cyber dissociative experiences. According to dissoanalysis developed by Ozturk as a modern psychotraumatology theory, individuals are forced to adapt by experiencing dissociative defenses on the axis of a traumatizing "hyper-digital stimulation". Successive dissociative defenses have brought "cyber dissociative experiences", "cyber alter personalities", "multiple memory systems" and "multiple consciousness systems" that individuals and societies are now controlled and even managed by dissociogenic digital network platforms. With the dominance of cyber communication over face-to-face communication, these cyber communications have begun to be perceived as more real by the maximal proportion of individuals. In this dysfunctional process, cyber alter personalities of individuals take control and transform cyber dissociative experiences into cyber dissociation. Today, "hysterical cyber blindness" that occurs after chronic cyber traumatization both interrupts the consciousness of individuals and causes them to be controlled by dissociogenic digital network platforms and social media applications. Ozturk's detailed and systematic scientific studies, emphasizing that digital communication networks and social media applications are used as a mass consciousness control strategy by oppressive systems and dictators, have brought the field of "dissoanalytic cyber psychology" to the fore. According to the dissoanalytic theory, it is only possible for cyber societies, which are managed by establishing oppression and control, to break their ties with their dominating systems through "dissociative revolutions". In the age of cyber dissociation, with the realization of dissociative revolutions, a development-oriented, creative, compassionate, fair and prudent new human and society profile is constructed by providing the psychosocial consciousness alliance of the masses. Cyber dissociative revolutions function through cyber dissociative experiences at the global psychodigital intersection of multiple consciousness and memory systems that emerged as an existential reaction against oppressive societies.

Keywords: Cyber dissociative experiences; cyber alter personality; theory of dissoanalysis; the age of cyber dissociation; mass dissociation; dissoanalytic cyber psychology; mass consciousness control; cyber traumatization; denial trauma; dissociative revolution

Theory of Dissoanalysis, The Age of Cyber Dissociation and Mass Consciousness Control

In oppressive societies, digital communication networks and social media applications are used to provide the mass consciousness control and almost impose cyber dissociative experiences on individuals. Anonymous dynamics, psychodigital delegations and mobile components that prevent individuals of modern society from being authentic and individualized cause a new dissociation phenomenon, defined by Ozturk as "cyber dissociative experiences", to be experienced by the masses, and digital communication networks function as a dissociogenic agent in this psychopathogenic process. According to the
theory of dissoanalysis, all societies of the world are now both controlled and managed through digital communication networks and social media that cause cyber dissociative reactions. Today, traumatized societies of the digital age, which encounter “cyber dissociative experiences” at maximal proportions, have now shown a psychosocial transformation and even started to create direction-oriented new human and society profiles. The “Age of Cyber Dissociation” began with the reign of the directed psychodigital focuses, which are closely related to the submissive identities of the new human and social profiles that emerged between the years 2000-2020, far from originality and creativity. Unless dissoanalysis of traumatized individuals, dissociogenic systems, societies controlled by oppression or cyber masses can be carried out, no person or nation can get rid of its violence-oriented and borderline psychopathogenic components, or even gain an orientation towards a developmental and integrative life organization. Ozturk’s Theory of Dissoanalysis is the whole of multidimensional scientific efforts, effective psychotherapy practices, and strategies to prevent short and long-term psychosocial traumatic experiences to end both intergenerational transmission of trauma and intergenerational transfer of psychopathology. In this context, dissoanalysis is a psychotraumatology theory and psychocommunal therapy that includes functional, interactive, and integrative psychotherapy methods. The dissoanalytic school, which also includes current psychohistory paradigms, importantly emphasizes that traumatic experiences are accompanied by dissociative reactions. According to Ozturk, the main purpose of dissoanalysis in terms of psychohistorical perspective is to create integrative individuals and societies open to development [1-5].

Dissoanalytical cyber psychology begins with Ozturk’s "Cyber Societies and Cyber Lives: Digital Communication Networks as a Dissociogenic Agent" study in 2020, which clearly emphasizes that digital communication networks and social media applications are used as a dissociogenic mass consciousness control agent by oppressive systems. The digital age almost imposes “adaptation by dividing” on individuals with the promise or illusion of an apparently more functional life experience. Digital network platforms cause the experience of harmony-oriented dual lives by creating a separation between the original or real identity of today's people and their digital identity. Adaptation by dividing serves a dual function as one of the defense mechanisms that individuals in modern societies use quite often on digital network platforms. While individuals in cyberspace adapt by dividing on the one hand, they develop cyber dissociative psychopathologies on the other hand [1-3,6]. This "adaptation by dividing" brought along individuals' “cyber dissociative lives”, “cyber alter personalities”, “multiple consciousness systems” and “multiple memory systems” and transformed the existing traditional society structure into a cyber society structure on a dissociogenic ground. Cyber dissociative experiences, which function with interruptions in consciousness and memory, show a mobile and dual psychosocial movement characterized by identity transitions. In postmodern oppressive societies, the individual and mass-oriented control and management process is carried out by creating cyber dissociation through digital communication networks and social media applications which are dissociogenic agents. Digital communication networks and social media applications, which are dissociogenic agents, dissociate individuals and societies by exposing them to cyber traumatization and overstimulation. Ozturk states that cyber society profiles are created with cyber icons, abusive dictators, authors, or artists allied with the oppressive system, so-called scientists and incompetent politicians. According to the theory of dissoanalysis, cyber dissociative experiences come into play as a defense system against the anonymizing and disinhibiting effects of modern society and turn into cyber dissociation over time [1-4,6-8].

**Digital Communication Networks as Dissociogenic Agents, Cyber Dissociative Experiences and Cyber Alter Personality**

Digital communication networks and social media applications, which are dissociogenic agents, shift the focus of attention to individuals by keeping people away from reciprocal dual communications or society, or even by controlling them. Individuals and societies, whose focus of attention is shifted to themselves, can easily take on an obedient nature by moving away from empathy and absolute reality that they now act directed and involuntarily with their traumatized narcissistic selves as if they were the servants of their iconic totems. Individuals, whose focus of attention is shifted to themselves, quickly shift towards a psychological nature that is exhibitionistic, voyeuristic, assertive, competitive, megalomaniacal, and directed. When the ratio of this directed and weak-minded mass in a society to the average increases, wars become inevitable, and childhood traumas and dysfunctional family dynamics begin to be experienced at very high levels. In the dissoanalytic psychohistorical perspective, the only way to prevent wars is to end childhood traumas and violence-oriented negative child-rearing styles [1,2,5,9,10]. According to the theory of dissoanalysis, digital communication networks and social media applications cause very important psychosociopolitical transformations and developments of individuals in today's society, both in psychological, sociological and political dimensions, and both normalize cyber dissociative experiences that bridge and transition between clinical dissociation and dissociation of actual life by revealing new human profiles that differ considerably from individuals in the recent past. Today, maladaptive, and dysfunctional uses of digital communications cause cyber traumas and cyber dissociative reactions. However, normal individuals can establish associative bonds between their cyber and actual lives and can optimally integrate dual lives with these two different thinking and behavior patterns. Adapting to the age of cyber dissociation, which is changing at an extremely rapid pace, is quite difficult or even impossible for most individuals. In societies where childhood traumas, wars and oppressive systems reign, cyber dissociative experiences, even cyber psychopathologies, and cyber addictions are experienced at maximal proportions [1-3,11-
Denial Trauma, Mass Dissociation and Hystorical Cyber Blindness versus Dissociative Revolution and Psychosocial Consciousness Alliance

According to the dissoanalytic school, most of the people in cyber societies try to both exist and become individualized with cyber realities. With the dissoiogenic effect of digital network platforms, especially social media applications, individuals and societies have entered in the process of a rapid transformation, and they have become managed by the technology itself, which is the inventor of this transformation process, and they have even moved away from their real selves. In today's digital age, individuals show a dual psychosocial movement between their real and cyber identities with the effect of both childhood traumas and cyber traumas. This dual psychosocial movement causes a phobic avoidance between their cyber identities and their real identities, and over time, “cyber dissociative experiences” begin to emerge in these individuals. Cyber dissociative experiences initially develop as a defense mechanism that protects the psychological stability of individuals against the exhibitionist, spectator and ruthless nature of digital network platforms and social media applications and eventually turns into cyber dissociation, so that after this process, individuals have a separate “cyber life” and a “real life”. Individuals can generally make healthy decisions at the optimal stimulation level in their current lives and continue their lives in a psychologically integrated manner. Living a life below or above the optimal stimulation level of individuals drags them into a dissociated life. Especially the overstimulation in social media applications can lead to “cyber dissociation” in individuals. Cyber dissociation and cyber dissociative experiences are actually an effort to adapt to hyperdigital stimulations in this digital space. The individual, who lost his self-control and turned into a submissive object due to chronic childhood traumas, psychosocial oppressions, dysfunctional family dynamics and cyber traumas, experiences an intense “psychodigital dissociation” between his/her cyber life and his/her real life. In digital-oriented communications, traumatized individuals cannot maximally integrate their cyber life with their real life, and they split. After this splitting, cyber life on behalf of the individual is more real than real life, and even cyber life becomes real life itself. Oppressive systems and dominant leaders or dictators have imprisoned individuals and societies in an age of “self-sabotage” and “mass control-oriented” cyber dissociation [1,3,4,6]!

According to Ozturk, digital communications, which continue to function by causing interruptions in consciousness and memory, and even enable the emergence of multiple consciousness and multiple memory systems, function concomitantly with cyber dissociative experiences. The number of individuals who can control consciousness and memory interruptions in cyberspace has increased. However, in the age of cyber dissociation, where face-to-face communication is less preferred over cyber communication, individuals with borderline personality organization can become an object or even a “cyber victim”
of fusion communications characterized by disruptions of consciousness and memory, which are further increased by the effects of cyber traumas and cyber abusers they encounter in the online space. Individuals can establish different cyber lives with the need for an unconscious, semi-conscious or conscious dividing to get rid of their intense anxieties associated with the oppressive system in their current lives and to be apparently free, and then these divided dual lives can lead co-consciously or unconsciously, and these individuals either try to be more functional by dividing, or they believe or are made to believe that they can be more functional. In clinical interviews conducted by psychotherapists with people who have experienced cyber dissociative experiences, it has been reported that they stated that they were associated or integrated while splitting, which Ozturk defined itself as “associative dissociation”. Psychodigital dissociation, which exists in a conjugate nature with associative dissociation, can strongly transform into cyber dissociation [1-3,6,15,16]. According to the theory of dissoanalysis, it is not surprising to come across profiles of people who can be functional or even creative while being divided today. Individuals of the age of cyber dissociation have now begun to learn the methods of optimally coping with their cyber or real-life traumas that when cyber dissociation against cyber traumas is experienced at a certain level, it allows people to maintain their functionality and reveal their creativity. Cyber alter personalities, which can emerge with the effect of cyber traumas and chronic childhood traumas that start at an early age, are actually an intense longing for individuals to process their traumatic experiences and eventually integrate, as well as a harsh reaction and existential struggle against the abusive system in which they exist. Digital network platforms have a structure that can be controlled, monitored, backed up and even recorded, and this structure seriously hurts, traumatizes, and dissociates individuals [1-4,6,7]. Individuals’ preference of communication in cyberspace to face-to-face communication causes them to experience “hysterical cyber-blindness”. Due to this hysterical cyber-blindness, individuals fail to realize that they are the real ones controlled on digital network platforms and have to experience cyber dissociative experiences! Dissociogenic digital communication networks impose a denial-oriented life on individuals and societies. While cyber traumatizations continue to create cyber victims in the space from individual to society, cyber dissociative experiences exist and mass consciousness control is carried out by oppressive systems using digital communication networks. In cyberspace, individuals can be both “abusers” and “victims” at the same time, and they deny the identity of the “abuser” while being a victim and the identity of the “victim” while being an abuser. The phenomenon of dissociative denial, experienced as a “dissociative oscillation” process, turns into a denial trauma; and cyber dissociative experiences, in fact, refer to the denial trauma itself [1-5].

Today, individuals and societies characterized by cyber dissociative experiences have already learned and even adopted to continue their lives in an apparently functional way by using dissociative defenses against negative child-rearing styles, childhood traumas, psychosocial oppressions, and authoritarian but ambivalent parents. Oppressive systems and dictators have succeeded in creating a directed and global cyber society, and they both manage and control this cyber society through cyber dissociation. According to Ozturk, just as childhood traumas are hidden in violence-oriented negative child-rearing styles, cyber traumas and digital abuses are also hidden in digital network platforms through cyber dissociative experiences [1,3-6,17-20]. Now, every right or wrong fantasy can easily turn into reality on behalf of all individuals in every age group in cyber societies that move away from the optimal control focus of the family at an early age and are stuck in the psychodigital control focus. Digital communication networks come into play as a dissociogenic agent when these limitless dreams or fantasies come true easily. Cyber dissociative experiences or cyber dissociation are both a challenge and an adaptation effort and a search for freedom developed against all kinds of oppressive systems that take away the subjectivity of individuals and societies. Digital visibility and popularity in cyber societies has become one of the most indispensable addictions of today. In the name of digital visibility and popularity, absolute reality, psychosocial reciprocity, and personal privacy no longer matter. Cyber societies allow individuals to express and (also) hide their real identities, feelings, and thoughts whenever they want to. What an individual hides is everything he/she does not present and share in the cyber space, which is the sum of his/her true personality, his/her true self, and the parts he/she cannot change. Freedom in cyberspace is a total utopia, even a dystopia! The new world order in cyber societies is achieved through “dissociative revolutions” that emerge with the simultaneous development-oriented reaction of the masses. Dissociative revolutions are the actions of individuals and societies that have been controlled and managed by oppression and traumatization for many years to cut their ties with their fascist leaders or dictators and to liberate them, and with these actions, psychosocial consciousness alliance is provided, a new human and society profile is created with a development-oriented and prudent [1-3,5-7]. On the other hand, cyber dissociative revolutions take place through cyber dissociative experiences at the global psychodigital intersection of multiple consciousness and memory systems that emerge as an existential reaction against oppressive societies. Cyber dissociation, the most prominent psychological phenomenon of the digital age, functions as a cyber revolution that makes it possible for the multiple consciousness system to prevail against the utopia of the singularity of consciousness.

Conflict of interests
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